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Symposia I:

Fruit Maturation Management
A PLURIENNIAL EXPERIENCE IN CONTROLLING PEACH FRUIT RIPENING USING DIFFERENT NATURAL SUBSTANCES

Guglielmo Costa1, Anna Maria Bregoli1, Massimo Noferini1, Vanina Ziosi2, Stefania Biondi2, and Patrizia Torrigiani2

ABSTRACT

AVG and PA were applied to Redhaven peach and Stark Red Gold nectarine trees grown under field conditions. Several concentration and application times were tested over a four years period of time. Both AVG and PA controlled ethylene emission, delayed fruit flesh softening and fruit abscission. The other fruit quality parameters studied were affected as related to substances, cultivar and application schedule. The obtained results were also investigated and tentatively discussed at biochemical and molecular levels.

Additional index word: aminoethoxyvinylglycine (AVG), polyamine (PA), fruit quality parameters, NIRs, 1-aminocyclopropane-1-carboxylate oxidase (ACO), 1-aminocyclopropane-1-carboxylate synthase (ACS)

INTRODUCTION

Fruit ripening is a complex process that involves molecular and biochemical events culminating in dramatic changes in flavor, aroma and texture of the flesh. Through these changes, fruit reaches its overall quality and becomes acceptable for consumption. In peach, a climacteric fruit, a rise in ethylene biosynthesis and respiration rate signals the start of the ripening and the hormone plays a key role in regulating the process (Kende, 1993; Lelievre, 1997, Mathooko et al., 2001). However, the ethylene climacteric in peach is an event occurring late when the fruit has already consistently softened (Tonutti et al., 1997). For this reason, peach fruits exhibit a short storage life that limit their commercial potential and induce farmers to anticipate the harvest date. As a negative consequence for this excessive early harvest, the overall peach fruit quality is reduced and does not fulfill the consumer’s expectation. A deeper knowledge of the physiological bases of the ripening should allow to develop strategies to control this phenomenon on the tree and to slow down post harvest decay processes. This was the main objective of a four-year project supported by Italian MURST (Ministry of University and Scientific and Technological Research) which involved four different Universities (Bologna, Padova, Udine, Viterbo). In particular, the research focuses on the relationships between ethylene and evolution of some parameters defining quality (Bologna, Udine), on the constitution of a repertoire of genes involved in fruit quality determination (Padova) and on the setting up of non-destructive methods to evaluate peach fruit quality (Bologna, Viterbo). To reach this goal and improve fruit quality, substances able to interfere with ethylene biosynthesis were chosen as a tool to manipulate peach fruit ripening. Fruits were treated in pre-harvest with aminoethoxyvinylglycine (AVG) or with polyamines (PA). AVG is a naturally occurring fermentation product which acts as a reversible inhibitor of ACC synthase (ACS) activity (Lieberman, 1975; Boller et al., 1979). ACS is a key enzyme in ethylene biosynthesis as it converts S-adenosylmethionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC), the

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immediate precursor of the hormone. AVG is the active ingredient of a new chemical, commercially available as ReTain®, that in field trials was shown to reduce fruit abscission and to improve fruit quality (Byers, 1997; Greene, 2002; Vizzotto et al., 2002). PA are well-known regulators of growth and differentiation (Bagni and Torrigiani, 1992), and may compete directly with ethylene for their common precursor S-adenosylmethionine (SAM). Since these effects were mainly defined in model plant system growing in controlled conditions, the objective of this research activity was also to verify the practical possibility to control ethylene, fruit quality and harvesting time in whole trees grown in open orchard. In order to monitor the ripening changes on the same fruit sample in planta a NIRs (near infrared spectroscopy), a non-destructive system, was used.

MATERIALS AND METHODS

Plant material and experimental design. During the first year, experiments were performed on 15-years old trees of cv Redhaven (Prunus persica, L. Batch), grafted on PSA5 seedling rootstock and trained to a free open-vase, while in the following years, experiments were performed on 7-year old Stark Red Gold nectarine (Prunus persica laevis DC) trees grafted on seedling rootstock, trained to a Y-shape. ReTain® (ABG 3178, Valent Biosciences, Chicago, IL, USA), containing 15% (w/w) AVG a.i., was applied as an aqueous solution, containing 0.05% (v/v) of a non-ionic surfactant (Silwet L-77®, ABG-7011, Valent Biosciences) and the three aliphatic PA, putrescine, spermidine and spermine (Sigma-Aldrich, Milano, Italy) were dissolved in 50 mM Tris-HCl buffer, pH 7. Concentrations and time of applications tested are listed in Table 1.

Fruit growth curve was used to establish the timing of chemical applications and fruit sampling to monitor ethylene emission, quality trait modifications and biochemical and molecular analyses. Diameter and fresh weight (FW) of the whole fruit were measured during the entire growth cycle at weekly intervals on 10 untreated fruits (homogeneous for size, color and position on the plant). The first derivative of the diameter and/or FW of the whole fruit was used to discriminate the four different growth stages (S1, S2, S3 and S4) (Costa et al., 1986; Bregoli et al., 2002).

Ethylene determination. Ethylene evolution was measured by placing the whole detached fruit in a 1-litre jar sealed with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 hour. A 10-ml gas sample was injected into a Dani HT 86.01 (Dani, Milan, Italy) packed-gas chromatograph as described by Bregoli et al., 2002.

Quality parameters evaluation

The main fruit quality traits, flesh firmness (FF), dry matter (DM), soluble solids content (SSC) and flesh acidity, were determined at all sampling times in control and treated fruits. FF was measured using a pressure tester (EFFE.GI, Ravenna, Italy). Dry matter was determined on mesocarp tissue after 48 h at 60°C. SSC was measured with an Atago digital refractometer (Optolab, Modena, Italy). Total acidity was determined on flesh juice by a semiautomatic instrument (Compact-S Titrator, Crison, Modena, Italy). Starting from one month before harvest, the evolution of these parameters was also determined “in planta” on 80 tagged fruits using the portable DCA-University of Bologna NIRs already described (Costa et al., 2002). To obtain a robust calibration equation, a sample of 15 fruits was collected for NIRs analysis and for the destructive determinations at a weekly interval.

Fruit abscission was determined by counting all the dropped and still attached fruits at commercial harvest, as well as 3 and 9 days later in both control and treated plants.
RNA extraction and northern analysis

Total RNA was extracted from ca. 10 g mesocarp fresh weight using the method described by Bonghi et al. (1998). RNA (18 µg per track) were transferred onto nylon membranes (Hybond-N, Amersham Pharmacia Biotech Italia) overnight according to standard methods (Sambrook et al., 1989). RNA blots were hybridized with $[^{32}P]dCTP$-labelled peach ACO and ACS probe as described in Biondi et al. (2001). The ACO probe and ACS probe were obtained by RT-PCR as previously described (Tonutti et al., 1997; Ruperti et al., 2001).

RESULTS AND DISCUSSION

Ethylene emission and fruit abscission

Both AVG and PA application delayed and reduced ethylene production, although differences were observed in relation to AVG application and to PA type.

Ethylene emission in Redhaven control fruits was detectable starting from 5 days before harvest (106 dAFB), and showed an increasing trend until the end of S4 (Fig. 1A-B). On the contrary, AVG-treated fruits, began to emit ethylene later (109 dAFB) and at much lower values than controls (Fig. 1A). All PA sprays strongly reduced or even nullified ethylene emission in the final days of fruit growth (109 and 111 dAFB; Fig. 1B).

In Stark Red Gold nectarine, ethylene emission reached its maximum at harvest (115 dAFB), and decreased thereafter (Fig. 2A-C). All treatments delayed the onset of and reduced (up to 90%) ethylene emission as compared to controls. All the late AVG applications were effective in reducing fruit ethylene emission at harvest time (Fig. 2A). However, the strongest effects were recorded with the lowest concentration applied late and with the highest concentration applied early. Both tested PA uniformly reduced fruit ethylene emission from the first day in which the hormone was detectable, up to 118 dAFB (Fig. 2B, C). During the following year of trials, similar data were obtained (data not shown) and thus present results confirm the capacity of these substances to control ethylene production during “on-tree” fruit ripening. As far as AVG is concerned, the time of application, more than its concentration, could play a critical role in controlling hormone production, as also observed in pear by Clayton et al., 2000. The higher efficacy of the earlier AVG application could be due to the fact that ethylene biosynthetic enzyme were already active or receptive to exogenous stimuli as observed in mesocarp of Springcrest peaches by Tonutti et al., 1997. As far as the PA is concerned, it is the first time that a clear effect on ethylene is observed in peach fruit under field conditions, beside the contribution in Japanese apricot obtained by Paksasorn et al., 1995, although with a different treatment technique.

Fruit abscission detected on Stark Red Gold control and treated fruits at harvest time and thereafter was significantly reduced by all AVG concentrations (from 40 to 70 % of control), independently by the application time as tested for the highest concentration (Fig. 3A). Instead, fruit abscission was slightly reduced by PA application: the most effective being spermidine at the highest concentration (2 mM) and 9 days later (Fig. 3B and C). Since ethylene–releasing chemicals are well known to induce fruit abscission in peaches (Byers et al., 2003), any chemical reducing ethylene production is expected to control abscission. Indeed, although a significant reduction in fruit drop was observed in AVG-treated fruits, only a very scarce effect was registered in PA-treated ones.
Fruit quality parameters

The AVG application reduced FF values of nectarine fruits at harvest, and the effect resulted directly related to the application time and the concentration (Fig. 4A). A similar effect was induced by both PA, although the most active appeared to be the two lower putrescine concentrations (5 and 10 mM) (Fig. 4B and C). These results are in agreement with the findings of several Authors that reported high sensitivity of flesh softening to ethylene. In Redhaven peaches, for example, propylene treatments dramatically enhanced endo-β-1,4-glucanase activity and transcript accumulation at ripening (Bonghi et al., 1998) and in ACO antisense Cantaloupe melon, transformed fruits exhibited strong inhibition of ethylene production, and did not undergo flesh softening (Guis et al., 1997).

AVG at all the concentrations and application times tested, reduced the SSC/acidity ratio as compared to control, mainly because of the high acidity levels induced. A similar effect was recorded also for all PA-treated fruits, although fruits treated with putrescine were slightly lower in SSC and higher in acidity levels as compared to spermidine treated ones (Table 2). Different results were obtained on Redhaven peach: the higher AVG concentration significantly increased the SSC (Fig. 5A), while both PA did not (Fig. 5B). Low SSC and high acidity levels are typical of fruits which are reaching the full ripening and the substances may have delay the progress of the ripening process as compared to controls. In any case, these two parameters seems to be less clearly dependent of ethylene than FF and our results, although adding new information, do not help to clarify the exact role of ethylene on sugars and acid metabolism. However information on the role of the hormone in controlling sugar and organic acid metabolism during fruit ripening is scarce compared with that on cell wall disassembly. Although there are recent studies on the expression of UDP-glucose pyrophosphorylase and sucrose phosphatase, obtained moreover on banana and kiwifruit, which demonstrated that the corresponding mRNAs increased in response to ethylene, suggesting a regulatory role of this hormone also on sugar metabolism during ripening (Langerkämper, 1998; Pua, 2000). The different results obtained in Redhaven peaches and Stark Red Gold nectarine might be related to the known different sensitivity of cultivars to growth regulators application as reported for fruit chemical thinning agents (Byers et al., 2003).

The detection of the quality parameters pattern in planta by the NIRs system was possible and the results achieved (i.e. for FF) were similar to those obtained with the standard methodology (Fig. 6). The NIRs also was able to detect the differences induced by the two substances discriminating treated and control fruits on the basis of the different ripening stage (Fig. 7).

Transcript levels of ethylene biosynthetic genes

Although biochemical data exist on ethylene and PA metabolism during fruit ripening and senescence, little is known at molecular level after AVG and PA application especially during “on-tree” peach fruit ripening. During this process in climacteric fruits, a positive correlation between ethylene production, biosynthetic activity and gene expression has often been found (Tonutti et al., 1997; Nakatsuka et al., 1998; Ruperti et al., 2001; Mathooko et al., 2001). In fact, in Stark Red Gold nectarines, we observed inhibition of ACO message accumulation by AVG and of both ACO and ACS transcripts by putrescine in accord with their effect on ethylene production (Fig. 8)

CONCLUSION

These results confirm for the first time in climacteric fruits, that both substances can inhibit ethylene production, affect fruit quality parameters (i.e. retained flesh firmness) and control abscission.
It is also interesting to point out that the constant ethylene inhibition obtained in all the tested experimental conditions could be related to the control exerted at transcript level. As a final remark, considering their mechanism of action and that both AVG and PA are naturally occurring substances, they may be candidate to represent a useful tool to modulate ripening in practical field condition also in the countries where a strict protocol for chemical registration is adopted.

ACKNOWLEDGEMENTS

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Table 1. Concentration and time of application of AVG and of three different PA on Redhaven peach and Stark Red Gold nectarine trees during different years of trials

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<th>Substances</th>
<th>Concentration in Redhaven</th>
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<th>Concentration in Stark Red Gold</th>
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<td>AVG</td>
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<td>7 (S4)</td>
<td>62.5 ppm (0.32 mM)</td>
<td>14 and 7 (S4)</td>
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<td>AVG</td>
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<td>7 (S4)</td>
<td>125 ppm (0.64 mM)</td>
<td>14 and 7 (S4)</td>
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<tr>
<td>AVG</td>
<td>250 ppm (1.28 mM)</td>
<td>7 (S4)</td>
<td>250 ppm (1.28 mM)</td>
<td>21(S3), 14 and 7 (S4)</td>
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<tr>
<td>Putrescine (Pu)</td>
<td>10 mM</td>
<td>19 (S3)</td>
<td>5 – 10 – 20 mM</td>
<td>30 (S3), 14 and 7 (S4)</td>
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<tr>
<td>Spermidine (Sd)</td>
<td>0,1 – 1 – 5 mM</td>
<td>19 (S3)</td>
<td>0,5 – 1 – 2 mM</td>
<td>30 (S3), 14 and 7 (S4)</td>
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<td>Spermine (Sm)</td>
<td>2 mM</td>
<td>19 (S3)</td>
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Table 2. Flesh titrable acidity, soluble solids content (SSC) and SSC-acidity ratio in nectarines treated with different concentrations of putrescine (Pu), spermidine (Sd) and AVG at harvest (115 dAFB). Data represent the means (n=10) and asterisks indicate significant differences relative to controls at P<0.05.

<table>
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<td>Control</td>
<td>6.5</td>
<td>12.5</td>
<td>1.92</td>
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<tr>
<td>5 mM Pu</td>
<td>7.3*</td>
<td>11.1*</td>
<td>1.52</td>
</tr>
<tr>
<td>10 mM Pu</td>
<td>7.6*</td>
<td>10.8*</td>
<td>1.42</td>
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<tr>
<td>20 mM Pu</td>
<td>8.6*</td>
<td>10.4*</td>
<td>1.21</td>
</tr>
<tr>
<td>0.5 mM Sd</td>
<td>7.7*</td>
<td>11.1*</td>
<td>1.44</td>
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<td>1 mM Sd</td>
<td>7.2*</td>
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<td>1.58</td>
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<tr>
<td>2 mM Sd</td>
<td>nd</td>
<td>11.3*</td>
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</tr>
<tr>
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<tr>
<td>125 ppm AVG</td>
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<td>1.57</td>
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<td>250 ppm AVG</td>
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<td>11.2*</td>
<td>1.28</td>
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<tr>
<td>250 ppm AVG (early)</td>
<td>8.4*</td>
<td>11.2*</td>
<td>1.33</td>
</tr>
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Figure 1. Ethylene emission from Redhaven peach fruits during stage 4. Effect of AVG (A) and polyamine (B) treatments. Commercial harvest date correspond to 111 dAFB. Data represent the mean ± SD. Pu, putrescine; Sd, spermidine; Sm, spermine.
Figure 2. Ethylene emission from Stark Red Gold nectarine fruits during stage 4. Effect of AVG (A), putrescine (B) and spermidine (C) treatments. Commercial harvest date correspond to 115 dAFB. Data represent the mean ± SD.
Figure 3. Nectarine fruit drop at harvest time (115 dAFB) and 9 days later (124 dAFB) after AVG (A), putrescine (B) and spermidine (C) applications. Data represent the mean ± SD of the percentage of abscissed fruits relative to total fruit number on the plants.
Figure 4. Effects of AVG (A), putrescine (B) and spermidine (C) treatments on FF pattern of Stark Red Gold nectarine fruits. Commercial harvest date correspond to 115 dAFB. Asterisks (*) indicate significant difference relative to controls at P<0.05.
Figure 5. Effects of AVG (A) and polyamine (B) treatments on SSC pattern of Redhaven peach fruits. Commercial harvest date correspond to 111 dAFB. Asterisks (*) indicate significant difference relative to controls at P<0.05. Pu, putrescine; Sd, spermidine; Sm, spermine.
Figure 6. Comparison between FF values determined by penetrometer and by NIRs

Figure 7. NIRs determination of SSC in Stark Red Gold fruits treated with AVG and PA
Figure 8. Transcript levels of ACS (A, A’) and ACO (B, B’) in nectarines at harvest (115 dAFB) after Putrescine (Pu), spermidine (Sd) and AVG treatments. (A, B) Northern blot analysis of total RNA (15 µg per lane) extracted from fruit mesocarp. (A’, B’) Respective band intensities normalised to ethidium bromide-stained loading controls. Numbers in abscissa indicate mM concentrations.
PREHARVEST PLANT GROWTH REGULATORS THAT INFLUENCE POME FRUIT MATURITY AND QUALITY

Duane W. Greene¹

ABSTRACT

Several plant growth regulators (PGRs) are used or have been used to influence ripening and enhance fruit quality. These include naphthaleneacetic acid (NAA), 2-chloroethylphosphonic acid (ethephon), succinic acid-2,2-dimethylhydrazide (daminozide), and aminoethoxyvinylglycine (AVG). NAA was one of the first drop control compounds identified. It can advance fruit ripening and reduce storage life under certain circumstances. Daminozide was an important PGR in the apple industry for over 25 years. It retarded preharvest drop and improved fruit quality. Registration of daminozide for use on apples was withdrawn in 1989 for regulatory reasons. Ethephon is an ethylene generating compound that also retards growth. It is applied as a preharvest spray to advance ripening and improve red color. AVG is the newest PRG that is used to retard preharvest drop and affects all aspects of fruit maturation by delaying ethylene biosynthesis.

INTRODUCTION

Plant growth regulators (PGRs) can be used to influence maturity and quality of pome fruit. These can be placed into two categories; those that are applied for other purposes but incidentally influence fruit maturity, and those that are applied primarily to affect fruit maturity. There are three primary PGRs used on pome fruits: naphthaleneacetic acid (NAA), 2-chloroethylphosphonic acid (ethephon, Ethrel), and aminoethoxyvinylglycine (AVG).

BRIEF HISTORY OF DEVELOPMENT

Auxins

_Naphthaleneacetic acid._

The auxins were the first group of plant hormones to be discovered. Because there were no protocols established, it took over half a century for indoleactic acid (IAA) to be discovered. It was soon discovered that a synthetic compound, NAA, that was very similar in structure and had strong physiological activity. NAA and several other synthetic auxins were found to cause fruitlet abscission when applied soon after petal fall, but retarded abscission when applied near harvest. NAA is the only one of these synthetic auxins used on pome fruit to survive rigorous regulatory scrutiny. NAA has been used since the late 1930's on apples and pears to influence abscission, making it the oldest commercially used on pome fruit.

Ethylene regulating compounds

The growth regulating properties of ethylene have been known for many years. Denny, Miller, and Crocker were fruit physiologists who in the 1930's recognized the regulatory activity of ethylene. However, its involvement in hormonal regulation of plant growth and development was not recognized by scientists in other disciplines for two reasons. First, there were no good bioassays to

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determine accurately and conveniently endogenous levels of ethylene. Second, they found it was very
difficult to accept major regulatory activity from a simple two-carbon compound that readily moved
both within and outside the plant. In the 1960's the gas chromatograph was widely adopted as the
method of choice to determine ethylene. It was only then that the growth regulation properties of
ethylene were fully recognized and it gained stature as a legitimate and important plant hormone.
There are two ethylene regulating PGRs that have become very important in influencing fruit
maturation and quality.

2-chloroethylphosphonic acid (ethephon, Ethrel)
Ethephon was the first ethylene-based PGR to become available. It was introduced in 1971
to stimulate latex flow on rubber trees. Since that time it has been registered to be used on over 20
crops, including such fruit crops as apple, cherry, pineapple and grapes.
The problems associated with administering ethylene in gaseous form to a plant under field
conditions are enormous. Ethephon simplifies this. It is a compound that is quite stable in aqueous
solutions at low pH, but at high pH levels, it becomes unstable. Ethephon is applied to tree fruit in an
aqueous spray where it is absorbed and moves into the cytoplasm that has a pH slightly below
neutrality (pH 7). Ethephon is unstable in this pH range and breaks down to liberate ethylene gas in
the cell. The ethylene liberated from the breakdown of ethephon frequently stimulates the plant to
produce additional endogenous ethylene.

Aminoethoxyvinylglycine (AVG, ReTain)
AVG is a naturally occurring PGR that was discovered by scientists at Hoffman LaRoche in the
early 1970's. Its primary mode of action is to inhibit ethylene biosynthesis, by blocking the enzyme
ACC synthase. AVG elicits many responses in apples but the most important one is that it inhibits
ripening. This compound was evaluated extensively in the late 1970s and early 1980s. Efforts to gain
registration of AVG were not pursued for economic reasons. Following the loss of daminozide as a
preharvest drop control compound, Abbott Laboratories resumed development of AVG for commercial
use. AVG received full label registration in the United States as the product ReTain in 1997 to control
preharvest drop and improve fruit quality of apples.

Direct and indirect effects and influences

PGRs that influence fruit ripening and fruit quality in storage fall into two categories; those that
are applied specifically to influence ripening and those that are applied for other purposes and
inadvertently affect ripening. Weather that occurs following application plays a dominant role in
whether the PGR influences the intended response and also the extent of the response. Therefore,
orchardist have only partial control over the eventual outcome because a major component in the
ultimate result, the weather, is somewhat unpredictable, is uncontrollable, and it occurs after the PGR
application is made.

PGRS THAT INFLUENCE FRUIT MATURITY AND RIPENING

Auxins

Naphthaleneacetic acid
The primary use for NAA late in the season is to delay preharvest fruit drop. It is most
effective on the majority of varieties when it applied before many sound fruit start to drop. If drop
does increase it may take 3 to 5 days for NAA to slow drop. During this period of time between application and retardation of drop, it is possible to have up to 25% of the crop abscise. Generally NAA is applied at a concentration of 10 mg l⁻¹. This lasts for 7 to 10 days. A second application may be made. NAA has the capability of advancing ripening and reducing storage life. This is most likely to happen if higher rates or multiple application of NAA are used, the time interval between application and harvest is long, hot weather follows application, or fruit are kept in storage for a relatively long period of time.

NAA may be used to advance ripening, but it is seldom used for this purpose when applied alone. The highest rate allowed on the label, 20 mg l⁻¹, will be most the effective rate. More frequently is applied with ethephon when the primary purpose is to advance fruit ripening.

**ETHYLENE REGULATING COMPOUNDS**

2-chloroethylphosphonic acid (ethephon)

The primary use of ethephon on apples is to advance ripening. This can be done to benefit both the grower and the consumer. The advantage to the consumer is that they will be able to purchase high quality apples of a particular variety earlier in the season, whereas growers will benefit because they can start to harvest and market earlier in the seasons. Ethephon is an extremely potent stimulator of ripening. There are several factors that affect the extent to which ripening is advanced and the resulting influence on storage life. These factors include: concentration of ethephon used, the time interval between application and harvest, temperature from the time of application until harvest, and the time from harvest until the internal fruit temperature is reduced to 0ºC.

**Intended use influences the concentration applied**

*Sales prior to normal harvest*

The purpose of this is to advance ripening to provide good-tasting fruit before normal harvest. We recommend 300 mg l⁻¹ ethephon plus 20 mg l⁻¹ NAA and make this application 2 to 3 weeks before normal harvest. Since fruit increase in size about 1% per day they remain on the tree, there is a reduction in fruit size and yield when fruit is harvested early.

*Fruit to be held for less than a month*

The strategy with this approach is to increase red color and flavor of fruit that are consumed during the normal harvest of a specific variety. We recommend 150 to 200 mg l⁻¹ ethephon, plus 20 mg l⁻¹ NAA applied 1 to 2 weeks before normal harvest.

*Fruit to be held at 0°C for an extended period of time*

The strategy with this approach is to maximize the amount of red color on the fruit while minimizing advancement of ripening and reduction in flesh firmness. We recommend 75 to 100 mg l⁻¹ ethephon plus 10 mg l⁻¹ NAA. The risk with this approach is that the weather will turn hot and cloudy, advancing ripening without the benefit of increased red color.

Aminoethoxyvinylglycine (AVG, ReTain)

AVG is normally applied about 4 weeks before anticipate harvest of untreated fruit. Applied at that time it retards the normal maturation which influences fruit quality at harvest and following storage.
Fruit quality at harvest

Flesh firmness of AVG-treated fruit is generally greater at harvest because AVG slows the loss of flesh firmness. This slowing of maturation is documented by a starch test, which gives an indication of the extent of hydrolysis of starch to sugar in the fruit. Red color is sometimes reduced on a calendar basis because ripening has been delayed. When evaluated at a similar stage of maturity, treated fruit generally have similar red color. Frequently red color of harvested fruit is greater because AVG allows fruit to remain on the tree longer without dropping, thus being exposed to more favorable coloring weather.

Storage effects of AVG

Fruit treated with AVG and kept at room temperature or on an open bench will lose flesh firmness less rapidly than untreated fruit. The reason for this is that these fruit produce little ethylene and they are not exposed to ethylene produced by untreated fruit. If these same fruit are placed in a regular air storage at 0°C with untreated fruit, differences between treated and untreated are substantially less. AVG inhibits ethylene biosynthesis in treated fruit but it does not make treated fruit immune to the ethylene produced by other fruit in the same storage. Consequently, large differences between AVG-treated and untreated fruit are seldom large in commercial, unsegregated storages. Some varieties of apples develop severe watercore. This is a malady that is caused by a large accumulation of sorbitol in the fruit. If enough watercore develops in fruit on the tree, fruit can develop breakdown in storage. AVG generally reduces watercore in susceptible varieties thus improving the chance that fruit will develop senescent breakdown.
MANAGEMENT OF CLIMACTERIC FRUIT RIPENING WITH 1-METHYLCYCLO-
PROPENE (1-MCP), AN INHIBITOR OF ETHYLENE ACTION

Jim Mattheis¹, Xuetong Fan², and Luiz C. Argenta³

ABSTRACT

Many of the postharvest management practices used to prolong storage life of climacteric fruit act by reducing the effects of the plant hormone ethylene. Refrigeration and controlled atmosphere (CA) storage both slow ripening in part by reducing ethylene production and activity. The discovery by Drs. Ed Sisler and Sylvia Blankenship at North Carolina State University that 1-methylcyclopropene (1-MCP) interferes with the ability of plants to respond to ethylene provides a potential new tool for postharvest management of climacteric fruits. Research worldwide using 1-MCP is providing insight into how control of ethylene action impacts (or does not impact) fruit ripening and how the use of this material can fit into commercial operations. This report summarizes some of the results obtained during research conducted over the past several years at the USDA, ARS laboratory in Wenatchee, Washington, USA.

A number of factors influence fruit responses to 1-MCP. These include 1-MCP concentration during treatment, duration of exposure, fruit maturity at the time of treatment, and the interval between harvest and when the treatment is applied. Like ethylene, 1-MCP is a gas and is applied in a sealed chamber or storage room. One of the desirable characteristics of 1-MCP is its activity is maximum when applied at the very low concentration of 1 ppm. At that concentration, residues are not detectable using standard analytical methods. Responses to 1-MCP can be induced by exposures of as little as 1 hour under laboratory conditions using relatively small volume chambers for treatment. In large storage rooms, longer treatment durations (i.e. 24 hours) are needed to ensure adequate distribution of 1-MCP throughout the room and sufficient contact time with fruit. In general, fruit temperature during the treatment is not critical assuming treatment concentration and duration are sufficient. However, riper fruit treated at low temperatures for relatively short periods may respond less compared to the same fruit treated at warmer temperatures. The duration between harvest and treatment within which maximal responses can be induced may vary with cultivar and fruit maturity. For example, to prevent development of superficial scald on ‘Granny Smith’ apples, treatment must be within 2 weeks of harvest. Fruit treated 4 or more weeks after harvest developed superficial scald and had lower firmness and titratable acidity compared to fruit treated at or within 2 weeks of harvest.

Both CA storage and 1-MCP treatment reduce fruit ethylene production (Figure 1). Comparisons of untreated apples stored in CA with 1-MCP-treated fruit indicate no differences in fruit quality for the first several months after treatment. For ‘Gala’ apples, values for firmness and titratable acidity were similar through 3 months after harvest. As storage duration increased beyond 3 months, fruit stored in CA had slightly higher firmness and TA compared to 1-MCP treated fruit stored in RA, however, 1-MCP treated fruit remained firmer with more titratable acidity than untreated fruit stored in air. The combination of 1-MCP treatment at harvest then storage in CA can provide the maximum benefit from both technologies for quality retention during long-term storage of apples. This is also the case for several cultivars of European pear including ‘Bartlett’, ‘Bosc’, ‘Comice’ and ‘d’Anjou’.

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However, as these pear cultivars are typically eaten when ripe, treatment with 1-MCP may delay ripening in a manner that does not provide flexibility for marketing within the first several months after treatment.

Ethylene regulates production of volatile compounds that contribute to apple and pear flavor. Long-term CA storage tends to reduce the capacity of fruit to produce these compounds, and 1-MCP treatment induces the same response. The duration of this 1-MCP response is determined by treatment conditions, particularly treatment concentration. Fortunately, when the effects of 1-MCP begin to fade, volatile production resumes and occurs at higher rates compared to fruit stored in CA. This and other effects of 1-MCP are not reversible by exposing 1-MCP treated fruit to ethylene.

While 1-MCP can be used to reduce the rate of ripening of fruit harvested at an advanced maturity, the responses obtained are relative to fruit condition at harvest. Experiments conducted using several apple and pear cultivars indicate fruit harvested past the optimum for long-term CA can benefit from the use of 1-MCP via reduced rates of firmness loss. While some measure of ripening control can be expected when late harvest fruit are treated with 1-MCP, the potential for long-term storage of these fruit remains low compared to fruit harvested earlier. This result may be exploitable for fruit to be marketed soon after harvest. Peach fruit harvested relatively mature showed slower ripening over a two week period when treated with 1-MCP at harvest.

Treatment with 1-MCP results in reduced respiration rate and ethylene production (Figure 1) for all climacteric fruit we have tested. These physiological responses are accompanied by impacts on fruit quality including reduced firmness and titratable acidity loss (Figure 2), control of many physiological disorders including superficial and soft scald, core flush and senescent flesh browning, and reduced development of peel greasiness and senescent decay. The reduction in greasiness indicates there is less production of cuticular components following treatment with 1-MCP. As these compounds play a role in reducing moisture loss, an increased potential for shrivel exists for 1-MCP treated fruit. The development of decay is slowed following 1-MCP treatment. This response is absent if 1-MCP treated fruit are wound inoculated with pathogens, indicating decay resulting from injuries during harvest and packing still requires the use of other decay control technologies.

While effective for extending the storage and marketing period of climacteric fruit, 1-MCP treatment does not slow all ripening processes or provide solutions to all postharvest problems. Starch breakdown and the accompanying increase in soluble solids are not affected by 1-MCP treatment. However, disappearance of watercore in apple is slowed following 1-MCP treatment. 1-MCP does not reduce fruit susceptibility to CO₂ and may in fact prolong the period of risk. For some slower ripening apple cultivars including ‘Braeburn’ and ‘Fuji’, delaying establishment of CA or control of CO₂ are means to reduce the risk. Delaying 1-MCP treatment is also an effective strategy to reduce the risk of CO₂ injury for these cultivars without excessive quality loss. Treatment with 1-MCP does not control disorders that are initiated prior to harvest including bitter pit or cork spot and delayed sunscald.

Research to date conducted worldwide indicates the use of 1-MCP can be consistently effective for extending the marketability of many climacteric fruit. Application of the material as a gas avoids the use of water and the potential risk of decay from inoculation via a re-circulating water drench. The low concentration at which 1-MCP is effective also assures a non-detectable residue after treatment. The reduction in firmness and titratable acidity loss following application of 1-MCP is comparable to that resulting from short- to mid-term CA storage. Inhibition of ethylene action also provides for slower ripening when fruit are not refrigerated, therefore, the use of 1-MCP can provide an additional level of quality management after fruit have left the warehouse and pass through the wholesale and retail marketing chain. Control of superficial scald and other physiological disorders following 1-MCP treatment may allow reduced use or elimination of other scald control technologies. While these benefits are likely, other management issues related to the use of 1-MCP will also need to be addressed. The increased tendency for shrivel in treated fruit will require proper humidity
management. Reduced production of volatiles that contribute to aroma can impact fruit quality, however, for apple fruit this response is comparable to what is induced by CA storage.

ACKNOWLEDGEMENTS

Technical assistance of David Buchanan and Janie Countryman, USDA, ARS, is gratefully acknowledged. We also thank Agrofresh, Inc. and the Washington Tree Fruit Research Commission for financial support.

Figure 1. Ethylene and CO₂ production by ‘Delicious’ apples. Fruit were treated at harvest with 1 ppm 1-MCP then held at 68 °F.
Figure 2. Firmness and titratable acidity of various apple cultivars. Fruit were treated with 1 ppm 1-MCP, then stored in air at 32 °F for 6 months followed by 7 days at 68 °F. Cultivars tested were: 1 - Delicious; 2 - Golden Delicious; 3 - Granny Smith; 4 - Fuji; 5 - Braeburn; 6 - Gala; 7 - Jonagold; 8 - Gingergold.
Session I

Flowering/Fruiting
FURTHER CHARACTERIZATION OF THE MANGO FLORIGENIC PROMOTER

T.L. Davenport1 and Z. Ying

ABSTRACT

Floral induction of mango is determined by interaction of a short-lived, florigenic promoter that is up-regulated in leaves during exposure to cool temperatures and an age-dependent vegetative promoter at the time that initiation of shoot growth occurs. Shoots are either vegetative or reproductive according to the ratio of these induction components at the time of initiation of growth. Research was conducted this flowering season in a commercial ‘Keitt’ mango orchard located in Homestead Florida. Flowering had already commenced when treatments were made, and the weather continued to be cool to insure floral-inductive conditions throughout the treatment period. Two to three cm diameter branches bearing at least 20 stem tips were isolated from the rest of the tree by girdling them using a pair of shears designed for the purpose. This removed the bark and cambium in a 4-mm wide ring around the branch. The inflorescences at the terminus of each stem were removed to stimulate growth of lateral buds. Because previous research had determined that one leaf per stem was sufficient to stimulate flowering in 100% of the shoots emerging from lateral buds, we tested the effects of 1, 1/2, and 1/4 leaf per stem. This was accomplished by removing all of the leaves on each stem except for one. The distal 1/2 or 3/4 portion of the final leaves was removed to achieve the 1/2 or 1/4 leaf treatments using pruning shears. Each treatment was replicated in 10 isolated branches. Results showed that one quarter of a leaf per stem was sufficient to stimulate flowering in 100% of the tested stems. Stems with no leaves produced only vegetative shoots.

In another experiment, branches were girdled as described above in ten replicate branches per treatment such that each isolated branch contained six stems. Each stem in the isolated branches was deblossomed as before to initiate new growth of lateral buds. A donor stem was designated at the end of a branch such that the five receiver stems were located at increasing distances from the donor. All of the leaves were removed from the five designated receiver stems. All leaves on the designated donor stems were removed except those required for each treatment. Treatments consisted of 0, 1/2, 1, 3, or 5 leaves retained on the donor stem. Flowering or vegetative response of each of the six stems were recorded after about 6 weeks when the new shoots (either vegetative or reproductive) were well developed. Three or more leaves on a donor stem resulted in 100% reproductive shoot formation on all six stems. One leaf on the donor stem was sufficient to induce flowering in all of the donor stems and most of the five defoliated stems regardless of distance, and 1/2 leaf on the donor stem stimulated flowering in that stem and in less than half of the defoliated stems. Stems that did not initiate reproductive shoots initiated vegetative shoots instead. Flowering occurred on those stems that were inserted into main branches in the same phylotaxic position as the leaf. The results indicate that leaves are capable of producing far greater amounts of florigenic promoter during strong floral inductive conditions than needed for induction of buds and that the promoter can move great distances (as far as 2 m) in phloem aligned in the same phylotaxic position of the source leaf.

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SPRAY VOLUME AND SURFACTANT EFFECTS ON NAA THINNING OF FLORIDA CITRUS

E. W. Stover and S. M. Ciliento

ABSTRACT

As with most tree fruits, high croploads in citrus can result in alternate bearing and production of small fruit of little or no value. Use of PGRs to overcome excessive citrus cropping has been studied for more than 30 years. There are many reports of reduced cropload and increased fruit size following application of 1-naphthaleneacetic acid (NAA) during early fruit development to various citrus cultivars in diverse growing regions. NAA has been labeled for thinning of many Florida citrus varieties for more than twenty years, but has received little commercial use. Almost all published reports have used high spray volumes requiring US$400-1600 ha\(^{-1}\) for NAA at current prices and the rates of 250-500 ppm required to thin Florida citrus. This contrasts with US$12-45 ha\(^{-1}\) for NAA to thin most apple cultivars. Since annual spray material costs for fresh Florida citrus currently average US$500 ha\(^{-1}\), high PGR cost appears to be a major limitation to more widespread NAA thinning of Florida citrus. We conducted five trials to determine whether lower spray volumes and/or inclusion of different surfactants would permit adequate thinning at much lower cost per hectare. Experiments were conducted using a randomized complete block design with 6-12 trees per treatment. Sprays were applied using a commercial airblast orchard sprayer when fruitlets averaged 10-15 mm in diameter, during physiological drop. NAA was used in the formulation FruitFix 800. Surfactant was always included at 0.05% v/v. At commercial maturity, all fruit per tree were harvested and were counted and sized using a portable optical sizer. Weight and diameter were determined for each fruit in a 30-50 fruit subsample, and the calculated regression was used to convert diameter data to weight estimates. In three experiments, one with ‘Murcott’ (at 250 ppm NAA) and two with ‘Sunburst’ (at 500 ppm NAA), comparison of spray volumes of 560 (only 2 of 3 experiments), 1100, or 2300 L ha\(^{-1}\) demonstrated significant fruit size enhancement from all NAA applications with increases in production of the largest and most valuable fruit. Substantial cropload reductions resulted from most NAA treatments but with no statistically significant difference between NAA treatments. There was a marked linear effect of spray volume on all crop-value parameters (cropload, yield, mean fruit size, and production of largest fruit sizes) in one ‘Sunburst’ experiment, but no differences between NAA treatments in the other ‘Sunburst’ experiment or the ‘Murcott’ trial. The effect of three surfactants (non-ionic, Silwet L-77, and LI-700) on NAA thinning was tested in both ‘Murcott’ and ‘Sunburst’. The most notable difference was greater cropload and yield reduction from L-77 compared to non-ionic surfactant in ‘Murcott’, with less increase in production of largest fruit sizes. The trend was similar but not significant in ‘Sunburst’. Based on these findings, our current recommendations for NAA thinning of citrus are use of spray volume of ~1000 L ha\(^{-1}\) on mature trees, with proportionally lower volume on smaller trees, and inclusion of a non-ionic surfactant. Because experience with NAA thinning of Florida citrus is limited, it is only recommended where the disadvantages of overcropping are perceived to substantially outweigh the potential losses from overthinning. Successful NAA thinning has been achieved routinely in ‘Sunburst’ and ‘Murcott’ in deep sand sites of the Central Florida Ridge but thinning is much more sporadic in coastal groves with shallow soils and perched water tables. We currently have experiments underway to determine how regional differences in water relations and spring high temperatures may influence thinning response.

REDUCING PEACH (*PRUNUS PERSICA*) FLOWER BUD NUMBERS WITH A MID-WINTER APPLICATION OF SOYBEAN OIL


ABSTRACT

Removal of peach flower buds, flowers or young developing fruit in early spring is important to increase final fruit size. Methods such as summer GA sprays, burning flowers with chemicals, or hand-thinning to remove buds and flowers are costly or inconsistent, thus growers wait until ~30 days after full bloom to start hand-thinning fruit, which often reduces the potential size and economic value of the packed fruit. Experiments were conducted from 1997 to 2003 in University and commercial peach orchards in South Carolina to determine the efficacy of soybean oil (SO) for pre-bloom thinning of difficult-to-thin peach cultivars. Previous work reported by Sams, Deyton, and others at the University of Tennessee showed that SO rates of 6 and 8% effectively thinned peach flower buds without over-thinning. Difficult-to-thin and small-fruited cultivars were selected and sprayed once each winter either in December, January or February for 2 to 4 years during the 7-year study period. Application rates of 4, 6, 7, 8, 10 and 12% v:v degummed or cooking grade SO were tested and applied at 1400 to 1555 L per hectare. Latron B-1956 was used as an emulsifier at a rate of 10% of the SO volume. Dormant oil at 2 or 3% was the control treatment. Bloom was either delayed or advanced several days by SO depending on cultivar and year. Experiments to determine the best time to apply SO to delay bloom gave inconsistent results across years. SO significantly reduced the number of live flower buds within 2 to 3 weeks after application. Rates of 4% did little thinning, whereas 10 and 12% tended to over-thin. The 6% SO rate thinned approximately 10-20% of the dormant flower buds while 8% SO thinned from 15 to 40% depending on cultivar and year. Cooking grade SO thinned flower buds as well as degummed SO but was more expensive to use. SO significantly decreased hand thinning costs per hectare and for some cultivars improved fruit size, commercial pack-out and economic return to the grower. The 8% SO rate did the best job of thinning without decreasing yield and also increased fruit size, which increased the average price per packed box of fruit. SO in place of dormant oil also did an acceptable job of controlling scale in the test orchards.

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Session II

Molecular Biology/Metabolism
REGULATION OF GIBBERELLIN BIOSYNTHESIS DURING SEED GERMINATION IN ARABIDOPSIS

Yukika Yamauchi, Mikihiro Ogawa, Yuji Kamiya and Shinjiro Yamaguchi

ABSTRACT

Regulation of Gibberellin (GA) Biosynthesis by Cold Temperature

Seed dormancy and germination are controlled by endogenous and environmental factors. For example, GA induces seed germination, while ABA plays a crucial role in maintaining seed dormancy. In Arabidopsis, seed germination is critically controlled by light and temperature. The effects of light on seed germination are primarily mediated by the red and far-red light photoreceptor phytochromes (Phy). We have previously shown that GA biosynthesis is regulated by light via Phy during Arabidopsis seed germination (Yamaguchi et al. 1998). In this study, we analyzed the effect of cold treatment (imbibition at 4°C in the dark for several days) on GA biosynthesis.

During Arabidopsis seed germination, two genes encoding GA 3-oxidase, \textit{AtGA3ox1} and \textit{AtGA3ox2}, are mainly expressed in the same cell-types. We found that the \textit{AtGA3ox1} gene, but not the \textit{AtGA3ox2} gene, is upregulated in response to cold treatment for stratification. Consistent with \textit{AtGA3ox1} expression, endogenous GA4 levels were elevated following cold treatment. Using a loss-of-function mutant (\textit{ga4}) of \textit{AtGA3ox1}, we showed that \textit{AtGA3ox1} is essential for the promotion of seed germination by cold treatment. Affymetrix GeneChip (8.3K genes) analysis identified a new set of GA-responsive genes that appear to be unique to the cold temperature environment. \textit{In situ} hybridization illustrated that the \textit{AtGA3ox1} transcript was detectable in additional cell-types after cold treatment. These results suggest that GA distribution and response are modulated under different environmental conditions during seed germination. Combined with our previous findings, these data illustrate part of the complex mechanisms by which active GA levels are determined through the regulation of \textit{AtGA3ox1} and \textit{AtGA3ox2} genes.

GA-responsive Gene Expression during Arabidopsis Seed Germination

We have analyzed GA-regulated gene expression using GeneChip as mentioned above. in the non-germinating \textit{gal-3} seeds imbibed at 22°C in the light. Over 100 genes were determined to be differentially expressed between GA-treated and non-treated seeds within 6 h. These genes were further classified into sub-groups based on their expression profiles during wild-type seed germination. This analysis allowed us to identify a group of GA-responsive genes of which transcript levels correlate well with an increase in endogenous active GA levels analyzed by GC-MS. Ogawa et al. Plant Cell, 15, 1591-1604 (2003).

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THE ENDOGENOUS GIBBERELLINS IN A GERMINATED BARLEY DWARF MUTANT

Kumala Dewi\textsuperscript{1} and Peter. M. Chandler

ABSTRACT

In germinated grains of ‘Himalaya’ barley (\textit{Hordeum vulgare}, L.), new synthesis of gibberellins (GAs) occurs rapidly. When grains were imbibed at 4 °C in the dark and then transferred to constant 20 °C with low intensity light, the content of bioactive GA (GA\textsubscript{1}) increased about 70 – fold in the first two days, and remained constant for a further three days. We have evaluated the endogenous GAs and the expression of mRNAs for both GA 20-oxidase and GA 3-oxidase in germinated grains of a dwarf mutant (\textit{grd2-430}) of barley. This mutant contains a 3ox2 gene with a single nucleotide substitution compared to the wild type, resulting in a non-conservative amino acid substitution in the principal vegetative 3-oxidase. The grains were germinated in the same conditions as those above, and the contents of a range of GAs were determined by GC-MS on purified extracts of samples harvested at day 4. The content of endogenous GA\textsubscript{1} in the dwarf was about 1/50 of the tall (8 pg/dwarf seedling and 405 pg/tall seedling). The leaf elongation rate of the dwarf seedling measured during the first three days of growth was considerably lower than tall seedlings (8.5 mm/day and 25 mm/day for dwarf and tall respectively). In another experiment it was also found that α-amylase production was lower in the dwarf compared to the tall. These results showed that seedling growth requires new synthesis of GA. The contents of \textit{GA\textsubscript{53}}, \textit{GA\textsubscript{44}}, \textit{GA\textsubscript{19}} and \textit{GA\textsubscript{8}} were lower in dwarf compared to tall seedlings, whereas \textit{GA\textsubscript{20}} and \textit{GA\textsubscript{29}} were slightly higher in dwarf compared to tall seedlings. The low content of active GA precursors, namely \textit{GA\textsubscript{53}}, \textit{GA\textsubscript{44}} and \textit{GA\textsubscript{19}} found in the dwarf seedlings suggest that in \textit{grd2-430} homozygotes, expression of the GA20-oxidase protein and / or activity is higher than tall seedlings. On the other hand, we expect the 3β-hydroxylase activity to be lower in dwarf compared to tall. As there is less conversion to GA\textsubscript{1} in the dwarf the content of its immediate precursor (GA\textsubscript{20}) is slightly higher compared to tall, as is the content of a GA\textsubscript{29} catabolite, namely GA\textsubscript{29}. The expression of both GA20-oxidase and GA3-oxidase was evaluated using RNA blots. Scutella were dissected and collected for RNA extraction and poly(A\textsuperscript{+}) selection. The samples were the scutella of ‘Himalaya’ grains (tall) and homozygous mutant \textit{grd2-430} (dwarf). The results indicated that both GA 20-oxidase and GA 3-oxidase mRNAs were expressed at higher levels in the scutella of dwarf compared to tall seedlings. The low GA\textsubscript{1} content and high expression of mRNA 20-oxidase suggests that feed-back regulation of gibberellin biosynthesis occurs in this dwarf mutant of barley.

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PHYTOHORMONE PROFILES YIELD POSITIONAL INFORMATION TO BUDS IN NORD-MANN FIR, *ABIES NORDMANNIANA*

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**ABSTRACT**

Positional information is a concept used to explain the coordinated activity of cells or larger body parts during ontogenesis, but little is known about its physico-chemical background. *Abies nordmanniana* Spach. is a tree with regular architecture, all growth points of the crown having predictable fates depending on their position. The purpose of the study was a) to develop a technique for simultaneous analysis of a range of phytohormones, cytokinins, auxin and gibberellic acids on small samples, and b) to compare homologous growth points with known differing growth potential during natural and manipulated crown development. This paper reports preliminary results on seasonal and spatial variation in cytokinin contents of growth points, reflecting the possible involvement of phytohormones in positional information.

By electrospray LC-MS techniques we identified cytokinins in buds at specified positions in the tree crown as well as in stem and root samples. They were quantified with picomol sensitivity and ±5% precision. The technique enabled us to distinguish between glycons and aglycons, but not to discriminate compounds from their corresponding monophosphates.

The compounds detected were trans-zeatin (Z), trans-zeatin-riboside (ZR), dihydrozeatin (DHZ), dihydrozeatin-riboside (DHZR), N\(^6\)-(\(\Delta^2\)-isopentenyl)adenine (iPA) and –adenosine (iPAR) and small amounts of 7 and 9-glucosides. Remarkably absent were O-glucosides (ZOG and ZROG). All buds held about the same Z/ZR and DHZ/DHZR ratios but at very differing levels, that changed in a consistent manner from autumn to spring. The absolute levels (per g FW) of Z+ZR, DHZ+DHZR and iPA+iPAR varied according to bud position/role and so did the relative abundance of these 3 groups of compounds. A subapical stem sample showed lower levels than the apical bud, with relatively more of the cytokinin in ribosidic forms. The cytokinin content in root samples was close to detection limit.

A profile of cytokinin forms thus emerged for each site that could yield a rather detailed spatial information to the cells of that site.

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DEVELOPMENT OF NOVEL PLANT TRANSFORMATION METHOD USING ArF EXCIMER LASER MICRO ABLATION

Akio Kobayashi and Shin’ichiro Kajiyama

ABSTRACT

Plants have hard and thick cell wall around the plasma membrane, and it is difficult to access inside the cells without pernicious effect on cell viability. To overcome this problem, protoplast cells are usually used for introduction of foreign materials such as DNAs. However, protoplasts are labile, and the regeneration of cell wall and the subsequent cell proliferation are often faced with difficult problems. Moreover, the integrity of the tissue is totally destroyed in the protoplasts, making it difficult to investigate the physiological roles of a specific single cell in the tissue. Here, we report a novel microsurgery technique for the partial removal of rigid cell wall of a targeted single cell in the intact plant tissue. The cortical structure, including the cuticle and cell wall layer, was removed partially (ca. 10% of cell surface area) by the irradiation of 193 nm pulsed UV laser (ArF excimer laser) without acute damage to the cell viability. Using the microprojection with the size-variable slit, which is placed in the middle of the laser path, a sophisticated microsurgery technique capable of controlling the area and depth of the hollow on the cell surface was established. The cell viability of the treated cells (Torenia epidermal cells) was retained in more than 98% of the cells 24 hrs after the irradiation (40 mJ/cm² in the area of 25 µm²). The SEM (scanning electron microscope) observation revealed that the cuticle layer of the irradiated site was completely ablated, and the cellulose microfibril of the secondary cell wall was shaved off partially. The regeneration of cell wall fibril was observed at the fourth day after laser treatment. As a model experiment, the plasmid DNA harboring CMV 35S promoter drove sGFP (synthetic Green Fluorescent Protein) was introduced by the microinjection through the “aperture” on the cell surface, carved by the laser irradiation. The transient expression of the gene was seen with high efficiency (max 14% of the treated cells). This method can minimize the damage to the treated cells and should be a powerful tool for introducing huge genetic materials such as chromosome or organelles into the targeted cells.

PLANT METABOLITE ANALYSIS USING LASER-ASSISTED SINGLE CELL SAMPLING

Shin’ichiro Kajiyama¹ and Akio Kobayashi²

ABSTRACT

In order to understand the physiology of a whole organism, it is necessary to understand the function of a single cell as its fundamental unit. The analysis of bulk tissue samples only gives the average information about metabolite profiles of different cell types, and this does not reflect either the fine scale metabolic profiles among individual cells or provide informations about physiologically important processes, regulated on the single cell basis. In this context, not only a reliable sampling technique from a specified single cell but also a reliable analytical method for the limited sample has been sought-after to investigate the precise metabolic profiling of different cell types. In plant cells, it is more difficult to access the inside of the cells than in animal cells because plant cells are rather small in size and have rigid cell walls. Single plant cell sampling from a targeted cell is usually performed by a fine oil-filled glass microcapillary, mounted on a micromanipulator. However, this method is rather time consuming and the presence of rigid cell wall requires high skill. Recently we developed a unique method of laser-assisted single cell sampling and applied this to metabolite analysis at the resolution of the single cell. For this purpose, we adopted a flexible excimer laser irradiation system we recently developed. The rigid cuticle and cell wall layer were removed by laser irradiation before insertion of the sampling capillary, and this procedure enables us to obtain solute from the targeted cells smoothly. Some picoliter of cell contents were picked up from a single cell of GFP expressed transgenic torenia (Torenia hybrida) using this method³. The trans gene (GFP) mRNA was detected by RT-PCR, and the petal pigments i.e. peonidin-3,5-diglucoside and malvidin-3-glucoside-5-(p-coumaroyl)-glucoside were also identified using nano flow LC-ESI MS/MS and MALDI TOF MS. Pigment analysis of a single cell sample revealed that the total anthocyanin amount as well as the proportion of each anthocyanin was different among the targeted cells. More specifically, in the upper part of petal, the malvidin-3-glucosyl 5-(p-coumaroyl)glucoside was the dominant and in the middle and lower part of the petal, peonidin-3,5-diglucoside was the major pigment. This method would be one of the most powerful tools for studying metabolic profiling of individual plant cells.

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DEVELOPMENT OF A TRANSIENT RNAi ASSAY SYSTEM USING ARABIDOPSIS MESOPHYLL PROTOPLASTS

Chung-Il An1, Ei-ichiro Fukusaki, Aki Sawada, and Akio Kobayashi

ABSTRACT

Double-stranded RNA (dsRNA) induces sequence-specific gene silencing in eukaryotes through a process known as RNA interference (RNAi). RNAi is now used as a powerful tool for functional genomics in many eukaryotes, including plants. Various types of RNAi vectors have been developed to induce RNAi in plants. However, construction of RNAi vectors and generation of transformants require laborious work, and are time-consuming. Furthermore, commonly used RNAi vectors (e.g. CaMV 35S promoter-driven RNAi vector) cannot be applied to genes whose suppression may result in lethal phenotypes. Considering the simplicity of RNAi-trigger preparation and effectiveness of gene silencing, dsRNA administration would be the best manner for transient RNAi induction in plants, as in animals. To date, however, there is no report on dsRNA-mediated RNAi induction in plant protoplasts. We herein report a dsRNA-mediated transient RNAi assay system using Arabidopsis mesophyll protoplasts. To visualize dsRNA-induced gene silencing in Arabidopsis protoplasts, mesophyll protoplasts were cotransfected with 10 µg of GFP expression plasmid and 100 bp of dsRNA. Transfection of mesophyll protoplasts with dsRNA targeting GFP significantly reduced GFP expression. However, no effect was observed when luciferase-encoding dsRNA was introduced. These results demonstrate that the in vitro-prepared dsRNA induces specific gene silencing in Arabidopsis mesophyll protoplasts. To evaluate RNAi quantitatively, we applied the dual luciferase reporter assay system to transient RNAi assay. We first examined the effect of dsRNA length on gene silencing using this system. Mesophyll protoplasts were cotransfected with 0.25 µg of reporter and internal control plasmid, and 2.5 µg of different length of dsRNA (50, 100, 250, and 500 bp). More than 90% of gene silencing was observed in every case, and longer dsRNA induced stronger gene silencing. We next examined the effect of dsRNA amount on gene silencing. Mesophyll protoplasts were cotransfected with 0.25 µg of reporter and internal control plasmids, and a different amount of 100 bp dsRNA (weight ratios of dsRNA to the reporter plasmid were 0.1, 1, and 10, which correspond to 0.025, 0.25, and 2.5 µg, respectively). When luciferase-encoding dsRNA was introduced, the reporter luciferase activities were decreased by about 60%, 80%, and 90% respectively. These results indicate that larger amount of dsRNA induced stronger gene silencing. In conclusion, we have established a transient RNAi assay system using Arabidopsis mesophyll protoplasts.

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HOW WELL ARE THE PHOTORECEPTOR SYSTEMS CONSERVED IN NON-PHOTOSYNTHETIC PLANTS?

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ABSTRACT

Light is the most important environmental signal for plants. So it is necessary to understand the photoreceptor systems to control the growth of plants by light. But limited information on the systems is available because of their complexity to date. To get new information on the systems, we focused attention on non-photosynthetic (NP) plants. Our hypothesis is that the photoreceptor systems to control the photosynthetic ability such as shade-avoidance response are lacked but the systems to control the basic photomorphological responses such as flowering are conserved in NP plants. In this study, from four NP plants (Orobanche minor, Cuscuta japonica, Monotropastrum globosum, and Galeora septentrionalis) PHYA and CRY1 homologous cDNAs were cloned. Sequence analysis revealed that 14-42 amino acids conserved in PHYAs of photosynthetic plants were substituted or deleted in the PHYAs from NP plants (fig. 1). There are many mutations in the phytochrome domains, where are directly related to the signal transduction, comparing with other domains in all four PHYA homologs. Interstingly, HisKA domain was completely disappeared in MgPHYA.

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Figure 1. Numbers and Positions of substituted or deleted amino acids in photoreceptors from NP plants. Total Numbers of mutated amino acids are indicated above the sequences. Number of mutated amino acids in each domain is indicated below.
SESSION III

FRUITING/FRUIT QUALITY
DOES PREHARVEST APPLICATION OF NAA INDUCE RIPENING ON CLIMACTERIC TREE FRUIT?

Eric A. Curry

ABSTRACT

1-Naphthaleneacetic acid (NAA) has been an effective bioregulator for the control of preharvest fruit abscission on apples and pears for more than 60 years. Although it has been reported NAA at super-optimal rates for control of preharvest drop may advance ripening, data reported herein suggest this may not be the case. Like ethylene, NAA promotes fruit abscission in the early spring, presumably by an ethylene-induction mechanism. Because ethylene also stimulates fruit ripening in the fall, is it tempting to assume NAA acts similarly on mature fruit tissue, even though ethylene promotes whereas NAA inhibits fruit abscission. When applied to individual ‘Delicious’ spurs 2 weeks before commercial harvest, fruit treated with ACC produced ethylene sooner than untreated fruit, whereas those treated with NAA produced less than controls (Fig 1.). These data indicate both leaves and fruit retain the ability to produce ethylene from ACC. NAA does not appear to induce ethylene in these tissues, and in fact suppresses ethylene production in whole fruit. In a related trial, ‘Delicious’ trees receiving a 4X treatment of NAA (80 ppm) 2 weeks before commercial harvest were examined for changes in quality. Treated fruit had similar soluble solids, firmness, and rate of starch conversion, but a marked reduction in ethylene increase two weeks after initial treatment (Data not shown).

Opposing fruit abscission responses to preharvest ethylene or NAA, therefore, may be due to: 1) differential tissue sensitivity or response to NAA; 2) differential auxin status across the abscission zone created by the changing absorptive surface areas of leaves and fruit; or 3) NAA-induced inhibition of ethylene in the stem abscission zone as is evidenced in fruit tissue (Fig. 1). As seen in the figure to the right, when all ‘Delicious’ fruit were removed 2 weeks after treatment, mean starch rating was similar between those from treated and control trees. Apparent is the absence from the untreated controls of fruit with more advanced starch clearing, i.e., those fruit which probably dropped prematurely (Fig. 2). Indeed, that NAA advances ripening may be a perception based on the artificial retention of fruit of advanced maturity.

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RESPONSE OF ‘CRIOLLA DOMINICANA’ TABLE GRAPE (*VITIS VINIFERA*) TO NITROGUAIACOL, BRASSINOLIDE, AND ACETYLTHIOPROLINE

J. Pablo Morales-Payan¹ and William M. Stall²

ABSTRACT

A study was conducted in the Dominican Republic to determine the effect of the biostimulants acetylthioproline (AP) (0, 75, 150, 225, and 300 mg.L⁻¹), sodium salts of 5-nitroguaiacol + ortho-para-nitrophenol (NG) (125, 250, 375, and 500 mg.L⁻¹), and brassinolide (BR) (0.01, 0.02, 0.03, and 0.04 mg.L⁻¹) on the yield and marketable quality of ‘Criolla Dominicana’ table grape. The biostimulants were sprayed at the same rates once (at flowering), twice (at flowering and 15 days later), or three times (at flowering, plus 15 and 30 days later). Yield and marketable quality (appearance) were determined at harvest. When the biostimulants were applied once, yield and quality were not significantly affected, as compared to control plants. Two applications of the biostimulants significantly increased fruit yield as compared to control plants, but three applications did not result in further yield improvement. When BR was applied two or three times, fruit yield increased approximately 15% when rates increased from 0 to 0.02 mg.L⁻¹, and yield increase reached a maximum of approximately 19% at higher rates. BR increased yield by increasing berry size. However, BR did not lengthen the rachis, which lead to berries being too tight in the cluster, misshapen, and more prone to fruit rots. Two or three applications of NG increased yield approximately 17% when rates increased from 0 to 250 mg.L⁻¹, with maximum yield increase of approximately 22% at higher rates. Grape yield was 18, 24, and 27% higher than in control plants when AP was applied two or three times at the rates of 150, 225, and 300 mg.L⁻¹, respectively. NG and AP treatments did not adversely affect grape quality, since rachis elongated sufficiently to accommodate the increased berry size.

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SYMPOSIUM II

BIOCHEMICAL AND MOLECULAR ASPECTS OF PGR’S
USING PROTEOMIC TOOLS TO STUDY ACTIONS OF PLANT POLYPHENOLS ON DISEASE-RELEVANT PROTEINS

Helen Kim

ABSTRACT

In 2002, Americans spent $3 billion on over-the-counter dietary supplements. Many of these, including botanically-derived ones such as grape seed extract, are valued for their high proanthocyanidin content which is considered beneficial because of the anti-oxidant activity intrinsic to the proanthocyanidins. Oxidative stress is a risk factor for age-related neurodegenerative conditions, including Alzheimer’s disease. We hypothesized that dietary supplementation with grape seed extract enriched in proanthocyanidins would have neuroprotective actions in normal adult rodent brain that would be manifested as either enhancement of protein changes important for neuronal viability, and/or reduction of protein changes linked with neuropathology. We utilized proteomics technology (2-dimensional electrophoresis, matrix-assisted laser desorption ionization time of flight mass spectrometry [MALDI-TOF MS] and liquid chromatography-tandem mass spectrometry [LC-MS/MS]) to assess protein changes in rat brains after dietary supplementation with grape seed extract (donated by Kikkoman Corporation, Chiba, Japan). 43 day old normal female rats were maintained on 5% grape seed extract-supplemented diets for six weeks, after which they were sacrificed, the brains dissected out, snap frozen in liquid nitrogen, and archived at –80°C. Homogenates of the rat brains were subjected to two dimensional electrophoresis (isoelectric focusing, followed by SDS-PAGE) using a linear pH 4-7 gradient for the first dimension, and electrophoresis in the second dimension on a 10-20% acrylamide gel. The resultant 2D gels were stained with Coomassie Brilliant Blue. The gel images were acquired with a densitometer, and subjected to software-assisted image analysis. The latter identified several (13) gel “spots” that quantitatively differed in intensity or in horizontal position between the two sets of gels representing the dietary groups. MALDI-TOF MS generated identities of the proteins were confirmed by LC-MS/MS. The quantitative differences indicated by the image analysis were confirmed in many cases by western blot analysis. With the exception of one novel protein, and a cytoskeletal protein, all the proteins identified in this study were previously implicated in either Alzheimer’s disease brain tissue, or brains from mouse models of neurodegeneration. Moreover, the differences determined in this study were in the opposite direction to those detected for these proteins in the disease tissues. These results strongly suggest that ingestion of components in grape seed extract was neuroprotective, and that efforts toward engineering plants that have enhanced proanthocyanidin-producing capabilities be given high priority.

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HORMONAL INTERACTIONS IN FRUIT DEVELOPMENT

Jocelyn Ozga and Dennis Reinecke

ABSTRACT

Fruit development involves a complex interplay of cell division, differentiation and expansion of sporophytic and gametophytic tissues that is carefully coordinated over time. Plant hormones are signals that regulate many processes of plant development including fruit development leading to mature fruit and viable mature seed. Auxins, gibberellins (GAs), cytokinins, abscisic acid, and ethylene have been implicated at various stages of fruit development. In the past hormone application studies and hormone analysis studies have supported that fruit development is in part regulated by hormonal interaction. More recently, biochemical and molecular studies are showing how hormones effect fruit development. This talk will focus on understanding the interaction between auxin and gibberellin in pea fruit development using physiological, biochemical, and molecular approaches.

In most fruits, normal ovary (pericarp) growth requires the presence of seeds and the final weight of the fruit is often proportional to the number of developing seeds (Nitsch 1970). This is the case in pea where pericarp growth (length, fresh weight and dry weight) was positively correlated with initial seed number (Ozga et al., 1992) and the removal or destruction of the seeds 2 to 3 days after anthesis (DAA) resulted in the slowing of pericarp growth and subsequent abscission (Eeuwens and Schwabe 1975; Ozga et al., 1992). Chemical signals originating from the seeds may be responsible for continued fruit development by maintaining hormone levels in the surrounding fruit tissue (Eeuwens and Schwabe, 1975; Sponsel, 1982). Developing pea seeds and pericarps contain gibberellins (GAs; Garcia-Martinez et al., 1991; Rodrigo et al., 1997) and auxins [4-chloroindole-3-acetic acid (4-Cl-IAA) and IAA; Marumo et al., 1968; Magnus et al., 1997]. During early pericarp growth (2 DAA), application of the naturally occurring hormones, auxin (4-Cl-IAA; Reinecke et al., 1995) and GA (Eeuwens and Schwabe, 1975; Ozga and Reinecke, 1999), to deseeded pericarp can substitute for seeds and stimulate pericarp growth.

Pea plants metabolize GAs by the early 13-hydroxylation pathway: GA$_{12}$ → GA$_{53}$ → GA$_{44}$ → GA$_{19}$ → GA$_{20}$ → GA$_1$ (Sponsel 1995). In pea, PsGA20ox1 codes for the multifunctional enzyme GA 20-oxidase that converts GA$_{53}$ to GA$_{20}$ (Martin et al., 1996; Garcia-Martinez et al., 1997) and PsGA3ox1 (Mendel’s LE gene) codes for the enzyme GA 3ß-hydroxylase (Lester et al., 1997; Martin et al., 1997) that converts GA$_{20}$ to the biologically active GA$_1$. Pericarps, from unpollinated pea ovaries (2 days before anthesis), contained high levels of PsGA20ox1 mRNA (van Huizen et al., 1997) and GA$_{20}$ (5 ng g$^{-1}$ fwt$^{-1}$ for emasculated ovaries at the equivalent to 0 and 1 DAA, Garcia-Martinez et al., 1991). However, pericarps from unpollinated ovaries (2 d before anthesis) contained minimally detectable levels of PsGA3ox1 mRNA (Ozga et al., 2003) and GA$_1$ (García-Martínez et al., 1991). At anthesis, pericarp and seed PsGA3ox1 mRNA levels increased 50-fold and 19-fold, respectively. By 1 DAA, message level had dropped rapidly to 9- to 10-fold above pre-pollinated levels and remained at this level through 4 DAA for pollinated ovaries. Although steady-state GA$_1$ levels were reported to be the same in pollinated and unpollinated ovaries at 0 DAA, GA$_8$ levels were two times higher in pollinated than unpollinated ovaries at this time (García-Martínez et al., 1991). Since GAs is the immediate biologically inactive product of GA$_1$ (as a result of 3β-hydroxylation), these data suggest that more GA$_1$ was synthesized in pollinated than unpollinated pericarps and/or ovules at 0 DAA. In
addition, the large increase in \( \text{PsGA3ox1} \) mRNA levels was not observed in emasculated pericarps at the equivalent to 0 DAA. These data show that pollination triggers the synthesis of pericarp \( \text{PsGA3ox1} \) mRNA message, and suggest that \( \text{GA1} \) is synthesized from the pool of \( \text{GA20} \) present in pre-pollinated pericarps by pericarp \( \text{GA3} \beta\)-hydroxylase. The resulting pulse of \( \text{GA1} \) could stimulate initial fruit set and development.

Both \( \text{PsGA20ox1} \) (van Huizen et al., 1997) and \( \text{PsGA3ox1} \) (Ozga et al., 2003) mRNA levels substantially decreased after pollination in the pericarp. This decrease in message levels during this stage of ovary development may be important for a multitude of factors, including hormones (auxins and GAs), to fine-tune regulate GA biosynthesis to coordinate ovary and seed development.

Studies using the pea split-pericarp assay (test compounds are applied to the inner walls of split, or split and deseeded 2-DAA pericarps) have shown that the presence of seeds is required to maintain the conversion of \( [14\text{C}]\text{GA19} \) to \( [14\text{C}]\text{GA20} \) (van Huizen et al., 1995), the expression of \( \text{PsGA20ox1} \) (van Huizen et al., 1997) and \( \text{PsGA3ox1} \) (Ozga et al., 2003) in the pericarp.

The effects of hormones (4-Cl-IAA, IAA and \( \text{GA3} \)) on the expression \( \text{PsGA20ox1} \) and \( \text{PsGA3ox1} \) message were also investigated. The application of 4-Cl-IAA to 2-DAA deseeded pea pericarp stimulated the conversion of \( [14\text{C}]\text{GA19} \) to \( [14\text{C}]\text{GA20} \) (van Huizen et al., 1995) and the expression of \( \text{PsGA20ox1} \) (van Huizen et al., 1997) and \( \text{PsGA3ox1} \) (Ozga et al., 2003) message in pea pericarp. IAA, which does not stimulate pericarp growth was ineffective in stimulating expression of pericarp \( \text{PsGA20ox1} \) (Ngo et al., 2002) or \( \text{PsGA3ox1} \) (Ozga et al., 2003) message. These data suggest that similar auxin-induced transcription regulatory elements may operate to coordinate regulation of this part of the GA biosynthesis pathway, and that biologically active auxin acts in a concerted fashion on the GA biosynthesis pathway to stimulate production of active GAs in the fruit.

In addition, \( \text{GA3} \) reduced 4-Cl-IAA stimulation of both \( \text{PsGA20ox1} \) and \( \text{PsGA3ox1} \) message levels in deseeded pericarp. The inhibitory effect of \( \text{GA3} \) on 4-Cl-IAA-stimulated increases of both \( \text{PsGA20ox1} \) and \( \text{PsGA3ox1} \) message levels could be a direct effect of elevated levels of GA on the auxin-stimulation pathway or an indirect effect through the GA feedback regulation pathway.

Interestingly, in intact fruit, pericarp \( \text{PsGA3ox1} \) message levels increased linearly from 6 to 10 DAA. The increase in \( \text{PsGA3ox1} \) mRNA levels coincided with the rapid increase in pericarp diameter (inflation) to accommodate the developing seeds (Ozga et al., 2003). A peak in auxin levels (IAA and 4-Cl-IAA, Eeuwens and Schwabe 1975; Katayama et al., 1988; Magnus et al., 1997) in the seeds precedes the increase in \( \text{PsGA3ox1} \) mRNA levels in the pericarp at this stage. The steady-state \( \text{GA1} \) levels in the pericarp (approximately 1 ng · g fw·1 from 4 to 12 DAA; Rodrigo et al., 1997), however, do not increase with increasing \( \text{PsGA3ox1} \) message level during this period. Yet, Sponsel (1982) found that 1 µg of \( \text{GA3} \) applied to the pericarp of “seed-killed” ovaries (seeds killed 2 DAA by pricking them with a needle) was sufficient to restore pericarp elongation growth, whereas 10 µg of \( \text{GA3} \) per pericarp was required to restore normal pericarp inflation. The greater amount of \( \text{GA3} \) required for stimulation of pericarp inflation coincides with a peak in \( \text{GA3ox} \) message expression during this time. Therefore, it is possible that the turnover rate of \( \text{GA1} \) is substantially higher during this period, resulting in similar steady-state \( \text{GA1} \) levels from 4 to 12 DAA, or that a tissue-localized increase in GA biosynthesis is required for pericarp inflation.

The following paragraph describes a working model for hormonal-directed fruit set, and seed and pericarp coordinated development based on pea fruit development (Figure 1). Pollination stimulates \( \text{GA1} \) synthesis via an increase in \( \text{GA3ox} \) mRNA levels (possibly via stimulation of auxin synthesis in the ovary) in both the seeds and pericarp resulting in initial fruit set and growth of both tissues. Subsequently, seeds maintain growth in the pericarp at least in part by transporting auxin (4-Cl-IAA) to the pericarp, where it stimulates both \( \text{GA20ox} \) and \( \text{GA3ox} \) message levels maintaining a critical level of \( \text{GA1} \) for pericarp growth. Biologically active GA (\( \text{GA1} \)) also can feed-back regulate its synthesis in the pericarp by reducing \( \text{GA20ox} \) and \( \text{GA3ox} \) message levels. In addition, auxin (4-Cl-IAA) affects fruit
growth directly through auxin-mediated responses (van Huizen et al., 1996). Therefore, an auxin-GA interaction is required for coordination of fruit and seed development. In other plant systems (tomato, Arabidopsis, etc.), IAA would replace 4-Cl-IAA in the model since 4-Cl-IAA in plants has been localized (with one exception) to the Vicieae tribe from the Fabaceae family (legumes, Reinecke 1999).

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Figure 1. Model for GA-auxin interactions during pea fruit growth. 4-Cl-IAA transported from the seeds stimulates the pericarp GA biosynthetic pathway [GA20ox (GA19→GA20) and GA3ox (GA20→GA1, active GA)]. GA1 may feedback regulate levels of GA20ox and GA3ox message. 4-Cl-IAA also effects pericarp growth directly (independently of GA biosynthesis).
SESSION IV

GENE EXPRESSION/METABOLISM
A KINASE HIGHLY HOMOLOGOUS TO PKA FROM PARASITIC PLANT

K. Uematsu¹, K. Yomeyama², Y. Sugimoto³ and Y. Fukui¹

ABSTRACT

To understand the germination mechanism of root parasitic plant (*Orobanche minor*), we searched for genes coding protein kinases, expressed in the conditioned seed. One of them showed significant homology to catalytic subunits of PKAs (cAMP-dependent protein kinase) from other organisms, which was named as PKA1. PKA has not been found in higher plants. We cloned the entire sequence of this gene. The gene product expressed in *E.coli* exhibited the kinase activity on the synthetic PKA substrate (kemptide). To confirm that PKA1 can be defined as a PKA, we also searched for a PKA regulatory subunit. The entire coding region of one gene was obtained. The gene was named REG1. The gene product expressed in *E.coli* exhibited the cAMP binding activity. The recombinant PKA1 was mixed with the purified recombinant REG1 and its catalytic activity was examined. REG1 inhibited the activity almost completely. The enzyme activity was restored by addition of cAMP. These results suggest that PKA1 is regulated by the cAMP level through REG1.

For a candidate for the substrate of PKA1, pyruvate kinase genes were cloned. Two fragments were obtained by PCR. These genes were named PYK1 and PYK2. When these recombinant proteins incubated with recombinant PKA1, not PYK2 but PYK1 was phosphorylated. This result suggests that PYK1 can be regulated by PKA1. We analyzed the cAMP level during the conditioning period, when PKA1 was expressed. The cAMP level was undetectable when conditioning started. However, as the incubation time became longer, the level rose to about 0.04 pmol/mg seeds after 3 days. This result suggests that the cAMP level is regulated in *O. minor* seeds.

In this study, we have shown that PKA is present in *O. minor*. Because the cAMP level changes during the conditioning period, the PKA activity may be regulated in this period. In addition, a pyruvate kinase was found to be a putative substrate of PKA1, which can be regulated by PKA1. These results suggest that cAMP-PKA system is present and may play an important role in *O. minor* seed.

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ISOLATION AND CHARACTERIZATION OF ETHYLENE INSENSITIVE 2 GENE IN PETUNIA (PETUNIA HYBRIDA)

Kenichi Shibuya¹, Kris Barry¹, Joseph Ciardi², Holly Loucas¹, Harry Klee³ and David Clark¹

ABSTRACT

The EIN2 protein plays a central role in the ethylene signal-transduction pathway. Loss-of-function mutations in EIN2 result in complete ethylene insensitivity in Arabidopsis. However, the role of this protein has not been well examined in species other than Arabidopsis. To gain a better understanding of EIN2 function, we have isolated a homologue of the Arabidopsis EIN2 gene (PhEIN2) from Petunia hybrida. The predicted sequence of PhEIN2 showed significant identity to Arabidopsis EIN2 throughout the entire protein, and there is a particularly highly conserved region in the carboxyl-terminus. Transgenic petunia plants with reduced expression of PhEIN2 exhibited ethylene-insensitive phenotypes in all responses examined. In the transgenic plants, flower longevity after exogenous ethylene treatment or pollination increased up to 5-fold compared to wild-type plants. The PhEIN2 transgenic plants also showed delayed fruit ripening. An inhibition of adventitious root formation of vegetative cuttings in the transgenic lines was also observed. PhEIL1 expression was induced by exogenous ethylene treatment in wild-type plants but it was lessened or not induced in the transgenic lines with reduced PhEIN2 expression. Furthermore, a greater reduction of PhEIN2 expression resulted in stronger ethylene insensitivity in all phenotypes examined. These results indicate that PhEIN2 positively regulates a wide range of ethylene responses in petunia.

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3’ UNTRANSLATED REGION SPECIFIC RNAi AND ITS APPLICATION TO FACILE HUNTING OF LOW EXPRESSING GENE HOMOLOG

E. Fukusaki1, K. Kawasaki, C.-I. An, A. Sawada, S. Kajiyama, A. Kobayashi

ABSTRACT

RNAi induction for chalcone synthase gene (CHS) of torenia, *Torenia hybrida* Blue was performed in order to elucidate the effect of target region that was introduced and expressed as double strand RNA in plant. Each inverted repeat DNA construct for a coding region and 3’UTR region of *CHS* was introduced into torenia plant. The transformant targeting a coding region represented a complete disappearance of follower color. In contrast, petals of the transformant for 3’UTR showed pale blue color. These results suggest that all the homologous genes were silenced in case of RNAi with coding region and the target gene was specifically down regulated with 3’UTR. Furthermore, we succeeded in the cloning of a minor CHS homologue gene with low expression level from the cDNA of the CHS 3’UTR RNAi transgenic plant in which a major CHS was thought to be specifically silenced. This result opened up the possibility that RNAi would be used for the efficient cloning of the low expression homologous genes.

Figure.  a) The upper line indicates a schematic outline of the *Torenia hybrida* chalcone synthase (CHS) mRNA (1465 base). The arrow 1 represents the coding sequence which was used in the CHS antisense construction and CHS CDS RNAi construction; the arrow 2 is for the CHS 3’UTR sequence which was used in CHS 3’UTR RNAi. Black bar indicates the probe sequence which was used in Southern and Northern analysis. The shaded bar shows the amplified region in the quantitative RT-PCR analysis. The second, the third and the bottom line indicate the intermediate construct pCHSint, pCHSint2 and pCHSint3. b) Schematic outline of the T-DNA constructs. Nos-pro, nopaline synthase promoter; 35S pro, CaMV 35S promoter; Nos-ter, terminator of nopaline synthase gene; NPT2, neomycin phosphotransferase; arrow CHS in antisense construction, ‘pCHSanti’ indicates CHS partial coding sequence (nt 304-912); arrow CHS in the CHS CDS RNAi construction pCHSRNAi also indicates CHS partial coding sequence (nt 304-912) and the spacer is 1870 bp GUS sequence; arrow CHS in the 3’UTR RNAi construct, ‘pCHS3’UTRRNAi’ indicates CHS partial 3’UTR sequence (nt1275-1406) and the spacer is its upstream region (nt 840-1274).

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ROLES OF BRASSINOSTEROIDS AND THEIR BIOSYNTHESIS GENES IN SEED GROWTH AND GERMINATION OF PEA (PISUM SATIVUM L.)

Takahito Nomura1*, Masaaki Ueno2, Suguru Takatsuto3, Yasutomo Takeuchi2 and Takao Yokota1

ABSTRACT

To investigate the roles of brassinosteroids during seed growth/maturation and germination, we quantified the endogenous BRs and the transcripts of BR synthesis (LKB, LK, PsDWF4, PsCPD1, PsCPD2, DDWF1, PsD), metabolism (PsBAS1) and receptor (LKA) genes in seeds and seedlings of pea (Pisum sativum L.). As pea seeds rapidly grow, the levels of brassinolide and castasterone were increased but, in fully-expanded seeds, were decreased drastically, indicating brassinolide and castasterone are important for seed growth. In support of this, the PsD expression was increased in conjugation with the increase of castasterone and brassinolide. 6-Deoxocastasterone was accumulated high in fully expanded seeds but rapidly decreased through desiccation presumably by the action of the PsBAS1 enzyme. The levels of upstream precursors, 6-deoxocathasterone, 6-deoxoteasterone, 3-dehydro-6-deoxoteasterone and 6-deoxotyphasterol were not changed much from immature to mature stages. 6-Deoxocathasterone was the major brassinosteroid in mature seeds and is likely to be an important storage form. Through seed growth, the LK transcript levels remained constant but those of other genes were fluctuated. In mature seeds, the PsCPD1 gene level increased markedly while the levels of LKB, PsCPD2, DDWF1, PsBAS1 and LKA were considerably high although much lower than in immature seeds, suggesting that the mRNAs of these genes may be stored as informosomes and utilized to generate brassinosteroids when seeds germinate. In imbibed seeds, neither castasterone nor brassinolide were detected but the transcripts of PsD, DDWF1, PsDWF4, PsBAS1 and LKA were increased. One and three days after the imbibition, castasterone was detected in germinating seeds. In these plantlets, the level of castasterone as well as the PsD expression was high in shoots and roots but low in seeds, indicating that the PsD protein seems to be a key enzyme for seed germination.

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A PRELIMINARY COMPARISON OF GENE EXPRESSION BETWEEN VEGETATIVE AND FEMALE BUDS OF BLACK SPRUCE (Picea mariana) USING SERIAL ANALYSIS OF GENE EXPRESSION (SAGE)

R.F. Smith¹, J. Letourneau¹ and S. Sauer²

ABSTRACT

Significant progress has been made in identifying the genes associated with flowering in many herbaceous plants. However, by comparison, little progress has been made in conifers. Serial Analysis of Gene Expression (SAGE) is a relatively new technique which offers several advantages in that it can be used both to detect and identify ‘novel’ genes that are being expressed and to provide a quantitative assay on the abundance of known transcripts. Further, SAGE allows for the screening of more genes at a time than do techniques such as RT-PCR. This paper will briefly describe the SAGE technique, and provide an overview of some preliminary results from a comparison between SAGE-tag libraries produced from post-dormancy (spring) female and vegetative buds of black spruce (Picea mariana).

INTRODUCTION

The events that lead to the successful formation of a flower or reproductive organ requires a coordinated series of steps involving both the activation and inactivation, and the up- and down-regulation, of a series of genes over time. It is only when these genes interact in a coordinated manner that flowering can be successful (cf Carpenter et al 1995, Theissen et al 2000). The ABC model of flowering (see Weigel and Meyerowitz 1994, Yanofsky 1995) was developed to help explain both the positioning of the floral structures (sepals, petals, stamens and carpels) and to classify the genes involved in the formation of each of these organs. However, it is now recognised that some floral homeotic genes cannot be classified as ‘canonical’ A-, B-, or C-class genes (Eckardt 2003). More recently, the ‘Quartet Model’ was proposed to better explain the genetic control of floral organ identity. In the Quartet model, four classes of proteins form ‘higher order’ multiprotein complexes to control floral organ identity (Theissen 2001).

Comprised of approximately 20 billion base pairs, pines (Mann and Plummer 2002), as well as other conifers, possess genomes approximately 100-fold larger than Arabidopsis (TAI 2000). Similarly, the number of genes is unknown, and, compared to many crop plants, very few expressed sequence tags (ESTs) have been identified. The scarcity of baseline genetic information for conifers compared to ‘model’ herbaceous species such as Arabidopsis thaliana, poses considerable difficulties in genetic studies. Of the few studies targeted at elucidating the genetic control mechanisms of reproductive development in conifers, most have focussed on identifying homologues to flowering genes in other plants (cf Rutledge et al 1998, Tandre et al 1995).

Serial Analysis of Gene Expression (SAGE), developed at John Hopkins University (see Velculescu et al 1995, 1997), is a relatively new technique for both revealing (identifying) genes that are being expressed and quantifying their levels of expression. Using SAGE, it is possible to effectively compare gene expression between two cell or tissue types, detect and identify novel genes that are being expressed, and to provide a quantitative assay on the abundance of known transcripts. A basic principle of SAGE is that a short sequence comprised of 9 to 11 bp (base pairs) contains sufficient information to identify a unique transcript and that by joining a number of these tags together

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(concatemerization) these long DNA sequences can then be read in a single sequencing event, thereby providing information on many genes at once. Detailed descriptions of SAGE methodologies can be found at the following web sites: a) www.sagenet.org and b) www.embl.org/info/sage.

The ability of SAGE to measure, in one step, both quantitative and qualitative aspects of gene expression, distinguishes it from other techniques currently available such as expressed sequence tags (ESTs) which take a one gene at a time approach, and microarrays which are limited to quantifying the expression level of a predetermined and presently ‘incomplete’ set of genes (Madden et al 2000). Few studies have used SAGE with plants, and even fewer on conifers (cf Lorenz and Dean 2002). This paper presents some preliminary results of a comparison between SAGE-tag libraries from vegetative and seed-cone buds in black spruce (Picea mariana (Mill.) B.S.P.).

MATERIALS AND METHODS

Tissue Collection: Post-dormancy female and vegetative buds from black spruce were collected in April 2003, placed immediately into liquid nitrogen in the field and placed into a –80°C freezer until RNA was extracted.

RNA Isolation: Frozen buds were ground to a fine powder in liquid N2 using a mortar and pestle. Total RNA was isolated using the method described in by Chang et al (1993) with the following modifications; the volume of extraction buffer was reduced to 6 mL for each 1 g of plant tissue, the tissue was incubated in the extraction buffer for 15 minutes @ 65°C with mixing at 5 minute intervals and the pellet was washed with 500uL of 70% EtOH after the ethanol precipitation step. Chloroform:IAA extractions were done using phase lock gel (PLG) tubes (Brinkman, Westbury, NY) to maximize sample recovery and extraction efficiency.

Serial Analysis of Gene Expression: Serial Analysis of Gene Expression Libraries were generated using the protocol as described by Jasper et al 2001 (in Supplemental Data) with the exception that PLG tubes were used for all phenol:chloroform extractions. All cDNA sequencing was done by a commercial sequencing facility (DNA Landmarks, Montreal, Quebec, Canada).

Data analyses: SAGE tag sequences were compared to the NCBI Arabadopsis database using a Basic Local Alignment Searches Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST). A preliminary comparison between the two libraries was made using both actual and normalized datasets. Data were normalized to 20,000 tags using SAGE software (Genzyme Molecular Oncology, Framington, MA, USA).

RESULTS

Of the total of 20209 and 4326 SAGE tags produced from the seed-cone (female) and vegetative buds, 39.8% and 40% were unique or single-copy tags respectively (Figure 1). Of the 20 most abundant tags found in the female library, three were identified as putatively corresponding to flowering-related genes/proteins and seven others as transcription regulators (F-box) (Table 1). Three of the most abundant tags from the reproductive library coincided with flowering-related genes in the Arabadopsis database, whereas none of these flowering-gene tags were observed in the tags from the vegetative library. Conversely, although putative transcription regulators were present in the vegetative library (4/20) the majority of the tags (10/20) matched genes/sequences associated with compounds normally involved in maintaining bud hardiness over winter storage (disease-resistance/storage proteins etc.).
Figure 1. Summary of the numbers of different SAGE tags (no. genes in class) versus the percentage of the total tags observed in the library. N=20,209 and 4326 for the seed-cone (reproductive) and vegetative bud libraries respectively.

There were tags unique to each library that were consistent with the bud-type e.g., transcripts associated with vegetative and reproductive growth exclusively. Of the 22 tags with 10 or more copies present in the female and not present in the vegetative library, 5 had matches with ‘flowering’ genes, 7 were transcriptional regulators, and the other 10 ‘housekeeping’ genes e.g., plant growth regulator response genes, general storage/metabolism. Conversely, of the 17 comparable tags present in the vegetative but not in the female library, 3 matched ‘needle/leaf’ development genes, 9 were transcriptional regulators, and 5, ‘housekeeping’ genes, comparable, but different from those observed in the female library (data not presented).

DISCUSSION

In black spruce, as well as other spruces, reproductive buds develop one to two weeks before comparable vegetative buds on the same tree. The female buds at the time of sampling in this study were entering into a rapid expansion phase (see description in Owens and Blake 1985) whereas the vegetative buds, although undergoing some cell division, had not yet significantly began to increase in size. The greater abundance of transcription factor related tags (e.g., F-box) present in the female buds, and the greater abundance of tags associated with compounds normally associated with maintaining bud hardiness over winter storage (disease-resistance/storage proteins etc.) in the vegetative buds, would be consistent with the different physiological stages of development for the two bud-types.

The Unusual Floral Organs (UFO) gene in Arabidopsis, which is expressed in the shoot apical meristem throughout development, encodes for an F-box-containing protein that is essential for the proper patterning and identity of both petals and stamens (Durfee et al 2003). Although functional analyses of the F-box genes in this study have not been done, their apparent greater abundance in the female than the vegetative library is a further indication of the potential for SAGE as a diagnostic tool for transcription profiling.
Table 1. Summary and descriptions (selected matches following BLAST) of the most abundant SAGE tags from the A) Seed-cone (female) bud library (n=20,209) and B) vegetative library (n=4,326). The abundance represents the percent of the total tags in a given library. Accession no. and identification are from NCBI.

### A. Seed-cone (female) buds

<table>
<thead>
<tr>
<th>Rank</th>
<th>Tag sequence</th>
<th>Abundance (%)</th>
<th>Accession no.</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GGGAACTGCG</td>
<td>0.98</td>
<td>NM_118530</td>
<td>Auxin response transcription factor</td>
</tr>
<tr>
<td>2</td>
<td>GTCAGGTAAT</td>
<td>0.83</td>
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<td>3</td>
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<td>NM_123141</td>
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</tr>
<tr>
<td>4</td>
<td>GGGAGGTAGT</td>
<td>0.53</td>
<td>NM_124207</td>
<td>Expressed protein</td>
</tr>
<tr>
<td>5</td>
<td>CGGCTGGGA</td>
<td>0.48</td>
<td>NM_124097</td>
<td>Disease resistance protein</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>7</td>
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<td>F-box protein family (transcription regulator)</td>
</tr>
<tr>
<td>8</td>
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<td>Cellulose synthase, catalytic subunit</td>
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<tr>
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### B. Vegetative buds

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<th>Abundance (%)</th>
<th>Accession no.</th>
<th>Identification</th>
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</tr>
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<td>Cellulose synthase, catalytic subunit</td>
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<td>CGGCTGGGA</td>
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<td>11</td>
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<td>Multidrug resistance, P-glycoprotein</td>
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<td>CCCGGTGCGA</td>
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<td>NM_111292</td>
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</table>
Screening SAGE tags against existing databases often yields tags with no matches e.g., no corresponding ‘hits’, partly, because many of the sequences, in GenBank and similar databases do not necessarily contain the 3’-most sequence, often resulting in less than 50% of the SAGE tags ‘matching’ known genes or ESTs (van den Berg et al 1999, Zhang et al 1997). Similarly, in loblolly pine (*Pinus taeda*), approximately 50% of over 59,700 ESTs from wood-forming tissues exhibited no apparent homologs in *Arabidopsis* or any other angiosperm in public databases (Kirst et al 2003). However, in this study, BLAST searches against the *Arabidopsis* database produced multiple positive ‘hits’ for all of the SAGE tags examined (>2 copies). Although multiple matches in the databases creates difficulties associated with determining which is the ‘correct’ gene associated with the tag, these preliminary results did demonstrate sufficient qualitative and quantitative differences to indicate that the SAGE technique could be used to distinguish between the two tissues.

**CONCLUSIONS**

Considerable work remains to be done in the production and identification of SAGE tags. As the number of SAGE tags produced increases, it will become possible to estimate the number of unique transcripts from these tissues. We hope to produce approximately 50,000 SAGE tags from each tissue type. Depending on the level of redundancy observed, this may allow us to determine a first estimate of how many genes are being expressed in each bud-type. The relatively small number of conifer, and more specifically spruce ESTs in the public databases will continue to be problematic in that the number of SAGE tags that correspond to known ESTs (or genes) are limited. We have produced and sequenced over 10,000 ESTs from black spruce buds and are now comparing the SAGE tags to this database. A Genome Canada /Genome B.C. program for forestry (http://www.bcgsc.ca/ge/poplart), which has as one of its major goals to produce 100,000 to spruce ESTs, should over the next few years, further reduce our reliance on linking SAGE tags with genes from other plants.

**ACKNOWLEDGMENTS**

We wish to thank Dr. Vladimir Benes and the staff at the Genomic Core Facility at EMBL for helpful discussions and technical support.

**LITERATURE CITED**


Theobroxide stimulates lipoxygenase activity and level of jasmonic acid in short-day plants

Xiquan Gao, Chisato Minami, Qing Yang, Atsuo Kimura, Hideyuki Matsuura and Teruhiko Yoshihara

ABSTRACT

Theobroxide spraying completely reversed the inhibitory effect of night break (NB) on potato (Solanum tuberosum L.) tuberization and flower buds formation in Pharbitis nil. Theobroxide also stimulated lipoxygenase (LOX) activity in potato and Pharbitis nil plants. Theobroxide-treated potato plants had an increased level of endogenous jasmonic acid (JA) under LD, and it suggested that JA was metabolized to tuberonic acid (TA) during 2 weeks after treatment. Spraying with salicylhydroxamic acid (SHAM), however, repressed the effect of theobroxide on potato tuberization and flower bud formation in Pharbitis nil. LOX activities and endogenous contents of JA and TA were also reduced in theobroxide+SHAM-treated plants. These results suggest that theobroxide has a potent role in the developmental regulation of short-day plants, and the inductive effect of theobroxide on tuberization and flower bud formation is possibly achieved by stimulating JA cascade.

INTRODUCTION

Under short day (SD) conditions, potato plants produce tubers and Pharbitis nil forms flower buds, whereas in long days (LD) they do not. Some potato species do not form tubers when grown in SD supplemented with a night-break (SD+NB) (Ewing and Struick, 1992; Jackson, 1999), and NB also represses flower bud formation in Pharbitis nil. Although some endogenous plant hormones in the regulation of potato tuberization and flower bud formation in Pharbitis nil have been well reported, the exogenous roles of them are not clear (Wijayanti et al., 1995; Xu et al., 1998; Jackson, 1999).

It is well documented that exogenously applied jasmonic acid (JA) and its methyl ester (MeJA) can induce tuberization of potato stolons cultured in vitro (Koda and Kikuta, 1991; Pelacho and Mingo-Caster, 1991; Castro et al., 2000). 12-hydroxy jasmonic acid glucoside (named tuberonic acid glucoside, TAG) isolated from the leaves of Solanum tuberosum L. cv. Irish Cobbler (Yoshihara et al., 1989) was also found to be capable of inducing tuberization. Both TA (tuberonic acid) and TAG are structurally related to JA.

In plants, JA is formed from linolenic acid (LA). Lipoxygenase (LOX; EC 1.13.11.12) is the first enzyme that converts LA to JA (Yoshihara et al., 1999). Salicylhydroxamic acid (SHAM) is an efficient JA biosynthetic inhibitor that blocks LOX activity and gene expression (Parrish and Leopold, 1978). Suppression of LOX activity reduced tuber yield, decreased average tuber size and disrupted tuber formation (Kolomiets et al., 2001), suggesting that LOX is involved in the control of potato tuber development.

Recently, theobroxide, a novel compound isolated from the culture filtrate of a pathogenic fungus Lasiodiplodia theobromae has been reported to exhibit potent activity in potato tuber formation and flower induction in Pharbitis nil under non-inductive conditions (Yoshihara et al., 1999).

1 Laboratory of Bio-organic Chemistry, 2Laboratory of Molecular Enzymology, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, North 9, West 9, Sapporo, 060-8589, Japan
2000). Moreover, theobroxide showed a similar inducing activity as that of JA on in vitro micro-tuber formation (Nakamori et al., 1994). However, little is known about the relationship between theobroxide and JA in these processes. In this study we investigated the interactive effects of theobroxide and SHAM on potato tuberization and flower bud formation in Pharbitis nil. Endogenous levels of JA and TA and activity of LOX in the plants treated with theobroxide and SHAM were also examined.

MATERIALS AND METHODS

Plant materials and photoperiodic treatments Potato (Solanum tuberosum L. cv Irish Cobbler) and Pharbitis nil (cv. Violet and Sunsmile) were planted under LD (18L/6D) and treated with chemicals as described in our previous report (Yoshihara et al., 2000). The LD photoperiod was 18L/6D or 14L/10D, and the SD was 10L/14D. Night break is performed with 1-hour exposure with white light in the middle of dark of SD period. The chambers were equipped with 20 fluorescent lamps to provide a light intensity of 90 μmol m⁻² s⁻¹ and set at 25 °C and 60% relative humidity.

Preparation and application of test solutions Theobroxide and SHAM were each dissolved in 100 ppm of Tween 20 in H₂O to give the desired concentrations. A solution of 1 mM of theobroxide and SHAM was sprayed to leaf surfaces at two-day intervals. The control plants were sprayed with 100 ppm of Tween 20. For the theobroxide+SHAM treatment, theobroxide solution was sprayed 30 min after SHAM treatment. For LOX activity measurement, theobroxide was sprayed alone or done with SHAM simultaneously. The leaves were harvested at designated times, ground immediately to a fine powder in liquid nitrogen, and then stored at –80 °C until use.

Determination of endogenous contents of JA and TA Quantification of endogenous JA and TA levels in potato and Pharbitis nil leaves were carried out using LC-MS, as described by Matsuura et al. (2000).

Measurement of lipoxygenase activity About 0.1 g of leaf powder was homogenized in 1 ml of 0.1 M PBS buffer (pH 7.3). The homogenate was centrifuged for 20 min at 15,000 g, and LOX activity in the supernatant was analyzed immediately using a Hitachi U3210 spectrophotometer with 0.1 M LA as the substrate in 0.1 M phosphate buffer solution (PBS, pH 6.0), and following the increase in absorbance at 234 nm to monitor the formation of conjugated–diene compound. One unit of LOX activity is defined as 1 μmol of product formed mg⁻¹ protein min⁻¹. Protein contents were determined by Bradford method using BSA as standard. All extraction procedures were carried out at 4 °C.

RESULTS AND DISCUSSION

Effects of theobroxide on potato tuberization and flower bud formation in Pharbitis nil under night break condition. Previously we observed that theobroxide exhibited inducing effect on tuberization and flowering in Pharbitis nil under LD conditions (Yoshihara et al. 2000). In present study, we examined the effect of theobroxide under NB conditions. When the plants were exposed with NB, a 15% decrease in total fresh weight of tubers was found in 66.7% of treated plants, compared with that under SD conditions. Theobroxide treatment, however, reversed the inhibitory effect of NB on tuber formation, with 21.12 g of tubers in 100% of treated plants (Table 1). In Pharbitis nil, 100% of SD-grown plants produced flower buds at day 10 (Fig. 1). No flower bud formed in control plants, however, under LD conditions until day 26 after treatment. Flower bud formation was delayed to day 20 under NB conditions, with 80% of plants producing average 3.9 of flower buds, about 35% of that in SD-grown plants, even till day 26. However, theobroxide brought flower bud formation forward to day 12, with 100% plants producing average 13.8 of flower buds at
day 26 (Fig. 1). These data indicate that theobroxide can reverse the interruption of potato tuberization and flower bud formation of *Pharbitis nil* by night break, suggesting an absolute inducing effect of theobroxide on the regulation of some developmental processes in SD plants.

**Table 1. Effects of theobroxide on potato tuber formation under different photoperiod conditions.**

<table>
<thead>
<tr>
<th>Photoperiod regime</th>
<th>Treatment</th>
<th>Percentage tubering plants</th>
<th>Total number of tubers</th>
<th>Total fresh weight of tubers (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LD</td>
<td>Control</td>
<td>0% (0/6)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Theobroxide</td>
<td>100% (6/6)</td>
<td>5</td>
<td>6.91</td>
</tr>
<tr>
<td>SD</td>
<td>Control</td>
<td>100% (6/6)</td>
<td>7</td>
<td>9.27</td>
</tr>
<tr>
<td>NB</td>
<td>Control</td>
<td>66.7% (4/6)</td>
<td>7</td>
<td>7.65</td>
</tr>
<tr>
<td></td>
<td>Theobroxide</td>
<td>100% (6/6)</td>
<td>8</td>
<td>21.12</td>
</tr>
</tbody>
</table>

The tubers were harvested 4 weeks after treatments started. The LD and SD indicated were 18L/6D and 10L/14D, respectively. Six plants were used for each treatment.

**Effect of SHAM on theobroxide-induced potato tuberization and flower bud formation in *Pharbitis nil***  As our previous report (Yoshihara et al. 2000) showed that theobroxide induced potato tuberization and Kolomiets et al. (2001) showed that an inhibitor of LOX activity, naproxen, disrupted potato tuber formation, we examined the effect of another inhibitor of LOX, SHAM, on theobroxide-induced potato tuberization. As expected, theobroxide stimulated tuberization under LD (14L/10D) conditions, with 25% (2 of 8) of control plants producing 3 tubers with a total weight of 1.01 g (Table 2). Theobroxide-treated plants produced 20 tubers with a weight of 5.79 g in 87.5% (7 of 8) of tubering plants. The fresh weight of tubers produced in theobroxide+SHAM-treated plants (4.94 g) was 15% less than that of theobroxide alone (5.79 g) (Table 2). Thus theobroxide-induced tuber formation was inhibited by SHAM.

SHAM also showed inhibitory effect on theobroxide-induced flowering in *Pharbitis nil*. Theobroxide significantly stimulated flowering in *Pharbitis nil*. The number of flowers in theobroxide+SHAM-treated plants, however, was reduced to about 56% of that in theobroxide treatment alone (Fig. 2).

It was reported that naproxen inhibited potato tuberization *in vitro* (Kolomiets et al., 2001). Recently, SA (salicylic acid) was reported to be involved in the stress-induced flowering of *Pharbitis nil* (Hatayama and Takeno 2003). It was also found that ASA (Acetylsalicylic acid), which applied to cotyledons of the short day plant *Pharbitis nil* prior to an inductive 16-h dark period inhibits flowering by 90% (Groenewald et al., 1998). Here we showed that SHAM is involved not only in reduction of tuberization, but also inhibition of flower bud formation induced by theobroxide.
Figure 1. Effect of theobroxide on flower bud formation in *Pharbitis nil* under different photoperiods. (○), LD (18L/6D); (■), LD+theobroxide; (▲), SD (10L/14D); (○), NB (1 hour exposure with light in the middle of dark of SD photoperiod); (●), NB+theobroxide.

**Influence of SHAM on the inductive effect of theobroxide on LOX activity**

SHAM is the inhibitor of LOX expression and activity, and it repressed the inductive effect of theobroxide-induced tuberization, we examined if theobroxide and SHAM have interactive effects on LOX activity. LOX activity was steadily increased in both control and theobroxide-treated plants after thirty minutes of the treatment, while it was approximately 2-fold higher in theobroxide-treated plants than the control one hour after the treatment (Fig. 3). The activity in theobroxide-treated plants was decreased to the same level as that in control two hours after treatment. LOX activity in theobroxide+SHAM-treated plants, however, was maintained at a much lower level than that in theobroxide-treated plants (Fig. 3). It was reported that when naproxen was applied in an in vitro tuberization system, tuber formation was disrupted and such disruption was correlated with a suppression of LOX activity (Kolomiets et al., 2001). Here, we showed that the inhibition of LOX activity by SHAM is related to a reduction of tuber formation in vivo (Table 2 and Fig. 3). The results in present study suggest that theobroxide stimulates LOX activity, and that SHAM reduces the stimulative effect of theobroxide.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage tubering plants</th>
<th>Total number of tubers</th>
<th>Number of tubers/plant</th>
<th>Total FW of tubers (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25% (2/8)</td>
<td>3</td>
<td>1.5</td>
<td>1.01</td>
</tr>
<tr>
<td>Theobroxide</td>
<td>87.5% (7/8)</td>
<td>20</td>
<td>2.86</td>
<td>5.79</td>
</tr>
<tr>
<td>SHAM</td>
<td>25% (2/8)</td>
<td>4</td>
<td>2</td>
<td>0.22</td>
</tr>
<tr>
<td>Theobroxide+SHAM</td>
<td>87.5% (7/8)</td>
<td>19</td>
<td>2.71</td>
<td>4.94</td>
</tr>
</tbody>
</table>

The plants were grown under LD (14L/10D) conditions, and potato tubers were harvested four weeks after spraying started. Eight plants were used for each treatment.
Figure 2. Effect of SHAM on the flowering in *Pharbitis nil* (cv. Sunsmile) induced by theobroxide under long-day (18L/6D) conditions. (○), control; (□), theobroxide (10^{-3} M); (●), theobroxide+SHAM (10^{-3} M). The data is indicated with a total number of flowers in six treated plants.

Figure 3. Effects of theobroxide and SHAM on LOX activity in potato leaves under LD (16L/8D) conditions. The leaves were sprayed with theobroxide (10^{-3} M) and a mixture solution of theobroxide and SHAM (10^{-3} M), and then collected at various times in darkness. Data are the means±SE of three assays.

**Interactive effects of theobroxide and SHAM on the endogenous levels of JA and TA**

Because theobroxide showed similar activity to that of JA and its analogue TA in tuberization (Nakamori et al., 1994), we assumed that these chemicals might work in the same pathway. To test this hypothesis, we investigated whether theobroxide can affect the endogenous levels of JA and TA in potato leaves during tuber formation. We found that the endogenous JA levels reached a peak at day 3 after being treated with theobroxide, and then decreased to a relatively low level similar to the controls. However, it increased slightly again at day 28 (Fig. 4a). Endogenous TA content on day 1, 3 and 7 in theobroxide-treated plants were almost the same as in control plants (Fig. 4b). However, a sharp increase of TA content was observed 14 days after treatment. In theobroxide+SHAM-treated plants, the peak of JA content was delayed up to day 7 (Fig. 4a), while no peak of TA was observed (Fig. 4b). Consistent with the observations on tuber formation in theobroxide- and theobroxide+SHAM-treated plants, the partial depression of theobroxide-induced tuber formation by SHAM was correlated with a reduction of endogenous JA and TA levels (Table 2 and Fig. 4). Since JA can be metabolized to TA or TAG (Yoshihara et al., 1996), these results suggest that the metabolism of JA to TA was partially delayed, which caused a reduction of tuber formation.

In summary, theobroxide stimulates LOX activity and affects endogenous levels of JA and TA, while it induces potato tuberization and flower bud formation in *Pharbitis nil*, and the inductive effects of theobroxide are inhibited or delayed by exogenously applied SHAM. It suggests that the inductive effect of theobroxide on potato tuberization and flower bud formation in *Pharbitis nil* is probably achieved by stimulating JA cascade.
Figure 4. Effects of theobroxide and SHAM on the endogenous levels of JA (a) and TA (b) in potato leaves. Plants were treated with (○), theobroxide (10^{-3} M) and (△), theobroxide+SHAM (10^{-3} M) as described in Materials and Methods. (○), control plants received 100 ppm Tween 20.

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SESSION V

REGULATION OF GROWTH AND DEVELOPMENT I
WHEAT (*TRITICUM AESATIVUM* L. cv. USU APOGEE) GROWTH ONBOARD THE INTERNATIONAL SPACE STATION (ISS): GERMINATION AND EARLY DEVELOPMENT

G.W. Stutte1, O. Monje and S. Anderson

ABSTRACT

A series of experiments to determine the effects of microgravity on growth and development of wheat were conducted during a 73-day period onboard the International Space Station. The experiment relied upon telescience for the remote operation, monitoring, and management of the experiment. Growth and development of wheat was measured directly by the ISS crew, estimated from analysis of telemetry data, and quantified with digital image analysis. On-orbit germination rates of stowed root modules containing seed cassettes were greater than 95%. Absolute growth rates of ~4.25 cm per day were observed in both the flight and ground control plants. Final height of plants on orbit was approximately 10% greater than comparable ground controls. The results suggest that early growth and development of wheat can be consistently achieved in long-duration space experiments.

BACKGROUND

Plants have long been proposed as components of bioregenerative life support systems (BLSS) for long duration space missions in order to regenerate the atmosphere, purify water, and produce food. An understanding of the fundamental physiological and developmental effects of microgravity on photosynthesis is essential to the success of these missions (Wheeler et al., 2002). The Photosynthesis Experiment and System Testing and Operations (PESTO) experiment was designed to directly measure photosynthesis of a developing wheat canopy. These data will have application in design and management of a bioregenerative life support system for long duration missions (e.g. ISS Salad Machine, Lunar surface habitation module; Mars transit and/or surface mission) could be made (Stutte et al., 2001). Inherent in the use of higher plants in a BLSS is the assumption that the plant growth and development will function the same under microgravity (µg) conditions as under 1-g conditions. Collectively, plant-based microgravity experiments has demonstrated that the primary biological events associated with plant development- germination, seedling orientation, shoot development, flowering, pollination, fertilization, seed development, and senescence- occur successfully in microgravity (Halstead and Dutcher, 1987; Nechitailo and Mashinsky, 1993; Musgrave, et al., 1998.).

While numerous plant experiments have been performed in microgravity, they are often limited by the lack of replication, poor environmental control, and limited on-orbit access to data. In fact, there have been relatively few opportunities to systematically evaluate the microgravity impacts on regulating initial germination, growth and development of a crop plant under well-controlled, replicated, environmental conditions in space. The International Space Station provided such an opportunity. Specialized plant growth chambers are required to conduct plant experiments onboard ISS. The Biomass Production System (BPS), a system consisting of four independently controlled plant growth chambers, was recently flown in ISS for 73 days. The initial flight of the BPS consisted of two experiments, the Technology Validation Test (TVT) (Morrow et al., 2001) and the Photosynthesis Experiment and System Testing and Operations (PESTO). The TVT objectives were to validate the plant growth system hardware functionality in microgravity (Morrow et al., 2001; Iverson et al., 2002). The PESTO objectives were to determine germination, growth, photosynthesis and transpiration of dwarf wheat in microgravity (Stutte et al., 2001, 2002, 2003). We report in this paper the effects of microgravity on seed germination and early growth.

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MATERIALS AND METHODS

Launch and Landing. The PESTO experiment was launched in the BPS to the International Space Station (ISS) on 8 April, 2002, onboard STS-110 ( Atlantis), transferred to ISS on 13 April, 2002 midway through ISS Increment IV, and returned to Earth onboard STS-111 (Endeavor) on 19 June, 2002, for a total of 73 days in space (Figure 1).

Growth Conditions. Triticum aestivum L. cv. USU Apogee were grown in a series of experiments under controlled environment conditions during Increment IV of the International Space Stations (8 April –19 June, 2002). The plants were grown in a controlled environment chamber, the Biomass Production Chamber (BPS) developed by Orbital Technologies, Inc., (Madison, WI) to support plant growth in space (Iverson et al., 2003). The BPS has four independently controlled plant growth chambers (19 cm x 16 x 19.5 cm) with growing area of 254 cm² per chamber. Three of these chambers grew wheat during ISS Increment IV. Each plant growth chamber had 32 plants. There were a total of 7 harvests/6 replantings of wheat on orbit.

Environmental conditions were nominally maintained at 280 µmol m⁻² s⁻¹ photosynthetically active radiation on a 20 hr light/ 4 hr dark cycle at 24°C and 70% RH. Carbon dioxide concentration was nominally controlled to 1500 µmol mol⁻¹ during the light cycle; there was no CO₂ control during the dark cycle. Root zone nutrients were provided from slow release nutrient pellets (Osmocoteᵀᴹ) mixed into rooting matrix of 1-2 mm calcined clay (Profileᵀᴹ). Moisture content in the root zone was controlled to ~50% water holding capacity by using stainless steel porous tubes embedded in the media and maintaining negative pressure of ~0.3 kPa on the tube (Stutte et al., 2000, 2001).

A continuous record of the environmental conditions (CO₂, temperature, and relative humidity) experienced by the plants was obtained from the ISS and BPS using CDS 1.02 client software. These data were used to maintain a BPS ground control chamber in the Orbiter Environmental Simulator at Kennedy Space Center, FL. The ground controls were harvested following same procedures as those on orbit and processed separately from the flight tissue. The wheat plants were harvested when they were 21 days old, except when operational events required an early or late harvest.

Data and Image acquisition. Digital images were acquired from the BPS video system (Hanse Electronics MB series MB-1050C) for each chamber at two-hour intervals and stored for later retrieval. A total of 3,018 images were obtained from the flight chamber and 3,065 images were obtained from the ground control chamber. These images were remotely accessed through the NASA Ames Research Center’s (ARC) Telescience Support Center (TSC) using custom developed Communication and Data System software (CDS 1.02). The CDS 1.02 software developed through
NASA ARC TSC was designed as client software to view/track real-time data from the BPS. This included health and status, science specific data demands, hardware specific monitoring demands, and operations logs in addition to images captured by the CDS (Figure 2). These images were downloaded from ISS on a regular basis and posted on a secure FTP site for distribution.

Figure 2: Display of NASA ARC’s Telescience Support Centers interface for selection of digital video images obtained and stored on BPS (left). Display of digital image retrieved at a remote site using NASA ARC’s CDS v.1.02 software. These images could be viewed in near real-time and/or downloaded for additional analysis.

Image analysis. Digital images obtained from the BPS were used to determine the height of specific plants during the experiment. The digital video camera had wide-angle lens that distorted the image of plants in the growth chambers. The parallax distortion was determined to be <5% for plants in the position number 8 for each row (Figure 3). Thus, plant growth analysis was limited to those plants. The plants were identified and height determined using reference grid in the chamber to calibrate each image. No additional correction for image parallax was performed. Each plant was measured until the leaf tip was out of the field of view. This typically occurred 4 to 6 days after imbibing the seeds. This procedure was repeated for each of the seven wheat plantings on ISS in the BPS flight unit, and the 9 plantings in the BPS ground control unit. Height data was normalized for variation in germination time of each seed within a replicate by synchronizing the “0” time with first appearance of the shoot.

Figure 3: Sequence of digital video images as downloaded though NASA ARC’s CDS v. 1.02 client software. Plants in row 4, position 8 (see arrow) for each row were used in the analysis using the internal plant growth chamber grid for calibration. A grid had been overlaid to show the image distortion associated with parallax from the wide-angle lens of the BPS digital video camera. Data was acquired on each plant until the growth tip was no longer visible in the image.
Growth analysis. Growth analyses using data obtained from these images was combined and standard error and mean separation between treatments was determined using Students t-test. Dan Bursch, ISS Increment IV crewmember, photographed each row of the growth chamber at harvest and recorded the number of plants per row. He then down-linked these images and from ISS to Earth. Germination rates and final plant height were then determined. The same procedure was followed with the ground control treatments.

RESULTS

Initial growth and development was comparable to that observed during germination and early development of the ground controls (Table 1). Wheat germination in all chambers started on orbit was greater than 95%. Once seeds emerged, the growth rate of both the flight and ground control plants was ~4.25 cm per day. The ISS grown plants was approximately 10% taller ground control plants, although there was difference in total biomass between the flight and ground grown plants (Stutte et al., 2002). These results are comparable to growth rates observed during pre-flight bioengineering and hardware biocompatibility tests in the BPS flight hardware (Stutte et al., 2000, 2001).

Growth rates of the initial leaves were the same between the flight and ground control plants following initial germination. The growth rates were linear for both the ISS {height=0.173*(hr) + 2.337; r²=0.99} and ground control {height=0.176*(hr)+2.21; r²=0.99} treatments for up to 60 hours of initial growth (Figure 4). The standard deviation was less than 10% for both treatments. The chronology of divergence in growth rates between the flight and ground control is the subject of additional analysis.

Figure 4: Growth of wheat germinated on ISS (▁) or Ground Control (▃) BPS root modules. Data show standard deviation (SD) of plant height for ISS (n=6) and ground (n=9) plants at approximately two-hour intervals. Average SD was 7.1% for ISS samples and 9.8% for ground samples.
Table 1: Microgravity effects on early germination and growth rate of wheat.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)&lt;sup&gt;z&lt;/sup&gt;</th>
<th>Growth rate (cm d&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Final Height (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight</td>
<td>96.8 (6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.25 (7)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.4 (6)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ground</td>
<td>95.7 (9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.26 (9)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.2 (7)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>z</sup> Percent of 32 seeds per chamber.  
<sup>y</sup> data for 0 to 60 hr of growth normalized for time of germination.  
<sup>x</sup> mean value () where (=n).  
<sup>x</sup> values followed by a different letter are significantly different as determined by Student’s t-test at P<0.05.

**CONCLUSION**

The advanced image acquisition and environmental control systems incorporated into the BPS plant growth hardware provided an excellent environmental control condition to determine effects of microgravity on early stand establishment of wheat. The video imaging capabilities allowed for near-real time monitoring of plant performance during the mission and the telescience tools developed by allowed for images to be rapidly accessed on the ground for analysis.

The capability to obtain high resolution, non-destructive measurement of growth from the ISS was also demonstrated. This experiment validated the performance of the environmental control and image acquisition capabilities of the BPS during spaceflight. This research utilized telescience to manage and interpret data from an experiment being performed on the ISS.

This analysis has clearly demonstrated the feasibility of obtaining high germination rates of wheat seeds that have been stored under ambient conditions for long periods prior to imbibing. These analyses have also revealed that growth of the initial leaves of wheat were the same in both the flight and ground treatments. This information will have potentially broad application in the design and management of “seed cassettes” for plant production systems on the ISS or on longer duration space missions (e.g., Mars transit/surface mission).

**ACKNOWLEDGEMENTS**

This research was supported by a grant from the National Aeronautics and Aerospace Administrations Office of Biological and Physical Research Fundamental Biology Program (NCC10-027). The authors express sincere thanks to the ISS Increment IV crew for their support of the PESTO experiment and the efforts of the BPS/PESTO mission support teams at Ames Research Center, Moffett Field, CA, Kennedy Space Center, FL, and Orbitec, Madison, WI.

**REFERENCES**


EFFECTS OF PLANT GROWTH REGULATORS ON AGRONOMIC CROP YIELDS IN THE DESERT SOUTHWEST

Michael D. Rethwisch¹

ABSTRACT

A series of field experiments were conducted on irrigated alfalfa and cotton in the U.S. low desert during 2001-2002 which documented the effects of plant growth regulators on yields and other crop growth parameters. Testing on alfalfa in 2001 included three plant growth regulators. One product is a gibberellic acid inhibitor (Apogee®, active ingredient = prohexadione calcium, marketed by BASF) while the other two (Messenger®, active ingredient = harpin protein, Eden Biosciences; AuxiGro® WP, active ingredients = 29.2% L-glutamic acid, 29.2%gamma aminobutyric acid, Emerald BioAgriculture) are both marketed as biopesticides that have plant growth enhancing properties. Testing on cotton in 2001-2002 included only Messenger® and AuxiGro® WP.

In alfalfa all treatments consistently resulted in lower yields than the untreated check over multiple harvests. Reduced yields were expected for the Apogee® treatments, however, such results were unexpected for the plant growth enhancer treatments (Messenger® and AuxiGro® WP). Plant mapping data obtained at June 2001 harvest indicated that Messenger® treatments had ~10% less plant material remaining at second stem node than untreated check (Rethwisch unpublished).

All four treatment timings of Messenger® applied to cotton in 2001 resulted in lower lint yields when compared with the untreated check at end of season (Rethwisch et al, 2003). Plant mapping was done in both June and July, and bracketed a high temperature event in early July. Data indicated that the bottom fruiting node remained the same for the untreated check in July as in June, but Messenger® treated plots abscised approximately two nodes of earliest fruiting structures.

In 2002 AuxiGro® WP was applied alone and in combination with foliar fertilizers on cotton. Usage of AuxiGro® WP by itself resulted in a yield reduction compared with the untreated check (Rethwisch et al., 2003b). AuxiGro® WP usage in combination with fertilizers increased yields slightly, but greatest economic effects were as a result of improved cotton quality with cotton values/acre increasing by $195/acre from the best treatment when compared to the untreated check.

Results from these differing experiments indicate that usage of plant growth enhancers are initiating increased growth, and are therefore increasing the immediate plant nutrition needs. It is thought that plants by themselves are unable to supply this sudden nutrient demand via the roots, and senesce available foliar growth (as evidenced in alfalfa) or lose fruiting structures (as in cotton) if needed available nutrients are inadequate. Foliar fertilizers in combination with plant growth enhancing products appear necessary to realize potential of such plant growth regulators in these situations.

LITERATURE CITED


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A MYCOHERBICIDE AND SELECTED PLANT GROWTH STIMULANTS ENHANCE THE COMPETITIVENESS OF PEPPER (CAPSICUM ANNUUM) WITH AMARANTHUS LIVIDUS

J. Pablo Morales-Payan1, William M. Stall2, Raghavan Charudattan3, and James T. DeValerio4

ABSTRACT

A study was conducted in Gainesville, Florida, to determine the effects of integrating the potential mycoherbicide Phomopsis amaranthicola and the growth stimulants gibberellic acid (GA3, 20 mg L-1), acetylproline (AP, 200 mg L-1), triterpenic acid (TTA, 40 mg L-1), and a Norwegian kelp (Ascophyllum nodosum) derivative (NKD, rate equivalent to 30 mg L-1 of kinetin-like activity), to improve the competitive ability of bell pepper with livid amaranth (Amaranthus lividus), by simultaneously suppressing weed growth with the mycoherbicide and enhancing crop performance with the growth stimulants. Bell pepper was sprayed with GA3, AP, NKD, or TTA one day before transplanting. P. amaranthicola was applied over the crop/weed canopy (1.0 x 10^6 conidia ml^-1) 10 and 20 days after weed emergence (16 and 26 days after transplanting bell pepper). When P. amaranthicola was not applied and bell pepper was not treated with growth stimulants, livid amaranth was more competitive than bell pepper, and crop growth and yield were <50% as compared to weed-free bell pepper. The competitiveness of bell pepper with livid amaranth was increased when the crop was treated with GA3, AP, and NKD, but not with TTA. When livid amaranth was sprayed with the mycoherbicide, the weed developed typical Phomopsis blight, premature leaf abscission, and stem lesions, resulting in lower biomass accumulation and reduced shading by livid amaranth on the bell pepper canopy, as compared to disease-free livid amaranth. When livid amaranth treated with P. amaranthicola competed with bell pepper treated with GA3, AP, or NKD, bell pepper was more competitive with livid amaranth than when P. amaranthicola was not applied, and the bell pepper losses were <10%.

INFLUENCE OF GROWTH SUPPRESSION ON PANICLE GROWTH, PLANT STATURE, AND CROP PRODUCTION IN RICE

R. Dunand

ABSTRACT

Imazethapyr and trinexapac-ethyl have been shown to suppress growth in grasses. In rice production, growth suppression of red rice, a noxious weed in rice, and tall rice varieties have potential. Panicle suppression of red rice limits seed production. Height suppression of rice improves lodging resistance. The recent releases of imidazolinone-tolerant rice and several tall rice varieties have raised interests in the potential for growth suppressants to affect red rice and lodging in rice.

Rice was drill-seeded on 7-inch row spacings at 90 lb/A. Plot size was 9 x 25 ft. Standard agricultural practices for all plots were utilized as recommended for pest control, adequate soil fertility, and enhancement of seedling vigor. CL121, an imidazolinone-tolerant variety, was planted in an area naturally infested with red rice. Imazethapyr (Newpath, BASF Corporation, Research Triangle Park, NC) was applied at 28 g/A during panicle differentiation (PD) and heading (HD) of red rice. Timings corresponded to 79 and 105 days after planting (DAP). Francis, a tall commercial rice variety, was treated with trinexapac-ethyl (Palisade, Syngenta Crop Protection Inc., Greensboro, NC) at 6 and 12 g/A during internode initiation (II) and late boot (LB). Timings corresponded to 53 and 80 DAP. Growth stages in both studies coincided with the elongation of internodes and panicle development on the main stem. Experimental design was a randomized complete block with four replications, and applications of growth suppressants were made using a CO2-driven backpack sprayer with a delivery rate of 15 gal/A.

Response of red rice to imazethapyr varied, depending on time of application. Panicle density was decreased by the PD application from 185 to 4 panicles/10 yd². Abnormal or malformed and unproductive panicles (little or no seed set) were observed with the mid- and late-season applications. The appearance of abnormal panicles in the control was fewer than 5 panicles/10 yd² compared with 4 and 120 panicles/10 yd² with imazethapyr applied at PD and HD. Overall grain yields were unaffected and were 6118, 6266, and 5703 lb/A for the control and PD and HD treatments, respectively.

Response of crop development to trinexapac-ethyl was dependent on time and rate of application. At maturity, plant height (distance from the soil surface to the tip of the panicle extended vertically) averaged 107, 95, 110, and 109 cm for the high and low rates at II and LB, respectively (control=114 cm). Lodging ranged between 0 and 8% in the trinexapac-ethyl treatments compared with 38% in the control, and the high rate of trinexapac-ethyl delayed maturity by 5 and 2 days with applications at II and LB, respectively. Grain yield averaged 8430, 7357, 8046, and 8160 lb/A for the high and low rates applied at II and LB, respectively (control=7102 lb/A). Grain yield with the low rate of trinexapac-ethyl applied at II and the high rate applied at LB were significantly different from the control.

Growth suppressant activity of imazethapyr and trinexapac-ethyl can have a significant impact on rice production. Reducing seed production in red rice with mid- and late-season applications of imazethapyr can improve grain quality, serve as a component part of a weed resistance management program to prevent transfer of herbicide tolerance from the crop to the weed, and reduce future red rice infestations. Reducing plant height in tall rice varieties with mid- and late-season applications of trinexapac-ethyl can improve lodging resistance, which can result in an increase in grain yield, an improvement in grain moisture and quality, an optimization in combine operation, and more standing stubble for subsequent crawfish production.

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SESSION VI

REGULATION OF GROWTH AND DEVELOPMENT II
ENHANCEMENT OF PROPAGULE FORMATION OF *HEMEROCALLIS* AND *HOSTA*

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ABSTRACT

Bare-root perennial field production is relatively new to Nova Scotia, but it presents a potential for economic growth for the region if done efficiently. In the commercial system under consideration, bare-root plants are field-grown for one season to produce as many divisions as possible from the plants harvested. The objective of this study is to evaluate the effects of practical manipulations on the propagule formation of *Hemerocallis* and *Hosta*. Greenhouse and field experiments were designed to achieve this objective.

An experiment to evaluate the effects of Rossizing and Benzyladenine (BA) was performed in a greenhouse at the Nova Scotia Agricultural College, on two cultivars of *Hemerocallis* (‘Happy Returns’ and ‘Chicago Apache’) and two cultivars of *Hosta* (‘Francee’ and ‘Patriot’). Benzyladenine was applied as a root dip at four different concentrations: 0, 125, 250 and 500 ppm. Plants that received the Rossizing treatment were cut with a sharp knife through the stem. The cut was initiated about 1 cm above the basal plate and went vertically through the crown. Results from this experiment showed that, for *Hemerocallis*, the Rossizing technique increased the production of growing points. Depending on the size of these growing points, a plant may be divided more than once. Rossizing was more efficient at high BA concentration (500 ppm). For *Hosta*, increasing BA concentration produced more compact plants. Photosynthates may be reallocated towards propagule formation.

A first season of field experiments (2002) was performed at Balamore Farm Ltd., Great-Village NS, on *Hosta* ‘Gold Standard’ and *Hemerocallis* ‘Happy Returns’. Five plant growth regulators at three rates and two untreated checks were evaluated: BA (1250, 2500 and 3750 ppm), Cycocel (1000, 2000 and 3000 ppm), Ethephon (300, 600 and 900 ppm), Apogee (75, 150 and 225 ppm) and a seaweed extract/0.10% IBA/0.05% BA mixture (1000, 2000 and 3000 ppm). PGRs were applied as a foliar spray at two timings: July 1\(^{st}\) and August 1\(^{st}\). Results from this first season showed that some plant growth regulators favor propagule formation. For *Hemerocallis*, plants that received the application of the seaweed mixture and the application of Cycocel produced a significantly higher number of divisions compared to the controls. For *Hosta*, the application of the seaweed mixture also produced a higher number of divisions compared to the controls.

The results from the first field season stimulated another greenhouse experiment. Sequential applications of plant growth regulators were evaluated. Each crop was sprayed with one of two plant growth regulators: Seaweed mixture and BA for *Hosta* ‘Gold Standard’ and Seaweed mixture and Cycocel for *Hemerocallis* ‘Happy Returns’. The plants received 1, 2 or 3 applications at two-week interval. The rates were the same as for the field experiments. Results showed high variability and no conclusive trend has been observed.

A second field experiment evaluating Cycocel, BA and the Seaweed mixture is in progress to verify the results from the first season. The sequential applications experiment is also being repeated. Stress measurements (transpiration and stomatal conductance) will be recorded and an experiment using carbon\(^{14}\) will be conducted in order to trace the effects of the plant growth regulators on partitioning in relation to propagule formation.

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PRODUCTION APPLICATIONS OF PACLOBUTRAZOL AND ANCYMIDOL AFFECT LANDSCAPE PERFORMANCE OF PANSIES

Michael A. Arnold and Garry V. McDonald

ABSTRACT

Germination of cool season annuals scheduled for fall sales may occur as early as August. This often necessitates applications of plant growth regulators (PGR) to control excessive stem elongation during greenhouse production. In response to observations of stunted, but otherwise healthy, cool season annuals in landscape plantings, a study was initiated to determine if residual effects of growth regulators used in production might be responsible for the observed responses. Viola × wittrockiana H. Gams ‘Crown Yellow’ seeds were germinated in plug trays. Pansies were sprayed with paclobutrazol (Bonzi) or ancymidol (A-Rest) at plug stage, 10 days after transplant from plugs to jumbo 6-packs, or at both stages. Paclobutrazol was applied at 0, 5, 10, or 15 mg L⁻¹ and ancymidol at 0, 2, 4, or 8 mg L⁻¹. Plants were transplanted on 11 Nov. 2002 to landscape beds to access residual effects on growth. Increased suppression of growth with increased PGR concentrations occurred during greenhouse production with paclobutrazol in response to all application times. Similar reductions were present for ancymidol, but plug stage applications were less effective in growth reduction. Plug stage applications of ancymidol did not adversely impact landscape growth, and only the 8 mg L⁻¹ applications during 6-pack production had a negative impact on landscape growth. Conversely, all tested rates of paclobutrazol reduced subsequent landscape growth of pansies. In general, later production stage applications and greater concentrations induce greater post-transplant growth suppression in the landscape than lower concentrations or plug stage applications. Dual applications of paclobutrazol at the plug and 6-pack stages increased residual effects in the landscape compared to single stage applications. Excessive plug and/or greenhouse production PGR applications appeared more likely to reduce bedding plant growth in landscapes than soil accumulation of paclobutrazol.

INTRODUCTION

Germination of cool season annuals scheduled for fall sales may occur as early as August. This often necessitates applications of PGR to control excessive stem elongation during greenhouse production. Applications of PGR for this purpose are applied to many annuals at plug stage, greenhouse stage, or both (Ball, 1991; Fletcher et al., 2000). Ball (1991) cautions that post-production residuals in the landscape can be a problem without careful choice of chemicals and application techniques. With increased production of cool season bedding plants in warm climate portions of the country this potential problem may be of increased significance.

Most studies with PGR in greenhouse and nursery corps are primarily concerned with effects of PGR on the crop during production. Rarely are plants followed through the marketing channels or are post-production residuals tested in landscape settings. Occasional positive responses in flowering or more compact, but still vigorous, plants such as reports Ipomoea carnea B. von Jacquin subsp. fistulosa (K. Von Martinus ex J. Choisy) D. Austin or Verbena canadensis L. ‘Hometead Purple’ (Arnold, 1998; Arnold et al., 2002). Arnold and Lang (1996) found substantial effects of various production practices on subsequent landscape responses of pansies and petunia (Petunia × hybrida Hort. Vilmorin-Andrieux). Keever and Foster (1991) reported residual post-transplant reductions in

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growth of Pelargonium × hortorum L.H. Bailey ‘Ringo Deep Scarlet’, Tagetes erecta L. ‘Inca Orange’, Vinca × wittrockiana ‘Blue Shades’, Impatiens L. × ‘Zenith’ and Salvia farinacea Benth. ‘Victoria Blue’ in response to production sprays with various concentrations of uniconazole. Hence, the objectives of this study were to 1) investigate the impact of typically recommended paclobutrazol and ancymidol applications during plug and greenhouse production on subsequent landscape growth of pansies and 2) to begin testing the potential for paclobutrazol present in landscape soils repeatedly planted with PGR treated bedding plants to induce growth reductions in pansies.

MATERIALS AND METHODS

Greenhouse production. Viola × wittrockiana ‘Crown Yellow’ seeds (Ball / Pan Amer. Seed, West Chicago, IL) were planted in plug trays (molded plastic propagation trays with 1.5 cm³ inverted cone-shaped pockets, TLC Polyform Inc., Morrow, Ga.) containing ProMix BX (Premier Horticulture, Quebec, Canada) on 6 September 2002. Trays were placed in a growth chamber set at 23/21°C day/night temperatures and with 18/6 hr day/night photoperiods. Seedlings were divided into three groups. One group was treated at the plug stage, one at 10 days after transplant to 6-packs, and one group at both stages. On 3 Oct. 2002 (plug stage), two-thirds of the seedlings were divided into equal groups and sprayed to runoff with 0, 5, 10, or 15 mg L⁻¹ paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl-pentan-3-ol, formulated as Bonzi, Uniroyal Chemical Co., Middlebury, CT] or 0, 2, 4, or 8 mg L⁻¹ ancymidol [α-cyclopropyl-α(β-methoxyphenyl)-5-pyrimidinemethanol, formulated as A-Rest, SePro Corp., Carmel, IN). On 4 Oct. 2002, all seedlings were transplanted from the plug trays into jumbo 6-pack trays (158 cm³ six-cell molded plastic tray inserts, Kord Products Inc, Bramalea, Ont., Canada) and placed in a production greenhouse. On 14 Oct. 2002 (day 10 after transplant), the one-third of the seedlings that were not previously treated with PGR and half of those that had been treated at the plug stage were sprayed with 30 ml per 6-pack of the same PGR treatments as described for the plug stage. The end result was three 6-packs of pansies that had been treated either at plug stage, day 10 after transplant, or at both stages with one of the eight PGR and concentration combinations (432 total plants). Heights and canopy diameter in two perpendicular directions were measured at the end of plug and 6-pack production. A plant index was calculated as height × width 1 × width 2 to serve as a pseudo-volume estimate of canopy size.

Landscape trials. On 11 Nov. 2002, plants were transplanted to raised landscape beds to assess residual effects on growth. The 12.2 m long × 3.7 m wide raised trial beds were constructed using 10 cm diameter exterior treated landscape timbers. Trial beds contained a Silawa fine sandy loam (siliceous, thermic ultic haplustalfs, 73% sand, 9 % clay, 18 % silt) soil and were crested from 30 cm in the center to 15 cm at the edges to ensure drainage. Plots were fertilized at planting and monthly thereafter with a 13N-5.7P-10.8K granular fertilizer (Pursell Industries, Inc., Sylacauga, AL) at the rate of 0.45 kg of actual N per 92.9 m² of bed surface. Composted pine bark mulch was maintained at a depth of approximately 5 cm during the landscape trial. Beds were hand cultivated weekly to control weeds. Irrigation was applied as needed to maintain soil moisture via stationary overhead risers. Growth of the pansies was monitored monthly in the landscape from December 2002 through April 2003.

Analysis of variances were determined for the greenhouse and landscape trials and the highest order interaction found significant for a given PGR is discussed. Means and standard errors were determined using the least squares means procedures. Regression equations and response surfaces were constructed when the analysis of variances indicated a significant interaction involving concentration or time in the landscape. Only those models significant at P ≤ 0.05 are presented.

Mimicking soil accumulation. In an attempt to mimic the effects of accumulation of PGR in the soils from continual incorporation of rootballs from PGR treated rootballs, 1.7 m² sections of
planting beds were drenched with 1.08 L of 0, 2, 4, or 8 mg·L⁻¹ of paclobutrazol (formulated as Bonzi). After drenching the blocks were tilled to a depth of approximately 20 cm, then Viola × wittrockiana 'Crown Yellow' seedlings grown in 0.7 L black plastic containers (TLC Polyform Inc., Moorow, Ga.) without PGR applications were planted in the plots to determine effects on plant growth. Each drench was represented by three blocks with twelve individual plant replications per block. Height and width perpendicular and parallel with the row were measured monthly from November 2002 through March 2003, and a plant index calculated as previously described.

RESULTS AND DISCUSSION

Greenhouse production. No significant $P \leq 0.05$ differences were present among seedlings at the plug stage (Fig. 1), which was expected as PGR applications were made near the end of plug production. Increasing concentrations of ancymidol applied at day 10 after transplant to 6-packs or at both plug and day 10 resulted in increased suppression of pansy growth at the end of greenhouse production (Fig. 1A). Applications of ancymidol at plug stage alone were much less effective at suppressing canopy size, with a less pronounced response to increasing concentrations (Fig. 1A). Ancymidol applications at day 10 after transplant of 2 to 8 mg·L⁻¹ resulted in commercially acceptable levels of growth suppression regardless of the plug stage treatment, while plug stage applications of ancymidol appeared to provide inadequate growth suppression for commercial purposes.

In contrast, all but the lowest levels of paclobutrazol applied to plugs or 6-packs resulted in excessive growth suppression in the greenhouse phase (Fig. 1B). This would be consistent with the observed practice of using the higher level applications of paclobutrazol as a late production stage treatment to arrest growth of plants that need to be held past optimal times in production.

Landscape trials. Plug stage applications of ancymidol did not adversely impact landscape growth, and only the 8 mg·L⁻¹ applications during the 6-pack production stage (day 10 or plug + day 10) had a negative impact on landscape growth (Fig. 2). Interestingly, 4 to 8 mg·L⁻¹ plug stage applications of ancymidol appeared to have a promotive effect on later landscape growth of pansies (Fig. 2). Growth spurts of plants after initial suppression with growth regulators has been reported in some species of plants (Fletcher et al., 2000). Promotive effects of paclobutrazol at low doses (5 mg·L⁻¹) during container production on subsequent flowering of the woody shrub, Plumbago auriculata Lam., have been reported (Arnold and McDonald, 2001). Conversely, all tested rates of paclobutrazol reduced subsequent landscape growth of pansies (Fig. 3) and growth suppression increased when this PGR was applied later in production (Fig. 3B and 3C versus 3A). Dual applications of paclobutrazol at the plug and 6-pack stages increased residual effects in the landscape compared to single stage applications (Fig. 3C versus Fig. 3A and 3B). Paclobutrazol drenches with rates of as high as 40 to 80 mg·L⁻¹ during container production produced a more compact canopy on bush morning glories, without adverse residual effects in the landscape (Arnold et al., 2002). While in general woody nursery crops require high rates for adverse residual responses than herbaceous species, landscape growth of P. auriculata was reduced in some trials by as low as 10 mg·L⁻¹ paclobutrazol production drenches (Arnold and McDonald, 2001). Bir et al. (1997) reported residual reductions in stem elongation of Kalmia latifolia L. following 20 mg·L⁻¹ sumagic (uniconizol) sprays during container production. Ball (1991) states that Petunia × hybrida growth may be severely reduced by 15 mg·L⁻¹ paclobutrazol applications during production.

Mimicking soil accumulation. One potential explanation for observed poor growth of transplanted pansies into high quality landscape beds would be an accumulation of PGR in the soil with repeated incorporations of rootballs of PGR treated plants when color plantings are rotated seasonally. No significant ($P \leq 0.05$) interaction was observed between the tested rates and time in the landscape, but main effects of time in the landscape and paclobutrazol concentration were evident (Fig.
4). A mild reduction in growth occurred across tested rates, with greatest reductions occurring with 4 and 8 mg·L⁻¹ applications. However, even at the highest rates, growth reductions from soil incorporated paclobutrazol were less than 10% of controls, suggesting that residual levels in the soil would need to be substantially greater in order to induce similar growth reductions as associated with the residual effects of PGR applications during production.

CONCLUSIONS

Growth suppression of pansies at mid- and high ranges of paclobutrazol tested herein resulted in very unacceptable growth in the landscape and would have been deemed commercially unacceptable. These adverse residual effects of both PGR were obtained under highly favorable growth conditions in well maintained, fertilized, and irrigated trial beds. Growth may have been even less acceptable under more stressful home-owner conditions. Unfortunately, flowering was not measured in this study, but there did not appear to be any noticeable effects on flowering that were not proportionally reflected in growth index responses to the PGR. Testing is needed to determine if similar responses occur on other cool season species and if recommended production applications to warm or transitions season annuals also exhibit noticeable residual effects in landscapes. Arnold and Lang’s (1996) work with pansies and petunias found cultivar specific residual responses in the landscape to common production practices such as various size containers or copper container treatments, suggesting that PGR effects may vary with the production system and cultivar selection. Timing of applications can also have substantial impacts on responses to PGR applications (Arnold, 1998). Results of experiments presented in this paper suggest that no PGR rates for bedding plant production should be recommended without a prior assessment of post-production residual response risks in landscapes. Concern would normally be for potential negative residual effects, but some residual growth suppression may be desirable in certain settings, such as with hanging baskets or patio containers where the potential for plants to over growth the intended size is possible. Reduced growth responses of pansy in landscape beds might potentially be associated with accumulation of residual paclobutrazol from repeated incorporation of treated rootballs into the soil, but growth reductions appear to be much more readily associated with carry-over effects from excess applications of PGR during plug or greenhouse production.

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LITERATURE CITED


Figure 1. Interactions for plant index of *Viola × wittrockiana* ‘Crown Yellow’ among stage of application (plug, day 10 after transplant, plug + day 10), growth regulator concentration [ancymidol (A), paclobutrazol (B)], and size at the end of plug stage and greenhouse production in 0.16 L 6-pack containers; $R^2$ at 6-pack stage were 0.94, 0.97, and 0.82 for ancymidol and 0.89, 0.98, and 0.75 for paclobutrazol at plug, day 10, and plug + day 10, respectively.
Figure 2. Interaction among application times and ancymidol concentration during greenhouse production on subsequent landscape growth of *Viola × wittrockiana* ‘Crown Yellow’. Symbols represent the mean (± standard errors) of 90 observations; second order polynomial regression equations across ancymidol concentrations, $R^2=0.97$, 0.33, and 0.95 for plug, day 10, and plug + day 10, respectively.
Figure 3. Interaction among application times (A = plug stage, B = day 10, C = plug + day 10) and paclobutrazol concentration during greenhouse production on the subsequent growth of Viola × wittrockiana ‘Crown Yellow’ over time in the landscape. Response surfaces were generated, $R^2$ = 0.53, 0.64, and 0.69 for plug, day 10, and plug + day 10, respectively; all significant at $P \leq 0.0001$. 
Figure 4. Main effects ($P \leq 0.05; R^2 = 0.74$) of paclobutrazol soil incorporations on the growth of transplanted 0.7 L container-grown *Viola × wittrockiana* ‘Crown Yellow’ across time in the landscape; symbols represent means (± standard errors) of twelve observations.
EFFECT OF RETAIN® IN REDUCING PISTILLATE FLOWER ABORTION IN SERR WALNUT

Robert H. Beede

For over thirty years, scientists with the University of California Pomology Department at Davis, in cooperation with their colleagues in Cooperative Extension, have researched the cause and solution for pistillate flower abortion (PFA) in walnut (Polito et al.). Included in this dedicated team are Dr. Vito Polito, Pomology Department Chair and Professor, Dr. Peter Catlin, Professor Emeritus who spent much of his career studying multiple cultural factors suggested as the cause for PFA, and Farm Advisors Steven Sibbett (Tulare County, retired and Emeritus), Joe Grant (San Joaquin County) and Kathy Kelley (Stanislaus County).

PFA is the loss of nut-producing pistillate flowers early in the season, typically 2 to 3 weeks after bloom. This was first noted in the Serr cultivar soon after the earliest plantings came into production in the early 1970’s. Originally referred to as the Serr drop problem, flower loss due to this phenomena sometimes exceeded 90 percent in certain orchards and years. Determining the cause of the disorder proved extremely difficult. By the late 1980’s the above researchers had eliminated mites, walnut blight, numerous nutritional deficiencies including nitrogen, calcium, and boron, tree age, shading, pruning practices, water stress, intratree competition for nutrients, incompatible pollen and lack of pollination. Cherry Leafroll virus, the cause of Blackline, was also studied as a possible cause and eliminated (Polito et al.).

Researchers then turned their attention to testing the possibility that excessive pollen might be the cause. Early tests with flowers receiving high pollen doses were promising. In the 1990’s, field-based research lead by UC Davis Pomology faculty confirmed from detailed tagging of flowers and collection of yields from individual trees varying in distance from a pollen source that PFA is always associated with high numbers of pollen grains present on the receptors (stigmas) of female flowers. PFA also decreased with distance from a pollen source. Reduction of the pollen load in test orchards by catkin removal decreased PFA and increased yield. Tests on cultivars other than Serr (Chandler, Vina and Chico) showed the presence of PFA but not at levels which typically resulted in economic loss.

This UC research changed the routine practice of planting pollenizers in most new walnut orchards unless they were isolated from surrounding pollen sources or the cultivar suffered consistently from poor overlap between female receptivity and catkin bloom. The discovery of excessive pollen affecting walnut flower abortion also lead to the UC recommendation that Serr growers remove the pollenizer trees from their orchards and shake the catkins from the remaining Serr trees during the early bloom period. Growers accomplish this with the mechanical shaker normally used for harvest.

During the early years of PFA study, Serr growers in northern California became so frustrated with the poor yields received from this potentially high-producing variety that most orchards were removed. This left the majority of the state Serr acreage in southern California where it enjoyed popularity as an early maturing, high edible yield variety which helped extend the harvest period and set marketing conditions. However, from 1998-2003, Serr has yielded very poorly in orchards which typically produced about two ton of in-shell walnuts per acre. Use of the catkin shaking recommendation has also been met with frustration because a single shake has been insufficient to adequately reduce the

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pollen load. Many Serr growers also do not own shakers and therefore find timely coordination of this practice difficult. Growers who have shaken also report mixed results depending upon their proximity to surrounding orchards with varieties adding to their pollen load.

Research by Dr. Polito (unpublished) showed that the excessive pollen tubes growing down the style of the female walnut flower produce excessive amounts of ethylene, a natural plant hormone associated with organ senescence. Elevated ethylene levels are likely the cause of flower abortion. Polito also field tested non-commercial compounds which either promoted or inhibited ethylene production and observed corresponding increases and reductions in PFA.

Recently, Retain®, a commercially available ethylene inhibitor developed by Valent BioSciences, has been widely tested on stone fruit for improving post harvest shelf life. This season, a simple test of its effect on Serr PFA was conducted in Kings County. A nine-year old block of Serr walnuts south of a Chandler orchard was selected. Ten fruiting shoots in each of twenty trees 120 to 150 feet south of the Chandler orchard were then tagged. The selected shoots each had two female walnut flowers in an early stage of pollen receptivity. Five of the shoots on each tree were treated with the equivalent of 11.7 ounces per acre of Retain®. The other five shoots per tree were left untreated. On May 5, 2003, the number of fruit set per shoot were recorded and analyzed as a randomized complete block. The shoots within each tree were treated as subsamples and the trees were evaluated as replications. Visual and statistical results showed a consistent improvement in fruit set from the Retain® application. Overall, Retain® treatment resulted in 89.0 percent set of the potential 200 flowers. Only 21.5 percent set was recorded in the untreated shoots. This was highly significant statistically.

Although exciting, much additional field work must be done to confirm these initial results and demonstrate that the response is consistent from year to year and among orchards with varying PFA levels. Growth regulators have long been likened to witchcraft, but there are several examples such as gibberellin in table grapes (Weaver,1959), NAA in apples (Wash. State), and Dormex® (George) in cherries where they significantly benefit the grower. Hopefully, Retain® is the product Serr walnut growers have been waiting for to recover orchard productivity and fiscal soundness.

**LITERATURE CITED**


HEIGHT CONTROL OF HERBACEOUS PERENNIALS FORCED USING NIGHT-INTERRUPTED LIGHTING UNDER NURSERY CONDITIONS

Gary J. Keever and J. Raymond Kessler, Jr.1

ABSTRACT

Increased customer demand for herbaceous perennials has resulted in large-scale production of herbaceous perennials by the nursery industry. However, customers tend to demand that plants be in flower at the time of purchase, and largely make purchasing decisions in the spring. This creates a production problem for many herbaceous perennials that naturally bloom in the summer, usually in response to long-day lengths. In nurseries in coastal areas of the South, ambient temperatures from mid winter to early spring are mild enough to promote early vegetative growth. Application of night-interrupted lighting (NIL) using incandescent lamps outdoors in a nursery setting has been used to force summer-blooming perennials into flower for an earlier, spring market. In those studies, time to flower decreased with no effect on flower numbers in qualitative long day plants while flower numbers increased with no effect on time to flower in quantitative long day plants. However, excessive stem elongation under NIL reduced plant quality. The objective of this study was to control plant height of summer-blooming herbaceous perennials grown under NIL with plant growth retardants (PGR).

Coreopsis grandiflora 'Early Sunrise' and Rudbeckia fulgida 'Goldsturm' were given night-interrupted lighting from 10:00 PM to 2:00 AM outdoors in a nursery setting at the Ornamental Horticulture Substation in Mobile, Alabama, U.S.A. beginning February 1, 2002. When plants of each cultivar began to elongate vigorously, PGR treatments were applied as a foliar spray at 2 quarts/100 ft2 (0.2 liters/m2). PGR treatments were 50, 100, or 150 ppm flurprimidol; 2,500, 5,000, or 7,500 ppm daminozide; 2,500, 5,000, or 7,500 daminozide plus 1,500 ppm chlormequat; and 50, 100, or 150 ppm paclobutrazol. A second application of 2,500 and 5,000 ppm daminozide and all daminozide plus chlormequat combinations were made one week later. There was also a NIL control and a natural photoperiod control.

Daminozide and daminozide plus chlormequat increased days to visible bud and flower in Coreopsis grandiflora 'Early Sunrise' compared to the NIL control. At the higher rates, many of these treatments were similar to the natural control. Therefore, the flower delay nullified the decrease in time to flower achieved by application of NIL. Daminozide and daminozide plus chlormequat reduced plant height by up to 49% and 54% compared to the NIL control but higher rates reduced flower and flower bud numbers. Paclobutrazol and flurprimidol decreased days to visible bud and flower, and reduced plant height by up to 9% and 34%, respectively, compared to the natural control. Flurprimidol at 50 and 100 ppm, paclobutrazol, and NIL resulted in taller plants compared to natural control.

Quality ratings were similar to the natural control with flurprimidol at 50 and 100 ppm while all other treatments were less than the natural control. All PGR treatments and the NIL control reduced time to visible bud and flower compared to the natural control in Rudbeckia fulgida 'Goldsturm'. All PGR treatments and the NIL control increased plant height and many PGR treatments and the NIL control increased flower and flower bud number compared to the natural control. Quality rating was greater than the natural control for daminozide at 5,000 and 7,500 ppm, all daminozide plus chlormequat, and flurprimidol at 150 ppm. The highest quality ratings occurred with daminozide at 7,500 ppm and daminozide plus chlormequat at 2,500 and 1500 ppm, respectively.

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SEAWEED-DERIVED STIMULANT AFFECTS YIELD OF BELL AND CUBANELLE PEPPERS (*CAPSICUM ANNUUM*) COMPETING WITH PURPLE NUTSEDGE (*CYPERUS ROTUNDUS*)

J. Pablo Morales-Payan¹ and William M. Stall²

ABSTRACT

A greenhouse experiment was conducted to determine the effects of a Norwegian kelp (*Ascophyllum nodosum*)-derived biostimulant (NKD) on the yield of weed-free and purple nutsedge-infested cubanelle and bell peppers. NKD (30 mg active ingredients per L) was applied to pepper transplants (15 cm-tall, four or five true leaves) 24 hours before transplanting. ‘Wizard’ bell pepper and ‘Cubanelle’ pepper were grown in plastic containers measuring 30 cm in diameter and 30 cm in height, filled with sandy soil from Gainesville, Florida. Nutsedge tubers were planted in the containers the same day as pepper was transplanted, emerging three days later and growing with the crop season-long. Purple nutsedge density ratios were 0, 3, 6, 9 and 12 nutsedges per pepper plant. Increasing purple nutsedge density in the containers resulted in reduced pepper growth and yield in both NKD-treated and control plants. However, in plants treated with NKD, leaf area was larger and yield losses were less severe than in untreated pepper. Increased leaf area in NKD-treated pepper increased shading on the nutsedge canopy, which in turn may have reduced the competitive ability of nutsedge with the crop. In general, at all nutsedge densities bell pepper losses were higher than in cubanelle pepper. For example, the yields of cubanelle and bell peppers competing with 25 purple nutsedges per container were reduced approximately 25 and 33%, as compared to the yields of weed-free cubanelle and bell peppers, respectively. In contrast, when transplants were treated with NKD, yield losses were approximately 12 and 20%, respectively, when competing with the same nutsedge density. These results indicate that under greenhouse conditions NKD-treated bell and cubanelle peppers plants may be more competitive with purple nutsedge, reducing potential yield loss due to purple nutsedge interference. Further studies must be conducted under field conditions to better assess the practical applications of the results of these greenhouse results.

Symposia III:

PGRS IN NURSERIES OF TREES AND WOODY PLANTS
PLANT GROWTH REGULATION IN ORNAMENTAL NURSERIES - UNREALIZED OPPORTUNITIES

Gary J. Keever

ABSTRACT

Sales of floriculture and nursery crops reached $13.3 billion in 2001. Of this total $8.6 billion was from sales of nursery crops and $488 million from herbaceous perennials, with four states, California, Florida, Texas and North Carolina, accounting for one half of the total crop value. Consumer perception of nursery crops demands compact, well branched shrubs and herbaceous perennials with dark green foliage and, if of ornamental value, the presence of flowers or flower buds. From propagation to liner and field production to shipping and marketing, production of high quality ornamental crops is labor-intensive and requires attention to detail. While numerous opportunities exist for the use of plant growth regulators (PGRs) in production, PGRs are rarely used in nursery production, with the exception of the use of the synthetic auxins indolebutyric acid and naphthalene acetic acid in vegetative shoot propagation. Manufacturers= concerns of a minor return for a major investment and potential product misuse may account for the limited number of labeled PGRs for nursery use and the few nursery crops on the labels. Growers= concerns relate to limited registration, inexperience in PGR use, inconsistent results, and cost effectiveness. Detailed crop protocols and experience in using PGRs in the controlled environment of greenhouses have resulted in their widespread use in floriculture. However, uncontrolled and unpredictable environmental conditions, different cultural practices, including growing media, irrigation practices, and larger crops in larger containers, have resulted in little or no response, or occasionally a negative response, to PGR application. If these limitations can be addressed, there are numerous opportunities in ornamental crop production where effective PGRs would be beneficial, including as a substitute for hand or mechanical pruning, to promote or inhibit flowering, to reduce shoot elongation resulting in greater compaction, to inhibit sprout formation on tree-form and grafted plants, to promote growth leading to shorter production cycles, and to improve post-harvest quality during shipping and marketing.

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BIOREGULATOR APPLICATIONS IN NURSERY FRUIT-TREE PRODUCTION

Don C. Elfving¹ and Eric A. Curry²

ABSTRACT

Fruit-tree nurseries in the USA produce many millions of trees each year. In the past, unbranched whips were the production standard for those fruit-tree species which do not readily branch, e.g., apple, pear, and sweet cherry. In the days of seedling rootstocks and extensive planting systems, nursery whips provided acceptable trees for new orchard plantings. Conversion to the use of clonal, size-controlling rootstocks for apple made possible high-density plantings of many more trees per acre. Higher establishment costs associated with such plantings placed increased emphasis on accelerating the onset of productivity in such orchards. One method for encouraging earlier fruiting is to stimulate branch development as early as possible. The interest in feathering (development of lateral branches on current-season’s shoots in the nursery) of apple trees stimulated considerable research over the last 20 years in chemical methods for interrupting apical dominance in the growing shoot in order to produce formation of lateral branches from axillary buds. Cytokinins were found to successfully induce lateral branching in apple; products containing cytokinins and gibberellic acid (GA) have been registered for this use. A few nurseries in Washington now use proprietary mixtures of 6-benzyladenine and gibberellins A₄ and A₇ to stimulate feathering on apple trees destined for high-density plantings as well as for back-yard plantings. One nursery in California reports the use of a foliar nutrient product for stimulation of branch development in apple trees. However, available cytokinin or cytokinin/GA mixtures do not work well on all apple cultivars. Development of size-controlling rootstocks for sweet cherry is fueling interest in producing feathered sweet cherry trees in the nursery. However, these same cytokinin/GA products are not effective for inducing branching in such species. Recent research underway in Washington has suggested that a new bioregulator product, cyclanilide® (Bayer CropSciences), may have considerable promise for induction of lateral branch development in fruit trees under nursery conditions. Cyclanilide can interrupt apical dominance and stimulate lateral branching in apple, pear, and sweet cherry trees in the nursery. Initial tests suggest that it can be used on several of the most important cultivars in all three species. Height at which branching develops depends on the height of the nursery tree at the time of treatment, so timing represents one method of controlling where branching occurs. Cyclanilide appears to act on the apical dominance system in plants by interfering in some way with auxin movement or metabolism. However, its precise mode of action remains unknown. Two characteristics of cyclanilide that appear to make it a very promising bioregulator for nursery use are: 1) it appears to be active on a range of species, although we do not know how many tree-fruit and ornamental species will respond to treatment; and 2) cyclanilide induces lateral branching without damaging or otherwise inactivating the terminal meristem. In the past, several so-called “chemical pinching” agents were tested for efficacy for lateral branch induction in apple. None of these products became commercial because they produced too much damage to the terminal meristem, thereby damaging the structure of the young tree and often producing poor-quality branches. Cyclanilide causes lateral buds to develop into branches without impairing the continued growth of the central axis. The result is a well-feathered tree with a strong, dominant central axis, the ideal tree configuration for introduction into any high-density planting system. More research is needed to understand the dynamics of the cyclanilide effect on tree growth and branch development, and the range of plant species that respond positively to this product.

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One Billion Seedlings and Growing

Steve Pelton

About Pelton Reforestation Ltd
  Founded 1968
  Tree planting, planting tools, tree packing services
  Seedling Nursery started 1978 when private growers allowed
  Now Ship 60 million seedlings/year from seed
  Located in Maple Ridge BC
    25 ha greenhouse
    40 ha outdoor growing area

Our Products
  1 year and 2 year old container grown seedlings of
    Spruce, Pine, Fir, Hemlock, Aspen, Alder, Willow

Customers
  We are a contract grower providing growing services.
  We serve the Pacific Northwest Forest Industry including:
    Forest Companies
    Governments
    Woodlots
    Christmas Tree Industry

Seed
  The client supplies seed since it is collected from the area to be reforested and is specific to that geo-climatic zone.
  When we receive the seed we document the weight, appearance, packing and any notable characteristics of the shipment.
  We then assess quality of the seed lot and prepare a protocol to improve the seed quality before sowing.
  This protocol requires approximately 12 wks to complete.

Seed Preparation - Techniques
  Often the seed is contaminated with debris or disease so we try to upgrade the seed to improve efficiencies throughout the nursery.
  We use the following techniques:
    Density separation
    Sizing
    Disinfection
    Priming

Seed Preparation - Services
  Other seed services we provide include:
    Drying
    Stratification
    Storage – long term freezer

1 Pelton Nursery, 12930 203 St., Maple Ridge, British Columbia V2X 4N2, CANADA
Growing Containers
The Forest Nursery Industry in BC uses the Styroblock for production. These containers are made from Expanded Polystyrene (EPS) and have zero ozone depleting potential. There are many sizes available and they have the following characteristics:
- Width and Length constant
- Height varies
- # Cells varies
- Volume varies from 20cc to 1000cc
See www.beaverplastics.com

Sowing – Growing Medium
The growing medium consists of the following:
Peat, sawdust, coir (milled coconut husk), and fertilizers in varying proportions specified by our experience with each species.
Example: dry zone species have less peat component and more sawdust to create a dry, free-draining growing medium.

Sowing Line – Growing Medium Mixing
The growing medium is mixed on a continuous soil line system capable of producing 35 yd³/hr. We prefer the rotating drum mixer type since it is gentle and does not destroy medium structure during operation.

Sowing Line - Loader
Our soil loader is a high speed model modified to ensure even compaction within the styroblock from cell to cell. It must also be consistent from day to day and from batch to batch of medium. This is a very important factor required for high quality seedling production.

Sowing Line - Tamper
The Tamper prepares the cells in the styroblock for seed by gently pushing the medium down 1.5cm. It also is responsible for spacing into the seeding machine.

Sowing Line – Seeding Machines
Seeding Machines require the following characteristics:
- High accuracy (98% +)
- Ability to center seed in cavity
- Ability to handle different sizes of seed
- Capable of planting approximately 1 million cells/day
We have 3 machines capable of this standard.

Sowing Line – Gritting Machine
The Gritting Machine applies small stones to the styroblock cells after the seed is placed. These small stones protect the seed during germination from drying, bird damage and direct sunlight. Grit also provides weight for the seed to push against during germination so the radicle can anchor in the medium.
Grit gives some moss and liverwort control during the growing cycle.
After the grit is applied, a water bar soaks the top of the cells to keep the seed hydrated during handling.
**Sowing Line – Blocks Off**
A person removes sown styroblocks from the sowing line and stacks them on wagons. These wagons are then transported to the greenhouse for growing.
The wagons also serve as a counting mechanism.
The sowing line typically produces 5000 blocks per 8 hour shift.

**Growing - Facilities**
Pelton Reforestation has a number of varied greenhouse facilities including glass, poly, and retractable-roof houses.
Throughout the nursery is an internal transport system called ‘cassettes’. These are wooden pallets with wheels. They roll on wooden benches inside the greenhouse bay and can be moved from bay to bay by trailers pulled by tractors. We have 50,000 cassettes in the nursery. A cassette can be moved to any area of the nursery.

**Growing - Germination**
The germination phase is one of the most critical in growing conifer seedlings. Temperatures must be maintained at approximately 20C average over 24 hours for quick germination. This phase takes place starting in January, so heating is a major component. Large natural gas boilers generate hot water which is distributed through steel piping underneath the cassettes. CO₂ is also extracted from the flue gas and used for photosynthesis. We employ heat retention curtains for further energy savings. There are also high pressure sodium lights installed for photoperiod control.

**Growing – Environment, Internal Transport, Central Database**
Environmental computers control all aspects of the nursery including: heating, cooling, lighting, CO₂, energy curtains and irrigation.
Our internal transport system based on the wooden cassette is critical to the nursery operation. The cassettes allow us to group crops by treatment rather than take the treatments to the crops. The system allows for flexibility, which is important in the nursery setting.
All operations in the nursery are tagged to specific crops and logged into a central database. Crop irrigations, movements and labor inputs are logged by order number for costing and data analysis. The server also produces reports for inventory, shipping schedules and crop treatments which are available on the web via http://.

**Growing - Specifications**
As contract growers, we are paid only for seedlings meeting specifications set out in the contract. In other words we have a very large incentive to make quality and quantity targets. Our growers use tissue analysis, growth curves and scatter diagrams of seedling height and diameter to modify cultural regimes to meet these objectives.

**Growing - Irrigation**
Irrigation is a critical function in the nursery requiring constant attention. All water supply is from the local city. We store water in our lake, which acts as a buffer during large pumping demands. Especially critical is the period of April – May when we experience night frosts, which can damage the crops outside. During this period we pump water over the seedlings to prevent frost forming.

**Growing – Maintain Growth**
Our quickest crops take 26 weeks to complete, and our longest 70 weeks. During this time operational decisions are mainly based around the weather. We must work in concert with the weather to achieve
our growth objectives. During the crop cycle we take tissue samples weekly and compare results to standards based on successful past crops. We practice Integrated Pest Management using biological control as our first line of defense, followed by chemical treatment should the biological approach fail. We have been very successful with IPM by moving crops to the best environments and minimizing the opportunity for insects/disease to get established.

**Growing – Root Systems**
A successful seedling requires a strong root system to get established in the woods. Much attention is placed on this aspect. We want to produce balanced seedlings that have a top large enough for strong growth but not large enough to overpower the roots for transpiration demand should the planting site be dry. Also, the physiology of the seedlings must match the planting site at the time of planting. In other words, the seedlings must be hardy and able to resist drought, frost and sun desiccation and be able to grow when conditions permit.

**Growing Issues – Yield Critical**
The nursery operates on revenue per square meter. Overall, we must be concerned with space efficiency, fuel costs, seed conservation, labor, uniformity of crops, and harvest costs. Unfortunately the current production system has problems in these areas and five years ago we began investigating other technologies that would help us improve in these areas.

**Transplanting – Why**
Plug to Plug transplanting technology borrowed from the horticultural sector is one way we are taking on these issues.
Transplanting allows us to achieve 100% cavity fill, remove poor seedlings from the cycle automatically, increase uniformity and vigor, use less space per given order and create new stock types previously not possible. We believe this technology has great potential.

**Transplanting – Mini Plugs**
Growing seedlings in mini plugs allows us to produce a large quantity in a small area. This permits high inputs (heat, assimilation lighting, humidity control) that would otherwise be too costly. The system can accommodate the young seedlings for 8 to 12 weeks during the most costly and critical time of year.
We are also able to single-sow the cavities, conserving expensive and rare seed.

**Transplanting - Gapping**
Gapping is the process of removing blanks or undesirable seedlings from the mini trays and refilling to 100%. The system uses computer vision based on color and luminance. A camera takes an image of the mini tray and the computer interprets the image based on the operator’s settings. Cells containing seedlings not meeting the criteria are removed automatically by pneumatic nozzles. Another machine looks at the emptied tray, decides which cells are missing and fills them in automatically with a high speed robotic arm. This process take one second per empty cell.

**Transplanting**
Transplanting machines take the 100% filled mini trays and physically move them row by row to the destination tray. These machines have no intelligence for detecting blank cells, hence the starter tray must be 100% filled or someone will have to patch the destination tray by hand. This is the reason for gapping.
Quality of the transplanting process is critical. The type of machine used, cell configurations and soil type will impact the quality. It is very important that the mini cell be placed gently into the destination container without damaging the root system in any way.

**Transplanting – 100%**

Once transplanted, the 100% filled styroblocks are checked by a person for planting quality and then placed on the cassettes for growing.

**Growing - Outside compounds**

At some point in their growing cycle, all of the seedlings are moved outside to the compounds. These areas are designed to give the seedling exposure to the elements. High light levels, ultraviolet light, thigmomorphogenesis and meristem night radiation losses are important signals the seedling responds to by building waxy needles, strong roots and larger stem diameter.

**Growing – Day Length Control**

Most plants use the length of night to determine time of year, and we use this technique to control the growth of our seedlings. By lengthening the night artificially with blackout curtains, we can induce the seedlings to set the terminal bud and begin the hardening process.

**Growing - Hardening**

Hardening is the final phase we do in the growing cycle. This prepares seedlings for shipment, storage and the rigors of out planting to the forest. Seedlings must be able to withstand any conditions that nature provides, including rain, frost, sun scalding, wind, animal browsing, hail, snow etc.

**Harvest**

Most of our harvests are on demand by the customer. When conditions are correct for out planting, we are contacted and a harvest is organized. Our current capacity is 1.3 million seedlings per day or approx 6000 boxes. This requires about 200 people. Our two busy planting seasons are June 20 to July 20 in the summer, and November 1 to January 15 in the winter.

**Shipping**

Shipping of seedlings is by truck with refrigerated semi trailers. During the summer harvest window, we typically load 10 trucks/day with the trucks returning on a three-day turnaround. In the winter the pace is less hectic, as the stock will be frozen-stored for up to 6 months. We initially freeze at -5C and then store at -2C. Boxes are then shipped to freezer storage facilities closer to the planting site.

**Out planting**

Our target is the creation of high value forests for future generations. As an industry forest plantation survival was poor in the early 1980’s, but, through diligent efforts, three-year survival has risen to 93%, according to the BC Ministry of Forests. Today the challenge for seedling growers is not survival - it is improving the growth performance of seedlings to achieve the highest quality growing forests of the future.
CONTRIBUTED PAPERS
CYP72B1 INACTIVATES BRASSINOSTEROIDS TO POSITIVELY MODULATE
PHOTOMORPHOGENESIS

Shozo Fujioka¹, Edward Turk,² Hideharu Seto,¹ Yukihisa Shimada,¹ Suguru Takatsuto,³ Shigeo
Yoshida,¹ and Michael M. Neff²

ABSTRACT

We investigated CYP72B1, an Arabidopsis cytochrome P450, to determine the biochemical and
physiological functions of this enzyme. We expressed CYP72B1 in the WAT11 yeast strain by
engineering the cDNA into the pYeDP60 yeast-expression vector, and compared by GC-MS the 26-
hydroxybrassinolide (26-OHBL) standard to the metabolite that resulted from feeding brassinolide
(BL) to yeast expressing CYP72B1. The 26-OHBL standard and the resulting metabolite had identical
retention times and mass spectra, demonstrating that CYP72B1 protein has the ability to convert BL to
26-OHBL. Castasterone (CS), the immediate precursor to BL, was also shown to be a substrate for
CYP72B1 conversion to the 26-hydroxy derivative, 26-OHCS. To test the endogenous function of
CYP72B1 in Arabidopsis, seedlings of a CYP72B1 null mutant (cyp72b1-1), the wild type (Ws-2), and
an over-expressor (cyp72b1-ox8) were fed BL or CS, and assayed for conversion to 26-OHBL or 26-
OHCS by GC-MS. Reduced levels of 26-OHBL and 26-OHCS were detected in cyp72b1-1,
and increased levels were detected in cyp72b1-ox8, indicating that 26-hydroxylation of brassinosteroid
(BR) is an endogenous biochemical function of CYP72B1.

To determine the activity of 26-OHBL in Arabidopsis, we compared it to the activity of BL, by
utilizing the previously described observation that exogenous BL stimulates hypocotyl elongation of
light grown Arabidopsis seedlings. cyp72b1-1, Ws-2, and cyp72b1-ox8 were unresponsive to 26-
OHBL compared to BL. cyp72b1-1 was hyper-responsive to 26-OHBL, while cyp72b1-ox8 was
less responsive when compared to Ws-2. These results suggest that 26-OHBL is an inactive BR in
Arabidopsis, and provides physiological evidence that conversion of BL to 26-OHBL by CYP72B1 is
an inactivation mechanism.

A negative regulatory role for BRs in photomorphogenesis is suggested. To determine if
CYP72B1 is involved in photomorphogenesis, we assayed the hypocotyl response to BL in the dark or
in white light. There is no difference in BL responsiveness between cyp72b1-1 and Ws-2 in the dark,
but in the light, cyp72b1-1 is hyper-responsive to increasing concentrations of BL. The light dependent
phenotype of cyp72b1-1 provides genetic evidence of CYP72B1 involvement in photomorphogenesis.
We measured the hypocotyl length of plants grown at various white light intensities, and at various BL
concentrations, and at every combination. The combination with the greatest photomorphogenic
response is at the highest white light intensity and the lowest concentration of BL, while the
combination with the least photomorphogenic response is at the lowest white light intensity and the
highest concentration of BL, supporting the hypothesis that the BR pathway has a negative regulatory
role in Arabidopsis photomorphogenesis. cyp72b1-1 resulted in reduced responsiveness to light
removal of a positive modulator) and hyper-responsiveness to BL (removal of an inactivating enzyme)
at every condition tested, which resulted in a same basic pattern as WS-2, but raised. cyp72b1-ox8
showed the opposite response. These results indicate that CYP72B1 is modulating the
photomorphogenic response through BL inactivation.

In conclusion, we have demonstrated that CYP72B1 is a steroid C-26 hydroxylase that converts
BL to 26-OHBL and CS to 26-OHCS, and CYP72B1 is a positive modulator of photomorphogenesis.

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BRASSINOSTEROID INDUCE IAA GENES AND AN AUXIN-RESPONSIVE ELEMENT, DR5

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ABSTRACT

Brassinolide (BL) induced some of the early-auxin-inducible genes, including IAA5 and IAA19. The genes were induced with different kinetics by indole-3-acetic acid (IAA) and BL. We found that BL induce a fusion of DR5, a synthetic auxin-responsive element, and β-glucuronidase (GUS) gene with similar kinetics to that of the IAA genes. Endogenous IAA levels per gram fresh weight did not increase when arabidopsis wild type and brassinosteroid (BR)-deficient mutant det2 were treated with BL. These results suggested that BR signaling pathway cross-talk with auxin signaling pathway at or upstream from the transcriptional system of the IAA genes via same cis-element without increase endogenous IAA level.

INTRODUCTION

It has been suggested that the actions of BRs are related to auxin action (Mandava, 1988; Sasse, 1999). Several authors have proposed that BR-induced effects might be via auxin, with BR treatment altering the levels of endogenous auxin or enhancing sensitivity to auxin. Although numerous physiological studies have been addressed the interactions between BRs and auxins, little is known about the underlying molecular mechanisms. Recently, we found that a number of early-auxin-inducible genes are quickly induced by BL treatment in Arabidopsis seedlings (Goda et al., 2002). Here we show that IAA5 and IAA19 genes are induced with different kinetics by auxin and BL. We used DR5-GUS reporter system, which has been widely used as a marker to study auxin distribution to gain further insight. We also analyzed endogenous IAA levels using gas chromatography-mass spectrometry to discern when BL activates the DR5-GUS and IAA genes most efficiently. Based on these results, the signaling interactions between auxin and BRs are discussed.

MATERIALS AND METHODS

Plant Materials

Arabidopsis thaliana ecotype Columbia was used as the wild type (WT) in this study. The arabidopsis det2 was used as the BR-deficient mutant (Chory et al., 1991). The DR5-GUS transgenic plant has been described by Ulmasov et al (Ulmasov et al., 1997). Seedlings were grown for seven days in half-strength MS liquid medium supplemented with 1.5% sucrose under continuous fluorescent light. The seedlings were then treated with hormones or mock-treated with 0.1% dimethyl sulfoxide.

Real-time Quantitative RT-PCR

Total RNAs were extracted from arabidopsis seedlings using the guanidine-hydrochloride method. The RNAs were then treated with DNasel and converted to cDNA with random primers. Quantitative RT-PCR was performed using real-time-monitoring TaqMan technology. The gene-specific TaqMan probes and PCR primers were designed avoid homology to other members of the IAA gene family.
Histochemical Localization of GUS Activity and GUS Activity Assay

Histochemical GUS staining was performed by incubating whole seedlings in GUS staining buffer containing 50 mM sodium phosphate (pH 7.0), 0.5 mM potassium ferrocyanide, 10 mM EDTA, 0.1% (v/v) Triton X-100, 2% (v/v) DMSO and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide at 37°C for 24 h. GUS activities were measured by using the GUS activity kit (SIGMA) according to the manufacturer’s instructions. The resulting fluorescence was measured with a multi-label counter, the Wallac 1420 ARVOsx2 (Perkin Elmer Life Science) at 355 nm (excitation) and 460 nm (emission). Protein content was determined according to the Bradford method to normalize GUS activity.

Quantification of endogenous IAA

Fresh plant material was carefully weighed, and then free IAA was quantified by gas chromatography single-ion-monitoring mass spectrometry analyses.

RESULTS

Induction of IAA5 and IAA19 genes

We studied expression of the IAA5 and IAA19 genes in response to treatment with exogenous auxin and BR. Seven-day-old det2 seedlings were treated with 10 nM BL, and the induction kinetics was analyzed. IAA5 was induced 34-fold and IAA19 12-fold, and levels of both transcripts peaked at 12 h after BL treatment.

To compare the induction kinetics of the IAA genes by BL treatment with those in response to IAA, WT seedlings were treated with 10 nM, 100 nM and 1 µM BL or IAA for up to 24 h. Both IAA genes were quickly induced by IAA treatment and showed stronger responses with higher IAA concentrations. The IAA5 and IAA19 genes were induced by over 80- and 40-fold, respectively. And transcript levels peaked 1 h after IAA treatment. In contrast, BL induced both IAA genes with much different kinetics than did IAA. The maximum induction of these genes by BL ranged from 3- to 5-fold. The BL induction kinetics in the WT was similar to that of the det2.

DR5 responds to BL

DR5 containing TGTCTC auxin response element (AuxRE) has been widely used as a marker to monitor endogenous IAA distribution. The IAA5 and IAA19 genes have one and three TGTCTC element, respectively, in the 500-bp fragments upstream of their start codons. To gain insight into the mechanisms of BL induction of primary auxin-responsive genes, we studied the involvement of the DR5 AuxRE in BL-induced gene expression. Transgenic arabidopsis seedlings containing the DR5-GUS reporter gene were treated with either 1 µM IAA or 10 nM BL, and the abundance of GUS transcripts was analyzed. IAA quickly induced GUS transcript, with similar kinetics to the induction of the IAA5 and IAA19 genes. GUS transcript abundance peaked 1 h after IAA treatment with a maximum induction of about 100-fold. In contrast, BL induced the DR5-GUS gene with peak induction of about 10-fold at 12 h of treatment.

The DR5-GUS transgenic seedlings were treated with either 10 nM to 1 µM BL or IAA, or 10 µM 1-aminocyclopropane-1-carboxylate (ACC), ABA, zeatin, methyl jasmonate or GA. GUS activities were measured at 24 h treatment. BL induced GUS activity dose independently between 10 nM to 1 µM. GUS activities in BL-treated plants were about 3-fold higher than those in the mock treated plants. In contrast, IAA induced GUS activity dose-dependently between 10 nM and 1 µM. The GUS activation of 10 nM BL was equivalent to that of 50 nM IAA treatment. These results indicated that BL was most effective hormone, behind auxins, to stimulate the DR5-reporter gene construct.
Endogenous IAA levels following BL treatment

The above findings prompted us to determine the endogenous free IAA levels in response to treatment with exogenous BL. Seven-day-old det2 and WT seedlings were treated with 10 nM BL for 12 h and then analyzed for fresh weight and endogenous free IAA levels. The fresh weight per seedling of BL-treated det2 or BL-treated WT seedlings was 150% of 115%, respectively, that of mock-treated seedlings. The amount of IAA per plant increased as the fresh weight increased. However, IAA levels per gram fresh weight did not change in response to BL treatment of either det2 or WT seedlings.

Histochemical analysis of DR5 induction

To analyze the organ specificity of the DR5-GUS activity induced by BL or IAA treatment, seven-day-old DR5-GUS transgenic seedlings were treated with either of these hormones for 12 h and then stained for GUS activity. In the control plants, GUS activity was detectable only at the edges of cotyledons and root tips. When seedlings were treated with 1 µM IAA. When seedlings were treated with a lower concentration of IAA, 50 nM, roots stained more strongly than shoots. In contrast, treatment with 10 nM BL induced GUS activity mainly in cotyledons, whereas GUS activity was not detectable in roots.

DISCUSSION

The fresh weight per seedling of BL-treated det2 mutant of WT reached 150% or 115%, respectively, that of mock-treated seedlings after a 12-h BL treatment. Since the IAA5 and IAA19 genes are induced by BL as quickly as the genes involved in cell elongation and cell wall organization (Goda et al., 2002), it is possible that these IAA genes are involved in cell elongation and cell wall organization. A precise dose-dependent kinetics study of the IAA5 and IAA19 genes after IAA treatment revealed that induction of IAA genes was as rapid as observed in previous studies (Conner et al., 1990; Abel et al., 1995). In contrast, BL induced IAA genes more moderately and more continually than IAA treatment in both det2 and the WT. The timing and the magnitude of maximum transcript accumulation were independent of BL concentration between 10 nM and 1 µM. From these observations, it is apparent that IAA and BL induce the two IAA genes with different kinetics and different dose-dependency. In our study, both the BL and IAA induction kinetics of the DR5-GUS gene were very similar to those of the IAA5 and IAA19 genes in the timing of the peaks, the relative peak heights, and the dose-dependency. We therefore speculate that BL activates these IAA genes at the transcriptional level, at least in part through the TGTCTC AuxRE.

Endogenous free IAA levels per gram fresh weight did not change in either det2 or WT seedlings. One possibility is that endogenous IAA was increased in response to BL treatment, but the increase was under detectable level. Another possibility is that endogenous IAA was not increased in response to BL treatment. If this is the case, the AuxRE is not specific to auxin, but responds to both auxins and BRs. It is noteworthy that endogenous IAA levels are higher in det2 mutant than in the WT. This finding indicates that the lower endogenous BRs in the det2 mutant do not result in lower endogenous IAA levels. It should also be noted that the abundance of IAA5 and IAA19 transcripts in det2 seedlings was lower than in WT seedlings, suggesting that decreased BRs down-regulate IAA5 and IAA19 gene expression not with decreased endogenous IAA level, but with increased IAA levels in the det2 mutant.

The most remarkable physiological response to treatment with higher IAA concentration is growth retardation, which is most significant in roots. IAA induces DR5-GUS mainly more strongly in roots than in shoots. In contrast, application of BL induce shoot growth promotion as well as root growth retardation. Since BL induces DR5-GUS activity mainly in cotyledons, activation of the DR5 element by BL might be related to promotion of growth in cotyledons.
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A DWARF MUTANT STRAIN OF JAPANESE MORNING GLORY (*Pharbitis nil*),
UZUKOBITO, HAS DEFECTIVE BRASSINOSTEROID BIOSYNTHESIS

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**ABSTRACT**

Japanese morning glory (*Pharbitis nil*) is a model plant characterized by a large stock of spontaneous mutants. The recessive mutant, Uzukobito, shows strong dwarfism with dark-green rugose leaves. The phenotype was rescued by the application of brassinolide, a bioactive brassinosteroid (BR), indicating that Uzukobito was a BR-deficient mutant. A detailed analysis of the endogenous BR levels in Uzukobito and in its parental wild-type plant showed that Uzukobito had a lower level of BRs downstream of (24R)-24-methylcholestan-3-one and (22S, 24R)-22-hydroxy-24-methylcholestan-3-one than those in the wild-type plant, while their immediate precursors, (24R)-24-methylcholest-4-en-3-one and (22S, 24R)-22-hydroxy-24-methylcholest-4-en-3-one, accumulated relatively more in Uzukobito. These results indicate that Uzukobito had a defect in the conversion of (24R)-24-methylcholest-4-en-3-one and (22S, 24R)-22-hydroxy-24-methylcholest-4-en-3-one to their 5α-reduced forms, which is catalyzed by DET2 in Arabidopsis. The *P. nil* ortholog of the DET2 gene (*PnDET2*) was cloned and shown to have the greatest similarity to DET2 among all the putative genes in Arabidopsis. Uzukobito had one amino acid substitution from Glu⁶² to Val⁶² in the deduced amino acid sequence of *PnDET2*. Recombinant *PnDET2* expressed in COS-7 cells was found to be a functional steroid 5α-reductase converting (24R)-24-methylcholest-4-en-3-one to (24R)-24-methylcholestan-3-one, while *PnDET2* with the mutation did not show any catalytic activity. This is the first indication that a plant steroid 5α-reductase can convert an intrinsic substrate. All these results clearly demonstrate that the Uzukobito phenotype resulted from a mutation on *PnDET2*. This is the first successful characterization of a morphological mutant at the molecular level among a large stock of *P. nil* mutants.

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THE INTERACTION BETWEEN BIOSYNTHESIS AND RESPONSE OF ABA DURING SEED GERMINATION IN *ARABIDOPSIS THALIANA*

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ABSTRACT

Abscisic acid (ABA) plays an important role in seed dormancy, stomata closure, and plant adaptation to environment stresses. ABA action is accomplished by regulation of both ABA content and its sensitivity. Although numerous studies have been reported the identification of genes involved in ABA biosynthesis and its response, the mechanism underlying the interaction between ABA biosynthesis and its sensitivity remains unclear. To reveal this molecular interaction during seed germination, we have analyzed ABA content in dry seeds using two ABA-insensitive mutants (*abi4*, *abi5*). *ABI4* encodes an APETALA2 (AP2) domain protein whereas the *ABI5* gene encodes a basic Leucine Zipper (bZIP) transcription factor. *ABI5* expression is most abundant in dry seeds and decreases during imbibition, while *ABI4* expression is enhanced during imbibition. The *abi5* mutant contained higher levels of ABA in dry seeds compared to that of wild type. Moreover, when we monitored transcript levels of the *NCED* genes, which encode key enzymes involved in ABA biosynthesis, only *NCED6* showed higher transcript levels in the *abi5* mutant than wild type. These results indicate that ABI5 is involved in the feedback regulation of ABA biosynthesis in the seed. By contrast, the ABA content in the *abi4* mutant imbibed seed was lower than that in wild type. Moreover, the reduction of *NCED6* mRNA levels in the *abi4* mutant was significant compared to that observed in wild type during imbibition. These results suggest that ABI4 is the positive regulator of *NCED6* during imbibition.

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A NEW DIRECT PATHWAY FOR BIOSYNTHESIS OF ABA

Higher plants biosynthesize abscisic acid (ABA) via the non-mevalonate pathway being followed by the carotenoid pathway. In fungi, the biosynthetic pathway of isopentenyl diphosphate (IDP) for ABA is different from that of higher plants. Fungi use IDP derived from the mevalonate pathway to form ABA. However, after IDP, two pathways, the carotenoid pathway involving oxidative cleavage of carotenoid by dioxygenase, and the direct pathway forming a C_{15} precursor from farnesyl diphosphate (FDP), have been proposed. This controversy has not come to a conclusion.

^{18}O-Labeling experiment of ABA with ^{18}O_{2} can distinguish between the carotenoid and the direct pathways. In the carotenoid pathway, one oxygen atom at C-1 would be labeled with ^{18}O, and, in the direct pathway, oxygen at C-1 would not be labeled since C-1 of FDP seems to be oxidized by oxidase. Analysis of ABA produced by *Botrytis cinerea* cultured under ^{18}O_{2} showed that ^{18}O was incorporated at C-1, 1 and 1' of ABA. 1',4'-trans-Diol-ABA, a direct precursor for ABA in the fungus, incorporated ^{18}O from ^{18}O_{2} at C-1, 1, 1' and 4'. This labeled positions suggested that C-4' of ABA had been labeled with ^{18}O. In fact, 4'-oxygen of ABA was easily changed *in vitro* with oxygen from H_{2}O under acidic conditions. The medium pH changed between 2.7 and 5.8 during culture, strongly supporting the exchange of 4'-^{18}O with ^{16}O from H_{2}^{16}O. These findings confirmed that C-1, 1, 1' and 4' of ABA were oxidized with molecular oxygen. This result was not consistent with that expected not only from the carotenoid but also from the direct pathway. A precise analysis of extracts from mycelia of the fungus revealed that the fungus did not produce carotenoid except for a trace of phytoene, but produced C_{15}-compounds, 2E,4E,6E-allofarnesene (1), 2Z,4E,6E-allofarnesene (2), 2E,4E-\alpha-ionylideneethane (3) and 2Z,4E-\alpha-ionylideneethane (4). A feeding experiment with 2-^{13}C labeled 1-4 showed that the fungus converted these compounds to ABA with incorporation ratios of 0.7, 0.2, 2.6 and 17.0%, respectively. Thus, we propose a new direct pathway for ABA of *B. cinerea* in which FDP is reduced at C-1 to give 1, cyclization following isomerization at C-2 of 1 gives 4 via 2, and then 4 is oxidized to ABA by monooxygenase with molecular oxygen (Figure).

Another ABA-producing fungus, *Cercospora crucenta*, cultured under ^{18}O_{2} incorporated ^{18}O at all oxygen atoms of ABA and of the direct precursor, 2Z,4E-1',4'-dihydroxy-\gamma-ionylideneacetic acid. The fungus produced 2Z,4E-\gamma-ionylideneethane along with 1. These findings and a feeding experiment with [2-^{13}C]-2Z,4E-\gamma-ionylideneethane suggest that the new direct pathway is working also in this fungus.

![Figure](image)

**Figure** Proposed New Direct Pathway in Fungi

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METABOLISM OF THE LONG-LASTING ANALOG OF ABSCISIC ACID IN RADISH
(Raphanus sativus)

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ABSTRACT

Abscisic acid (ABA) is an essential mediator in triggering the plant response to environmental stresses such as desiccation and freezing. Little genetic information of ABA metabolism is obtained so far, probably because no mutant for ABA metabolic enzymes is found. ABA is inactivated by two major pathways: the conjugation to give the glucosyl ester of ABA (ABA-GE) and the oxidation to give 8′-hydroxyabscisic acid (8′-HOABA) and its more stable isomer phaseic acid (PA). The conjugation pathway is a reversible inactivation to regenerate free ABA, whereas the oxidation pathway is irreversible and therefore intrinsic. The 8′-hydroxylation is believed to be catalyzed by ABA 8′-hydroxylase, which may be a microsomal cytochrome P-450 monoxygenase. The biological activity of 8′-HOABA is suggested to be more than 1/10 that of ABA, whereas that of PA is less than 1/10 to 1/100 that of ABA in most of the assays.

In 1996, we reported the synthesis and biological activity of 5′α,8′-cycloabscisic acid (CycloABA), and discussed the conformational requirement for ABA activity based on the conformation-activity relationships of four ABA analogs with a cyclopropane fused to the six-membered ring of ABA (Todoroki², 1996). CycloABA was the most effective among ABA and its analogs in a long-term assay, rice seedlings elongation inhibition, whereas it was as effective as ABA in a short-term assay, stomata opening inhibition. Moreover, CycloABA kept with the ABA activity over longer period than ABA. These results suggested that CycloABA is a metabolically stable analog of ABA.

In the present paper we developed the fast and easy ABA metabolism assay system with radish seedlings, and investigated the metabolism of CycloABA using this system. The populations of ABA-related compounds found in radish seedlings fed 1.0 mg of ABA: PA, 40%; DPA, 10%; ABA-GE, 30%; and unmetabolized ABA; 20%. The conversion ratio to the metabolites was 80%, and the oxidation/conjugation ratio was 10:7. On the other hand, incorporation of CycloABA into the seedlings was 60% of applied amount (1 mg); it was less than that of ABA (90%). This may have been caused by stronger inhibition of CycloABA against transpiration compared to that of ABA. The significant oxidative products of CycloABA were not able to be found, whereas the glucose conjugate of CycloABA (CycloABA-GE) was found as a major metabolite. The 40% of CycloABA incorporated was converted to CycloABA-GE. The net amount of CycloABA-GE was equivalent to that of ABA-GE, although incorporation of CycloABA into the seedlings was less than that of ABA, meaning that the ABA glucosyltransferase activity increased to inactivate CycloABA that was not inactivated by ABA 8′-hydroxylase. The perfect resistance of CycloABA against the 8′-hydroxylation suggests that the biological activity of CycloABA depends on ABA glucosyltransferase activity of a tested plant. The present findings showed that CycloABA is a useful probe for the mechanism of ABA metabolism in plants.

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DEUTERIUM LABELED PHASEIC ACID AND DIHYDROPHASEIC ACIDS FOR INTERNAL STANDARDS

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ABSTRACT

Concentration of abscisic acid (ABA) in plants is regulated not only by biosynthesis but also by metabolism. ABA is metabolized to 8'-hydroxy-ABA, and 8'-hydroxy-ABA is easily isomerized to phaseic acid (PA) by an intramolecular conjugated addition of 8'-oxygen to C-2' (Fig.). PA is converted to dihydrophaseic acid (DPA) and its epimer (epi-DPA). Quantitative analysis of these metabolites is important as well as that of ABA to understand changes in concentration of ABA in plants. However, internal standards of the metabolites suitable for the quantitative analysis have not been reported. We report preparation of deuterated metabolites of ABA, and application of the deuterated metabolites to the quantitative analysis.

Chemical synthesis of PA has been reported by three groups. Deuterium may be introduced into synthetic intermediates, but the procedures take time and the yields are not high. We planned to directly introduce deuterium into PA obtained from β-hydroxy-β-methylglutaryl ester of 8'-hydroxy-ABA by hydrolysis.

First, deuterium was introduced at C-3' and 5' of PA by a treatment with sodium deuteroxide. However, all of the deuterium was lost upon methylation by diazomethane. Second, the 4'-oxygen of PA methyl ester was labeled with ¹⁸O, but 4'-¹⁸O was easily substituted with ¹⁶O during alkaline hydrolysis in H₂¹⁶O. Finally, the introduction of deuterium at C-7' of PA was tried. A PA sample contains 8'-hydroxy-ABA at 2% of its weight due to equilibrium between PA and 8'-hydroxy-ABA. 8'-Hydroxy-ABA has an enone group as well as ABA, suggesting that deuterium can be introduced at C-3', 5', 7', 1'-O and 8'-O of 8'-hydroxy-ABA by treatment with sodium deuteroxide to give 8'-hydroxy-ABA-d₈. 8'-Hydroxy-ABA-d₈ is isomerized to PA-d₈ possessing deuterium at C-3', 5', 7' and 1'-O in a sodium deuteroxide solution. The alcoholic deuterium at 1'-O is exchanged with hydrogen during extraction by partitioning between ethyl acetate and water to give PA-d₇. Short-term treatment of PA-d₇ with sodium hydroxide would substitute four deuterium at C-3' and 5' with hydrogen, and the remaining three deuterium at C-7' to give PA-d₃. PA-d₃ with deuterium content of 86% was prepared with this method. The decrease in the deuterium content of PA-d₃ kept in buffer solutions at pH 3, 5, 7 and 8 for 3 h was less than 2%. 7'-Deuterium labeled DPAs, DPA-d₃ and epi-DPA-d₃, were obtained by reduction and hydrolysis of PA-d₃ methyl ester. Contents of ABA and its metabolites in fruit, leaves, and buds of apples and satsuma mandarins were assayed using ABA-d₆, PA-d₃, DPA-d₃ and epi-DPA-d₃ as internal standards. The metabolite contents of the organs varied, suggesting a different regulation of metabolism of ABA among the organs.

Fig. Metabolic Pathway of ABA

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USE OF THE TREE GROWTH REGULATOR PACLOBUTRAZOL TO CONTROL APPLE SCAB

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ABSTRACT

Paclobutrazol as a systemic fungicide for control of apple scab (*Venturia inaequalis*) was investigated in mature (cv. Hopa and Snow Drift) and young sapling (cv. Indian Magic) crabapples (Malus spp.). Treatments consisted of a control and paclobutrazol applied to mature trees at one or two times the recommended rate in April 2002 using the basal drench method. Saplings received both foliar and soil drench applications of paclobutrazol or propiconazole. Disease assessments of mature trees showed apple scab symptoms in treated trees were as severe as in untreated ones in the year of treatment, but were reduced slightly the second year in Hopa. Growth reduction occurred in all treated trees, suggesting that paclobutrazol levels needed for growth reduction were not sufficient to control apple scab in the year of treatment. In contrast, a one-time foliar application of paclobutrazol reduced apple scab incidence to levels found in saplings treated every two weeks with propiconazole, a fungicide and application method commonly recommended for apple scab control. Delayed uptake and transport of sufficient quantities of paclobutrazol to the crown of mature trees may account for the lack of apple scab control the year of treatment even though growth suppression occurred.

INTRODUCTION

Paclobutrazol (PBZ) inhibits both sterol and gibberellin biosynthesis in plants and fungi by interfering with cytochrome P₄₅₀ dependent oxidations of the isoprenoid pathway (Fletcher and Hofstra, 1988; Rademacher, 2000). Many of the most popular fungicides used today rely on the same mode of action, sterol biosynthesis inhibition, for their high degree of fungicidal activity. Paclobutrazol is a molecule possessing two asymmetric carbons (two chiral centers) and therefore may exist as one of four isomers, each with a unique stereochemical configuration that influences the growth inhibiting and fungicidal properties of the molecule. Formulations of paclobutrazol consist of a mixture of two stereoisomers, each isomer has its own unique biological activity (Lurrsen, 1988).

Fungal diseases which are chronic and caused by pathogens that survive in woody plants during the dormant season are often more difficult to treat and control than are annually occurring foliar diseases. In addition, woody plant diseases such as cankers, root rots, and wilts frequently have few control options, yet can often have the most severe effects on trees (Jacobs and Berg, 2000). Paclobutrazol, a triazole plant growth regulator, may have sufficient fungicidal activity to control certain woody plant diseases.

The potential for paclobutrazol to act as a systemic fungicide along with its apparent longevity in plants would make it a unique tool in controlling a wide variety of woody plant diseases. The purported benefits of protection from fungal disease and other environmental stresses, along with tree growth regulating activity, are making paclobutrazol a potentially valuable tool in the management and maintenance of trees in urban areas (Chaney, 2001; Davis and Curry, 1991; Fletcher et al., 1999).

MATERIALS AND METHODS

On April 1, 2002, 99 mature crabapples (average diameter five inches) of two different

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cultivars, Hopa and Snow Drift, growing at the Purdue University Horticulture Farm were treated with paclobutrazol using the basal drench method and the natural occurrence of apple scab was monitored. Paclobutrazol was applied at both the recommended rate, 1X PBZ (2.0 g a.i. inch⁻¹diameter) and twice the recommended rate, 2X PBZ (4.0 g a.i. inch⁻¹diameter). The occurrence of apple scab was monitored on the leaves of terminal shoots for 14 months following the application of paclobutrazol, allowing adequate time for uptake and translocation of the triazole growth retardant to the foliage. Observations were made periodically through the spring and summer of 2002 until the conditions necessary for secondary infections were no longer available. By late June most trees in the orchard had lost almost all of their leaves due to high disease severity, so further assessments could not be conducted. Disease incidence was assessed for the final time on June 3, 2003. Wet and cool conditions early in the spring of 2002 and 2003 resulted in high disease incidence and severity on both cultivars.

Reductions of terminal shoot growth induced by the compound also were measured to verify the presence of paclobutrazol in the shoots. These measurements were taken June 11 and November 11, 2002 and for the final time on June 3, 2003.

On April 18, 2002, 150 crabapple saplings (whips), cultivar ‘Indian Magic’, were planted at the Horticulture Farm. The whips were randomly assigned to one of six treatment groups to determine the effectiveness of paclobutrazol applied by the basal drench method or by foliar spray in controlling apple scab, and to compare paclobutrazol to propiconazole, a fungicide commonly used in the prevention of apple scab.

Treatments for Indian Magic Whips
1) Control
2) A basal drench with PBZ at the recommended rate (2.0 g a.i. inch⁻¹diameter), April 19, 2002.
3) Standard spray program: Foliar application of propiconazole every two weeks beginning at ½ inch green tip stage.
4) One time foliar application of PBZ (0.02 g a.i. ml⁻¹) at ½ inch green tip stage. Sprayed until leaf drip. May 2, 2002.
5) One time foliar application of PBZ (0.02 g a.i. ml⁻¹), 4 days after a heavy infection period occurred, to assess eradicative abilities. Sprayed until leaf drip. May 15, 2002
6) One time foliar application of propiconazole, four days after a heavy infection period occurred. Sprayed until leaf drip. May 15, 2002.

Shoot growth and disease incidence for the Indian Magic whips were assessed following application of paclobutrazol through the 2002 growing season. Evaluations could not be conducted in 2003 because the whips were mistakenly killed with an herbicide during routine maintenance in the orchard.

All data for both the mature trees and saplings were analyzed using ANOVA and differences between means were determined using Tukey’s w procedure (α=0.05)

RESULTS AND DISCUSSION

Shoot growth was assessed at the end of the first year of treatment in an attempt to determine if paclobutrazol was present in the upper canopy of treated mature trees. In the treated Hopa, shoot growth was significantly reduced in both 1X and 2X PBZ treatments compared to control trees, whereas shoot length of Snow Drift was affected only by treatment with 2X paclobutrazol (data not shown). Although the difference in shoot length was small, it indicated that paclobutrazol was already present at sufficient levels in the tree canopies to reduce shoot growth in the year of treatment.
On June 3 of the year following treatment both Hopa and Snow Drift showed significant reductions in shoot growth in response to both 1X and 2X treatments of paclobutrazol (Fig. 1 and 2).

Paclobutrazol applied in the spring of 2002 did not effectively control apple scab on either Hopa or Snow Drift varieties of crabapple in the summer of that year (data not shown). The weather conditions of the 2002 growing season were very conducive to high levels of scab infection. Disease incidence was nearly 100% on all trees in the orchard by the beginning of July, followed by almost total defoliation of those trees by the end of the month. When treatments were compared, no difference was observed in disease incidence that would suggest that paclobutrazol, even at the 2X rate, was successful in controlling apple scab during the year of treatment. In the year following application of paclobutrazol, disease incidence was significantly lower in treated Hopa when compared
to the control on June 3, 2003 (Fig. 3). A similar trend, but statistically insignificant difference was observed in Snow Drift in the second year following treatment (Fig. 4). The reduction in apple scab incidence for Hopa was not substantial enough to suggest that paclobutrazol when applied as a basal drench could be used to effectively control apple scab.

Growth assessment of the Indian Magic whips showed that of the six treatments only the foliar applications of paclobutrazol reduced shoot growth (Fig. 5). Treatments 1, 2, and 6, which include the control, paclobutrazol basal drench, and the one-time treatment with propiconazole after infection, respectively, had higher disease incidence than treatments 3, 4, and 5, which include the standard spray program with propiconazole and the foliar applications of paclobutrazol at green tip or after heavy infection, respectively (Fig. 6).

Figure 3. Percent disease incidence on June 3, 2003 of Hopa treated with 1X (2.0 g a.i. inch⁻¹ diameter) or 2X (4.0 g a.i. inch⁻¹ diameter) paclobutrazol (α=0.05).

Figure 4. Percent disease incidence on June 3, 2003 of Snow Drift treated with 1X (2.0 g a.i. inch⁻¹ diameter) or 2X (4.0 g a.i. inch⁻¹ diameter) paclobutrazol (α=0.05).
Figure 5. Terminal shoot growth (cm) on June 22, 2002 of Indian Magic treated with 1) control; 2) soil drench with paclobutrazol (2.0 g a.i. inch⁻¹ diameter); 3) foliar application of propiconizole (0.016% by volume) every two weeks beginning May 2, 2002; 4) foliar application of paclobutrazol (0.02 g ml⁻¹) at ½ inch green tip stage; 5) foliar application of paclobutrazol (0.02 g ml⁻¹) following a predicted heavy infection period; 6) foliar application of propiconizole (0.016% by volume) following a predicted heavy infection period (α=0.05).

Figure 6. Percent disease incidence on June 11, 2002 of Indian Magic treated with 1) control; 2) soil drench with paclobutrazol (2.0 g a.i. inch⁻¹ diameter); 3) foliar application of propiconizole (0.016% by volume) every two weeks beginning May 2, 2002; 4) foliar application of paclobutrazol (0.02 g ml⁻¹) at ½ inch green tip stage; 5) foliar application of paclobutrazol (0.02 g ml⁻¹) following a predicted heavy infection period; 6) foliar application of propiconizole (0.016% by volume) following a predicted heavy infection period (α=0.05).
CONCLUSIONS

Paclobutrazol should be thought of as two separate compounds because it is a mixture of stereoisomers, one which results in reduced plant growth and the other having fungicidal activity. The results in this study show that at the rates tested, paclobutrazol applied as a basal drench was not an effective systemic fungicide to control apple scab on Snow Drift or Hopa crabapples, although paclobutrazol application did result in a statistically significant but small decrease in disease incidence in Hopa the year following application (Fig. 3). Even when sufficient time was given for the compound to reach significant levels in treated trees to reduce shoot growth, apple scab occurrence was not sufficiently reduced to justify the use of paclobutrazol to control apple scab on these two varieties of crabapple in the landscape.

However, one foliar application of paclobutrazol to Indian Magic saplings (treatment 4 and 5) was very successful in controlling disease incidence, while the one-time application of propiconazole did not effectively control apple scab (treatment 6) (Fig. 6). This strongly suggests that paclobutrazol, in comparison to propiconazole, is more persistent on the leaf surface (paclobutrazol residue was still visible on the plant surface into October), has greater eradicator ability, is more effective in preventing infection, or any combination of the three. Paclobutrazol applied to the foliage for control of apple scab could be a unique tool in the management of landscape or orchard trees where growth regulation is acceptable or desired.

LITERATURE CITED


ACKNOWLEDGMENT

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EFFECT OF FASCINATION™ ON POSTHARVEST PERFORMANCE OF CUT RACEMES OF BIGBEND BLUEBONNET

W. A. Mackay¹, N. Sankhla, S. M. L. Rahman and T.D. Davis

ABSTRACT

Attractive racemes of Big Bend bluebonnet (Lupinus havardii Wats.) hold considerable promise as a new specialty cut flower crop. As a result of our breeding and selection efforts, we have developed several lines of improved germplasm with different flower colors. This study was conducted to evaluate the effect of Fascination™ (FAS), a commercial formulation of 1.8% GA₄+7 plus 1.8% BA (Valent BioSciences), on parameters related to postharvest performance and longevity of cut racemes of two blue flowered lines (Blue Select 2002, BS; ‘Texas Sapphire’, TS), two white flowered lines (White Select 2002, WS; ‘Texas Ice’, TI) and a dark pink (DP) flowered line of Big Bend bluebonnet. An aqueous solution of FAS (6, 12, 24, 48 and 96 ppm) was supplied through the base of the cut racemes placed in glass vases at 22±2°C under fluorescent lamps. The results indicated that in the improved germplasm, the senescence of flowers during vaselife constituted a key component of the display life. The response of the different lines varied considerably to the presence of FAS. In WS, TI and DP, low concentrations of FAS (6, 12 ppm) delayed the senescence of flowers, but a promotion was recorded in BS and TS. Except in WS, and to certain degree in TI, high concentrations of FAS (48, 98 ppm) exhibited toxic symptoms both in flowers and inflorescence axis. In a separate study, GA₃, alone was found to be relatively more effective, and less toxic, than BA in influencing flower senescence in cut racemes of bluebonnet.

INTRODUCTION

Promalin, a proprietary mixture containing 1.8% GA₄+7 and 1.8% BA was introduced in 1979 as a PGR for use in apples. It is now widely used to improve fruit quality and obtain larger and ‘typier’ fruits that have a greater market acceptance (Valent Bio Science, Illinois, USA). Fascination™ (FAS) contains the same combinations of GA₄+7 and BA as promalin. Currently, FAS is labeled for Easter lily, LA hybrid lily and Asiatic lily as an effective prophylaxis for postharvest leaf yellowing (Valent BioSciences, IL, USA).

Han (3) first reported that a combination of GAs and BA reduced yellowing in excised Easter lily leaves. Comparative studies, involving various lilies, established the effectiveness of GA₃, BA, Promalin and Accel (GA₄+7: BA of 1:10) in reducing leaf senescence, but Promalin was more effective in all cases (2, 6, 10). Application of Promalin sprays before cold storage increase flower longevity, prevent bud abortion and improve leaf quality in ‘Stargazer’ hybrid lilies (6). In cut Asiatic and Oriental lilies addition of mixture of BA and GA₄+7 to the preservative solution completely prevented leaf yellowing but induced bud blasting (4). Recently, it has been shown that in Oriental lilies pretreatment with Promalin and 1-MCP maintained superior quality under postharvest conditions encountered in commercial handling (1). It is clear that most of the research on the effect of Promalin on ornamental plants is limited to lilies. However, with the introduction of FAS research is underway for geranium, poinsettia and a variety of other ornamental plants.

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Over the years, as a result of our breeding and selection efforts, we have developed several lines of improved germplasm of Big Bend bluebonnet (*Lupinus havardii* Wats.) with different flower colors. The attractive racemes of Big Bend bluebonnet hold great promise as a new specialty cut flower crop (9). This study was conducted to evaluate the effect of FAS on parameters related to postharvest performance and longevity of cut racemes of some recently introduced cultivars and advanced lines of bluebonnet.

**MATERIALS AND METHODS**

*Lupinus havardii* plants, with three novel colors (deep blue, pink and white) were grown in non-shaded greenhouses at Dallas. The racemes were harvested in the morning and brought to the laboratory for experimentation. An aqueous solution of FAS (6, 12, 24, 48 and 96 ppm) was supplied through the base of the cut racemes placed in glass vases at 22±2°C under cool white fluorescent lamps (30 µmol m⁻² sec⁻¹). Racemes of two blue flowered lines (Blue Select 2002, BS; Texas Sapphire, TS), two white flowered lines (White Select 2002, WS; Texas Ice, TI) and a dark pink flowered line (DP) were used in the current study.

**RESULTS AND DISCUSSION**

Big Bend bluebonnet is a winter annual native to Chihuahuan desert. Over the years, in addition to the blue flowered lines, we have identified and developed pink and white flowered lines as well. In the initial studies, especially with the more commonly occurring ‘blue flowered’ lines, abscission of flowers in the cut racemes was determined as a key component of postharvest longevity (8), although flower senescence was also observed. The improved lines, as used in the current study, were developed by additional phenotypic selection and exhibit relatively low flower shattering than the earlier germplasm during vase life. However, senescence of flowers continues to affect the display life of the cut racemes. The response of different lines varied considerably to the presence of FAS. In WS, TI and DP, low concentrations of FAS (6, 12 ppm) slightly delayed the senescence of flowers, but a promotion were recorded in BS and TS. Although as compared to TI flowers, the flowers in BS exhibited almost no abscission, but the banner spot appeared to be very sensitive to the presence of FAS. Increasing concentration of FAS induced browning and blackening of the banner spot, and even the tip of the standard petal indicated wilting leading to senescence.

Puglisi et al. (5) observed that in *Clematis spp.*, FAS treatments resulted in linear increases in branch number with increasing rate. However, the treatment caused phytotoxicity, including tip dieback. In bluebonnet also, except in line WS, and to a certain degree in TI, the high concentrations of FAS (48, 98 ppm), which are considerably lower than the levels used by Puglisi et al. (5), exhibited toxic symptoms both in flowers and inflorescence axis. In a separate study, we found that GA alone was relatively more effective, and less toxic, than BA in influencing flower senescence in cut racemes of bluebonnet. Additionally, incorporation of sucrose in the vase solution counteracted the toxic effects of FAS (unpublished results). The beneficial effect of sucrose on vase life has been attributed to maintenance of low pH by sugars which improved water uptake (7). Further studies on the effect of FAS and sugars in combination on postharvest performance of cut racemes of bluebonnet are in progress.
LITERATURE CITED

CHLOROPHYLL FLUORESCENCE, PHOTOSYNTHESIS AND ENZYME ACTIVITIES IN BER (Ziziphus rotundifolia) UNDER SALINITY STRESS

R. Choudhary¹, S. Joshi¹, H.S. Gehlot¹ and N. Sankhla²

ABSTRACT

One year old plants of ber, when subjected to salinity stress, indicated reduced growth and elevated levels of proline, but no effect was observed on leaf pigments. Treatment with salt resulted in an immediate inhibition of net photosynthetic rate, but at low concentrations of salt the plants exhibited considerable recovery with time. However, at the highest salt concentration used, not only the net photosynthetic rate, but also the chlorophyll fluorescence characteristics were also drastically affected. The efficiency of photochemistry declined, and as a result of lowered efficiency of photochemistry and the highly reduced state of Q/A quantum yield of the electron transport also decreased. Eventually, the photoinhibition of assimilation rate coupled to the toxic effects of salt on cellular metabolism resulted in death of the plants. Under salt stress, the different enzymes responded quite differently, and their activities depended on the concentration of salt and the duration of the treatment.

INTRODUCTION

The development of accurate, non-intrusive, portable modulated fluorometers, and gas exchange systems and imaging techniques have opened new vistas in rapid monitoring of photosynthetic functioning in response to a variety of abiotic and biotic stresses (2, 3, 5). We have been interested in characterization and elucidation of the processes limiting growth, potential for survival, and productivity of plants under the environmental constraints that exist in the Great Thar desert of India. In this region, plants often face an acute shortage of water, extremes of temperatures, high thermal and irradiation load and, at specific sites, supra-optimal levels of salt in the soil (1, 6).

The specific objective of the current study was to evaluate the effect of imposed salinity stress on photosynthesis, chlorophyll fluorescence and enzyme activities in seedlings of Ziziphus rotundifolia (Ber) - a drought tolerant native fruit tree. Currently, it is extensively used as root stock for improved clones of Z. mauritiana which bears relatively bigger fruits.

MATERIALS AND METHODS

One year old seedlings of Ziziphus rotundifolia, growing in 12" earthenware pots, were used for experimentation. Salinity levels (S₁= 12 dS.m⁻¹; S₂= 24 dS.m⁻¹; S₃= 32 dS.m⁻¹) were maintained by adding sodium chloride (NaCl) to the pots. The chlorophyll fluorescence parameters were measured using a modulated fluorescence measuring system (Hansatech MSMF MK II) as described earlier (3). The rate of transpiration, stomatal conductance, net photosynthesis and intercellular CO₂ concentration was measured using a portable photosynthesis system (LI-6200 PPS, LI-COR Inc.) fitted with a TR-gas analyzer (LI-6250) with closed leaf chamber.

Enzyme activities were assayed in leaf samples as described previously (3). The experiments were suitably replicated and repeated.

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RESULTS AND DISCUSSION

High salinity, like water deficit, is also one of the most crucial factors for the growth of plants in Indian arid zone. Earlier we observed that in *Z. rotundifolia* imposition of water stress induced a fast decline in stomatal conductance, rate of transpiration and net photosynthesis. However Fv/Fm ratio and the quantum yield decreased only under severe moisture deficit (4). Additionally, a clear cut enhancement was also observed in basal and non-photochemical quenching of chlorophyll fluorescence. In contrast, S1 and S2 treatment, by and large, did not affect photosynthesis and electron transport significantly. Most of the effects were observed only initially on the fourth day after treatment resulting possibly from an osmotic shock. S3 treatment, however, proved to be almost lethal. Not only net photosynthesis rate, but chlorophyll fluorescence characteristics were also drastically affected (Table 1). The quenching of basal fluorescence increased and photochemical quenching declined indicating increase in energy transfer from PSII to PSI and decline in reoxidation of QA. Analysis of non-photochemical quenching based on theoretical Fv/Fm indicates that photoinhibitory quenching constitted the major portion of non-photochemical quenching, indicating photoinhibition of photosynthesis. The efficiency of photochemistry also declined drastically. The quantum yield of electron transport decreased as a result of lowered efficiency of photochemistry and highly reduced state of QA. It can, therefore, be concluded that photoinhibition was the main factor responsible for drastic reduction in net photosynthesis rate and coupled with a toxic effect of salt ions on cellular metabolism made the survival of the plants impossible under high salinity. Under salt stress, the different enzymes (alpha-amylase, acid phosphatase, protease, RNase, catalase, peroxidase, superoxide dismutase) responded quite differently, and their activities depended on the concentration of salt and the duration of the treatment (data not presented).

Overall, these results indicate that *Z. rotundifolia*, unlike water stress, is unable to tolerate high levels of salt in the soil.

REFERENCES

Table 1. Effect of salt on photosynthesis and chlorophyll fluorescence characteristics in leaves of ber.

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Hours after treatment</th>
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<td>M</td>
<td>N</td>
<td>M</td>
<td>N</td>
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<td>N</td>
</tr>
<tr>
<td>A</td>
<td>WW</td>
<td>6.9</td>
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<td>S3</td>
<td>0.18</td>
<td>0.23</td>
<td>0.26</td>
<td>0.26</td>
<td>0.30</td>
</tr>
</tbody>
</table>

* Assimilation rate (A, µmolCO₂ s⁻¹), intercellular CO₂ (Cᵢ, ppm), stomatal conductance (gₚ, cm s⁻¹), basal (q₀), photochemical (qₚ), non-photochemical quenching (qₙₚL in light, qₙₚD in dark), efficiency of photochemistry (Fᵥ/Fₘ) and quantum yield (dF/Fₘ) of electron transport via PSII (relative units). M=Morning; N=Noon; WW=Well watered; S₃=NaCl 32 dS.m⁻¹).
IN VITRO STUDIES ON CHICKPEA SEEDLINGS: EFFECT OF SALT ON GROWTH AND ANTIOXIDANT ACTIVITY

H.S. Gehlot¹, R. Dinesh¹, R. Choudhary¹, S. Joshi¹ and N. Sankhla²

ABSTRACT

Plants with high levels of antioxidants, either constitutive or induced, are reported to have greater potential to cope up with an array of abiotic stresses. Chickpea (Cicer arietinum L.) is one of the most important grain legumes of Asia, Africa and Latin America. However, like many legumes, it is sensitive to salinity. We have studied the effect of salt on growth and antioxidant activity in cultivars of chickpea differing in sensitivity to salt. Depending upon the concentration used (50-200 mM) although salt inhibited the growth, but promoted the activities of catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase and glutathione reductase. A slight increase was also recorded in malondialdehyde content in the shoots at high salt concentration. Depending on the cultivars, simultaneous incorporation of paclobutrazol in the culture media, considerably modulated the effect of salt on growth and activities of enzymes related to antioxidant metabolism. Thus, it appears that paclobutrazol may have a role in salt tolerance of chickpea seedlings. Overall, the results indicated that cv. C-235 is relatively more sensitive to salt than cv. H-208.

INTRODUCTION

Salinity constitutes a major abiotic stress and reduces plant productivity worldwide. High salt concentration induces ionic imbalance and hyperosmotic shock, and affects whole plant coordination, signaling pathways, detoxification response pathways, and plant growth and productivity (3, 6, 7, 8). Although salt tolerance is a multifaceted quantitative trait difficult to dissect and incorporate into salt sensitive plants, spectacular progress had been made in our understanding of salt tolerance mechanisms in plants (3, 6, 7, 8).

Chickpea (Cicer arietinum L.) is one of the most important grain legumes of Asia, Africa, and Latin America. It is the third largest pulse crop cultivated worldwide after bean and pea. Desi and Kabuli chickpea types have also found an important niche in the northeast grain belt of Australia where they are grown extensively. Although it can grow with very few inputs, like many other legumes, chickpea is sensitive to salinity.

There is a notable lack on specific information on the effect of salt on oxidative stress in chickpea. This study was carried out to study the effect of salt on early seedling growth and antioxidant activity in chickpea cultivars differing in salt sensitivity.

MATERIALS AND METHODS

Seedlings of chickpea (Cicer arietinum) were grown in vitro in MS medium containing 3% sucrose and 0.25% Phytagel (5). During the experimental period, the cultures were maintained at 25±1°C in light (40-50 μmol.m².sec⁻¹) for 16 hr/day. Three week old shoots were excised and processed for enzyme assays as described previously (5).

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RESULTS AND DISCUSSION

Four cultivars (P-109, RSG-44-C/31, H-208 and C-235) of chickpea were used to evaluate the relative effect of salinity (50-200mM) on germination and early seedling growth. In salt (150mM) the seeds of cv. H-208 indicated 65% germination, while less than 10% germinated in cv. C-235. Thus, cv. H-208 appeared more salt tolerant than cv. C-235. Elongation of both shoot as well as root was also inhibited in salt (Table 1). In cv. C-235, at concentrations of salt higher than 100mM, the lateral roots, by and large, failed to emerge at all. During growth, the roots progressively became black and even shoot necrosis and drying of leaflets was also clearly visible.

It is presumed that the deleterious effects of salt may at least be partially due to the generation of reactive oxygen species (ROS) and/or inhibition of the ROS-detoxification system (3, 6). Plants detoxify activated oxygen via a complex defense system consisting of a variety of antioxidants and ROS detoxifying enzymes (1, 2, 4). SODs, a family of multiple enzymatic forms catalyse the dismutation of the superoxide anion by forming $\text{H}_2\text{O}_2$ which may be disposed by CAT and PER. In chickpea, an increased activity of CAT, PER, and SOD was clearly evident at high salt concentration (Fig. 1).

In chloroplasts the initial reaction in scavenging $\text{H}_2\text{O}_2$ is catalysed by ASC-PER (1). In this pathway, the regeneration of ASC may proceed by either a reaction catalysed by NADPH-MDHAR or by coupled reactions involving GSH-DHAR and GR (1). Chickpea shoots indicated elevated activities of ASC-PER, MDHAR and GR even at low salt concentration (Fig. 1).

In addition to enzymes involved in ROS detoxification, plants possess an array of antioxidants which are efficient oxy-radical scavengers (1). In chickpea, increasing concentrations of salt brought about a reduction in the level of both ASC and GSH (Table 2), and induced enhanced peroxidation of lipids as evidenced by an increase in MDA content. Earlier, we observed that paclobutrazol, a triazole plant growth retardant, promoted antioxidant activity in chickpea seedlings. It also partially counteracted the effects of salt on antioxidants as well as the activities of enzymes involved is ROS detoxification( our
unpublished results). The significance of these results in protection of chickpea seedlings from deleterious effects of salt is being further evaluated.

REFERENCES


Table 1. Effect of salt on in vitro growth of chickpea cultivars

<table>
<thead>
<tr>
<th>Treatment (mM)</th>
<th>Seedling growth (mm) after 2 weeks*</th>
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<tr>
<td></td>
<td>H-208</td>
</tr>
<tr>
<td></td>
<td>Rt (L)</td>
</tr>
<tr>
<td>Control</td>
<td>70±5</td>
</tr>
<tr>
<td>Salt 50</td>
<td>50±5</td>
</tr>
<tr>
<td>Salt 100</td>
<td>30±2</td>
</tr>
<tr>
<td>Salt 500</td>
<td>15±3</td>
</tr>
</tbody>
</table>

* Rt(L) = length of main root; LRt(N = number of lateral roots; St(L) = length shoots
Table 2. Effect of salt on ascorbate, glutathione, proline and malondialdehyde content

<table>
<thead>
<tr>
<th>Treatment (mM)</th>
<th>ASC</th>
<th>DHA</th>
<th>GSH</th>
<th>GSSG</th>
<th>PROL</th>
<th>MDA</th>
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<tbody>
<tr>
<td>CONTROL</td>
<td>317±20</td>
<td>35±6</td>
<td>386±16</td>
<td>87±8</td>
<td>272±10</td>
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<tr>
<td>SALT 100</td>
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<td>93±8</td>
<td>226±8</td>
<td>89±10</td>
<td>302±12</td>
<td>75±4</td>
</tr>
</tbody>
</table>

* ASC = ascorbate; DHA = dehydroascorbate; GSH = reduced glutathione; GSSG = oxidized glutathione, PROL = proline, MDA = malondialdehyde (values of MDA are in nmol.g⁻¹ fresh weight, while values of all other constituents are µg.g⁻¹ fresh weight)
EFFECT OF GIBBERELLIN BIOSYNTHESIS INHIBITORS ON IN VITRO GROWTH AND ANTIOXIDANT ACTIVITY IN CHICKPEA SEEDLINGS

R. Dinesh, H.S. Gehlot, R. Choudhary, S. Joshi and N. Sankhla

ABSTRACT

Gibberellin biosynthesis inhibitors (GBI’s) are the most potent group of growth retardants which act primarily by inhibiting GA-biosynthesis followed by secondary modulation of ABA, ethylene, cytokinins and polyamines. In fact, they have been credited as multiple “stress-protectants”. We have studied the effect of paclobutrazol (PACLO), uniconazole (UNI) and prohexadione-Ca (PROHEX) on in vitro growth and antioxidant activities in chickpea (Cicer arietinum L.) seedlings. Of the three GBI’s, PACLO and UNI were found to be more effective than PROHEX in retarding growth. In PACLO and UNI, the lateral roots continued to grow equally in the medium, became thick and imparted a characteristic ‘brush-like’ appearance. The leaves also became thick and developed an intense dark green color. PACLO was found to be substantially much more effective in promoting the activities of catalase, peroxidase, ascorbate peroxidase, monodehydroascorbate reductase and glutathione reductase than the other two GBI’s. An increase in the level of ascorbate and glutathione was also observed. Overall, the effects of PROHEX were found to be the mildest in comparison to PACLO and UNI.

INTRODUCTION

Since the late 1960’s, several synthetic gibberellin biosynthesis inhibitors (GBI’s), which reduce shoot elongation without affecting phenology or being phytotoxic, have been evaluated for their ability to regulate growth and development in plants (5, 6, 7). To date four different types viz., onium compounds, compounds with N-containing heterocycle, acyliclohexanediones and daminozide, and 16, 17-dihydrogibberellins, have been shown to block distinct steps of GA metabolism. Although the primary action of GBI’s is mediated by a reduction of GA’s, they often affect formation of ABA, ethylene, sterols, flavonoids or other plant constituents, and add to the benefit of plant growth retardation. Protection of plants by these compounds from apparently unrelated stresses is often associated with increased antioxidant potential(5, 7). In fact, GBI’s have been credited as multiple “stress-protectants”(5).

We have evaluated the effect of two triazoles (paclobutrazol, PACLO; uniconazole, UNI) and the structural mimic of 2-oxoglutaric acid prohexadione-ca (PROHEX-Ca) on in vitro growth and antioxidant activities in chickpea (Cicer arietinum L.) seedlings which is one of the most important grain legumes of Asia, Africa, and Latin America.

MATERIALS AND METHODS

Chickpea (Cicer arietinum L.) seedlings were grown in MS medium containing 3% sucrose and 0.25% phytagel. Shoots were excised and processed for antioxidants and enzyme essays as described previously (4). The activities of enzymes of ascorbate-glutathione cycle (ascorbate peroxidase [ASC-PER]; monodehydroascorbate reductase [MDHAR]; dehydroascorbate reductase [DHAR]; glutathione reductase [GR]), other enzymatic antioxidants (catalase [CAT]; peroxidase [PER]; superoxide dismutase [SOD]), and the levels of reduced and oxidized forms of ascorbate [ASC,
DHA], glutathione [GSH, GSSG], malondialdehyde [MDA], a known product of lipid peroxidation, and proline [PROL] were determined as described earlier (4).

**RESULTS AND DISCUSSION**

The most pronounced effect of GBI’s on plants is reflected in a sizable reduction in elongation growth, primarily due to decreased internodal elongation, and the treated plants become greener and more compact (5, 6, 7). In chickpea, both PACLO and UNI were found quite effective than PROHEX in retarding shoot growth (Table 1). A characteristic feature of the lateral roots in PACLO and UNI was that all the lateral roots elongated equally, became thick and imparted a brush-like appearance. Although PACLO and UNI retarded shoot elongation, the leaves developed intense dark green color and became much stouter and thicker than in the control. The growth inhibitory effect of GBI’s that is coupled to promotion of leaf pigments, has been reported earlier also (5, 7).

Table 1. Effect of GA-biosynthesis inhibitors on *in vitro* growth of chickpea seedlings

<table>
<thead>
<tr>
<th>Treatment (µM)</th>
<th>Seedling growth (mm) after 2 weeks*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rt(L)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>40±5</td>
</tr>
<tr>
<td>PACLO 7</td>
<td>50±4</td>
</tr>
<tr>
<td>PACLO 14</td>
<td>45±5</td>
</tr>
<tr>
<td>UNI 7</td>
<td>40±4</td>
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<tr>
<td>UNI 14</td>
<td>35±5</td>
</tr>
<tr>
<td>PROHEX 7</td>
<td>48±4</td>
</tr>
<tr>
<td>PROHEX 14</td>
<td>42±2</td>
</tr>
</tbody>
</table>

* Rt(L) = length of main root; L Rt(N) = number of lateral roots; St(L) = length shoots; Cst(N) = paclobutrazol; UNI = uniconazole; prohex = prohexadione-Ca

The coordinated activities of plant defense systems, consisting of antioxidants (ASC, GSH, alpha-tocopherol, carotenoids) and enzymes (CAT, PER, SOD, ASC-PER, MDHAR, DHAR and GR) can effectively scavenge the reactive oxygen species produced by plants during normal metabolism as well as under oxidative stress (2, 3). Therefore, plants with high levels of antioxidants, either constitutive or induced, are reported to have greater potential to cope up with an array of abiotic stresses (1). Enhanced antioxidant activity following treatment with GBI’s has been reported in several plants (5). All the GA biosynthesis inhibitors significantly promoted the activities of CAT and PER in chickpea seedlings (Fig. 1). PACLO was found to be substantially much more effective than UNI and PROHEX. However, the effect of these growth retardants was found to be different on the activity of SOD. In the presence of UNI, SOD activity remained unaffected, but both PACLO and PROHEX were found to slightly lower the activity of SOD.
The shoots of chickpea seedlings growing in the presence of PACLO exhibited considerably high activity of ASC-PER (Fig. 2). However, with the passage of time, the magnitude of the effect tapered off. In comparison to PACLO and UNI, the effect of PROHEX was found to be much milder on the activity of ASC-PER. All the chemicals also exhibited a profound effect on the activity of MDHAR. PACLO was found to be more effective than UNI and PROHEX which did not elicit a sustained effect on the activity of this enzyme. In general, PACLO was found to be the most effective growth retardant in influencing the activities of both DHAR and GR. The relatively low efficacy of PROHEX may be due to its rapid degradation whereas PACLO and UNI are quite stable (5, 6).

In addition to ROS detoxifying enzymes, plants possess a variety of antioxidants which are efficient oxy-radical scavengers (1). In chickpea, treatment with PACLO also resulted in a promotion of ASC and GSH levels and simultaneously a reduction was recorded in DHA and GSSG. However, no difference could be detected in MDA content that reflects the extent of lipid peroxidation, although the roots of PACLO-treated plants consistently indicated low levels of MDA than the control.

Overall, taken together, these results indicate that the chickpea seedlings treated with GBI’s elicited higher antioxidant potential than the control. This concerted increase in antioxidant potential following treatment with GBI’s may have important consequences in plant protection under stress.

REFERENCES

Fig. 1. Effect of GBI's on the activity of oxy-stress enzymes

Fig. 2. Effect of GBI's on the activity of oxy-stress enzymes
REDUCTION OF ETHYLENE-INDUCED FLOWER ABSCISSION AND LEAF YELLOWING IN CUT INFLORESCENCES OF PHLOX BY THIDIAZURON AND SUCROSE

N. Sankhla1, W.A. Mackay and T.D. Davis

ABSTRACT

In cut flowerheads of perennial phlox (Phlox paniculata L. ‘John Fanick’), a recently identified superior selection for Texas landscapes, the display life and longevity is primarily influenced by abscission of flowers, opening of flower buds during display and the quality of leaves on the axis. Flower abscission may be initiated within 72 hours via ‘corolla shedding’, whereas yellowing of leaves is a late event seen after 7-10 days. Addition of 2-chloroethylphosphonic acid (CEPA) in the vase medium (10-200 µM) promoted flower abscission as well as leaf yellowing. The effect of CEPA (10-50 µM) on flower abscission and leaf senescence was considerably reduced if thidiazuron (TDZ) and sucrose were present in the vase solution. TDZ and sucrose, when added together, were found to be more effective than if added separately. Earlier, we reported that both 1-MCP and STS also counteracted CEPA-induced flower abscission in phlox. Thus, it is possible that the effects of TDZ and sucrose are, at least partially, mediated via their effect on ethylene production/sensitivity.

INTRODUCTION

Phlox paniculata L., the perennial phlox bearing dense flowerheads in shades of attractive colors, is a popular plant for midsummer color in home or landscapes. P. paniculata ‘John Fanick’ is a relatively heat tolerant superior selection of phlox that blooms for an extended period during the hot summer in Texas (9). Its dense terminal clusters of flowers in shades of deep red-pink and white with a dark red eye have great potential as a cut flower crop.

Previously, we reported that thidiazuron (N-phenyl-N1-1,2,3-thiadiazol-5-ylurea, TDZ), a phenylurea derivative with strong cytokinin-like activity reduced flower abscission and leaf senescence in cut phlox stems (9). This study was conducted to evaluate the role of sucrose, alone and in combination with TDZ, on parameters associated with postharvest performance of cut flowerheads of phlox ‘John Fanick’. Experiments were also conducted to study the interaction between 2-chloroethylphosphonic acid (CEPA), which greatly hastens flower abscission, sucrose and TDZ.

MATERIALS AND METHODS

Stock plants of ‘John Fanick’ were clonally multiplied in vitro, acclimitized, and transplanted in the Texas A&M University trial garden at Dallas (9). Flowerheads, with freshly recut stems, were placed in glass vases containing 400 ml water or an equivalent amount of the test solution containing sucrose (1-8%), CEPA (10-50µM) and TDZ (5-45 µM), alone and in combination with each other. The vases were kept at 22-24°C and received 30 µmol·m⁻²·sec⁻¹ irradiance from cool-white florescent lamps. Observations on parameters affecting postharvest performance were recorded regularly. Anthocyanin content was measured by recording the absorbance at 530 nm of petal extracts prepared in 1% HCl in methanol.

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RESULTS AND DISCUSSION

In cut flowerheads of ‘John Fanick’ phlox abscission of flowers, which is limited to ‘corolla shedding’ only (9), may be initiated within 72 hours. Simultaneously, additional buds begin to open into new flowers. Interestingly, as the vase life progresses, the initial deep red-pink-white corollas of fully open and mature flowers turn blue/violet. The new flowers opening in vases exhibit either light blue color or fail to develop any color, and visibly appear to be almost white.

In ‘John Fanick’ incorporation of sucrose (2-4%) in the vase solution not only reduced the flower abscission, but significantly enhanced the opening of flower buds, and greatly extended the longevity of the flowerheads. The addition of sucrose to the medium also enabled the new flowers to develop the original pink/red-white color by promoting anthocyanin formation and greatly helped maintain the original flower color in the fully open flowers. Sucrose is known to significantly enhance anthocyanin content and the intensity of petal color in several cut flowers (2, 3). The stimulation of anthocyanin production by sucrose may involve either increased phosphorylation by hexokinase which is related to anthocyanin synthesis or an induction of expression of anthocyanin biosynthesis (2, 3, 4, 5). Furthermore, a supply of sugars is necessary for the glycosylation of anthocyanins. The perceived color of many flowers is a result of a complex interaction among anthocyanins, co-pigments, pH of the vacuole, changes in the concentrations of metals, and the shape of the petal cells (6, 7), all of which may also cause a shift in the absorbance spectra of anthocyanins leading to a change in the reflective color of the flowers from an array of red to blue (1). Recently, it has been reported that the change in pH occurring in the petal is the main cause of the blueing effect in rose and sucrose delayed blueing and increased cell sap pH (7).

As with sucrose, TDZ also bought about a reduction in flower abscission. However, unlike sucrose, TDZ failed to maintain the original color of the petals in already open flowers as well as in the newly opened flowers which were either light blue or white (9).

In contrast to TDZ and sucrose, incorporation of CEPA in the vase solution strongly promoted flower abscission, inhibited opening of flower buds and greatly reduced the flower size. Both TDZ as well as sucrose counteracted the effect of CEPA. Sucrose considerably reversed the effect of CEPA on flower abscission and maintenance of flower color whereas TDZ was more effective in reversing the effect of low concentrations of CEPA on flower abscission, flower size and leaf senescence.

Both sucrose as well as purine cytokinins are known to affect ethylene production, ethylene sensitivity and longevity of cut flowers (4, 10). Earlier, we also reported that both STS and 1-MCP, which are known to interfere with ethylene action, also counteracted CEPA-induced flower abscission (8). Thus it is possible that the effects of TDZ and sucrose are, at least partially, mediated via their effect on ethylene production/sensitivity.

REFERENCES


ABSTRACT

Big Bend bluebonnet (*L. havardii* Wats), a winter annual native to the Chihuahuan desert, produces attractive flowers on a long raceme. Over the years, by recurrent phenotypic selection, we have identified and developed blue, white, and pink flowered lines of bluebonnet which differ in their sensitivity to ethylene. In this study, we have evaluated the effect of nitric oxide (NO*) a highly bioreactive endogenous molecule which is reported to down regulate ethylene production, on flower abscission and senescence of bluebonnet racemes. Sodium nitroprusside (SNP) was used as the source of NO* donor. Depending upon the concentration of SNP (10-200 µM) used, the various lines of bluebonnet exhibited differential response which varied from promotion of flower abscission to senescence. In general, the blue flowered lines were found to be more sensitive to NO* than the white flowered lines. The pink flowered lines exhibited an intermediate response. Visible signs of flower senescence included wilting of the tip of the standard petal and a change in the color of the banner spot. In Blue Select-2002 germplasm the color of the banner spot changed from light yellow to muddy-brown/black in the presence of high SNP concentrations. Addition of sucrose and thidiazuron, or pretreatment with 1-MCP/STS, reduced the effect of NO* on flower abscission and senescence.

INTRODUCTION

Nitric oxide (NO*) is a highly bioreactive and versatile molecule that targets either a redox or an additive chemistry, and exerts an unprecedented diversity of biological effects (1, 4). Over the last decade, extensive research relating to the role of NO* has been conducted in animal systems, although its role in plants remain very limited. Recent studies indicate that there is a cross talk between NO*, ethylene, IAA, ABA, GA calcium, calmodulin, GMP and cADPR (1, 4) and NO* may be a key signaling molecule in plants mediating responses to various abiotic and biotic stresses. Exogenous application of NO*, by inhibiting ethylene production, has been shown to extend the storage and marketing life of vegetables, flowers, and fruits (5).

Earlier we observed that the key determinants of postharvest longevity and performance in *L. havardii* are flower abscission and flower senescence (6). As ethylene is involved in promoting flower abscission and flower senescence in *L. havardii* this investigation was initiated to evaluate the effect of NO* on parameters affecting postharvest performance.

MATERIALS AND METHODS

Two blue flowered lines (an advanced breeding line, Blue Select 2002, BS; ‘Texas Sapphire’, a cultivar released in 1996, TS), two white flowered lines (an advanced breeding line, White Select 2002, WS; ‘Texas Ice’, a cultivar released in 1996, TI) and a dark pink flowered advanced breeding line (DP) representing germplasm from the Big Bend bluebonnet breeding program were used for this study.

Sodium nitroprusside (SNP) was used as source of NO* donor. Cut racemes were transferred to glass vases containing either 400 ml water or an equivalent amount of freshly prepared solution of

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SNP of desired concentration (10-200µM) at 22±2°C under cool white florescent lamps (30 µmol·m⁻²·sec⁻¹). Observations on parameters related to postharvest display were recorded regularly.

RESULTS AND DISCUSSION

Blue, white and pink flowered lines of bluebonnet differ in their sensitivity to ethylene (6). The sensitive lines indicate increased abscission of flowers in comparison to those which are relatively more tolerant to ethylene (6). In the current study, improved lines developed through recurrent phenotypic selection were used. These lines exhibit relatively low flower shattering, but flower senescence continues to affect the quality of display life.

Depending upon the concentration of SNP used (10-200µM), the various lines of bluebonnet exhibited differential response which ranged from promotion of flower abscission and senescence to almost no effect. In blue flowered TS, even at low concentrations, SNP slightly promoted flower abscission. However, high SNP concentrations generally promoted senescence of flowers in TS, BS, and DP, while in WS and TI senescence of flowers was only affected at the highest concentration of SNP. Visible signs of flower senescence included wilting and burning of the tip of the standard petal and a change in the color of the banner spot. In BS, the banner spot was found to be highly sensitive to the presence of SNP in the vase solution. Increasing concentrations of SNP bought about a clear change in the color of the banner spot from light yellow to muddy-brown/intense black. Even the white petals in WS, which is the least sensitive to ethylene, developed black streaks and spots at high concentrations of SNP.

NO-mediated toxicity is mainly due to its reaction with superoxide anion (O₂⁻), leading to the formation of strong oxidant peroxinitrite, which can oxidize thiol residues to sulfenic and sulfonic acids (3). However, in soybean the HR cell death appears to be activated following interaction of NO* with H₂O₂, rather than O₂⁻ (2). Furthermore, the release of NO* into solution depends on the characteristics and concentrations of the NO* donor, the pH, temperature and concentrations of NO* target molecules (4). Thus, it becomes difficult to discriminate between the pharmacological effects and physiological relevance of the role of NO* donors and modifications induced by endogenous NO*.

In separate experiments, we observed that the effects of SNP on flower abscission, flower senescence and the color of the banner spot are partially counteracted in the presence of sucrose and TDZ, or by pretreatment with 1-MCP (our published results). The relevance of these results await further research.

REFERENCES

FLURPRIMIDOL FOLIAR SPRAYS CONTROL GROWTH OF EIGHT NEW GUINEA IMPATIENS (IMPATIENS HAWKERII) CULTIVARS

Brian E. Whipker¹, Ingram McCall¹, Brian A. Krug¹, and James L. Gibson²

ABSTRACT

Foliar sprays of 10 mg·L⁻¹ flurprimidol were determined to be the recommended concentration during initial New Guinea impatiens trials, in which only 'Pure Beauty Fuchsia' was used and cultivar response differences are known to exist. In a follow-up study, flurprimidol foliar sprays of 5 to 20 mg·L⁻¹ were applied to eight New Guinea impatiens cultivars. Cultivar response varied with the use of flurprimidol foliar sprays. Five cultivars were >20% shorter than the untreated control with 10 mg·L⁻¹ flurprimidol. Plant diameters of six cultivars were >20% smaller with 10 mg·L⁻¹ flurprimidol when compared to the control, with the range of all treatments being between 13% and 30%. Results confirm earlier recommendations that 10 mg·L⁻¹ flurprimidol produced compact, marketable plants. Cultivars differences existed, therefore growers will need to conduct their own trials to determine optimal concentrations for their operation and cultivars used. To account for cultivar and geographically variation, flurprimidol concentrations between 5 to 10 mg·L⁻¹ should be used by growers when beginning trials.

INTRODUCTION

Plant growth retardants (PGRs) are commonly applied in order to produce high-quality, compact plants (Tayama et al., 1992). Flurprimidol (SePRO, Carmel, Ind.) has been labeled as Cutless for ornamental crop use in the U.S. and will be commercially introduced as Topflor for greenhouse crops. Topflor has been tested on a number of greenhouse plants such as argyranthemum (Argyranthemum frutescens) (Cavins et al., 2003) and bedding plants (Whipker et al., 2003a). Whipker et al. (2003b) found that flurprimidol foliar spray of 10 mg·L⁻¹ provided comparable plant growth control and limited flowering delay of 'Pure Beauty Fuchsia' New Guinea impatiens as the recommended uniconazole foliar spray of 5 mg·L⁻¹ or 10 mg·L⁻¹ paclobutrazol (Corr, 1995; Pasutti and Weigle, 1980). Variation in cultivar response has been reported in New Guinea impatiens (Bailey, 1995). Therefore, this study was conducted to confirm earlier recommended flurprimidol concentrations and determine if a New Guinea impatiens cultivar response existed.

MATERIALS AND METHODS

Rooted cuttings of 'Pure Beauty Fuchsia' New Guinea impatiens were transplanted into 750-mL (12.5-cm diameter) round, plastic containers on 27 Mar. 2003 using Berger® BM6 root substrate (Berger Peat Moss, St. Modeste, Quebec, Canada). Plants were fertigated with 150 mg·L⁻¹ N from Excel® 15-5-15 Cal-Mag (Scotts, Marysville, OH) (15N-2.1P-12.5K). Greenhouse temperature day/night set points were 24/18 °C and the plants were grown under natural daylength. The PGR foliar

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Sprays were applied two weeks after transplanting using a volume of 204 mL·m⁻²: flurprimidol at 5, 10, and 20 mg·L⁻¹; or an untreated control. The experiment was a completely randomized design with 4 single-plant replications of the four treatments. On 26 May, total plant height (measured from the pot rim to the uppermost part of the inflorescence) and plant diameter (measured at the widest dimension, turned 90°, and averaged) were recorded. Plant height and diameter values for flurprimidol were regressed using the PROC REG procedure (SAS Inst.) to determine the best-fit linear or quadratic model. Terms of the model were judged to be significant or nonsignificant and included in the final model based on a comparison of F values at α = 0.05.

RESULTS AND DISCUSSION

Plant height. The response to flurprimidol foliar sprays of 5 to 20 mg·L⁻¹ varied by cultivar (Table 1). The three taller cultivars responded quadratically to increasing concentrations of flurprimidol, while the other five responded linearly. Five cultivars were >20% shorter than the untreated control with the use of 10 mg·L⁻¹ flurprimidol. 'Sonic Red' was the most responsive cultivar, being 36.5% shorter than the control and concentrations lower than 10 mg·L⁻¹ flurprimidol should be trailed. 'Petticoat Red Star Improved' was the least responsive cultivar (5.8% shorter than the control when 10 mg·L⁻¹ flurprimidol was applied), but the need of a PGR application is questionable for this compact cultivar. In the earlier study, flurprimidol foliar sprays of 10 mg·L⁻¹ resulted in plants 16% shorter than the untreated control (Whipker et al., 2003b). The greater degree of control in this study may be due to timing. The earlier work was conducted during the summer while this experiment occurred during the spring when plant growth may be slower due to cooler growing temperatures.

Plant diameter. Flurprimidol foliar sprays were also effective in controlling plant diameter (Table 1). Flurprimidol at 10 mg·L⁻¹ provided >20% smaller plant diameters for six of the cultivars when compared to the untreated control, with the range for all treatments being between 13% and 30%. 'Pure Beauty Fuchsia' New Guinea impatiens plant diameter was 14% smaller with the use of 10 mg·L⁻¹ flurprimidol (Whipker et al., 2003b). Similar to plant height, the greater control in this study may be due to seasonal variation in which the studies were conducted.

CONCLUSIONS

Cultivar response varied with the use of flurprimidol foliar sprays, which is in agreement with Bailey (1995). Our results indicate that growers will need to conduct their own trials to determine optimal concentrations for their own operation and cultivars used. Compact, marketable plants were produced with 5 to 10 mg·L⁻¹ flurprimidol and when beginning trials, growers should use that concentration range.

ACKNOWLEDGEMENTS

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<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Vigor class¹</th>
<th>Plant height</th>
<th>Plant diameter</th>
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<tr>
<td></td>
<td></td>
<td>Regression (cm)</td>
<td>Control (%)²</td>
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<tr>
<td>Super Sonic Cherry Cream</td>
<td>T</td>
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<tr>
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<tr>
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¹ Vigor class based on tall (T), medium (M), and short (S) cultivars.
² Values represent the percent of shorter height or smaller diameter, when compared with the untreated control. Based on the use of a 10 mg L⁻¹ flurprimidol foliar spray.
RESPONSE OF MULTIFLOWERING LINES OF PEA (*PISUM SATIVUM*) TO LIGHT QUALITY AND GIBBERELLINS

Sonja L. Maki¹, Heidi J. Mullen and Susan R. Singer

ABSTRACT

The number of flowers produced per node is an important characteristic in both agronomic and ornamental legumes. The inflorescence of pea is one step more complex than the simple racemes of *Arabidopsis*. Inflorescence development is intriguing from both agricultural and evolutionary perspectives and is regulated at several stages of development. Environmental and autonomous regulation of flowering time, branch complexity (i.e. number of iterations of branching prior to flowering), number of flowers per node and per branch, and regulation of determinacy in all branch orders affect the overall inflorescence pattern.

In pea, one to several flowers can be produced from a secondary inflorescence (I2) meristem at each node. We have been studying lines which vary in indeterminancy of the I2 meristem. A multi-flowering line has been identified in our lab from a cross between the Proliferating Inflorescence Meristem (*pim-1*) mutant and the Determinate (*det*) mutant which we have named *ultramulti*. Under a long day (LD) photoperiod (18 h light/6 h dark provided by 8 h high pressure sodium and metal halide lights followed by 10 h of incandescent lights) a single application of 25 micrograms of GA3 or GA4 to the *ultramulti* line resulted in an increase in yield. The number of flowers per node was not altered (about 3 flowers per node), however there was an increase in axillary growth resulting in the overall production of additional flowers and fruits.

The objective of this study was to characterize the flowering response of the *ultramulti* line grown under a short day (SD) photoperiod (8 h light/16 h dark, maintained with high pressure sodium and metal halide lights) and in a far-red light deficient growing environment (obtained by photoselective film provided by N. Rajapakse, Clemson University, Clemson, SC). The average node of flower initiation (NFI) of the *ultramulti* line was greatly increased under short days (NFI=90) and to a lesser extent under the -FR light environment (NFI=34) when compared to long days (NFI=18). The number of flowers produced at the node of floral initiation was also increased under short days and the -FR light environment. Up to 12 flowers per node were observed in -FR light deficient environments and as many as 15 flowers have been observed under SD growth chamber environments. Evidence for the separability of the node of flower initiation and multi-flowering phenologies was also observed when far-red light was excluded (-FR) from the growing environment. When grown under the photoselective film which excludes far-red light, the *ultramulti* line produced up to twelve flowers at each node and the NFI was increased, but not to the extent of short day grown plants.

Our characterization of the *ultramulti* line in this study suggests that it will be useful in revealing interactions between the photoperiod dependent pathway and the floral and infloral developmental pathways. Future studies will focus on determining whether PIM expression is altered in the *ultramulti* line when grown under environments which result in altered inflorescence branching patterns.

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EFFECTS OF THE COMBINED APPLICATION OF ETHEPHON AND GIBBERELLIN ON THE RICE SEEDLING GROWTH GROWING UNDER DIRECT SEEDING CONDITIONS

Hajime Watanabe and Masahiko Saigusa

ABSTRACT

Seedling establishment is one of the most important agronomic traits in direct seeding rice cultivation. We investigated the effects of two plant growth regulators (PGRs), including gibberellic acid (GA₃) and ethephon (ET), on seedling growth under flooded soil conditions. Seedling growth was increased by a single treatment of GA₃ or ET over that of the control. However, effects of combined applications of GA₃ and ET were more pronounced than that of GA₃ or ET alone at both growing temperatures (15 and 20 °C). The growth of different organs of rice seedlings, such as coleoptiles, first leaves and second leaves were also increased by PGRs treatment. In conclusion, a high seedling establishment rate in direct seeding cultivation in the cold regions of Japan will be possible by using the proper combinations of PGRs.

INTRODUCTION

Seedling establishment is one of the most important agronomic traits in direct seeding rice cultivation. It has been considered that plant hormones, such as gibberellin (GA), ethylene and abscisic acid (ABA), have promotive effects on rice seedling organs. However, the effects of these plant hormones are occasionally diverse due to the several environmental factors, including temperature, flooding depth and so forth. In addition, synergistic (plus) or counteracting (minus) plant hormone interaction can be found in several growth systems of plants (Davies, 1995). A notable case of the former interaction in the point is internode elongation in deep-water rice. Ethylene promotes the growth of internodes tissue of deep-water rice, which respond to flooding by a rapid elongation induced by ethylene formation (Metraux and Kende, 1983). Ethylene promotes growth in part by increasing the responsiveness to the internodes tissue to GA, and appears to do so by causing a reduction in the endogenous levels of ABA, so that the growth rate is determined by the relative levels of endogenous GA and ABA, potent antagonist of GA (Kende et al., 1998). Here, we investigated the effects of single or a combined application of ethylene and GA on the growth of different organs of rice seedling growing under different temperatures and flooding conditions.

MATERIAL AND METHODS

The cultivar used in the present study was Kokoromachi (Oryza sativa L.), belongings to japonica type cultivar. The seeds were sterilized with a Benlate solution and then immersed in water, subsequently washed by water, and then soaked in the test solution. Ethephon (2-chloroethylphopanic acid, Ishihara Sangyo Kaisha, LTD, Osaka, Japan) was used as an ethylene-releasing agent, and GA₃ (Sigma Chemical Co., MO, USA) was used for gibberellin. The components of test solution was as follows; 1) Water alone (control), 2) 50 ppm ethephon (ET), 3) 100ppm GA₃, 4) 50 ppm ET+100ppm GA₃. After treatment with plant hormone solution, the seeds are again immersed in water to remove the excess test solution. The imbibed seeds were germinated in water at 30 °C in the dark. The germinated seeds were sown at 1 cm of seeding depth in seedling pot with small compartments containing nursery soil (Kureha Chemical Co., LTD. Tokyo, Japan). The seeds were allowed to grow.
at 15 or 20 °C in continuous light conditions. The flooding depth (FD) was 2 and 5 cm in each experiment. The experiments were done with 4 replications.

RESULTS AND DISCUSSION

In case of 15 °C of growing temperature

The plant height was significantly increased by all PGR treatments tested compared with that of control at 2cm of FD (2FD) (Fig. 1A), whereas, the effect of combined application of ET+GA3 was more pronounced than those of ET and GA3 alone at 5cm of FD (5FD) (Fig. 1B). ET or GA3 alone at 2FD did not stimulate the mesocotyl growth, however, a combination of ET and GA3 treatments increased the mesocotyl growth significantly at 2FD (Fig. 1A). At 5FD, all PGR treatments increased the mesocotyl length significantly and, the maximum elongation was caused by a pair of ET and GA3 (Fig. 1B). In coleoptile, the trends of effects of PGR treatments on the elongation were similar to the case of mesocotyl at both flooding depths (Fig. 1). Interestingly, synergistic effect of a combined application of ET and GA3 was also observed as with the case of mesocotyl growth (Fig. 1A and B). In the first leaf, which mainly consists of a leaf-sheath, ET alone and ET+GA3 treatments showed the significant increase in the growth at both flooding depths (Fig. 1). In the second leaf, significant growth-promoting effects were observed by GA3 alone and ET+GA3 applications over that of the control at the 2FD (Fig. 1A). In addition to these treatments, GA3 alone also increased the 2nd leaf growth significantly at 5FD (Fig. 1B). No significant difference in the ratio of shoot dry weight to shoot length (RWL) was observed among the PGR treatments at both FD (Table 1), indicating that promotive effects of PGR treatments on the rice seedling is not mere a spindly growth; an increase in shoot growth by PGRs treatments were accompanied by the enhancement of dry weight.

In case of 20 °C of growing temperature

All PGR treatments increased the plant height significantly compared with that of control, but maximum elongation was induced by ET+GA3 treatment at 2FD, whereas (Fig. 2A), no significant growth-promoting effect with respect to plant height was observed with GA3 treatment at 5 FD (Fig. 2B). In the mesocotyl, only ET+GA3 treatment gave significant growth-promoting effect over that of the control at 2cm FD as is the case with the 15 °C (Fig. 2A), however, both GA3 alone and ET+GA3 treatments increased the mesocotyl elongation significantly at 5 FD (Fig. 2B). In the coleoptile, significant elongation was occurred by ET alone and ET+GA3 treatments at 2FD (Fig. 2A), whereas, no significant growth-promoting effect was obvious in any treatment at 5FD (Fig. 2B). In the first leaf, only ET+GA3 treatment had significant stimulating-effects on elongation at 2FD (Fig. 2A), but all PGR treatments showed the significant increase of the elongation at 5cm FD (Fig. 2B). In the second leaf, both GA3 alone and ET+GA3 treatments showed the prominent growth stimulating effects at both 2 and 5 FD, but the maximum elongation was obtained with the combination of ET+ GA3 at both FD (Fig. 2). No significant difference in the RWL was observed among the all PGR treatments at both flooding depths as shown in the case of the 15 °C growing temperature (Table 2).

In present studies, rice seedling growth growing under direct seeding condition was increased by the single treatment of GA3 or ET over that of the control in some cases. However, growth- promoting effects were diverse according to the differences in target organs of rice seedlings and in environmental conditions, such as temperature and flooding depth. However, effects of the combined applications of GA3 and ET were more pronounced than those of GA3 or ET alone, and these growth-promoting effects were more stable than single treatments of each PGR in spite of environmental conditions. These results suggest that the ET and GA3 acted additively or synergistically. This synergism was observed almost all the cases in this experiment except for the coleoptiles grown under at 20 °C at 5FD. This might be because that coleoptile growth had already reached a maximum at the sampling date. In
the series of the experiment, coleoptile growth rate of ET+GA3 treatment was observed quicker than another treatments during the early stage of the seedling growth (data not shown).

Suge (1974) and Takahashi and Kaufman (1992) pointed out that synergistic action of ethylene with GA was seen in the growth of rice seedlings. However, PGRs were applied continuously in the culture medium, and growth temperature was relatively high (30 °C) in their experimental system for considering the direct seeding cultivation in cold region. The aim of our experiment is to enhance the early growth of rice seedlings in the direct seeding cultivations in the cold regions, including Tohoku district of Japan, by chemical control using various PGRs. It has been considered that physiologically critical temperature for the germination and early seedling growth is around 17 °C (Nishiyama 1978, Sasahara and Ikarashi 1989). An early seedling growth, including seedling establishment is one of the most crucial agronomic issues in direct seeding rice cultivations. In fact, we set up the growing temperature (15 and 20 °C) in consideration for these facts and actual situation where direct seeding cultivation was conducted in the Tohoku district of Japan. Furthermore, the rice seeds were pre-soaked for uniformity of germination in the most direct seeding cultivation method conducted in the Tohoku district of Japan. We applied PGRs during the seed soaking process. Our application method of PGRs to the rice seeds is relatively simple and would be easily accepted to the practical direct seeding system. From an agronomical point of view, our experimental system might be closer to the practical direct seeding cultivation than another previous experiment in terms of its experimental system.

In conclusion, high seedling establishment rate in the direct seeding cultivation in the cold regions of Japan will be possible by using the proper combinations of PGRs.

**LITERATURE CITED**


EFFECT OF 5-AMINOLEVULINIC ACID ON GROWTH AND NUTRIENT UPTAKE OF LEAF VEGETABLES IN ALKALINE SOIL

R. Yoshida¹, S. Watanabe², Y. Fukuta¹, Y. Kusaka¹, K. Iwai³ and T. Tanaka²

ABSTRACT

5-aminolevulinic acid (5-ALA) is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme. We examined the effects of 5-ALA alone and PKV on yield quantity in spinach and komatsuna grown under alkaline soil. The decreasing yield in alkaline soil was retard by the folial application of 5-ALA and PKV solutions. Both the compounds increased the uptake of nitrogen in spinach or komatsuna. On the other hand, 5-ALA and H₂O₂ increased markedly the dry yield of petiole tissue in komatsuna and this increasing rate was retard by the folial application of antioxidants, especially by glutathione and ascorbic acid. From these results, we suggest that the physiological function of 5-ALA is closely related to the promotion of H₂O₂ biosynthesis.

INTRODUCTION

5-aminolevulinic acid (5-ALA) is a key precursor in the biosynthesis of porphyrins, such as chlorophyll and heme. The folial application of 5-ALA to crop plants has often been reported in relation to chlorophyll biosynthesis and plant greening. We examined the effects of 5-ALA alone and of the combination of 5-ALA plus chelating iron or 5-ALA plus PKV on yield quantity in spinach and komatsuna grown under alkaline soil. The effects of oxidant and antioxidant on dry yield of komatsuna were also studied.

MATERIALS AND METHODS

Alkaline soil (pH 8.5) was prepared by an addition of natural calcium carbonate (Kaikaseki). Both spinach and komatsuna were grown in the alkaline soil.

RESULTS AND DISCUSSION

Growth of vegetables grown under alkaline soil  When spinach and komatsuna were grown in the alkaline soil, the growth and fresh or dry yield decreased remarkably as compared with those in natural soil. This decreasing growth rate in alkaline soil was retard by the folial application of 5-ALA solution alone. The applications of chelating iron or 5-ALA plus chelating iron had no effect.

The rate of nitrate per total nitrogen decreased in spinach treated with 5-ALA, but the rate of nitrate in spinach treated with chelating iron alone or 5-ALA plus chelating iron increased.

On the other hand, the foliar application of pentakeepV (PKV) was done in komatsuna. The decreasing growth rate in alkaline soil was retard by the application of 0.01% solution and this trend was found in petiole tissue. An increase of crop growth rate (CGR) or net assimilation rate (NAR) was obvious in plant tissue treated with PKV.

¹ College of Technology, Toyama Prefectural University, Toyama 939-0398, Japan; ²R&D Center COSMO OIL CO., LTD, 1134-2 Gongendo, Satte-shi Saitama, 340-193, Japan; ³SEIWA CO., LTD, 262-10 Shiba, Shimotsuga, Tochigi 329-0412, Japan

The chemicals used were 5-ALA (30ppm), chelating iron (55ppm), pentakeepV (PKV, 0.01%), hydrogen peroxide (H₂O₂, 10mM), glutathione (GSH, 10mM) and ascorbic acid (AsA, 10mM). Testing solutions of all chemicals were sprayed only to foliage of vegetables. The solution contains 0.1% of Approch as a wetting agent.
Oxidant and antioxidant  In case of komatsuna, 5-ALA and oxidant(H\textsubscript{2}O\textsubscript{2}) increased markedly the dry yield of petiole tissue, and The increasing rate of both the chemicals was retard by the application of antioxidant, such as glutathione and ascorbic acid(Table2). The content of H\textsubscript{2}O\textsubscript{2} in plant tissues treated with 5-ALA was higher than that in plant tissues treated with water(Table1).

CONCLUSION

In conclusion, from these results presented here the authors with to emphasize that 5-ALA and PKV increase the fresh and dry yield in spinach and komatsuna, especially in alkaline soil and these chemicals is very useful for the production of crop plants in alkaline soil. We also suggest that the physiological function of 5-ALA is closely related to the promotion of H\textsubscript{2}O\textsubscript{2} biosynthesis.

LITERATURE CITED


Table 1. Effect of 5-ALA on H\textsubscript{2}O\textsubscript{2} content of komatsuna.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh yield (g/ stable)</th>
<th>H\textsubscript{2}O\textsubscript{2} (µmol/ gFW)</th>
<th>H\textsubscript{2}O\textsubscript{2}/ stable (µmol )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.(Water)</td>
<td>19.37</td>
<td>13.10</td>
<td>253.70</td>
</tr>
<tr>
<td>5-ALA</td>
<td>26.81</td>
<td>20.30</td>
<td>544.19</td>
</tr>
</tbody>
</table>

Concentration: 5-ALA; 30ppm

Table 2. Effect of 5-ALA and antioxidant on yield of Komatsuna.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh yield (g/ 10stables )</th>
<th>Dry yielded (g/ 10stables )</th>
<th>Dry matter ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.(Water)</td>
<td>70.33± 10.9(100.0)</td>
<td>3.28± 0.1(100.0)</td>
<td>4.66</td>
</tr>
<tr>
<td>5-ALA</td>
<td>73.46± 8.5(104.5)</td>
<td>4.25± 0.3(129.3)</td>
<td>5.79</td>
</tr>
<tr>
<td>5-ALA + GSH</td>
<td>58.10± 8.0( 82.6)</td>
<td>3.84± 0.5(117.1)</td>
<td>6.61</td>
</tr>
<tr>
<td>5-ALA + AsA</td>
<td>65.30± 7.1( 90.3)</td>
<td>3.35± 0.2(102.1)</td>
<td>5.28</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2}</td>
<td>59.49± 9.0( 84.6)</td>
<td>4.02± 0.8(122.6)</td>
<td>6.76</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2} + GSH</td>
<td>62.02± 4.5( 88.2)</td>
<td>3.56± 0.5(108.5)</td>
<td>5.74</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2} + AsA</td>
<td>59.89± 5.3( 85.2)</td>
<td>3.85± 0.6(117.4)</td>
<td>6.43</td>
</tr>
</tbody>
</table>

Concentrations: 5-ALA;30ppm, glutathione(GSH);10mM, ascorbic acid(AsA);10mM, H\textsubscript{2}O\textsubscript{2};10mM
A FUNCTIONAL FERTILIZER “PENTAKEEP-V” CONTAINING 5-AMINOLEVULINIC ACID (ALA) AS THE EFFECTIVE COMPONENT

Shigeyuki Watanabe¹, Tohru Tanaka¹, Kazuya Iwai², Koichi Yoneyama³ and Yasutomo Takeuchi³

ABSTRACT

5-Aminolevulinic acid (ALA) is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme. We have found promotive effects of ALA on the growth and the yields of several crops and vegetables at lower concentrations. In addition, ALA was found to increase salt tolerance and cold resistance. We have developed a new functional fertilizer "PENTAKEEP-V" containing ALA as the effective component. In this poster, we introduce physiological activity of ALA and an excellent performance of "PENTAKEEP-V".

INTRODUCTION

5-Aminolevulinic acid (ALA) is a keto-amino acid with a molecular weight of 131. As a common precursor of tetrapyrrole compounds such as chlorophyll, phycobilin, heme, and vitamin B₁₂, it is an important compound in vivo that exists in all biological organisms. It was recently discovered that ALA improved photosynthesis, growth, yield, sugar content and tolerance against environmental stresses (Tanaka et al., 1992, Yoshida et al., 1996, Hotta et al., 1997a, 1997b, 1998; Watanabe et al., 2000). We have developed the new functional fertilizer "PENTAKEEP-V" which contains ALA as the effective components.

MATERIALS AND METHODS

This study was conducted from 2001 to 2003 using turf, primula, grape, cherry and paprika. Foliar application of "PENTAKEEP-V" (Seiwa co., ltd Japan) was done at concentrations of 0.04 to 0.05 wt% (spray volume corresponding to 2000 to 4000 L/ha). Rhizosphere application of "PENTAKEEP-V" was done at 700 mL/ha for Rock Wool hydroculture of paprika.

RESULTS AND DISCUSSION

Cherry plants were treated 4 times once a week with "PENTAKEEP-V" by foliar application of after flowering stage. The treatment increased the fruit weight by 7.9% (Table 1). The brix of the control (24.1%) was good enough and "PENTAKEEP-V" further increased the brix (26.6%) of the fruits. In the case of Kyoho grape plants, "PENTAKEEP-V" was foliar-applied 4 times in two weeks after the flowering stage. In terms of the cluster weight of Kyoho grape at harvest, there was no significant difference between the controls and the treated plants, but the berry weight was increased by 17% in the treated plots (Table 2). These results indicated that the fruit quality was improved by "PENTAKEEP-V". Figure 1 shows the results of "PENTAKEEP-V" treatment on paprika. The treatment increased the yield of paprika fruit by 13% during 29 weeks after planting. In addition, "PENTAKEEP-V" was found to maintain leaf color in turf and to improve cold tolerance in primula. Consequently, "PENTAKEEP-V" containing ALA is an effective material for agricultural use.

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LITERATURE CITED


<table>
<thead>
<tr>
<th>Table 1 Effect of PENTAKEEP-V on fruit quality of cherry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>PENTAKEEP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2 Effect of PENTAKEEP-V on fruit quality of &quot;Kyoho&quot; grape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster weight (g)</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>PENTAKEEP</td>
</tr>
</tbody>
</table>

Significance at p<0.01

Figure 1 Effect of PENTAKEEP-V treatment on the yield of paprika
EFFECT OF SALICYLATES ON AFRICAN VIOLET

Martín-Mex, Rodolfo2, Villanueva-Couoh, Eduardo1, Herrera-Campos, Teresita1 and Alfonso Larqué-Saavedra2*

ABSTRACT

Aqueous solutions of $10^{-6}$ to $10^{-10}$ M concentrations of salicylic acid were sprayed on ornamental vitroplants of African violet grown under greenhouse conditions to estimate its effect on the bioproductivity of the plant. These solutions were sprayed on the shoots of the plant on three occasions, 21, 28 and 35 days after being potted. Results showed that the best treatment was that of $10^{-10}$ M where significant results were found in the growth development pattern and in the number of leaves, in which an increase of 19% over the control was detected. Similarly, a 50% increase in the number of flower was recorded. It is of great importance to stress again that low concentrations of salicylic acid have the best effect, as we have reported in recent years.

INTRODUCTION

African violet (*Saintpaulia ionantha* Wendl.) is a commercially popular plant, considered to be one of the 15 most popular house plants in Europe and the United States (Jungnickel and Zaid, 1992). Salicylic acid is involved in diverse physiological processes of the plants such as: stomatal closure (Larqué, 1979), induction of flowering (Cleland *et al.*, 1974), an increase of somatic embryogenesis in tissue culture of *Coffea arabica* (Quiroz *et al.*, 2001) an increase in the accumulation of nitrates in roots of *Pinus patula* (San Miguel *et al.*, 2002) induction of systematic acquired resistance (SAR) in *Arabidiopsis* spp (Edwards, 1994) and inhibition of the biosynthesis of ethylene and seed germination (Leslie and Romani, 1988).

MATERIALS AND METHODS

The present study was carried out under greenhouse conditions in Conkal, Yucatán, Mexico, with an mean annual temperature of 30 °C and 70% relative humidity; under natural light conditions 650 μmol m$^{-2}$ s and a short day photoperiod (11 + 13 h light/darkness). Eighty vitroplants of African violet (*Saintpaulia ionantha* Wendl.) with six leaves and a rosette diameter of 25 mm were planted in four inch plastic pots. These were filled with sustrate 2:1 (Cosmopeat®, Cosmocel & Agrolite®, Dicalite). The plants were fertilized weekly during irrigation with 170 mg.L$^{-1}$ of nitrogen, phosphorous and potassium using the fertilizer 19N-19P-19K (Haifa Chemicals Ltd. Electrical conductivity was 2.5 mS cm$^{-1}$ and the pH at 6.0.

The salicylic acid solutions were prepared as described previously (Gutiérrez *et al.*, 1998) and applied as treatments at concentrations of $10^{-6}$, $10^{-8}$ and $10^{-10}$ M, distilled water was applied as a control. The solutions were applied to the foliage with a manual spray, in the afternoon, 18, 25 and 32 days after transplanting to the pots. A completely random design was used with 20 repetitions. The measurements of number of leaves were carried out each week after the application of the treatments and the last measurement was taken at the onset of flowering, number of flower primordia
was registered. The data was analyzed by ANOVA and the means were separated by Tukey’s HSD in $p \leq 0.05$.

RESULTS AND DISCUSSION

Results are shows in Table 1 were it can be seen that there were significant statistical differences obtained in the variables evaluated in the experiment as affected by SA treatments. The number of leaves of African violet increased by 19% with the application of the concentration at $10^{-10}$ M, SA, and by 13% with the concentrations at $10^{-6}$ and $10^{-8}$ M, SA, in comparison with the control, thus confirming results observed in *Pinus patula* (San Miguel *et. al.*, 2003) and *Clitoria ternatea* (Martin and Larqué-Saavedra, 2001) where an increase in fresh and dry biomass and in the number of branches was registered. In tobacco, applications of SA stimulated two leaves in comparison with the control (Gutiérrez, 1997).

In relation to the number of flower, there was an increase of 75% with $10^{-10}$ M SA, in comparison with the control. The treatments at concentrations of $10^{-6}$ M and $10^{-8}$ M surpassed the control by 25% and 37% respectively.

CONCLUSION

We consider number of flowers per plant, to be the most remarkable contributions in this work, since there are no reports in literature on the effect of salicylates on the growth of flowers of flowering in plants.

LITERATURE CITED


ACKNOWLEDGEMENTS

Thanks to Ing. Angel Nesticapan Garcés, QBB Mirbella del R. Cáceres Farfán and Silvia Vergara Yoisura for their technical support. CONACYT grant No. 33647-B.

Table 1. Effect of salicylic acid in vitro plants of African violet (*Saintpaulia ionantha* Wendl.) under greenhouse conditions, data are the mean value of 20 replicate plants. ± standard error.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of leaves</th>
<th>Number of flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testigo</td>
<td>16± 1b</td>
<td>8± 2b</td>
</tr>
<tr>
<td>10^{-10} M AS</td>
<td>19± 2a</td>
<td>14± 1a</td>
</tr>
<tr>
<td>10^{-8} M AS</td>
<td>18± 3ab</td>
<td>11± 2ab</td>
</tr>
<tr>
<td>10^{-6} M AS</td>
<td>18± 2ab</td>
<td>10± 3ab</td>
</tr>
</tbody>
</table>

Means with the same letter are statistically equal (Tukey’s 0.05).
POSITIVE EFFECTS OF SALICYLIC ACID ON THE FLOWERING OF GLOXINIA PLANTS

Martín-Mex1, Rodolfo, Villanueva-Couoh, Eduardo2, Uicab-Quijano, Veronica2 and Alfonso Larqué-Saavedra1*

ABSTRACT

Gloxinia is an ornamental plant of economic importance that has been selected for indoor environments due to the beauty of its flowers. Micropropagated plants of this variety were treated with salicylic acid to test its effect on the quality and the flowering expression of the plant. Salicylic acid at concentrations of 10⁻⁶ to 10⁻¹⁰ M were sprayed on gloxinia vitroplants cultivated under greenhouse conditions, this was repeated on four occasions. Results showed that SA at concentrations of 10⁻⁸ M increased the leaf area by 49% and the number of flower.

INTRODUCTION

Exogenous applications of salicylic acid affect the physiological processes of plants such as, promotion of stomatal closure (Larqué, 1978, 1979), increase of the biomass in soya and Pinus (Gutiérrez, 1998; San Miguel, 2001); increase of somatic embryogenesis in tissue cultures (Quiroz, 2001).

The participation of salicylic acid in the flowering process has been reported since the 70s; for example, by substituting the stimulus of the photoperiod (Oota, 1975 and Cleland, 1979). Recent studies in our laboratory have demonstrated significant effects on the flowering in the petunia (Martin-Mex and Larqué-Saavedra, 2000, data not published) when low concentrations of salicylic acid are applied. With this antecedent, it was decided to evaluate the effect of salicylic acid applications on flowering in other ornamental plants.

MATERIAL AND METHODS

Fifty vitroplants of gloxinia (Sinningia speciosa) “Ultra” with 6-7 leaves exposed, were transplanted to six inch plastic pots and grown under greenhouse conditions. The pots were filled with Cosmopeat®, Cosmocel & Agrolita®, and Dicalite (3:1). Minimum and maximum temperatures in the greenhouse were 19/30°C night and day respectively and a relative humidity of 70% was maintained. The plants were grown under natural light conditions (650 µmol m⁻² s⁻¹) with a photoperiod of 13 h. In this experiment the plants were maintained in good conditions of humidity and were fertilized weekly with a solution of 170 mg.L⁻¹ of soluble fertilizer (19N-19P-19K, Haifa Chemicals, Ltd.).

The solutions of salicylic acid (Merck, Co.) were prepared as described previously (Gutiérrez et al., 1998) and applied as treatments at concentrations of 10⁻⁶, 10⁻⁸ and 10⁻¹⁰ M with distilled water applied as a control. Tween 20 was added to the solution as a surfactant. The solutions were applied to the foliage of the plants in the afternoon using a manual spray, 18, 25 and 32 days after being transplanted to the pots. A completely random design was used with 10 replicates for each treatment.

The parameters determined in the plants were: the number of leaves and the total leaf area (this

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was carried out using a Li-cor, Li-3000A leaf area meter) 100 days after the first SA application; The total number of flower was registered each week. The data were analyzed using a variance analysis.

RESULTS

The results of the effect of salicylic acid treatments on the variables of number of leaves, leaf area and number are shown in Table 1. We can observe that the number of leaves per plant did not increase with the treatments of salicylic acid. With the treatments at concentrations of $10^{-8}$ and $10^{-6}$ M the total leaf area was increased by 49% and 24% respectively in comparison with the control. Plants treated with $10^{-8}$ M SA showed an increase of 37% in the number of flowers and around 25% with the other treatments, in comparison with the control (Fig. 1).

LITERATURE CITED


ACKNOWLEDGEMENTS

Thanks to Ing. Angel Nexticapan Garcés, QBB Mirbella del R. Cáceres Farfán and Silvia Vergara Yoisura for their technical support. CONACYT grant No. 33647-B
Table 1. Effect of foliage sprayings of salicylic acid on the growth of *Sinningia speciosa* “Ultra”. Values are the means of 10 replicate samples per treatment, ± standard error.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of leaves</th>
<th>Leaf area (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testigo</td>
<td>14 ± 0.4a</td>
<td>456.4 ± 16c</td>
</tr>
<tr>
<td>10^{-10} M AS</td>
<td>13 ± 0.6a</td>
<td>496.2 ± 23bc</td>
</tr>
<tr>
<td>10^{-8} M AS</td>
<td>13 ± 0.4a</td>
<td>680.9 ± 12a</td>
</tr>
<tr>
<td>10^{-6} M AS</td>
<td>13 ± 0.6a</td>
<td>565.2 ± 16b</td>
</tr>
</tbody>
</table>

Means with the same letter are not significantly different at $P \geq 0.05$, Tukey’s.

Figure 1. Effect of foliage applications of salycilic acid on the number of gloxinia (*Sinningia speciosa*) “Ultra” flowers. Means with the same letter are statistically equal (Tukey, 0.05).
CULTIVAR EFFECTS ON RADISH SENSITIVITY/ RESISTANCE TO CHRONIC ETHYLENE EXPOSURE

I. Eraso and G.W. Stutte

ABSTRACT

The Radish Assimilation in Spaceflight Testbed Atmospheres (RASTA) experiment is being developed to determine the effects of volatile organic compounds on growth and development in microgravity. Three cultivars of *Raphanus sativus* L; Sora, Cherry Belle and Cherry Bomb Hybrid II, were evaluated for resistance to ethylene effects. Radish will be used as an indicator species to determine effects of biogenic and anthogenic volatile compounds within the International Space Station on plant growth.

Radishes were selected as a candidate crop because of their short stature (<15 cm), rapid growth cycle (14-24 days) and sensory characteristics (color, flavor and texture). The radish cultivars were exposed to ethylene concentrations of 0, 250 and 750 ppb for 21 days, which corresponded to control, maximal, and phytotoxic concentrations in Cherry Belle radish (Eraso et al., 2002). Sequential harvests at 7, 14 and 21 DAP were made and growth analysis performed.

All three cultivars exhibited characteristic symptoms of ethylene exposure, such as stem swelling, height reduction, and severe leaf epinasty at both 250 and 750 ppb concentrations of ethylene. Cherry Belle was more sensitive to ethylene exposure than cultivars Sora and Cherry Bomb, which were more resistant than Cherry Belle at a concentration of 250 ppb. Ethylene affected harvest index of Sora at both 250 and 750 ppb concentrations more than either Cherry Belle or Cherry Bomb. Additional evaluations are required to determine the sensitivity and resistance of biologically active volatile organic compounds found in spacecraft for these cultivars.

INTRODUCTION

The Radish Assimilation in Spaceflight Testbed Atmosphere (RASTA) experiment was selected for spaceflight to determine the effects of microgravity on carbon partitioning of a salad crop. Radish was selected as test species because of their rapid growth cycle (14-24 days), sensitivity to environmental changes, sensitivity to atmospheric contaminants, and sensory characteristics. One of the primary objectives of the RASTA experiment is to determine the effects of chronic exposure to atmospheric contaminants present in Spacecrafts (e.g. space shuttle, International Space Station and future habitats) on productivity of plants. Plant exposure to volatile organic compounds (VOC’s) may promote plant stress, which will affect the biomass production (Stutte, 1999) on radishes. Kostka-Rick and Manning (1993) showed that changes in harvest index is a good indicator of chronic stress associated with atmospheric contaminants on radish. This is due to a reduction of the hypocotyls while having differential effect on leaf growth suggesting that plant biomass should be determined at the ratio between the hypocotyls and the shoots.

The Radish Assimilation in Spaceflight Testbed Atmospheres (RASTA) experiment will determine the effects of microgravity on the growth and development of *Raphanus sativus* L. A major goal of the RASTA experiment is to separate the microgravity effects from the spaceflight environment effects on crop growth. Ethylene is a plant hormone that causes many effects on plants including chlorosis of leaves, elongation of roots, stems and cells, stem thickness, leaf epinasty, and flower senescence among many other effects (Abeles, et al., 1992). Current NASA guidelines for the Spacecraft Maximum Allowable Concentration (SMAC) of ethylene is ~ 294 ppm (James, 1995), a concentration almost 10,000X greater than the threshold response for radish (Eraso et al., 2002).
Accumulation on ethylene in a closed spacecraft environment has been implicated as primary cause of lack of seed development of wheat grown onboard the Russian Space Station MIR (Salisbury et al. 1997). Ethylene production studies on the growth of lettuce, tomato, soybean, and wheat in a large closed environment chamber were reported by Wheeler et al. (1996). The results indicated that concentrations in excess of 250 ppb could be expected to accumulate in the absence of an ethylene removal system.

These ground-based experiments were performed to determine the effects of chronic ethylene exposure on growth and development of three radish cultivars that are being evaluated for the RASTA spaceflight experiment. The experiments were designed to evaluate responses under simulated spaceflight environmental conditions of temperature, relative humidity and carbon dioxide concentration. Ethylene concentrations were selected to represent atmospherically scrubbed conditions (<50 parts per billion), possible intermediate to high concentrations observed during recent ISS experiments (Stutte, unpublished), and chronic exposures previously observed on Russian MIR module (750 ppb).

Three *Raphanus sativus* L cultivars: Cherry Belle, Cherry Bomb and Sora were used in these experiments. These cultivars were determined from previous studies on radish responses to temperature, light and CO₂ (Stryjewski et al., 2001) and their effects on growth, total biomass and sensitivity to ethylene (Eraso et al., 2002).

**MATERIALS AND METHODS**

**Plant Material**

Three radish cultivars, Sora from Johnny’s Selected Seeds (Albion, ME), Cherry Belle and Cherry Bomb from Burpee (Warminster, PA) were used in these experiments. Six seeds were planted in Oasis™ growing medium foam blocks, placed in magenta flasks (6.3 x 10 x 6.3 cm) and 1X Hoagland solution was added until saturation (approximately 250 ml per magenta). After draining excess nutrient solution, the container was weighed and placed into small clear specially constructed Lexan chambers (38 x 35 x 30 cm), which allowed for control of relative humidity and CO₂. These chambers were installed in a controlled environment chamber (CEC; Conviron, Winnipeg CA). Plants were then maintained at a constant temperature of 23°C, constant CO₂ (1500 ppm), relative humidity at 75%, 18 h light and 6 h dark photoperiod, and 300 µmol m⁻² s⁻¹ PAR with cool white fluorescent lamps-VHO. Following saturation of the Oasis foam at planting, the growth media was replenished daily to ~80% of the foam’s holding capacity starting at 7 days after planting (DAP). Two plants were harvested from each flask at 7, 14, and 21 days after planting. Each treatment consisted of 6 flasks containing 2 plants per harvest. Plant tissue was separated into shoots and roots, where shoot length, root length, root diameter, root and shoot weight and leaf area measurements were taken.

Chlorophyll content was measured nondestructively at each harvest date with a Minolta SPAD-502 chlorophyll meter (Monje & Bugbee, 1992). Measurements were taken from the first fully expanded leaf and from the top of the plant. The reading was taken approximately from a point half the distance between the leaf tip and halfway the edge of the leaf.

Chlorophyll fluorescence of PSII was also determined on the same portion of the leaf using a modulated fluorometer (OS5-FL) at the same location where the chlorophyll content was measured (Genty, Briantais & Baker 1989). To avoid stress, measurements were taken inside the chamber on dark-adapted leaves for 30 minutes acquiring values for Fₘ and F₀ to calculate maximum PSII photon yield (Fᵥ/Fₘ, Krause and Weis, 1991).

After fresh tissue was collected and measured, samples were placed in a drying oven at 75°C for 72 hours for dry matter determination.
Ethylene treatment
A previously described flow control system provided independent control of ethylene levels in each of the small chambers in the CEC (Eraso et al., 2001; 2002) and three ethylene concentrations (0, 250 and 750 ppb) were evaluated. Ethylene concentration was monitored with a Photovac 10S Plus Portable Gas Chromatograph and with a 6890 Plus GC system. The detector used for the ethylene concentrations were thermal conductivity and a flame ionization detector (FID). An HP PoraPlot™ column was installed in the GC (Q 25’ X 530 um film), the carrier gas was helium at 6.6 ml/min at a constant flow and the oven of the GC was set at 20 °C (0 minute hold) programmed at 10°C/min to 54 °C (3 min.hold).

Growth analysis
Growth analysis is very important for increasing the total yield crop photosynthesis and biomass production through increased or extended light interception. Total photosynthesis has increased as a result of increase of leaf area. Growth analysis parameters will help us to understand the increase or decrease of photosynthesis per unit growth chamber area (since spaceflight missions are limited by space). The understanding of plant growth analysis will provide an increase on total biomass partitioning and achieved an increased productivity.

Statistical analysis
Two-way analysis of variance (ANOVA) was obtained using GraphPad Prism software, version 2.01. The analysis of variation was tested with three replicate values and the test was performed at the 0.05 probability level. Values used for the growth analysis and ANOVA are the average of four values per treatment and the average of three duplicated per treatment.

RESULTS AND DISCUSSION
Exposure of the three radish cultivars to high concentrations of ethylene decreases total plant biomass (Table 1). Sora had the highest total biomass of the three cultivars in the control treatment, although this was not significantly different from Cherry Bomb. Total biomass of all three cultivars decreased with increasing ethylene concentrations. There were no statistically significant differences between cultivars at any concentration at harvest (Table 1). In contrast, there was a statistically significant difference in ethylene response between cultivars at the 7 and 14 day harvests. At 7 DAP Cherry Belle had greater sensitivity to ethylene than Cherry Bomb and Sora. This effect was also observed, but not to the same magnitude at 14 DAP and by the time they were harvested at 21 DAP there is not a significant effect of ethylene on the three cultivars by the end of the life cycle of the radishes because they enter a phytotoxic stage due to the high concentrations of ethylene (750 ppb). These results are similar to the ones obtained in a nutrient film hydroponic system (Yorio et al.), although cv Cherry Bomb had slightly greater dry mass than Sora in those studies.

Cherry Bomb also had higher Harvest Index (gm hypocotyls/gm total biomass) under control (<12 ppb) conditions than either Sora or Cherry Belle cultivars (Figure 1). Harvest index (HI) decreased with increasing ethylene concentration for all cultivars. Relative responses of ethylene on HI were similar for both Cherry Bomb and Sora, which both had greater relative sensitivity than Cherry Belle (Table 1). These results are consistent with previous results of ethylene effects on radish development (Eraso et al., 2002).

Although there were significant effects of ethylene on total biomass and HI of all cultivars, there was no apparent effect on either leaf chlorophyll concentration or the potential electron quantum yield of PSII (Fv/Fm). In all cases, the values of Fv/Fm were above 0.8 indicating no physiological effect of ethylene on photosynthetic potential of the leaves (see figure 2).
Figure 1: Harvest Index % and total biomass decreases for the Cherry Bomb, Sora and Cherry Belle when the ethylene. Of the three cultivars, Sora has the highest total biomass and Cherry Bomb shows the highest harvest Index. SoraC. BelleSoraC. BelleSoraBelleSoraBelle

Figure 2: Chlorophyll and electron quantum yield of PSII at 21 days after planting.
There was clearly an effect on total biomass of the cultivars, where total biomass decreased while increasing the ethylene concentration. The dry mass data obtained during the sequential harvests between 14 and 21 days was used to characterize the effects on absolute growth rate (AGR, g dm$^{-1}$), net assimilation rate (NAR, g dm cm$^{-2}$ leaf area d$^{-1}$), leaf area (LA, cm$^{2}$), leaf area index (LAI, cm$^{2}$ leaf area per cm$^{2}$ growth area), specific leaf area (SLA, cm$^{2}$ gm dm$^{-1}$), and leaf area duration (LAD, cm$^{2}$ d$^{-1}$), (Figure 3). These results are generally consistent with those observed with total dry mass and harvest index. There is a correlation between absolute growth rate and net assimilation rate showing a partitioning of photosynthesis into new leaf area as a component of growth when three radish cultivars were exposed to chronic concentrations of ethylene. NAR (net assimilation rate) is a primary function of photosynthesis particularly during the first two weeks (Potter and Jones, 1977) and these observations are consistent with the dry mass data also. During this 7-day (14-21 DAP) period, values for NAR AGR, RGR, LAD, LAI and leaf area decreased with increasing ethylene concentration, while the specific leaf area (SLA) increased when the ethylene concentrations increased. These results suggest two primary effects of ethylene on growth and development of radish. The first is a reduction in total leaf area, which resulted in less potential for conversion of photons to energy. As a result, the NAR was decreased. At the same time, while the plants are getting smaller, leaf area per dry mass, stems and leaves from the three cultivars are getting thicker, which increases the specific leaf area (SLA) of the plant and this response partially compensates for the reduction in the net photosynthesis rate by absorbing a higher concentration of light as the canopy area reduces overtime at higher concentrations of ethylene. However, radish cultivars are so sensitive to the high concentrations of ethylene that any differences in sensitivity are masked as the crops develop. In this experiment, there was not an interaction between ethylene concentration and radish cultivar for the growth cycle of radishes (21 days).

In conclusion, radishes are sensitive to ethylene at an early age, which is the time where radishes develop cotyledons and they are followed by the formation of the primary leaves and consequently thickness of the hypocotyls. There were cultivar differences during the first two weeks after planting, but it was not observed significance cultivar differences at 21 DAP. This suggests that the ethylene response between cultivars consists of early sensitivity to ethylene, not a resistance to the ethylene effect. On the other hand, ethylene concentrations affect cultivars differently during the length of their life cycle and increases stress responses when the ethylene concentrations are increased from 0 to 750 ppb. Basically, this response to chronic exposure to ethylene (750 ppb), suggests that when the three cultivars reach a similar phytotoxic stage, there is no difference among radish cultivars is detected.

Selection of the optimum radish during spaceflight experiments depends on many factors: rapid growth, uniformity, sensitivity to ethylene, germination rate, and sensitivity to VOC (currently being studied). Of the three radish cultivars exposed to ethylene, Sora had the greatest growth rate, highest a leaf area per total plant mass (LAR), highest rate of carbon gain of photosynthesis rate per unit of leaf area (NAR), highest photosynthethic potential of the plant (LAD) and highest total biomass of the three cultivars under low (<12 ppb conditions). Cherry Bomb had less biomass, but a greater harvest index than Sora at 0 and 250 and 750 ppb of ethylene. Cherry Belle had the lowest values for for each of the growth parameters measured except for specific leaf area, where Cherry Belle showed thicker leaves at each ethylene concentration (0, 250, and 750 ppb, see figure 3).

Cherry Belle is the most sensitive of the three cultivars when a ratio was taken for each one of the growth values between the highest ratio from the control experiment (0 ppb) to the highest concentration of ethylene (750 ppb, see table 1). Generally speaking, Cherry Belle had a greater relative sensitivity to ethylene concentration than either Sora or Cherry Bomb. This suggests that Cherry Belle would be the optimum cultivar for identifying growth responses to atmospheric contaminant. However, given the short duration of many spaceflight experiments, the rapid growth
rate of Sora will make it a highly desirable cultivar. Cherry Bomb had the highest hardest index and it could become the ideal radish as a diet supply on space missions. Future research efforts will be directed towards understanding the relative sensitivity of Cherry Belle, Cherry Bomb and Sora to other potential atmospheric contaminants in spacecraft atmospheres.

**Table 1.** Relative responses of three radish cultivars to ethylene exposure were observed. Ratio of growth response at 0 ppb ethylene to 750 ppb ethylene treatment shows that Cherry Bomb is the most resistant radish.

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**Figure 3:** Analysis of growth parameters obtained during sequential harvest of three radish cultivars between 14 and 21 days after planting. **Abbreviations:** Net assimilation rate (NAR), Leaf area duration (LAD), Leaf area Index (LAI), absolute growth rate (AGR), Specific leaf area (SLA).
REFERENCES


ACETYLTHIOPROLINE SEED TREATMENT AFFECTS VEGETATIVE BIOMASS AND GRAIN YIELD OF MAIZE

J. Pablo Morales-Payan¹ and William M. Stall²

ABSTRACT

A field study was conducted in San Cristobal, Dominican Republic, to determine the effect of seed treatments of the enzyme antioxidant acetylproline (AP) on the accumulation of shoot dry biomass and the grain yield of ‘Francés Largo’ maize. The AP rates tested were 0, 0.4, 1.2, 2.0, 2.8, and 4.0 g per Kg of seed. The treatments were arranged in a randomized block design with four replications. Maize was grown according to local recommendations, except for AP treatments. Regression analysis was performed on the resulting data. No phytotoxic effects from AP were detected. Maize shoot dry biomass and grain yield tended to increase as AP rates increased. When the AP rate of 4.0 g per Kg of seed was used, shoot dry biomass accumulation and grain yield were approximately 20% higher than in control plants. Increased shoot dry biomass resulted from increased leaf area and dry weight, not from increased leaf number. Yield increase was due to increased cob size and grain number per cob. These results show that AP treatment may enhance the yield of grain and/or vegetative tissue in maize. Future studies will explore AP seed and foliar treatment combinations.

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OKRA (*ABELMOSCHUS ESCULENTUS*) GROWTH AND YIELD AS AFFECTED BY GIBBERELLIC ACID TIME, FREQUENCY, AND RATE OF APPLICATION

J. Pablo Morales-Payan¹, Rafael Deño-Suero², and William M. Stall¹

ABSTRACT

Field experiments were conducted in San Cristobal, Dominican Republic, to determine the effect of gibberellic acid 3 (GA₃) treatments on the growth and yield of two okra cultivars in the spring and summer seasons. Okra plants (‘Clemson Spineless’ and ‘Cuerno de Chivo’) were sprayed with 0, 10, 20, and 30 mg L⁻¹ of GA₃. Each rate was applied once (one day after flowering, DAF), twice (1 and 7 DAF), or three times (1, 7, and 14 DAF). In the spring, there were no significant treatment effects in the okra cultivars. However, in the summer, both cultivars responded to GA₃. When ‘Clemson Spineless’ was sprayed three times with 30 mg L⁻¹ of GA₃, plants were significantly taller, and produced longer fruit and higher fruit weight yield than plants with other treatments. In contrast, ‘Cuerno de Chivo’ plants sprayed two or three times with 20 or 30 mg L⁻¹ of GA₃, had longer fruit and higher yield as compared to other treatments in the same cultivar. The results of this study suggest that growth and yield response of okra to GA₃ may be dependent on rate and frequency of application, cultivar, and seasonal environmental conditions.

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ROOTING AND EARLY GROWTH OF ‘CRIOLLA DOMINICANA’ GRAPE (VITIS VINIFERA) CUTTINGS ARE AFFECTED BY SELECTED GROWTH REGULATORS

J. Pablo Morales-Payan¹ and William M. Stall²

ABSTRACT

In the Dominican Republic (DR), the leading table grape (Vitis vinifera) variety is ‘Criolla Dominicana’, which is propagated primarily rooting branch cuttings. A wide range of napthaleneacetic acid (NAA) and indolebutyric acid (IBA) rates is utilized to promote grape rooting. An experiment was conducted to determine the rooting and early growth response of ‘Criolla Dominicana’ cuttings to various rates of IBA, NAA (500, 750, 1000, and 1250 mg Kg⁻¹) and the alternative rooting stimulants acetylthioproline (AP, 0, 75, 150, 225, 300 mg L⁻¹), and 5-nitroguaicol + ortho-para-nitrophenol (NG, 125, 250, 375, 500 mg L⁻¹). Partially lignified, 30-cm long cuttings were soaked in aqueous rooting stimulant solutions for three minutes (IBA, NAA) or 30 minutes (AP, NG) before planting them in growth substrate-filled bags. Cuttings were placed in a nursery with adequate conditions for rooting and shoot growth. Destructive sampling was conducted on grape plantlets at 15-day intervals for four months. Time to root formation and bud breaking, root dry weight, shoot length, shoot dry weight, and leaf area were measured. AP, IBA, and NAA, but not NG, accelerated root production and bud breaking in cuttings as compared to the control. Four months after treatment, the largest plantlets were those from cuttings treated with AP at the rates of 225 and 300 mg L⁻¹. Plantlets resulting from cuttings treated with IBA and NAA at the rates of 1000 and 1250 mg L⁻¹ were larger than those from NG-treated cuttings and from cuttings without root stimulant treatment.

PLANT GROWTH REGULATORS AND NITROGEN AFFECT RATOON PRODUCTION IN PAPAYA (*Carica papaya*)

J. Pablo Morales-Payan and William M. Stall

**ABSTRACT**

Papaya (*Carica papaya*) is a tropical fruit produced on relatively short peduncules on the stem of a herbaceous, erect plant with strong apical dominance. As a result of its strong apical dominance, papaya plants commonly have only one stem, which can reach several meters in length after two or three years of continuous growth. Stems may grow too tall for practical crop management and harvest, and growers may cut them about 75 cm from the soil. Removing the apex results in multiple branching from the stem stump, which may be manipulated to produce a ratoon crop. A field study was conducted in La Altagracia, Dominican Republic, to examine the effect of nitrogen (N) fertilization (20, 40, 60, and 80 g per plant) combined with monthly foliar applications of gibberellic acid (GA$_3$) (50 and 75 mg L$^{-1}$), acetylproline (AP) (100 and 200 mg L$^{-1}$), kinetin (KIN) (65 and 130 mg L$^{-1}$), and benzyladenine (BA) (250 and 500 mg L$^{-1}$) on the ratoon growth and fruit yield of ‘Sunrise’ papaya. Treatments with N but without plant growth regulators were included. The length and diameter of ratoon branches was positively correlated with fruit yield. As N rate increased, fruit yield increased. Combining N and KIN, AP and GA$_3$ resulted in further yield enhancement. Application of GA$_3$ promoted ratoon length, and KIN increased the number of ratoon branches. No significant differences were detected between rates in a given growth regulator.

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REGULATION OF BIOMASS PARTITIONING IN HYDROPONICALLY-GROWN POTATO BY ALTERING NITROGEN CONCENTRATIONS

N.C. Yorio¹, G.D. Goins, R.M. Wheeler², and G.W. Stutte

ABSTRACT

Potatoes have been grown hydroponically for a number of years as a crop as part of NASA’s Advanced Life Support systems research for long duration spaceflight or planetary habitation. However, a typical nutrient solution consisting of excess nitrogen (7.5 mM N) can result in excessive inedible biomass that may lower overall life support system efficiency. Three N management protocols were evaluated as a means of regulating excessive vegetative growth of potatoes. Tests included reduction of overall nutrients via electrical conductivity (EC) setpoint, reduction of only the nitrate, and providing a mixed-N (nitrate + ammonia) source. Additionally, each test included a phasic treatment in which the [N] was maintained at the control concentration (7.5 mM) for the first half of the test and then maintained at the lowest N treatment. The phasic treatments resulted in tuber yields comparable to control treatments. Reducing EC, nitrate, or using mixed-N resulted in lower tuber yields, but higher tuber N-use efficiency and significantly reduced vegetative growth and plant canopy were observed. Increasing plant density coupled with N management practices may be a feasible method to maintain high tuber yield while reducing vegetative biomass.

INTRODUCTION

The National Aeronautics and Space Administration (NASA) has been conducting controlled environment agriculture experiments to evaluate the use of potatoes (Solanum tuberosum L.) in a life support system for humans in space (Wheeler et al., 1990). In these experiments, potato plants were grown either in pots containing peat-vermiculite potting mixtures or within recirculating nutrient film technique hydroponic (NFT) systems (Jones, 1997). When photosynthetically active radiation (PAR), temperature, and CO₂ are maintained near optimal levels, plants cultured with this approach can produce tuber yields similar to record field yields (Wan et al., 1996; Wheeler et al., 1990). In our hydroponic potato studies, the form of nitrogen (N) is typically nitrate (NO₃⁻). The nutrient solution is managed with constant pH regulation using nitric acid (HNO₃) and daily adjustment of overall nutrient uptake via electrical conductivity (EC) monitoring (Wheeler et al., 1999).

During the early crop stages, N uptake favors canopy development (Mäck and Schjoerring, 2002). If N application is not excessive, N uptake at later crop stages favors tuber initiation and bulking. The rate of N uptake increases during development of photosynthetic tissues (e.g., leaf expansion), but then declines during storage organ development (Imsande and Touraine, 1994). Collectively, these findings suggest that management of N availability with respect to crop growth phase could be key factor in overall patterns of biomass partitioning between the shoot and tubers.

Simultaneous addition of ammonium NH₄⁺-N and NO₃⁻-N to the nutrient solution could be a means to moderate pH fluctuations by mitigating charge imbalances (Lea-Cox et al., 1996). A continuous excess supply of nitrogen to roots has been shown to delay tuber initiation and promote vegetative shoot growth (Krauss and Marschner, 1982).

Nitrogen supply has been suggested as a useful means to manipulate partitioning between shoots and tubers (Ewing and Struik, 1992; Sweetlove and Hill, 2000). Limiting N concentration in solution could increase N use efficiency and tuber yield by suppressing shoot growth while enhancing

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assimilate partitioning into tubers. For controlled environment applications, it would be beneficial to know the optimal N management regime and plant N requirement to achieve high tuber yields while avoiding inefficient use of N through excess inedible biomass production.

OBJECTIVE

To quantify the effect of various N management practices on biomass partitioning and nitrogen use efficiency in hydroponically-grown potato.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* L.) plants cv. Norland were all grown in a 1.8m x 2.4m walk-in controlled environment chamber using nutrient film technique (NFT) hydroponics. High-pressure sodium lamps in the chamber provided ≈550 μmol m⁻² s⁻¹ PPF. Lamps were cycled in the chamber to maintain a 12 hour light (day)/12 hour dark (night) photoperiod. Air temperature was maintained at 20 °C day / 16 °C night and relative humidity was controlled to a constant 65%. Plants were harvested at 84 days after planting (DAP) and separated into shoots, roots (roots + stolons), and tubers. Plant tissues were oven dried at 70 °C for 72 hours and dry mass (DM) determined. Three separate nutrient solution nitrogen (N) treatments consisted of the following:

- **Electrical Conductivity Management (Test I).** Overall inorganic nutrients were limited via electrical conductivity (EC) control at 1.2 dS cm⁻¹ (control), 0.3 dS cm⁻¹, 0.6 dS cm⁻¹, or 1.2 dS cm⁻¹ for the first 42 days and 0.3 for the remaining 42 days of the test. Inorganic constituents of the nutrient solution are described in Table 1. Nitrogen (NO₃⁻) concentration was proportional to the EC. pH was maintained at 5.8 with dilute HNO₃.

- **Nitrate Concentration Management (Test II).** Nitrogen (NO₃⁻) was limited via manipulation of the hydroponic solution constituents at 7.5 mM N (control), 3.0 mM N, 1.0 mM N, and 7.5 mM N for the first 42 days and 1.0 mM N for the remaining 42 days of the test. Inorganic constituents of the nutrient solution were altered to maintain consistent EC of the solutions and are described in Tables 1-3. EC was controlled to 1.2 dS cm⁻¹ for all treatments. pH was maintained at 5.8 with dilute HNO₃.

- **Mixed Nitrogen Management (Test III).** Mixed nitrogen was provided in the nutrient solution at a 1.5:1 ratio of NO₃⁻-N to NH₄⁺-N (Table 4) with or without pH control to 5.8 with dilute HNO₃. These mixed N treatments were compared to 7.5 mM NO₃⁻ treatments with (control) or without pH control. EC was maintained at 1.2 dS cm⁻¹ for all treatments.

**Table 1.** 7.5 mM nitrogen (NO₃⁻) hydroponic nutrient solution used as the control treatment in all three nitrogen treatment tests (Tests I, II, and III).
Table 2. 3.0 mM nitrogen (NO₃⁻) hydroponic nutrient solution used treatment in the NO₃⁻ limiting treatments (Test II).

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Concentrations
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Refill 28 10 56 12 10 15

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<td></td>
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</tr>
<tr>
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<td></td>
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</tr>
<tr>
<td>K₂SO₄</td>
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<td></td>
</tr>
<tr>
<td>CaCl₂</td>
<td></td>
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</tr>
</tbody>
</table>

Concentrations
Start-up 1.0 0.5 3.0 2.5 1.0 2.0
Refill 9.3 10 56 12 10 20

Table 3. 1.0 mM nitrogen (NO₃⁻) hydroponic nutrient solution treatment in the NO₃⁻ limiting treatments (Test II).

<table>
<thead>
<tr>
<th>Salt</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
</tr>
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<tbody>
<tr>
<td>KNO₃</td>
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<td>0.5</td>
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<tr>
<td>Ca(NO₃)₂</td>
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<td>1.0</td>
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</tr>
<tr>
<td>MgSO₄</td>
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<td></td>
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<td></td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>KH₂PO₄</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fe-EDTA</td>
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</tr>
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<td>Micros</td>
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<td></td>
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<td></td>
<td>1.75</td>
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<tr>
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<td>CaCl₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Concentrations
Start-up 1.0 0.5 3.0 2.5 1.0 2.0
Refill 9.3 10 56 12 10 20

<table>
<thead>
<tr>
<th>Salt</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
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<tbody>
<tr>
<td>KNO₃</td>
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<td>1.5</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>3.0</td>
<td></td>
<td></td>
<td>1.5</td>
<td></td>
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<tr>
<td>MgSO₄</td>
<td></td>
<td>3.0</td>
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<td></td>
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</tr>
<tr>
<td>KH₂PO₄</td>
<td></td>
<td>0.5</td>
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<tr>
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</tr>
<tr>
<td>Micros</td>
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<tr>
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<td>CaCl₂</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Concentrations
Start-up 7.5 0.5 3.0 2.5 1.0 1.5
Refill 70 10 56 12 10 15

Table 4. Mixed nitrogen (NO₃⁻ and NH₄⁺) hydroponic nutrient solution treatment in the mixed nitrogen with and without pH control vs. nitrate only with or without pH control treatments (Test III).

<table>
<thead>
<tr>
<th>Salt</th>
<th>N</th>
<th>P</th>
<th>K</th>
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<td></td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>3.0</td>
<td></td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO₄</td>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KH₂PO₄</td>
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<td>1.0</td>
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<td></td>
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<tr>
<td>Fe-EDTA</td>
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<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Micros</td>
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<td>1.0</td>
</tr>
<tr>
<td>K₂SO₄</td>
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<td></td>
<td></td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl₂</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
</tr>
</tbody>
</table>

Concentrations
Start-up 7.5 0.5 3.0 2.5 1.0 1.5
Refill 70 10 56 12 10 15

<table>
<thead>
<tr>
<th>Salts</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>Ba</th>
<th>Mo</th>
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</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>50</td>
<td>7.4</td>
<td>0.96</td>
<td>0.52</td>
<td>9.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td></td>
<td>7.4</td>
<td>0.96</td>
<td>0.52</td>
<td>9.5</td>
<td>0.01</td>
</tr>
<tr>
<td>MgSO₄</td>
<td></td>
<td>134.0</td>
<td>96.0</td>
<td>12.5</td>
<td>6.8</td>
<td>123.5</td>
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<tr>
<td>KH₂PO₄</td>
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<td>134.0</td>
<td>96.0</td>
<td>12.5</td>
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<td>Fe-EDTA</td>
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<td>96.0</td>
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<td>6.8</td>
<td>123.5</td>
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<tr>
<td>Micros</td>
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<td>134.0</td>
<td>96.0</td>
<td>12.5</td>
<td>6.8</td>
<td>123.5</td>
</tr>
</tbody>
</table>

RESULTS

Electrical Conductivity Management (Test I).

Reduction of overall inorganic nutrients through EC management resulted in significant reductions in tuber, shoot, and root DM (Figure 1). Though harvest index (% dry mass partitioning to edible tissues) was high in the control treatments, it was significantly lower than all the EC treatments. These results suggest that the maintenance of adequate nutrients in the early phases of potato growth result in sufficient canopy development and N reserves in the plant to maintain high yields comparable to that of using a constant EC maintenance approach.
Figure 1. Harvest parameters for 84 DAP potato plants grown with limiting nutrients as controlled by electrical conductivity (EC) of the hydroponic nutrient solution. Asterisks indicate significant difference between the control mean and the respective N treatment (n=4) according to Dunnett’s procedure ($\alpha=0.05$). Asterisks above columns indicate significant difference for total DM.

Nitrate Concentration Management (Test II). The two lowest $\text{NO}_3^-$ treatments resulted in significant reductions in shoot, tuber, and root DM (Figure 2). However, the treatment in which the $\text{NO}_3^-$ was reduced from 7.5 mM to 1.0 mM at 42 DAP resulted in similar tuber yields and harvest index, but significantly reduced shoot biomass. These results suggest that providing adequate N in the early phases of potato growth result in sufficient canopy development and N reserves in the plant to maintain high yields comparable to that of using a constant N maintenance approach.

Figure 2. Harvest parameters for 84 DAP potato plants grown with limiting nitrogen (N) as controlled by $\text{NO}_3^-$ concentration in the hydroponic nutrient solution. Asterisks indicate significant difference between the control mean and the respective N treatment (n=4) according to Dunnett’s procedure ($\alpha=0.05$). Asterisks above columns indicate significant difference for total DM.
Mixed Nitrogen Management (Test III). Regulation of nitrogen through using a mixed-N source (NO$_3^-$ + NH$_4^+$) resulted in reduced shoot and tuber biomass, regardless of pH control (Figure 3). It is interesting to note that the additional uptake NO$_3^-$ through pH control (Table 5) did not result in additional biomass accumulation as in the previous N management treatments. These results suggest that pH control and the additional system cost that is associated with it may not be beneficial to overall efficiency.

![Figure 3](image_url)

**Figure 3.** Harvest parameters for 84 DAP potato plants grown with mixed nitrogen (NO$_3^-$ + NH$_4^+$) with or without pH control of the hydroponic nutrient solution with dilute (0.4M HNO$_3$). Treatments were compared to NO$_3^-$ only nutrient solutions with (control) or without pH control. Asterisks indicate significant difference between the control mean and the respective N treatment (n=4) according to Dunnett’s procedure ($\alpha=0.05$). Asterisks above columns indicate significant difference for total DM.

**Table 5.** Nitrogen (N) supply, N uptake, and N used efficiency of 84 day old potato plants grown in nutrient solution with different electrical conductivities (EC), NO$_3^-$-N concentrations, or mixed N (NO$_3^-$ + NH$_4^+$) with and without pH control.

<table>
<thead>
<tr>
<th>N Supply (g plant$^{-1}$)</th>
<th>N Uptake (g plant$^{-1}$)</th>
<th>N Use Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot + Root</td>
<td>Tuber</td>
</tr>
<tr>
<td><strong>Test I</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 dS cm$^{-1}$</td>
<td>3.3</td>
<td>0.8</td>
</tr>
<tr>
<td>0.6 dS cm$^{-1}$</td>
<td>5.5</td>
<td>1.2</td>
</tr>
<tr>
<td>1.2 / 0.3 dS cm$^{-1}$</td>
<td>5.7</td>
<td>2.0</td>
</tr>
<tr>
<td>1.2 dS cm$^{-1}$ (control)</td>
<td>9.1</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>Test II</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mM NO$_3^-$</td>
<td>1.0</td>
<td>0.2</td>
</tr>
<tr>
<td>3.0 mM NO$_3^-$</td>
<td>1.6</td>
<td>0.6</td>
</tr>
<tr>
<td>7.5 / 1.0 mM NO$_3^-$</td>
<td>7.4</td>
<td>2.0</td>
</tr>
<tr>
<td>7.5 mM NO$_3$ (control)</td>
<td>9.1</td>
<td>3.9</td>
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<tr>
<td><strong>Test III</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed N w/ pH</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Mixed N w/o pH</td>
<td>2.4</td>
<td>0.9</td>
</tr>
<tr>
<td>NO$_3^-$-N w/o pH</td>
<td>3.1</td>
<td>1.2</td>
</tr>
<tr>
<td>NO$_3^-$-N w/ pH (control)</td>
<td>7.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Asterisks indicate significant difference between the control mean and the respective N nutrient management treatment means (n=4) within each column according to Dunnett’s procedure ($\alpha=0.05$).
DISCUSSION

Regardless of N manipulation treatment, total plant and tuber N use efficiencies decreased, and tuber yield increased as more N was supplied and taken up by the plants. However, compared to plants receiving constant high N supply (7.5 mM control), total plant and tuber N use efficiency increased significantly when plants were given an initial high constant N supply, lowered to a modest NO$_3^-$-N supply post-tuber initiation at 42 days. This suggests that high constant N supply during early growth was adequate for plants to accumulate sufficient internal N reserve capacity to sustain high tuber yields in the presence of low external NO$_3^-$-N supply post-tuber initiation. Treatments that lowered NO$_3^-$-N supply directly (Test II) or indirectly through lowering nutrient solution electrical conductivity (Test I), or disabling pH control, and/or supplying NH$_4^+$-N (Test III) did not significantly benefit tuber yield, but did influence N use efficiency indices, largely via reduced shoot biomass. These findings suggest that a high constant N supply in hydroponic culture promoted high tuber yield in combination with excess inedible biomass production, which lowered N use indices relative to tubers. Although reductions in tuber yield were observed in some of the N-limiting treatments, overall yield could be compensated through greater planting density in conjunction with earlier harvests for enhanced system efficiency. Additional study of the physiological factors influencing the uptake and utilization of N in potatoes will help develop more efficient management strategies of N supply in hydroponic culture.

LITERATURE CITED


ESTABLISHMENT OF A NEW IN VITRO SYSTEM OF LICORICE (GLYCYRRHIZA URALENSIS)

Mareshige Kojoma¹, Toshiya Muranaka, Shigeo Yoshida

ABSTRACT

The roots and stolons of some Glycyrrhiza plants (Licorice, Leguminosae) is one of the most important drugs in oriental traditional medicine for anti-inflammatory, anti-allergic and anti-ulcer activities. Licorice is also an important commercial product used as a sweetener and a flavor in the tobacco and confectionary industries in the worldwide. This study describes the establishment of in vitro stolon tissue culture system of Glycyrrhiza uralensis. The formation of in vitro stolon was induced from a stem node in Murashige-Skoog (MS) medium supplemented with 0.01 \( \mu \)M \( \alpha \)-naphthaleneacetic acid (NAA) liquid culture in the dark. Subsequently, the best growth was achieved by addition with 0.01\( \mu \)M of NAA. Moreover, the effects of sucrose on the proliferation of the stolon were examined. The best response was in medium with 6% of sucrose. Shoots regeneration and adventitious roots induction were easily achieved on solid MS medium under the light culture.

INTRODUCTION

The dried roots and stolons of some Glycyrrhiza plants (Licorice, Leguminosae) is one of the most important drugs in oriental traditional medicine for anti-inflammatory, anti-allergic and anti-ulcer activities. Licorice is also an important commercial product used as a sweetener and a flavor in the tobacco and confectionary industries in the worldwide. Habitats of licorice areas are semi-arid regions of southern Europe and Asia. Recently, exhaustion of the natural bioresources and the desertification are occurred. Most licorice is harvested from wild plant, or cultivated in extensive farming. So the quantity and the quality of the products are not stable enough to use in drugs. Therefore, demand of breeding for a modern cultivation of Glycyrrhiza plants which are used as licorice has increased recently. Commonly, the plant is propagated with the cuttings of stolon. But this conventional method is very slow and vulnerable to viral infection during propagation. The development of rapid in-vitro micropropagation is necessary in order to breed licorice. In potato (Solanum tuberosum L.), The in vitro stolon production was successfully achieved already. (Xu et al, 1998). The present study describes the establishment of the in vitro stolon tissue culture system of Glycyrrhiza uralensis.

MATERIALS AND METHODS

Plant Materials

Seeds of G. uralensis were washed with tap water for 3 hr and surface-sterilized in 70 % (v/v) EtOH for 1 min and then in a 2 % (v/v) sodium hypochlorite solution (10% commercial bleach) containing 0.2 % (v/v) Tween 20 for 15 min, followed by three rinses with sterile water. Seeds were placed onto Murashige and Skoog (MS) medium (Murashige et al, 1962) containing 3% (w/v) sucrose and solidified with 0.2% (w/v) gelrite in plastic tubes (Ø30 mm×115 mm) containing 10 ml of medium. The medium was adjusted the pH to 5.8, and was autoclaved at 120°C and 1.2 kg cm⁻² for 15 min. The cultures were incubated at 23°C and under 4,500 lx fluorescent light (16 h/day).

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In vitro culture of single-node cuttings

After one month of seed culture, the germinated shoots were elongated. In this time, we picked up only a shoot to propagate the explants, which have same genome. Single-node cuttings from stem segments with one axillary bud were cultured in vitro, essentially as the described report (koujyouma et al, 1995). The medium consisted of the MS medium containing 3% sucrose and 0.2% gelrite and supplemented with 0.1µM of α-naphthaleneacetic acid (NAA). Culture conditions were the same as those given above.

Effects of GA₃ or NAA on the formation of the stolon

Effects of phyto-hormones, gibbelleric acid (GA₃) or NAA, on the formation of the in vitro stolon tissue were examined. Stem single-node segments were cultured in MS liquid media containing 3% sucrose in a polycarbonate culture bottle(φ80 mm×102 mm, containing 100 ml of medium). The media were supplemented with GA₃ (0, 0.01, 0.1, 1, and 10µM) or NAA (0, 0.01, 0.1, 1, and 10µM) respectively. They were cultured at 26ºC in the dark at 100 rpm on rotary shaker. 15 stem segments were cultured in each culture bottle. Each treatment consisted of twice replicates. The samples were transferred to the same fresh media after 2 weeks. Then, the samples were recorded after 2 weeks (totally 4 weeks) of culture.

Effects of NAA on the proliferation of the stolon

Effects of NAA on the proliferation of the stolon tissue were examined. The stolon tissue segments (about 2g) were cultured in MS liquid media containing 3% sucrose in a polycarbonate culture bottle(φ80 mm×102 mm, containing 100 ml of medium). The media were supplemented with NAA (0, 0.01, 0.1, 1, and 10 M) respectively. The in vitro stolon produced in the medium supplemented with 3 % sucrose and 0.01 M NAA was used in the present experiment. About ten segments (2 g-fresh weight, about 5 cm) were cultured in each culture bottle. They were cultured at 26ºC in the dark at 100 rpm on rotary shaker. Each treatment consisted of twice replicates. The samples were transferred to the same fresh media after 2 weeks. Then, the samples were recorded after 2 weeks (totally 4 weeks) of culture.

Effects of sucrose on the proliferation of the stolon

Effect of sucrose on the proliferation of the stolon tissue was examined. The stolon tissue segments (about 2g) were cultured in MS liquid media without phyto-hormone in a polycarbonate culture bottle(φ80 mm×102 mm, containing 100 ml of medium). The media were supplemented with sucrose (1, 3, 6, and 9%) respectively. The in vitro stolon produced in the medium supplemented with 3 % sucrose and 0.01 M NAA was used in the present experiment. About ten segments (2 g-fresh weight, about 5 cm) were cultured in each culture bottle. They were cultured at 26ºC in the dark at 100 rpm on rotary shaker. Each treatment consisted of twice replicates. The samples were transferred to the same fresh media after 2 weeks. Then, the samples were recorded after 2 weeks (totally 4 weeks) of culture.

RESULTS AND DISCUSSION

Effects of GA₃ or NAA on the formation of the stolon

Effects of GA₃ or NAA on the formation of the in vitro stolon from the node segments were investigated. The highest frequency of stolon induction was obtained at 0.01 µM NAA (40.0 %). And at the same NAA concentration, elongation of stolon was achieved best results (9.60 cm) in 4 weeks of culture. More increase of NAA concentration decreased the frequency of stolon induction and the elongation of one. Stolon induction was failed treated with highest concentration 10 µM NAA. To compare the NAA treatment, stolon elongation was decreased treated under each GA₃ treatments.
Effects of NAA on the formation of the stolon

Effects of NAA on the proliferation of the in vitro stolon induced in MS medium supplemented with 0.01 µM NAA from the node segments were investigated. The best results for the stolon proliferation was obtained using on the medium supplemented with 0.01 µM NAA. The rate of proliferation was achieved 6.64 times (fresh weight) per 4 weeks of culture. In the present condition, the stolon grew vigorously and very rapidly. However, an increased concentration of NAA reduced the stolon growth. When the stolon cultured in the medium with 10 µM NAA, the growth of the stolon was almost stopped and some short adventitious roots (2-5 mm) were slightly observed.

Effects of sucrose on the proliferation of the stolon

Effects of sucrose concentration on the proliferation of the in vitro stolon were investigated. The best results for the stolon proliferation was obtained using in the medium supplemented with 6 % of sucrose. The rate of proliferation was achieved 6.34 times (fresh weight) per 4 weeks of culture. Now, we are studying an interaction between sucrose and NAA.

Shoots and adventitious regeneration were easily achieved on solid MS media with gelrite supplemented with NAA or without NAA (data were not shown). In conclusion, we have developed an in vitro stolon culture system. It is appeared that 0.01 µM of NAA is most effective to induce the in vitro stolon. Moreover, the same condition and 6 % of sucrose concentration were preferable for the stolon proliferation. The present system provides a rapid in vitro micropropagation for licorice, Glycyrrhiza uralensis. Furthermore, we think the results obtained in the present study would be a useful for the secondary metabolism research and the genetic improvement of the plant using a biotechnological approach.

ACKNOWLEDGEMENTS

The authors wish to thank A. Kohara, H. Seki, K. Okamura, K. Kobayashi, K. Ohyama, M. Igarashi, M. Suzuki, T. Nakajima, Y. Kamide, Y. Momose and Y. Hashinokuchi (Plant Science Center, RIKEN, Laboratory for Biochemical Resources) for their helpful support of the present study. We are also grateful to Dr. T. Shibata (Hokkaido Experimental Station for Medicinal Plants, National Institute of Health Sciences) for the supply of G. uralensis seeds.

LITERATURE CITED

Murashige T, and Skoog F 1962 A revised medium for rapid growth and bioassays for tobacco tissue cultures. Physiol Plant 97: 402-408
SCREENING OF POLLEN TUBE GROWTH INHIBITORS: IDENTIFICATION OF CLETHRAMYCIN FROM A PLANT-ASSOCIATED ACTINOMYCETE.

Y. Igarashi1*, T. Yamakawa1, R. Yoshida2 and T. Furumai1

ABSTRACT

The cytoskeletal proteins, actin and myosin, play a central role in pollen tube growth. The pollen tube growth is inhibited by cytochalasin, which interferes with actin polymerization. In the screening of pollen tube growth inhibitors, clethramycin was found from the fermentation broth of an actinomycete strain *Streptomyces hygroscopicus* TP-A0623. The producing strain was isolated from a root of *Clethra barbinervis* collected in Toyama, Japan. Clethramycin showed *in vitro* antifungal activity against yeast such as *Candida albicans* and *C. glabrata* with the MIC of 0.5~8 µg/ml.

INTRODUCTION

In pollen tube growth, actin/myosin cytoskeleton plays an important role in the transport of the vesicles containing precursors for cell wall biosynthesis from the sites of their synthesis to the growing pollen tube tip (Mascharenhas, 1993). This process is inhibited by cytochalasin or latrunculin B, an inhibitor of actin polymerization, and thus pollen tube growth is also inhibited (Gibbon, 1999). An inhibitor of cytoskeletal function is expected to be a tool to probe the cell function and further to be a lead for therapeutic agents. In addition, in this process, organelles in which the cell wall precursors are synthesized such as the endoplasmic reticulum and Golgi apparatus are involved, and therefore the discovery of bioactive molecules with an unknown mode of action is expected. In this study, we screened for the inhibitors of pollen tube growth and found a new compound, clethramycin (Fig. 1).

MATERIALS AND METHODS

Microorganism

Strain TP-A0623, the clethramycin-producer, was isolated from a wild plant of *Clethra barbinervis* collected in Toyama prefecture, Japan. The root of the plant was cut into pieces of ca. 3 cm in length. They were successively immersed in 70% ethanol and 1% NaClO solution for 3 min. Then, they were rinsed with sterilized water and incubated on an agar plate consisting of agar 1.5%, amphotericin B 0.005% and methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate 0.02% at 32°C for 30 days. A colony of the strain TP-A0623 that grew out of a piece of the root was isolated and purified on an agar plate.

Fermentation

A loopful of a mature slant culture of *S. hygroscopicus* TP-A0623 was inoculated into four 500-ml K-1 flasks containing 100 ml of the seed medium consisting of soluble starch 1%, glucose

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0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone 0.5%, K₂HPO₄ 0.1%, MgSO₄·7H₂O 0.05% and CaCO₃ 0.3% (pH 7.0). The flask was incubated at 30°C for 4 days on a rotary shaker (200 rpm). Three-ml aliquots of the seed culture were transferred into one hundred 500-ml K-1 flasks each containing 100 ml of the production medium consisting of soluble starch 2%, glucose 0.5%, glycerol 2%, Pharmamedia 1.5%, yeast extract 0.3% and HP-20 resin 1.0%. Fermentation was carried out for 5 days at 30°C on a rotary shaker (200 rpm).

**Isolation**

The fermented whole broth was extracted with 50% aqueous methanol and the filtrate was evaporated *in vacuo*. The residual aqueous solution was adjusted to pH 7.0 and loaded on a column of HP-20. The column was eluted with aqueous acetone and the acetone was removed by evaporation. The aqueous layer was lyophilized to afford a brown powder. A portion of the powder was dissolved in DMSO and applied onto an ODS gel column. The column was eluted with 20~80% acetonitrile in 0.15% KH₂PO₄ buffer (pH 3.5). The combined fraction was adjusted to pH 6, evaporated and resultant aqueous solution was lyophilized. The resultant powdery material was extracted with a small amount of methanol and the extract was concentrated *in vacuo* to give pure clethramycin. By repeating the chromatography, 112 mg of clethramycin was obtained from 10 liters of culture broth.

**Biological Assay**

Pollen of the Japanese pear (*Pyrus* spp. cultivar Imagawaaki) was used for the assay. An agar plate containing sucrose 10%, Na₂B₄O₇ 0.01% and agar 1% (pH 6.3) was prepared and the pollen was placed in a line of 18 mm in length on the agar plate by using one edge of an 18 mm square cover glass. At the one end of the line of pollen, an 8-mm paper disc containing the test sample was placed. A number of pollen tubes grow almost perpendicularly to the line during incubation at 30°C for 20 hours in the dark. After the incubation, the pollen tube growth inhibition was measured under a light microscope. The strength of the inhibition was defined as the length (mm) of the zone in which the pollen tube growth was completely inhibited (Fig. 2).

**RESULTS AND DISCUSSION**

**Screening**

About one hundred fermentation extracts of actinomycetes isolated from the plant were subjected to the screening of the pollen tube growth inhibition assay. Among the six hits, strain TP-A0623 was chosen for its strongest activity. In the HPLC analysis of the extract from the strain, two classes of antibiotics were dereplicated based on the UV-vis spectrum and molecular mass matching. One includes antibiotics TAN 420C and TAN 420E and herbimycin A, and the other azalomycin B and its 11-O-methyl derivative. Among these, herbimycin A and azalomycin B, and several commercially available antibiotics with the known mode of action were tested in this assay. Pollen tube growth was inhibited by protein kinase and actin polymerization inhibitors, but not by the inhibitors of tublin depolymerization (paclitaxel), DNA polymerase (daunomycin) and protein synthesis (cycloheximide). In addition, two macrolides, filipin and azalomycin B inhibited the pollen tube growth whereas amphotericin B not. Kinase inhibition activity of staurosporine and herbimycin A is accountable for their effect on pollen tube growth because the pollen germination is controlled by MAP kinases (Wilson, 2000). Therefore this assay system can be used for the detection of protein kinase inhibitors. In addition to the dereplicated metabolites, we noticed the production of a hexaene antibiotic which showed pollen tube growth inhibition and antifungal activity against *C. albicans* in the fermentation extract. Although the production of hexaene antifungal antibiotics has been reported several times so far, few of them were characterized structurally. Therefore we attempted the isolation and structure determination of the hexaene antibiotic produced by strain TP-A0623.
Structure Determination

Clethramycyin was obtained as a yellow powder, soluble in methanol and insoluble in chloroform and ethyl acetate. Its UV-vis spectrum showed a typical pattern of polyene antibiotics with the absorption maxima at 337, 356 and 378 nm, indicating the presence of a conjugated hexaene moiety. The FAB-MS measurement of clethramycyin gave the parent ion peak [M-H]- at m/z 1216.7 in negative mode and [M+Li]+ at m/z 1224.6 in the presence of lithium chloride in positive mode. In the negative mode FAB-MS/MS spectrum, distinctive fragment ions were observed at m/z 97 and 80, indicating the presence of a sulfate group.

The 1H and 13C NMR spectra of clethramycyin showed a characteristic feature of the reduced-type polyolefinic polyketide. Olefinic carbons and protons were observed in the narrow spectral regions and the peaks for more than twenty carbons were detected. In addition, fifteen methylene and twelve hydroxylated methine groups were detected. One singlet and three doublet methyl groups, presumably derived from the methylmalonate, were easily recognized in the spectrum. Since it was considered that most of the remaining carbons were derived from the malonate, 13C-labeled clethramycyin was prepared by the fermentation fed with 1,2-13C2-acetate. The 2D-INADEQUATE experiments in combination with HMQC and DQF-COSY confirmed the incorporation pattern of the acetate units. By precisely analyzing DQF-COSY, TOCSY, HMBC and ROESY, the overall carbon skeleton of clethramycyin was determined as shown in Fig. 1.

Perhaps linearmycins (Sakuda, 1996) are the closest known compounds. The major differences between clethramycyin and linearmycins are the presence of O-sulfate and guanidino functionalities in clethramycyin. These structural differences seem to reflect on the biological properties. Linearmycin A shows more potent antimicrobial activity against bacteria than yeasts, whereas the trend in clethramycyin is adverse.

Biological Properties

In addition to the pollen tube growth inhibition, clethramycyin showed strong activity against yeasts Candida albicans, C. glabrata, C. krusei, C. tropicalis, Cryptococcus neoformans and a fungus Aspergillus fumigatus, but very weak against Gram-positive and negative bacteria. Cytotoxic effect was observed at the rather higher concentrations. The IC50 was 57 µg/ml against HeLa cells and 120 µg/ml against WI-38 cells. Clethramycyin showed no toxicity for male ICR mice (4 weeks old) by intraperitoneal administration at a dose of 10 mg/kg, but no therapeutic effect was observed with the experimental intravenous infection with C. albicans at the same dose.

Linearmycins, structural analogs of clethramycyin, are reported to inhibit the spheroplast regeneration, namely the cell wall biosynthesis, of C. albicans. Although the site of action of clethramycyin is not elucidated, it might inhibit the biosynthesis or transport of the cell wall precursors.
both in the pollen and yeast, considering its potent antifungal activity and structural resemblance to linearmycin.

**LITERATURE CITED**

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DISTRIBUTION OF STRIGOLACTONES, GERMINATION STIMULANTS FOR STRIGA AND OROBANCHE AMONG HOST AND NONHOST PLANTS

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ABSTRACT

Seed germination of weedy root parasites, Orobanche and Striga, is triggered by germination stimulants (strigolactones) released from root of host plant. Strigolactones induce seed germination at picomolar or at even much lower concentrations. The first strigolactones, strigol and acetyl-strigol, were isolated from cotton, a non-host plant (Cook 1972). The other strigolactones characterized to date are released from host plants; orobanchol from red clover, alectrol from cowpea, and sorgolactone from sorghum. Recently, Yasuda et al. isolated strigol from aseptic root culture of Menispermum dauricum, a Chinese medicinal plant (Yasuda, 2003). These results suggest that strigolactones distribute widely among host and non-host plants.

Although germination tests with these parasitic weed seeds are highly sensitive and specific to strigolactones, it is difficult to quantify individual strigolactones in root exudates because some host plants produce more than one strigolactone, and in addition the others may produce germination inhibitors as well.

We have established an LC/MS/MS method for determination and quantification of strigolactones (Sato 2003). This method enables direct analysis of strigolactones in crude ethyl acetate extracts of root exudates without any purification.

By using this LC/MS/MS method, we identified and quantified strigolactones in root exudates of several plant species. For example, cotton was confirmed to produce strigol and acetyl-strigol but not orobanchol or alectrol. Time-course of strigol production by cotton seedlings was analyzed and higher production was observed for 5–7 days after germination.

Other host plants including Arabidopsis thaliana, carrot, sorghum, tomato, and soybean were found to produce known and novel strigolactones. These results indicate that strigolactones are not specific to host plants but rather common metabolites in the plant kingdom. It is thus important to examine biological roles of strigolactones in plants and also in relation to root-related physiological processes.

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METABOLIC PROFILING OF COUMARINS IN MORNING GLORY, *IPOMOEA TRICOLOR*, AFTER VARIOUS STRESSES

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ABSTRACT

Coumarins are secondary metabolites found in many plants and are accumulated by various stresses. Coumarins are biosynthesized via phenylpropanoid pathway. Details of the biosynthesis and biofunction of coumarins in plants are unclear. Neither genes nor enzymes related to the formation of lactone moiety of coumarins have been isolated yet. Metabolic profiling of coumarins in morning glory, *Ipomoea tricolor* cv. heavenly blue, subjected to various kinds of stresses was performed for clues to know the mechanism controlling the coumarin biosynthesis. Analysis of coumarins in morning glory was performed by HPLC and LC/ESI-MS. Hypocotyls of morning glory were subjected to the treatments with bud-cell suspension of *Fusarium* spp., pathogenic *Fusarium* (*F. oxysporum* f. sp. *batatas*: PF), a causal fungus of fusarium wilt, and non-pathogenic *Fusarium* (*F. oxysporum* 101-2: NPF). Scopoletin, scopolin, umbelliferone, skimmin, and esculetin were detected in the extract of hypocotyls of morning glory treated with NPF and PF. Coumarins in morning glory after fungus stress were quantified with HPLC. Scopoletin, scopolin, umbelliferone, and skimmin were induced by either PF- or NPF-treatment. Amounts of scopoletin and umbelliferone in morning glory were reached maximum 16 hours after treatments. Respective glucosides of these coumarins, scopolin and skimmin, increased constantly for 72 hours. Scopoletin and umbelliferone seemed to be glucosylated to scopolin and skimmin, respectively after treatments with PF and NPF. Glucosylation is thought to protect the highly reactive phenolic hydroxy group against cellular oxidases. Scopoletin and umbelliferone may be glucosylated to protect phenolic hydroxy group. There was no difference in contents of scopoletin and scopolin in morning glory treated with PF or NPF. Contents of umbelliferone and skimmin in PF-treated morning glory were more than those in NPF-treated. Umbelliferone content in PF-treated morning glory was ~8-fold of that of NPF-treated morning glory 8 hours after each treatment. Activity of phenylalanine ammonia-lyase (PAL), which is a key enzyme in phenylpropanoid pathway, increased by *Fusarium* treatments compared with water treatment. PF-treatment induced higher PAL activity than NPF-treatment did. Coumarin profile changed after infection stress treatments. Difference between NPF- and PF-treatment was shown in the change of the coumarin profile.

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SYNTHESIS AND CHEMICAL REACTIVITY OF (11E,13E)-LABDA-11,13-DIENE-8α,15-DIOL, A NOVEL SIGNAL TRANSDUCER IN DEFENCE RESPONSES IN TOBACCO PLANTS

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ABSTRACT

In recent years, we identified a novel labdane-type diterpene, (11E,13E)-labda-11,13-diene-8α,15-diol (1), from tobacco mosaic virus (TMV)-infected tobacco leaves (Nicotiana tabacum cv. Samsun NN) as a wound-induced protein kinase-activating factor-1 (WAF-1) [Seo et al., Plant Cell, 15, 863-873 (2003)]. This compound increases rapidly in tobacco leaves during a hypersensitive response to TMV-infection and after wounding, and activates the tobacco mitogen-activated protein kinases (MAPKs), WIPK and SIPK, at nanomolar levels (the maximum activities are observed by exogenous application at 0.1-1.0 nM, respectively). Both MAPKs are respectively located at control points in the signal transduction network of plant defense. Thus, WAF-1 is considered to be one of the critical signal transducers in defense responses in tobacco plants. In fact, application of WAF-1 to tobacco leaves enhanced the resistance to TMV-infection. Since the amounts available from natural sources was limited, the chemical synthesis of WAF-1 was needed for the structural identification and the comprehensive examinations of the plant functions. In addition, we faced the problem that WAF-1 was measurably labile, which on occasion led to the marked decomposition during storage. Thus, the profound examination of the chemical reactivity of WAF-1 was needed for its safety storage to make its bioassays precise and reproducible. Here we report the synthesis, structural elucidation, and chemical reactivity of WAF-1.

WAF-1 was synthesized from the known aldehyde (3), prepared from commercially available natural (+)-sclareolide (2), via the Honoer-Wadsworth-Emmons reaction using 3-ethoxycarbonyl-2-methyl-prop-2-enylphosphonate in an optically pure form. The structure of WAF-1 was completely determined by NMR analysis of the synthetic one which showed high biological activity comparable to that of natural WAF-1. WAF-1 was measurably air-sensitive: upon exposure to air, 1 was easily oxidized to the corresponding aldehyde (4), which then suffered oxidative-degradation, i.e., loss of 14- and 15-positioned carbons, to give (11E)-8α-hydroxy-14,15-dinorlabd-11-en-13-one (5) as a major product.

In this paper, we will also show that WAF-1 is a promising bio-probe for elucidation of the complicated and intractable signal network of plant defense.

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MOLECULAR CLONING AND CHARACTERIZATION OF cDNAs ENCODING DITERPENE CYCLASES INVOLVED IN BIOSYNTHESIS OF PHYTOALEXINS IN SUSPENSION-CULTURED RICE CELLS

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ABSTRACT

In suspension-cultured rice cells, diterpenoid phytoalexins such as momilactones and (-)-phytocassanes are produced in response to exogenous elicitors such as chitin oligomers and cerebrosides. We attempted to clone cDNAs encoding diterpene cyclases involved in the phytoalexin production by RT-PCR with the degenerate primers designed on the basis of conserved motifs of the plant diterpene cyclases, and found that at least two species of mRNA encoding putative diterpene cyclases were expressed in the elicited rice cells. These putative diterpene cyclases were tentatively named OsDTC1 and OsDTC2.

The cDNA encoding the full-length ORF of OsDTC1 was cloned by both 3' and 5' RACE and overexpressed in Escherichia coli as a fusion protein with glutathion S-transferase. The recombinant OsDTC1 converted ent-copalyl diphosphate (ent-CDP) into a diterpene hydrocarbon-like compound X and pimara-8,15-diene in ca. 10% and less than 1% yields, respectively. We also found that compound X accumulated in the elicited rice cells with known diterpene hydrocarbons, 9β-pimara-7,15-diene, stemar-13-ene, pimara-8,15-diene, aphidicol-15-ene, and aphidicol-16-ene. Considering the possibility that compound X is ent-cassa-12,15-diene, a hypothetical biosynthetic intermediate leading to (-)-phytocassanes, we synthesized ent-cassa-12,15-diene from (R)-Wieland-Mischer ketone. The retention time and full-scan mass spectrum of compound X in capillary GC-MS corresponded completely to those of the synthetic ent-cassa-12,15-diene. On the other hand, when syn-CDP was used as a substrate, the recombinant OsDTC1 converted the substrate into pimara-8,15-diene and aphidicol-15-ene in less than 1% yields. Both pimara-8,15-diene and aphidicol-15-ene are minor components in the elicited rice cells, and their metabolites have not been identified as rice phytoalexins. These results indicate that OsDTC1 functions as ent-cassa-12,15-diene synthase, although OsDTC1 might be involved in biosynthesis of the minor diterpene hydrocarbons.

The expression of OsDTC1 mRNA and the presence of ent-cassa-12,15-diene were also confirmed in ultraviolet-irradiated rice leaves. Cloning of the cDNA encoding the full-length ORF of OsDTC2 is now underway. This is the first report of the cloning of a cDNA encoding a diterpene cyclase involved in the biosynthesis of a diterpene hydrocarbon that is a putative precursor of diterpenoid phytoalexins in rice.

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IN SITU LOCALIZATION OF POLYISOPRENE IN A RUBBER PRODUCING PLANT, EUCOMMIA ULMOIDES OLIVER

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ABSTRACT

The localization of polyisoprene in young stem tissues of Eucommia ulmoides Oliver was investigated by histochemical staining and Fourier transform infrared microspectroscopy (FT-IR). The fibrous structures were stained with Oil Red O. FT-IR microspectroscopy analysis proved that the fibrous structures were indeed trans-polyisoprene. The presence of granular structures clearly stained with the dye and having a characteristic absorption at 2960 cm⁻¹ in FT-IR suggested that trans-polyisoprene accumulated in the vicinity of the cambium layer. We have successfully showed the localization of trans-polyisoprene in plant tissues for the first time, and our histological investigation allowed us to presume the main sites of biosynthesis and accumulation of rubber.

INTRODUCTION

E. ulmoides is one of the rare woody plants producing trans-polyisoprene (Hendricks et al. 1946; Shiu-ying 1978). This plant accumulates solid rubber (EU-rubber) (Tangpakdee et al. 1997; Bamba et al. 2000). The regions for rubber production and accumulation in the plant tissues have not been identified clearly. In this study, the regions of polyisoprene accumulation in young stems of E. ulmoides were elucidated by a combination of FT-IR microspectroscopy and a chemical staining technique.

It was difficult to study on a rubber-producing plant histology due to the lack of a suitable method for a preparation of well-preserved specimens of polyisoprene-producing organs and the chemical and/or a spectroscopic identification of polyisoprenoids. Conventional paraffin- and resin-embedding techniques are not applicable because the localization of the lipophilic components in the tissue would not be maintained with these techniques. In this study, our improved technique for section preparation enabled us to indicate the site of polyisoprene accumulation in the tissues.

MATERIALS AND METHODS

Oil Red O was purchased from Aldrich Chemical Company, and pyridine and acetic acid were obtained from Wako Chemicals (Osaka, Japan). Stems of Eucommia ulmoides Oliver were collected in July 2000 at the Hitachi Zosen Corporation Experimental Station (Habu 2264-1 Innoshima, Hiroshima, Japan).

For the preparation of tissue sections, portions of succulent stems with a diameter of ca. 7 mm were taken from shoots that sprouted after pruning a 15-year-old E. ulmoides. The stems were cut (appropriate size of a longitudinal section ca. 10 mm; of a cross section ca. 5 mm) and fixed using distilled water on an electronic freezing sample stage with a peltiert chip (EF-10; Nihon Micorrome Laboratory Co., Osaka, Japan). The electronic-freezing unit with frozen samples was placed on a Rotary Microtome (ST-101; Nihon Micorrome Laboratory Co.), and the sections were then cut to 25 µm thickness. The optimum temperature of the peltiert chip for well-preserved tissue sections was

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determined to be -5 °C. Lipids were removed from the tissue sections by three changes of preheated acetone at 40 °C for 10 min.

Staining of lipophilic compounds containing rubber was carried out using Oil Red O, which is used as a conventional lipid-staining reagent. Oil Red O solution (4 g Oil Red O, 70 ml pyridine, 30 ml distilled water) was used immediately after filtration. The sections prepared as described above were mounted on a glass slide and then stained with Oil Red O for 2-3 min. The excess staining reagent was removed using 45 % acetic acid.

FT-IR analysis was performed using a Continuum infrared microscope attached to a Nexus 670 infrared spectrometer equipped with a mercury-cadmium-telluride (MCT) detector (Thermo Nicolet). The microscope was equipped with an automated motorized stage that was operated by a micropositioning stage controller interfaced to a personal computer. Data collection, stage control and processing of mapping data were performed using OMNIC Atlas Software (Thermo Nicolet). The 25-μm-thick longitudinal sections prepared as described above were mounted on BaF2 disks. Spectra were collected using a 20 μm x 120 μm aperture, 256 co-added scans in transmission from 4000 to 700 cm–1 with a spectral resolution of 8 cm–1. Mapping experiments were designed so that 30 spectra were collected at a distance of 600 μm from epidermis to cambium. Individual spectra were collected using an automated motorized stage.

RESULTS AND DISCUSSION

Among the various devices tested for preparation of tissue sections, an electronic freezing sample stage, combined with a temperature-controlling device containing a peltier chip, was found to be the most effective. This freezing unit allowed delicate adjustment of conditions for slicing tissues. After many trials at different stage temperatures, an optimal temperature (-5 °C) was set for *E. ulmoides* stem samples, and intact 5- to 30-μm thin sections were successfully prepared from a young *E. ulmoides* stem. This technique for preparing frozen tissue sections was found to be very useful and widely applicable to a variety of samples for which resin- and/or paraffin-embedding techniques are not suitable.

Histochemical staining experiments were first performed with Oil Red O, which is used as a conventional lipid-staining reagent. Cross and longitudinal sections were prepared from soft pithless stems of *E. ulmoides*, mentioned above. Red-stained structures were observed along the parenchymal cells on the phloem side in cross-sections (Fig. 1a). In longitudinal sections, the fibrous structures thought to be EU-rubber were observed and also stained red with Oil Red O (Fig. 1b). The staining experiment indicated that the fibrous structures are distributed lengthwise in intercellular spaces. In addition, clearly stained granular structures were found around the cambium region on the phloem side. The structures were abolished after removal of lipids with an organic solvent.

It is difficult to clearly reveal polyisoprene deposition sites in the tissue by histochemical staining since there is no specific dye that stains polyisoprene selectively. To circumvent this problem, FT-IR microspectroscopy was applied to the stem sections. Infrared absorption spectra were obtained for fibrous and granular structures in the epidermis that were stainable with Oil Red O, and the spectra were then compared with that of authentic trans-polyisoprene. The fibrous structures from the mature *E. ulmoides* bark were spectroscopically identical to that of Library data for trans-polyisoprene (Aldrich polymers library). *Trans*-polyisoprene is generally known to have characteristic absorptions at 835 cm–1, 1,385 cm–1, 1,430 cm–1, 1,665 cm–1 and 2,960 cm–1. There is no report on in-situ microspectroscopic FT-IR analysis of polyisoprene. The absorption for the *trans* double bond, observed at 1,665 cm–1 in an authentic sample, gave a rather weak absorbance and in *E. ulmoides* sections the region overlapped with that of the C=O stretch of protein amide bonds. From the instrumental viewpoint it is impossible to isolate spectroscopically the contribution of the fragments
responsible for the absorptions at 835 cm$^{-1}$ and 1,385 cm$^{-1}$, due to trans C-H deformations from the corresponding chromatographic trace. The absorptions at 2960 cm$^{-1}$ (CH$_3$ group of trans-polyisoprene) and 1430 cm$^{-1}$ (C-H deformations) were reliable regions by which to identify the presence of trans-polyisoprene in *E. ulmoides* section. The spectra of fibrous and granular structures were almost identical to those of authentic trans-polyisoprene and the mature fibrous rubber from *E. ulmoides* bark (Fig. 2; traces F, C). In addition, the spectrum of the epidermal site supported the presence of fatty acids (Fig. 2; trace E).

Line-mapping analysis was performed on the region between the epidermis and cambium in the longitudinal section. Each acquisition spectrum was overlaid in three dimensions over the range 2700 to 3200 cm$^{-1}$, covering the characteristic absorptions of trans-polyisoprene (Fig. 3). The characteristic absorptions of trans-polyisoprene were clearly seen at the sites where the fibrous structures were stained with Oil Red O. The chemical and spectroscopic evidence support the proposition that the fibrous structures are indeed trans-polyisoprene, namely EU-rubber. Furthermore, the FT-IR spectra of the granular structures exhibited the typical absorption band (2,960 cm$^{-1}$) ascribable to trans-polyisoprene.

These findings suggest that low-molecular-weight rubber occurs in granular structures in the vicinity of the cambium, and that high-molecular-weight rubber occurs in fibrous structures in the vicinity of the epidermis. The fiber elongation or development of rubber is assumed to proceed outward from the cambium as the cells grow.

**CONCLUSIONS**

In this study, we established a method of preparing frozen tissue sections appropriate for microspectroscopic analysis. This is an excellent method of sample preparation for FT-IR microspectroscopy and is applicable to a variety of plant samples. The in situ localization of polyisoprene was successfully revealed by means of FT-IR microspectroscopy. The chemical and physicochemical studies on *E. ulmoides* yielded the important histological information that has not been obtained from the Para rubber tree which produces latex rubber. It was revealed that *E. ulmoides* does not have a specific organ, such as the so-called laticifer in the Para rubber tree, but accumulates fibrous rubber in intercellular spaces. This microspectroscopic analysis has high resolution and is a very useful tool for the in situ identification of polyisoprene.

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**REFERENCES**


FIGURES

Figure 1. Oil Red O staining of an E. ulmoides stem cross-section (a) and a longitudinal section (b). E Epidermis, C cambium; P pith
Figure 2. FT-IR microspectroscopy analyses at various sites on a longitudinal section of an *E. ulmoides* stem. *E* Epidermis, *F* fibrous structures, *N* non-fibrous structures, *C* vicinity of cambium. Triolein is a model compound of fatty acid triglyceride. *Arrows* in the spectrum of an authentic sample indicate the absorptions specific to *trans*-polyisoprene.

Figure 3. *In situ* line mapping by FT-IR microspectroscopy in a longitudinal section of an *E. ulmoides* stem. *E* Epidermis, *C* cambium.
HEAVY-ION BEAM MUTAGENESIS FOR HERBICIDE RESISTANT PLANTS.

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ABSTRACT

Heavy-ion beams are very effective tools of mutagenesis for seed embryos at a fertilization stage. This new technique was subjected to screening of herbicide-resistant mutants, using tobacco plants (*Nicotiana tabacum* L. cv. BY-4). Intact embryos were irradiated with N ions (135-MeV/nucleon, LET value is 28.5 keV/µm), and the plants were allowed to grow until M1 seed maturation and harvested. M1 plants were used in the resistance test against S-23142 (N-[4-chloro-2-fluoro-5-propargyl-oxy-phenyl]-3,4,5,6-tetrahydrophthalimide) and BZB-OH (3-(2-Chloro-4-mesylbenzoyl)-2-phenylthiobicyclo[3.2.1]oct-2-en-4-one hydrolysate).

M1 plants carrying dark green leaves were used for screening resistance against a photo-bleaching herbicide, S-23142. S-23142 inhibits protoporphyrinogen oxidase (Protox), which is the terminal enzyme of the common branch of heme and chlorophyll biosynthetic pathways that oxidizes protoporphyrinogen IX (Protogen) to protoporphyrin IX (Proto IX). Protogen is accumulated as a result of the inhibition of Protox by the herbicide, and leaks out of chloroplasts. Then a Protogen oxidizing activity that is located at the plasma membrane oxidizes Protogen into Proto IX. The herbicidal action is due to the accumulation of Proto IX, a strong phytotoxic photosensitizer bleaching pigments in chloroplasts. Young leaves of M1 plants were treated with a solution of S-23142 and were grown at 25°C under light for 2 days. Two resistant plants was selected from 94 M1 plants. And leaves of one strain were not bleached by 100µM S-23142, while wild-type leaves were suffered bleaching at 1µM S-23142. However Protox activity in the stromal fraction showed no difference between wild-type and the tolerant plant. The results suggest that the mechanisms of resistance include reduction of uptake of the herbicide or rapid metabolic destruction of excess Proto IX.

M1 plants were used in the resistance test BZB-OH, a herbicide inhibiting 4-hydroxyphenylpyruvate dioxygenase which is a key enzyme of the new bleaching herbicide families, sulcotrione and isoxasol. M1 seeds were placed on 1/2 MS medium containing 0.5 µM BZB-OH, and then were incubated for 4 weeks. BZB-OH-tolerant seedlings capable of germination under this condition were selected and planted in soil. A total of 23 BZB-OH-tolerant plants were selected from 5840 M1 seeds. Two strains of M2 progeny showed strong tolerance. Extremely low concentrations (0.01-0.5µM) of BZB-OH inhibited the biosynthesis of colored carotenoid in the wild-type plants, however the resistant plants of M3 progeny showed the effects at 0.5-1µM. The resistant strains were 2 times more tolerant to BZB-OH and sulcotrione than wild-type plants. In contrast, they were not resistant to norflurazon, an inhibitor of phytoene desaturase. Mutants induced with ion-beam irradiation have potential not only for practical use in the breeding of chemical resistant plants but also for gene analysis which will surely facilitate the molecular understanding of the mechanisms of resistance.

ANALYSIS OF GIBBERELLINS IN A TOMATO *(LYCOPERSICON ESCULENTUM)*
MUTANT WITH SHORT INTERNODE INDUCED BY SEED RADIATION WITH CARBON ION BEAM

H. Yamazaki¹ and M. Masuda²

ABSTRACT

Analysis of gibberellins (GAs) was conducted in a tomato mutant with short internode induced by radiation with carbon ion beam to seeds ‘First’. Stem length of the mutant was 60% shorter than that of wild type ‘First’, while there was little difference in leaf length between these two plants. Stem elongation of the mutant was severely inhibited especially in the internodes above the first true leaf. C-13-hydroxylated GAs (GA₁₉, GA₂₀, GA₂₉, GA₄₄, GA₅₃ and GA₉₇) and C-13-non-hydroxylated GAs (GA₉, GA₁₂, and GA₁₅) were identified in stems of the mutant by GC-MS. Stems of ‘First’ contained the same classes of GAs. Only C-13-hydroxylated GAs including GA₁ were identified in leaves of the mutant and ‘First’. There was little difference in GA-like activity in either stems or leaves estimated by the dwarf rice micro-drop assay between these two plants. As a result of quantitative analysis by GC-MS, the concentration of GAs in stems and leaves of the mutant tended to be higher than that of ‘First’. These results suggest that the mutant has the ability to produce GAs comparable to ‘First’. It is unlikely that internode shortening of the mutant is caused by the malfunction of GA biosynthesis.

INTRODUCTION

Mutation has been widely used for crop improvement. Many available mutants have been produced via radiation of gamma rays. Recently, radiation of ion beam is expected as a new technique for obtaining novel mutants since mutagenesis mechanism of ion beam is different from that of gamma rays. A new type of mutant in *Arabidopsis thaliana* has been produced by radiation of ion beam (Tanaka et al., 1997).

Masuda et al. (2003) have investigated the availability of ion beams as a mutagen in tomato, and obtained a mutant with short internode by the irradiation of carbon ion beam to tomato ‘First’ seeds. ‘First’ is high yielding, but lacks resistance to many damaging biotic and abiotic stresses. Short internode would be useful for production of indeterminate tomato cultivars with long harvesting period, because it can save training/harvesting labor (Yanokuchi and Okamoto, 2001).

GAs play an important role in the regulation of plant elongation. Several dwarf mutants of maize (Phinney, 1961), rice (Suge, 1990) and barley (Suge, 1983) are known to have lower concentration of GAs than corresponding normal plants. The tomato mutant with short internode has response to applied GA₃, and the short internode character is controlled by a single recessive nuclear gene (Masuda et al, 2002; Yuasa at al., 2003). In this paper, qualitative and quantitative analysis of GAs in the mutant with short internode was conducted to clarify the relationship between endogenous GAs and internode shortening of the mutant.

MATERIALS AND METHODS

A mutant with short internode was induced from tomato ‘First’ irradiated with 50 Gy of 220 MeV $^{12}$C⁵⁺ ion beam to their seeds (Masuda et al., 2003). Seeds of mutant (M5) and wild type ‘First’ were

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sown in 12-cm diameter pots filled with commercial soil on Dec. 5, 2002, and grown in a glasshouse in
which minimum temperature was maintained above 15C. At the 7th true leaf stage (Jan. 31, 2003),
leaves and stems of the mutant and ‘First’ were collected for the qualitative (50 - 100 g in fresh weight)
and quantitative (5 - 10 g in fresh weight) GAs analysis. Growth of the seedlings was investigated at
the same time.

In the qualitative analysis, GAs in stems and leaves were extracted with 80% aqueous methanol
and purified according to the method as described by Yamazaki et al. (2002). After fractionation by
reversed phase HPLC (32 fractions), GA-like activity in each fraction was estimated by the modified
dwarf rice micro-drop assay (Nishijima and Katsura, 1989). The GA-active fractions were methylated
followed by trimethylsilylation, and analyzed by GC-MS according to the method as described by
Yamazaki et al. (2002). GAs were identified from Kovats retention indexes (KRI) and mass spectra in
comparison with authentic GAs. Some GAs which we did not have authentic ones were tentatively
identified with reference to KRI and mass spectra data of GAs reported by Gaskin and Macmillan

Out of the identified GAs, the concentration of GA1, GA19, GA20, GA44 and GA53 was determined.
GAs in the tissues were extracted with 80% aqueous methanol and purified by the same method as that
for the qualitative analysis. The extraction solvent was spiked with [2H2]GA1, [2H2]GA19, [2H2]GA20,
[2H2]GA44 and [2H2]GA53 as internal standards. After the purification, the concentration of these GAs
was measured by GS-MS.

RESULTS AND DISCUSSION

The mutant seedlings had very short stem and thick stem diameter (Table 1). The stem elongation
was excessively inhibited in internodes above the first true leaf (26% of ‘First’ on the average), while
the shortening of hypocotyl was not so severe (Fig. 1). There was little difference in leaf length and
leaf number between these two plants.

The extract from stems of the mutant and ‘First’ contained several components with GA-like
activity (Fig. 2). By GC-MS analysis in stems of the mutant, six C-13-hydroxylated GAs (GA19, GA20,
GA29, GA44, GA53 and GA97) and three C-13-non-hydroxylated GAs (GA9, GA12, and GA15) were
identified from seven zones of HPLC fractions (Table 2 and Fig. 2). Stems of ‘First’ contained the
same classes of GAs. In leaves of the mutant, four C-13-hydroxylated GAs (GA19, GA20, GA29 and
GA44) were identified from four zones of HPLC fractions (Fig. 3). In addition to these GAs, 3-epi GA1
and GA17 were identified in leaves of ‘First’ (Table 2 and Fig. 3).

In most of the HPLC fractions, the GA-like activity in stems of the mutant was similar to or
higher than that of ‘First’ (Fig. 2). The similar result was obtained in the GA-like activity in leaves
(Fig. 3). According to the quantitative analysis by GC-MS, the concentrations of GAs in both stems
and leaves of the mutant were similar to or higher than that of ‘First’ (Table 3). The concentrations of
GA44 and GA53 in stems and of GA20 in leaves of the mutant were significantly higher than those of
‘First’. These results indicate that the mutant has GA-biosynthetic ability comparable to wild type
‘First’. Predominant GAs in stems of the mutant and ‘First’ were GA53, GA44 and GA19, which are
located at early steps of the early C-13 hydroxylation pathway, while the leaves contained GA1 and
GA20 at a relatively high level.

GA is one of the most important determinants of plant height. It has been known that dwarfism in
several crop plants is caused by defect in GA biosynthesis (Suge, 1990). Generally, 3β-OH-GAs, such
as GA1 and GA4, are considered as physiologically active GAs. GA-deficient mutants have trouble in
some step of the biosynthetic pathway to produce active GAs. We expected that the short-internode
phenotype in the tomato mutant was associated with malfunction of GA biosynthesis. Contrary to the
expectation, however, there was little difference in the concentration of endogenous GAs between the
mutant and wild type ‘First’. In this experiment, the concentration of active GAs in stems was not
determined because we could not identify them in stems of either the mutant or ‘First’. Although active GAs might be existed in the stems at a very low level, their levels would be too low to cause the difference in stem elongation between these two plants. As a result of the sensitive micro-drop assay, there was no difference in the GA-like activity in the HPLC fractions corresponding to the retention time of GA1 and GA4 (Fr. 2-3 for GA1, Fr. 22-23 for GA4) between these two plants (Fig. 2). From these results, it is not unlikely that internode shortening of the mutant is attributed to malfunction of GA biosynthesis.

**LITERATURE CITED**

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Suge H 1990 Survey of endogenous gibberellins in rice lines having different dwarfing genes and in autotetraploid lines. Japan J Breed 40:21-31

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*Significance*

ns, *, ** Not significant or significant by *t*-test at *P*=0.05 or 0.01, respectively.

*a* Diameter under the first true leaf.

*b* Length of the fourth true leaf.
Table 2. C-13-hydroxylated (A) and C-13-non-hydroxylated (B) gibberellins identified by GC-MS in stems and leaves of wild type tomato ‘First’ and a mutant with short internode.

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<tr>
<td>Wild type</td>
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</table>

+ : identified, - : not identified.
a tentatively identified.

Table 3. Gibberellins concentrations of wild type ‘First’ and a mutant seedlings with short internode. Values are means of three replications.

<table>
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<td></td>
</tr>
<tr>
<td>Wild type</td>
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<td></td>
</tr>
<tr>
<td>Significance</td>
<td>ns ns *</td>
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</table>

ns, *, ** Not significant or significant by t-test at P=0.05 or 0.01, respectively.
a Not identified.
GIBBERELLINS INDUCE $\alpha$-AMYLASE AND CONCERN THE DEVELOPMENT OF COTYLEDON IN IMMATURE SEEDS OF *P. NIL*

Masatoshi Nakajima$^1*$, Akira Nakayama$^1$, Zheng-Jun Xu$^2$, and Isomaro Yamaguchi$^1$

**ABSTRACT**

Immunohistochemical analyses for active gibberellins (GAs) and GA-responsive $\alpha$-amylase, PnAmy1, showed that they were localized around starch granules in integument of developing seeds of morning glory (*Parbitis nil* cv. violet), and that the appearance of active GAs preceded to that of PnAmy1. Following the disappearance of starch granules, development of cotyledon became clear, which suggests GAs possibly concern the development of cotyledon through the induction of $\alpha$-amylase in the seeds. We also studied the expression pattern of the *PnAmy1* gene by *in situ* hybridization, which showed signals were detected tissue-specifically in seed coat, where no signal for PnAmy1 was detected by immunohistochemistry. Then we pursued *in situ* hybridization and RNA blot analysis for GA biosynthetic genes to identify the tissue and the period that active GAs were synthesized at in the developing seed. We cloned the cDNA homologs of GA 20-oxidase and GA 3-oxidase from immature seeds with conventional degenerate-PCR and RACEs. Signals of these GA biosynthetic genes were not observed in integument but in seed coat by *in situ* hybridization. Their expression patterns overlapped with that of *PnAmy1* gene spatially and timely, suggesting that both GAs and PnAmy1 are synthesized in seed coat and then secreted to the neighboring integument selectively. The *in situ* hybridization analyses with various developing seeds also revealed that the expressions of the genes all started near the joint zone with placenta, as if some stimuli for their expressions came into the developing seeds via the zone.

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MOLECULAR PHENOTYPING OF ARABIDOPSIS WILD TYPE AND abi5 MUTANT DURING IMBIBITION BY MICROARRAY COMPREHENSIVE EXPRESSION ANALYSIS

Kazumi Nakabayashi1, Yuji Kamiya and Eiji Nambara

ABSTRACT

Abscisic acid (ABA) plays a crucial role in seed maturation and dormancy. Recent studies identified several loci encoding transcription factors that are involved in ABA signaling in the seed. However, only fragmentary information has been reported on how the expression profiles differ in those mutants. Loss-of-function of ABI5 results in reduced ABA sensitivity in the seed and ABI5 is one of the bZip transcription factors that binds to ABRE (ABA responsive element). We have performed molecular characterization of WT and the abi5 mutant by microarray expression analysis. A statistical search for any cis-element in highly expressed genes in WT dry seed revealed one of the most typical ABRE that contains ACGT-core sequence. More detailed distribution analysis with the genes having 2-copy ABRE in the promoter showed an enhanced effect of the ABRE on high-level expression. Combinational analysis of ABRE with another cis-element CE (Coupling element) also showed a more apparent effect for high expression in dry seed. On the other hand, the correlation between high-level expression and ABRE diminished in imbibed seeds. This result is consistent with the endogenous ABA level that increases during seed maturation and dramatically decreases upon imbibition. In abi5 dry seed, the correlation between ABRE and high expression level is largely weakened. Furthermore, the same ABRE was identified from down-regulated genes in abi5 dry seed. These results indicate that the selected ABRE is one of the main elements that provides ABA inducibility during seed maturation, and ABI5 plays an important role on expression of those genes. In this way, microarray comprehensive analysis is useful for molecular phenotyping of the mutants verifying in vitro analysis in an in vivo situation.

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CLONING AND CHARACTERIZATION OF TWO SOLANESYL DIPHOSPHATE SYNTHASES FROM *ARABIDOPSIS*

Kazutake Hirooka¹, Yoshikazu Izumi, Takeshi Bamba, Ei-ichiro Fukusaki, and Akio Kobayashi

EXPANDED ABSTRACT

*trans*-Long-chain prenyl diphosphate synthases catalyze the sequential condensation of isopentenyl diphosphate (C₅) units with allylic diphosphate to produce the C₃₀-C₅₀ prenyl diphosphates, which are precursors of the side chains of prenylquinones. Based on the relationship between the product specificity and the region around the first aspartate-rich motif in *trans*-prenyl diphosphate synthases so far characterized, we have isolated two cDNAs for a member of *trans*-long-chain prenyl diphosphate synthases from *Arabidopsis thaliana*. Each of the cDNAs was heterologously expressed in *Escherichia coli*, and the recombinant His₆-tagged proteins were purified and characterized. Product analysis revealed that both of the cDNAs encode solanesyl diphosphate (C₄₅) synthases (At-SPS1 and At-SPS2). At-SPS1 and At-SPS2 utilized farnesyl diphosphate (FPP, C₁₅) and geranylgeranyl diphosphate (GGPP, C₂₀) but did not accept either dimethylallyl diphosphate (C₅) or geranyl diphosphate (C₁₀) as an allylic substrate. Kinetic analysis indicated that both of At-SPS1 and At-SPS2 prefer GGPP to FPP as the allylic substrate. We also analyzed the subcellular localization of At-SPS1 and At-SPS2 by transient expression of the green-fluorescent-protein (GFP) fused proteins in the protoplasts prepared from the *A. italiana* leaves. As a result, the GFP-fused At-SPS1 was localized in endoplasmic reticulum and the GFP-fused At-SPS2 was localized in chloroplast, which suggest that At-SPS1 is devoted for the biosynthesis of ubiquinone side-chain and that At-SPS2 participates in the biosynthesis of plastoquinone side-chain.

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MORPHOLOGICAL AND HORMONAL RELATIONSHIPS IN SHOOTS OF PILLAR AND STANDARD PEACH TREES

Thomas Tworkoski

ABSTRACT

Fruit tree canopy size and shape are managed genetically and culturally to enable high density plantings and to facilitate orchard operations such as pest control. In peach trees (Prunus persica) different canopy shapes can be attained with scions of genetically-selected tree growth habits when grafted to seedling rootstock. Scion of Pillar peach tree growth habits have narrow canopies that may be amenable to high density plantings. Research is being conducted to determine relationships between morphological characteristics and auxin and cytokinin quantities in shoots of Pillar and Standard peach trees. Pillar and Standard ('Harrow Beauty') peach scion was budded to seedling rootstock and planted at the Appalachian Fruit Research Station in Dec. 1998. Bud break and growth components of 1-yr-old stems were measured on 28 March 2002. Branch angle and current-yr growth was then measured during the 2002 growing season. Auxin and cytokinin concentrations were measured in current-yr growth during the 2003 growing season. For auxin analysis, samples were extracted overnight with 80% methanol (fortified with stable isotope of indole-3-acetic acid as internal standard), dried, separated against ethyl acetate (pH 8, retaining aqueous phase), slurried with PVPP, decanted, separated on C18 (pH 3) columns, methylated with ethereal diazomethane, and quantified by gas chromatography-mass spectrometry (GC-MS), correcting for losses with the internal standard. For cytokinin analysis, samples were extracted overnight with 80% methanol (fortified with stable isotopes of trans-zeatin, isopentenyladenosine, dihydrozeatin, isopentenyladenine, trans-zeatin riboside, dihydrozeatin riboside, and trans-zeatin-9-glucoside as internal standards), dried, digested with acid phosphatase (EC 3.1.3.2) to convert nucleotides to nucleosides, loaded to a strong anion exchange column in series with a C18 column, eluted from the C18, loaded to strong cation exchange column (pH 3), eluted with 2 M ammonia in methanol, permethylated with dimethylsulphinyl carbanion and quantified by GC-MS, correcting for losses with the internal standards. As the 2002 season progressed, growth characteristics in Pillar and Standard trees appeared to be associated with hormone differences. Similar bud break patterns indicated weak apical dominance within a 1-yr-old shoot of either growth habit. Slower growth rates and longer distances from terminal buds to first sylleptic branch indicated a hormone-mediated apical control in Pillar trees. Orthotrophic growth also suggested an auxin-mediated response while delayed senescence may indicate a cytokinin effect as the growing season progressed. In 2003, no difference in auxin concentrations occurred in either growth habit early in the season but greater shoot auxin concentrations were found in Pillar than Standard trees as the season progressed. This supports the finding of weak apical dominance at bud break but more pronounced apical control (e.g. more orthotrophic growth and less sylleptic growth) in Pillar than Standard trees as the season progressed. Lower cytokinin/auxin ratios in Pillar shoots may have contributed to reduced sylleptic growth. Currently, research is being conducted to compare genotypes and establish the management practices for high density Pillar peach tree plantings.

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PRODUCTION OF SEEDLESS FRUITS IN SOME DATE PALM CULTIVARS

M.A. Shaheen¹

ABSTRACT

The effect of spraying unpollinated flowers of date palm cultivars (Nebut-Saeif, Sakaie, Seleg and Khudari) with GA₃, IAA and 2,4,5-T at various concentrations of single or combined regulators and in one or two sprays on the production of seedless fruits was studied.

Different cultivars responded to the treatments at various degrees. The treatments resulted in the production of seedless fruits, but the percentages obtained differed greatly with the concentration and the number of sprays. Most of the seedless fruits maintained color characteristic of the Khalal stage. Few of the seedless fruits reached the rutab stage. Weight of the seedless fruits was relatively less as compared with the normal fruits.

INTRODUCTION

Date palm (Phoenix dactylifera L.) is one of the oldest known and cultivated fruit trees in Saudi Arabia. It has both economic and cultural impacts on the lives of native inhabitants. With more than 14 million date palm trees Saudi Arabia is considered as one of the most important date producing countries. The tree is very well suited to environmental condition characteristics on almost all the regions of the kingdom.

As with many other plants, the use of plant growth regulators in date palm may increase the yield and induce seedless fruits. A good seedless date, approaching the pollinated one in quality, would be highly desirable either as a fresh fruit or for various purposes of date processing.

Few investigations were carried out in this respect, and their results were not promising. Thus, Sharpless and Hilgeman (1950) studied the effect of synthetic hormones (2,4-D, NAA and IBA at the rate of 20 and 500 ppm) on growth and ripening of "Sayer" date palm fruits. The treatments were applied on three dates representing different stages of fruit development. They concluded that the chemicals and concentrations used had no stimulatory effect on the growth and ripening of normally pollinated fruits in this cultivar.

Nixon (1959) applied GA₃ to 'Medjool', 'Deglet Noor', 'Halawy', 'Khadrawy' and 'Barhee' date palm fruits and found an increase in length of Deglet Noor fruits, but the effect on length of other cultivars was variable. Ketchie (1967) showed that GA₃ (20,50 and 100 ppm) applied to pollinated bunches produced larger fruit than on checks. This effect on unpollinated bunches was not certain. Seedless dates were obtained in unpollinated bunches treated with GA₃. Abd-Alaal et al. (1982) found that the use of 2,4-D, 2,4,5-T, 2,4,5-TP, IAA and GA₃ at the concentrations of 25-100 ppm resulted in formation of seedless dates in the "Khadrawi" date palm cultivar. The seedless dates were somewhat similar to the normal dates in size and shape. Abou Aziz et al. (1982) reported that unpollinated spadices treated once or twice with 50 or 100 ppm GA₃ produced some seedless fruits lighter in weight, longer and thinner than seeded fruits. Hussein et al. (1974) reported that GA₃ applied to pollinated fruits of "Barhi" dates had a very small effect on fruit physical characteristics.

The present investigation was carried out to determine the effect of GA₃, IAA and 2,4,5-T on inducing seedless fruits in four date palm cultivars.

¹ Department of Arid Land Agriculture, Faculty of Meteorology, Environment & Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia
MATERIALS AND METHODS

The four cultivars used; were 'Nebut-Seif', 'Sakaie', 'Seleg' and 'Khudari'. These cultivars are grown at commercial grove Riyadh area. Vigorous and almost uniform in size trees were chosen. Three trees per cultivar were used. The spathes formed on each tree were thinned to eight. The spathes were covered with paper bags before cracking. After cracking, the flower strands were thinned to 60 strands/spathe, then they were divided into three groups of 20 strands each. These groups were randomly treated with the different growth regulators used. Two different concentrations were used for each growth regulator. GA3 and IAA were used at the concentrations of 50 or 100 ppm, whereas, 2,4,5-T at 10 or 20 ppm. The growth regulators were used either alone or in different combinations and were applied either once or twice. In the two application treatments, the second application was applied one month after the first (Table 1).

At harvest time, the number of parthenocarpic fruits was counted, and the percentage of these parthenocarpic fruits was calculated for each treatment. The average weight of the fruit was determined.

Data were analyzed statistically using the analysis of variance. L.S.D. was calculated to test differences among treatment means (Snedecor and Cochran 1967).

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</thead>
<tbody>
<tr>
<td>1</td>
<td>GA3 50 ppm</td>
<td>Once</td>
</tr>
<tr>
<td>2</td>
<td>GA3 100 ppm</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IAA 50 ppm</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IAA 100 ppm</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,4,5-T 10 ppm</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2,4,5-T 20 ppm</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>GA3 + IAA (50 + 50 ppm)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>GA3 + 2,4,5-T (50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>IAA + 2,4,5-T (50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>GA3 + IAA + 2,4,5-T (50 + 50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Pollinated</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>GA3 50 ppm</td>
<td>Twice</td>
</tr>
<tr>
<td>14</td>
<td>GA3 100 ppm</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>IAA 50 ppm</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>IAA 100 ppm</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>2,4,5-T 10 ppm</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>2,4,5-T 20 ppm</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>GA3 + IAA (50 + 50 ppm)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>GA3 + 2,4,5-T (50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>IAA + 2,4,5-T (50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>GA3 + IAA + 2,4,5-T (50 + 50 + 10 ppm)</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Distilled water</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Unpollinated</td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Nebut-Seif

The production of seedless fruits with the use of different growth regulators differed with the treatment. A single application of 50 ppm IAA + 10 ppm 2,4,5-T gave the highest percentage of seedless fruits (47.00%). Two applications each of treatments 50 ppm GA3, 50 ppm IAA, 100 ppm GA3 or 50 ppm GA3 + 10 ppm 2,4,5-T gave percentages of seedless fruits relatively less than the previous treatment. These percentages were 43.27, 42.30, 40.80 and 40.77 %, respectively (Table 2).

Weight of seedless fruits was also affected by the treatment and by the number of sprays. The two spray treatments, generally, produced fruits heavier in weight than the single spray treatments. Results showed that treatments with GA3 (50 ppm and 100 ppm), IAA (50 ppm), GA3 + 2;4,5-T (50 ppm + 10 ppm, respectively), IAA (50 ppm) + 2,4,5-T (10 ppm) induced parthenocarpic fruits heavier in weight than in other treatments. The average fruit weight relevant to the above treatments ranged between 9.33 and 10.47 g/fruit (Table 2).

Table 2. Effect of some growth regulators on the production of seedless fruit (%) and fruit weight (g) in “Seleg” date palm cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Seedless fruits (%)</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of sprays</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once</td>
<td>Twice</td>
</tr>
<tr>
<td>GA3</td>
<td>50</td>
<td>1.37</td>
<td>1.47</td>
</tr>
<tr>
<td>GA3</td>
<td>100</td>
<td>0.84</td>
<td>1.15</td>
</tr>
<tr>
<td>IAA</td>
<td>50</td>
<td>12.73</td>
<td>8.77</td>
</tr>
<tr>
<td>IAA</td>
<td>100</td>
<td>0.65</td>
<td>0.43</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>10</td>
<td>0.39</td>
<td>5.70</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>20</td>
<td>0.32</td>
<td>3.57</td>
</tr>
<tr>
<td>GA3 + IAA</td>
<td>50 + 50</td>
<td>5.57</td>
<td>2.30</td>
</tr>
<tr>
<td>GA3 + 2,4,5-T</td>
<td>50 + 10</td>
<td>0.30</td>
<td>6.82</td>
</tr>
<tr>
<td>IAA + 2,4,5-T</td>
<td>50 + 10</td>
<td>5.50</td>
<td>5.51</td>
</tr>
<tr>
<td>GA3 + IAA + 2,4,5-T</td>
<td>50 + 50 + 10</td>
<td>9.73</td>
<td>9.33</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pollinated</td>
<td></td>
<td>----</td>
<td>0</td>
</tr>
<tr>
<td>Unpollinated</td>
<td></td>
<td>----</td>
<td>0</td>
</tr>
<tr>
<td>L.S.D.</td>
<td>5 %</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS: Not significant

Sakaie

Results showed that this cultivar was greatly affected with the treatments and that one spray treatment with IAA at 50 ppm was highly effective in the induction of seedless fruits which reached 79.12%, followed by one-spray treatment with GA3 (50 ppm) + IAA (50 ppm) which induced 56.67% seedless fruits. The two-spray GA3 (100 ppm) treatment and the two-spray treatment IAA (50 ppm) induced seedless fruits at lower percentages than the above treatments (54.43% and 49.00%, respectively). The rest of the treatments were less effective on induction of seedless fruits and ranged from 18.27 to 42.67% (Table 3).
Fruit weight was also affected with the treatment and with the number of sprays. However, in 11 of the different treatments the fruit weight averaged more than 15 g/fruit which was close to the average fruit weight of hand-pollinated fruits (Table 3).

**Table 3.** Effect of some growth regulators on the production of seedless fruit (%) and fruit weight (g) in “Sakaie” date palm cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Number of sprays</th>
<th>Seedless fruits (%)</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Once</td>
<td>Twice</td>
<td>Once</td>
</tr>
<tr>
<td>GA$_3$</td>
<td>50</td>
<td>26.30</td>
<td>4.23</td>
<td>15.76</td>
</tr>
<tr>
<td>GA$_3$</td>
<td>100</td>
<td>42.67</td>
<td>54.43</td>
<td>14.30</td>
</tr>
<tr>
<td>IAA</td>
<td>50</td>
<td>79.17</td>
<td>49.00</td>
<td>17.90</td>
</tr>
<tr>
<td>IAA</td>
<td>100</td>
<td>21.63</td>
<td>31.03</td>
<td>17.33</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>10</td>
<td>20.90</td>
<td>30.00</td>
<td>15.10</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>20</td>
<td>19.40</td>
<td>18.27</td>
<td>11.03</td>
</tr>
<tr>
<td>GA$_3$ + IAA</td>
<td>50 + 50</td>
<td>56.67</td>
<td>34.97</td>
<td>13.56</td>
</tr>
<tr>
<td>GA$_3$ + 2,4,5-T</td>
<td>50 + 10</td>
<td>39.20</td>
<td>22.57</td>
<td>15.73</td>
</tr>
<tr>
<td>IAA + 2,4,5-T</td>
<td>50 + 10</td>
<td>29.93</td>
<td>27.67</td>
<td>12.47</td>
</tr>
<tr>
<td>GA$_3$ + IAA + 2,4,5-T</td>
<td>50 + 50 + 10</td>
<td>19.57</td>
<td>19.57</td>
<td>----</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
<td>15.26</td>
</tr>
<tr>
<td>Unpollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
<td>----</td>
</tr>
<tr>
<td>L.S.D.</td>
<td></td>
<td>5 %</td>
<td>29.19</td>
<td>1 %</td>
</tr>
</tbody>
</table>

NS: Not significant

**Seleg**

The results showed that this cultivar was the least responsive to the treatments with growth regulators (Table 4). The percentage of seedless fruits did not exceed 12.73 % in any of the treatments. Fruit weight was considerably reduced as compared with normally pollinated fruits (Table 4).
Table 4. Effect of some growth regulators on the production of seedless fruit (%) and fruit weight (g) in “Khudari” date palm cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Seedless fruits (%)</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of sprays</td>
<td>Number of sprays</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once</td>
<td>Twice</td>
</tr>
<tr>
<td>GA₃</td>
<td>50</td>
<td>1.93</td>
<td>6.13</td>
</tr>
<tr>
<td>GA₃</td>
<td>100</td>
<td>6.70</td>
<td>2.53</td>
</tr>
<tr>
<td>IAA</td>
<td>50</td>
<td>16.30</td>
<td>4.53</td>
</tr>
<tr>
<td>IAA</td>
<td>100</td>
<td>0.67</td>
<td>6.60</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>10</td>
<td>0.67</td>
<td>2.27</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>20</td>
<td>0.90</td>
<td>0.39</td>
</tr>
<tr>
<td>GA₃ + IAA</td>
<td>50 + 50</td>
<td>3.97</td>
<td>16.07</td>
</tr>
<tr>
<td>GA₃ + 2,4,5-T</td>
<td>50 + 10</td>
<td>0</td>
<td>11.37</td>
</tr>
<tr>
<td>IAA + 2,4,5-T</td>
<td>50 + 10</td>
<td>4.30</td>
<td>3.20</td>
</tr>
<tr>
<td>GA₃ + IAA + 2,4,5-T</td>
<td>50 + 50 + 10</td>
<td>4.00</td>
<td>38.23</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unpollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>L.S.D.</td>
<td></td>
<td>17.80</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.79</td>
<td>2.13</td>
</tr>
</tbody>
</table>

NS: Not significant

Khudari

The response of this cultivar to treatments with GA₃, IAA and 2,4,5-T was very little (Tables 5). The highest percentage of seedless fruits obtained was 38.23 % in the two-spray combined treatment of GA₃ (50 ppm) + IAA (50 ppm) + 2,4,5-T (10 ppm). The average fruit weight was less as compared with the normal pollinated fruits (Table 5).

Table 5. Effect of some growth regulators on the production of seedless fruit (%) and fruit weight (g) in “Nebut Seif” date palm cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (ppm)</th>
<th>Seedless fruits (%)</th>
<th>Fruit weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number of sprays</td>
<td>Number of sprays</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once</td>
<td>Twice</td>
</tr>
<tr>
<td>GA₃</td>
<td>50</td>
<td>38.10</td>
<td>43.27</td>
</tr>
<tr>
<td>GA₃</td>
<td>100</td>
<td>34.67</td>
<td>40.80</td>
</tr>
<tr>
<td>IAA</td>
<td>50</td>
<td>27.33</td>
<td>42.30</td>
</tr>
<tr>
<td>IAA</td>
<td>100</td>
<td>30.60</td>
<td>32.80</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>10</td>
<td>27.10</td>
<td>34.20</td>
</tr>
<tr>
<td>2,4,5-T</td>
<td>20</td>
<td>27.50</td>
<td>33.63</td>
</tr>
<tr>
<td>GA₃ + IAA</td>
<td>50 + 50</td>
<td>29.47</td>
<td>26.00</td>
</tr>
<tr>
<td>GA₃ + 2,4,5-T</td>
<td>50 + 10</td>
<td>32.77</td>
<td>40.77</td>
</tr>
<tr>
<td>IAA + 2,4,5-T</td>
<td>50 + 10</td>
<td>47.00</td>
<td>36.10</td>
</tr>
<tr>
<td>GA₃ + IAA + 2,4,5-T</td>
<td>50 + 50 + 10</td>
<td>16.63</td>
<td>16.87</td>
</tr>
<tr>
<td>Distilled water</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unpollinated</td>
<td>----</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Concerning fruit maturity, it was observed that seedless fruits maintained a colour similar to Khalal stage (red in cv. Kudari and yellow in the other three cvs.). It was also noted that few seedless fruits reached the rutab stage (Fig. 1).

**DISCUSSION**

The results of the foregoing experiments indicated that spraying unpollinated flowers of the Nebut-Seif, Sakaie, Khudari and Seleg cultivars with GA₃, IAA and 2,4,5-T could be successful in inducing seedless fruits.

The results obtained in the present investigation concerning the induction of seedless fruits using growth regulators are more or less similar in trend to previous investigations (Abou Aziz et al. 1982 and Ketchie 1967). Abd-Alaal *et al.* (1982), working on the Khadrawi cv. in Iraq, ascribed the failure to obtain seedless fruits to the inadequacy of single sprays, since they succeeded in producing seedless fruits with three successive sprays, at monthly intervals using 2,4,5-T at the rate of 100 ppm.

Concerning fruit weight, it is indicated that seedless fruits generally tend to be less in weight as compared with normally pollinated fruits. This falls in line with the findings of Abou Aziz *et al.* (1982).
It was also observed that seedless fruits attained a colour similar to the Khalal stage. This again might be due to the inadequacy of the number of sprays and/or the concentration used (Abd Alaal et al. 1982).

SUMMARY AND CONCLUSIONS

From the foregoing discussion, the results together with those of other investigators clearly show that seedless fruits could be obtained by using different growth regulators. The response to the treatments varies with the cultivar and concentration. Trials in this respect should be carried out to determine the proper growth regulator, concentration and number of sprays for each cultivar before the treatment is recommended on a commercial scale.

REFERENCES


GROWTH CONTROL OF VEGETATIVE ANNUALS WITH ANCYMIDOL, FLURPRIMIDOL AND PACLOBUTRAZOL LINER DIPS

Brian E. Whipker1, Ingram McCall1, Brian Krug1, and Michelle Bell2

ABSTRACT

Commercially available plugs of 'Improved Charlie' vegetative petunia, 'Angel Mist Purple Improved' angelonia, 'Outback Purple' scaevola, and 'Twilight Blue with Eye' verbena were dipped for 10 minutes in 4, 8 or 16 mg·L\(^{-1}\) (ppm) solutions of ancymidol (0.264%; trademark A-Rest), flurprimidol (0.38%; trademark Topflor), or palcobutrazol (0.4%; trademark Piccolo). Ancymidol at concentrations up to 16 ppm were not effective for controlling growth of any of the four plant types trialed, therefore higher concentrations may be required. At the concentrations used (≤16 mg·L\(^{-1}\)), none of the PGRs (ancymidol, flurprimidol, or palcobutrazol) provided control of plant diameter of vegetative verbena. Higher concentrations of these PGRs or the use of uniconazole should be studied to determine optimal recommendations. Flurprimidol significantly controlled growth of angelonia and scaevola at 4 mg·L\(^{-1}\), while 8 mg·L\(^{-1}\) was required for vegetative petunia. Paclobutrazol provided similar control of plant diameter of vegetative petunia as flurprimidol, but higher concentrations would have to be used for comparable control on angelonia (>16 mg·L\(^{-1}\)) and scaevola (9.3 mg·L\(^{-1}\)). Although additional trials will need to be conducted to determine optimal concentrations, these results suggest flurprimidol and paclobutrazol liner dips are a suitable, cost-effective alternative for controlling plant growth of vigorous vegetative annuals.

INTRODUCTION

Plant growth retardants (PGRs) are commonly applied to container-grown plants to control stem elongation and produce high-quality, compact plants (Tayama et al., 1992). Limited scientific recommendations are available for growth control options for asexually propagated vegetative annuals. Commercial recommendations include foliar sprays of 10 to 25 mg·L\(^{-1}\) paclobutrazol [(2R, 3R+ 2S, 3S)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl) pentan-3-ol)] (Bonzi, Syngenta, Greensboro, NC) or 5 to 10 mg·L\(^{-1}\) uniconazole [(E)-1-(p-chlorphenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl-1-penten-3-ol)] (Sumagic, Valent USA, Marysville, OH)] (J. Williams, personal communication). Flurprimidol [((-1-methylethyl)-4-(trifluromethoxy)phenyl]-5-pyrimidinemethanol) (Topflor, SePRO, Carmel, IN)], has been trialed on osteospermum (Osteospermum ecklonis) (Olsen and Andersen, 1995), streptocarpus (Streptocarpus hybridus) (Pobudkiewicz, 2000), and Marguerite daisy (Argyranthemum frutescens) (Cavins et al., 2003). While PGR recommendations are available for finishing vegetative annuals, no scientific recommendations exist for a substrate soak prior to transplanting (liner dips). Therefore, this study was conducted to determine the efficacy of ancymidol, flurprimidol, and paclobutrazol liner dips on growth control of angelonia (Angelonia angustifolia), scaevola (Scaevola aemula), vegetative petunia (Petunia x hybrida), and vegetative verbena (Verbena x hybrida).

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2SePRO Corp., 11550 N. Meridian St., Carmel, IN 46032-4562.
MATERIALS AND METHODS

Commercially-available plugs (105 cell, with a cell size of 4 x 3 x 3.5 cm) of 'Improved Charlie' vegetative petunia, 'Angel Mist Purple Improved' angelonia, 'Outback Purple' scaevola, and 'Twilight Blue with Eye' verbena were thoroughly irrigated until the substrate was saturated and allowed to drain for 30 minutes. The root mass of the cuttings were then dipped for 10 minutes in 4, 8 or 16 mg·L⁻¹ (ppm) solutions of ancymidol, flurprimidol (0.38%), or paclobutrazol (0.4%; trademark Piccolo, Fine Agrochemicals, Worcester, UK). The cuttings were transplanted into 750-mL (12-cm diameter) round, plastic containers on 20 Feb. 2003 using Berger® BM6 root substrate (Berger Peat Moss, St. Modeste, Quebec, Canada), which contains 75 to 80% Canadian sphagnum peat and 20 to 25% perlite. Plants were fertigated with 150 mg·L⁻¹ N from Excel® 15-5-15 Cal-Mag (Scotts, Marysville, OH) (15N-2.1P-12.5K). Greenhouse temperature day/night set points were 24/18°C (75/65°F) and the plants were grown under natural daylength. The experiment was a completely randomized design with 6 single-plant replications of the ten treatments. Plant diameter (measured at the widest dimension, turned 90°, and averaged) and total plant height (measured from the pot rim to the uppermost part of the inflorescence) was recorded on 10 Apr. for angelonia. On 15 Apr., petunia, scaevola, and verbena plant diameter measurements were recorded. Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, NC) and means were separated by least significant differences at \( P \leq 0.05 \). Plant height and diameter values for flurprimidol and paclobutrazol were regressed using the PROC REG procedure (SAS Inst.) to determine the best-fit linear or quadratic model. Terms of the model were judged to be significant or nonsignificant and included in the final model based on a comparison of F values at \( \alpha = 0.05 \).

RESULTS AND DISCUSSION

**Angelonia.** Flurprimidol and paclobutrazol affected total plant height and plant diameter of angelonia (Fig. 1a&b). Plants treated with 4 mg·L⁻¹ flurprimidol were 23% shorter than the nontreated plants. The degree of height control with 16 mg·L⁻¹ paclobutrazol (14.5%) was less than with the flurprimidol application of 4 mg·L⁻¹. Higher concentrations of paclobutrazol should be studied to determine optimal concentrations. At the concentrations used (≤ 16 mg·L⁻¹), ancymidol did not provide control of plant height or diameter.

Plant diameter was 23.5% smaller with flurprimidol at 8 mg·L⁻¹ and 9.2% smaller with 8 mg·L⁻¹ paclobutrazol, compared to the nontreated control (Fig. 1b). The 8 mg·L⁻¹ flurprimidol liner dip resulted in greater control of plant diameter than paclobutrazol, which would offer an economic advantage for producing smaller diameter plants that can be spaced closer. This distributes the cost-per-square-foot-per-week of the greenhouse operation over more plants and results in lower per pot production costs.

**Vegetative Petunia.** Flurprimidol and paclobutrazol were similar in their effect on plant diameter (Fig. 2). Plants treated with 8 mg·L⁻¹ flurprimidol were 55.1 cm in diameter (15% smaller) and with 8 mg·L⁻¹ paclobutrazol were 55.8 cm in diameter (20% smaller), than the nontreated plants. At the concentrations used (≤ 16 mg·L⁻¹), ancymidol did not provide control of plant diameter.

**Vegetative Verbena.** At the concentrations used (≤ 16 mg·L⁻¹), none of the PGRs (ancymidol, flurprimidol, or paclobutrazol) provided control of plant diameter (data not shown). Higher concentrations of these PGRs or the use of uniconazole should be studied to determine optimal recommendations.
Scaevola. Both flurprimidol and paclobutrazol controlled plant diameter of scaevola (Fig. 3). Plants treated with 4 mg·L⁻¹ flurprimidol were 29.2% smaller than the nontreated plants. To achieve a similar degree of diameter control with paclobutrazol, 9.3 mg·L⁻¹ would be required. While the plants treated with 4 mg·L⁻¹ flurprimidol flowered and were proportional to pot size, higher concentrations stunted plant growth. These results were based on North Carolina growing conditions; therefore, concentrations would need to be adjusted for other locations or for growers desiring less control. Ancymidol at ≤16 mg·L⁻¹ did not provide control of plant diameter.

CONCLUSIONS

Flurprimidol significantly controlled growth of angelonia and scaevola at 4 mg·L⁻¹, while 8 mg·L⁻¹ was required for vegetative petunia. Paclobutrazol provided similar control of plant diameter of vegetative petunia as flurprimidol, but higher concentrations would have to be used for comparable control on angelonia (>16 mg·L⁻¹) and scaevola (9.3 mg·L⁻¹). At the concentrations used (≤16 mg·L⁻¹), none of the PGRs (ancymidol, flurprimidol, or paclobutrazol) provided control of plant diameter of vegetative verbena. Higher concentrations of these PGRs or the use of uniconazole should be studied to determine optimal recommendations. Ancymidol at concentrations up to 16 ppm were not effective for controlling growth of any of the four plant types trialed, therefore higher concentrations may be required. Although additional trials will need to be conducted to determine optimal concentrations, these results suggest flurprimidol and paclobutrazol liner dips are a suitable, cost-effective alternative for controlling plant growth of vigorous vegetative annuals.

ACKNOWLEDGEMENTS

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Tayama, HK, RA Larson, PA Hammer, and TJ Rolls, Eds. 1992 Tips on the use of chemical growth regulators on floriculture crops. Ohio Florists’ Assoc., Columbus, OH, 92 pp
Figure 1. The effect of flurprimidol (0.38% Topflor) and paclobutrazol (Piccolo) liner dips on 'Angel Mist Purple Improved' angelonia total plant height (a) and plant diameter (b). Plant height $r^2 = 0.77$ and 0.22, respectively, for flurprimidol and paclobutrazol. Plant diameter $r^2 = 0.61$ and 0.17, respectively, for flurprimidol and paclobutrazol.
Figure 2. The effect of flurprimidol (0.38% Topflor) and paclobutrazol (Piccolo) liner dips on 'Improved Charlie' vegetative petunia plant diameter. Plant diameter $r^2 = 0.48$ and 0.35, respectively, for flurprimidol and paclobutrazol.

Figure 3. The effect of flurprimidol (0.38% Topflor) and paclobutrazol (Piccolo) liner dips on 'Outback Purple' scaevola plant diameter. Plant diameter $r^2 = 0.76$ and 0.68, respectively, for flurprimidol and paclobutrazol.
COMPARISON OF BUNCHING ONION (Allium fistulosum L.) GROWTH UNDER HIGH-PRESSURE SODIUM AND COOL WHITE FLUORESCENT LAMPS

S.L. Edney¹*, N.C. Yorio¹, G.W. Stutte¹, O.A. Monje¹, and R.M. Wheeler²

ABSTRACT

A study comparing the growth of eight cultivars of onion (bunching onions or scallions, Allium fistulosum L.) was performed in separate tests in controlled environment chambers under either high-pressure sodium (HPS) or cool-white fluorescent (CWF) lamps. Air temperature, relative humidity, CO₂, PPF, and photoperiod were maintained for all tests at 23 ºC, 65%, 1200 µmol mol⁻¹, 300 µmol m⁻² s⁻¹, and 16-h light/8-h dark, respectively. Time-course growth measurements were made and final harvest was taken at 42 days after planting (DAP). Results showed that maximum biomass accumulation was not reached at the 42 DAP final harvest for any cultivar. This suggests that a longer cropping cycle may be necessary to achieve both maximum productivity and cropping efficiency under both lamp types. Only one cultivar, Deep Purple, showed a significant difference (P < 0.05) of edible dry mass and total dry mass between lamp types. This cultivar also had greater top mass and total dry mass under HPS than CWF. No effect on carbon partitioning (harvest index) was observed between lamp types for any variety. When data from both lamp types were combined and analyzed by ANOVA, cv. Guardsman had a greater plant height, edible dry mass, and total dry mass than all other cultivars. However, cv. Kinka appeared to be the best variety for use in potential space flight experiments because of its high germination rate, erect uniform growth and short plant height.

INTRODUCTION

The Advanced Life Support (ALS) Program at Kennedy Space Center (KSC) has been studying plants as components of bioregenerative life support systems for a number of years. Plants in these systems could provide food, oxygen, carbon dioxide removal and wastewater processing. However, near-term space missions such as the International Space Station (ISS) and early missions to Mars will be restricted in available area (volume) and electrical power, and the life support role of plant (crop) production systems will be limited. Still, the impact of plants on these early missions could be substantial in both enhancing diet diversity and providing the psychological benefit of fresh produce for the crew (1). Thus there is a need to study the effects of fresh food or “salad crop” production systems and their contribution to the diet and well being of crews living in confined habitats (2). Such studies could help assess the horticultural requirements for growing plants for supplemental food systems for the International Space Station (ISS), near-term Mars transit scenarios, and large-scale, integrated testbeds for ALS concepts (e.g., BIO-Plex) (3). To advance technology levels, baseline testing should be initiated to define horticultural approaches and environmental responses for these crops. Testing should include screening of cultivars using horticultural approaches and lighting concepts envisioned for near term ALS applications and test beds.

Several candidate “salad-crop” species have been studied extensively in controlled environments for ALS applications (4). Bunching onions (scallions) have recently been studied as one of the salad crop species. Tests evaluated the growth and development of eight commercial onion cultivars in a baseline environment typical of what might be used for a “salad crop” production system using high-

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MATERIALS AND METHODS

Plant Cultural Conditions: All plants were grown in plastic trays (0.25 m² growing area) using recirculating nutrient film technique culture. A modified ½-strength Hoagland’s solution was used. Solution pH was automatically controlled to near 5.8 with additions of dilute nitric acid (0.4 M HNO₃). Water depletion from the nutrient reservoirs due to evapotranspiration was monitored and manually replaced on a daily basis to maintain constant liquid level. Solution electrical conductivity was monitored and maintained near 1200 µS cm⁻¹ by daily addition of a complete stock solution. Four separate nutrient solution delivery systems were used for this study with each system circulating solution between two culture trays. One cultivar was planted per tray, therefore two cultivars shared each nutrient delivery system. Nutrient solution temperature was not controlled but was monitored; it remained around 25°C throughout the test.

Eight bunching or scallion onions (Allium fistulosum L.) cultivars were used in the test (Table 1). Seeds were sown onto white ABS plastic tray inserts at a density of 200 plants per tray (800 plants per m²). Seeds were sown on a strip of hydrophilic nylon (Nitex) fabric to serve as a nutrient solution wick. Trays were covered for the first four days with a white, translucent acrylic “germination” covers to maintain high humidity around the germinating seeds.

Environmental Parameters: Each test was performed in a walk-in controlled environment chamber (Model M-48, EGC, Chagrin Falls, OH). Lighting was provided from high-pressure sodium (HPS) lamps cycled to provide a 16-h light/08-h dark photoperiod. Light levels were maintained at 300 µmol m⁻² s⁻¹ PPF. Air temperatures were maintained at constant 23°C and relative humidity was maintained at a constant 65%. Duplicate tests were repeated in a similar chamber outfitted with VHO cool white fluorescent (CWF) lamps.

Plant Growth Measurements: Canopy height and light period PPF were measured manually each week. Light period PPF was measured at plant canopy with a LiCor quantum sensor (Model LI-189, LiCor, Lincoln, NE). Time–course growth measurements were performed at 14, 21, 28, and 35 DAP. Two representative plants were harvested per tray for growth rate analysis. Fresh mass was determined on tissues separated into tops and roots for all harvested plants. Plant tissues were oven-dried at 70°C for 72 h to determine dry mass. At 42 DAP, a final harvest was performed on the remaining plants in each tray.

RESULTS AND DISCUSSION

There was a lack of lighting treatment response for all cultivars except for Deep Purple, which had a greater top dry mass and total dry mass under HPS (Figure 1). However, lamp type did not have an effect on carbon partitioning (harvest index) in any cultivar. Growth curves from time course measurements showed that no cultivar reached maximum biomass accumulation under either lamp type (Figure 1), therefore a longer growth cycle may be necessary to achieve maximum cropping efficiency. Cultivars Guardsman and Deep Purple showed the greatest dry mass accumulation under both lamp types (Table 2). Although these cultivars achieved the greatest DM accumulation under either lamp type, they were more variable in growth than other cultivars. Among the shorter cultivars, Kinka had the highest DM accumulation. Because of size restrictions, cultivar Kinka appears to be the best choice for future space flight experiments because of its smaller, uniform height.
LITERATURE CITED


Table 1. Cultivars of bunching onion and seed suppliers.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Supplier</th>
<th>Type</th>
<th>200 seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinka</td>
<td>Kyowa Seed Co., Ltd</td>
<td>White</td>
<td>0.36</td>
</tr>
<tr>
<td>Kruncho</td>
<td>Kyowa Seed Co., Ltd</td>
<td>White</td>
<td>0.46</td>
</tr>
<tr>
<td>Choho</td>
<td>Kyowa Seed Co., Ltd</td>
<td>White</td>
<td>0.46</td>
</tr>
<tr>
<td>Choetsu</td>
<td>Kyowa Seed Co., Ltd</td>
<td>White</td>
<td>0.40</td>
</tr>
<tr>
<td>Guardsman</td>
<td>Territorial Seeds</td>
<td>White</td>
<td>0.92</td>
</tr>
<tr>
<td>Pacific Pearl</td>
<td>Territorial Seeds</td>
<td>White</td>
<td>0.74</td>
</tr>
<tr>
<td>Evergreen Hardy White</td>
<td>Johnny’s Seeds</td>
<td>White</td>
<td>0.38</td>
</tr>
<tr>
<td>Deep Purple</td>
<td>Johnny’s Seeds</td>
<td>Red</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Table 2. Measurement parameters for eight onion cultivars grown for 42 days under HPS and CWF lamps. Values represent means of data combined for each lamp type.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Plant Height (cm)</th>
<th>Edible FM (g plant-1)</th>
<th>Edible DM (g plant-1)</th>
<th>Total DM (g plant-1)</th>
<th>Harvest Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guardsman</td>
<td>63.99 (1.93) a</td>
<td>28.33 (4.17) a</td>
<td>1.42 (0.21) a</td>
<td>1.64 (0.25) a</td>
<td>86.08 (0.57) ns</td>
</tr>
<tr>
<td>Deep Purple</td>
<td>61.08 (1.40) a</td>
<td>28.03 (3.00) a</td>
<td>1.31 (0.13) a</td>
<td>1.51 (0.18) ab</td>
<td>87.51 (2.19) ns</td>
</tr>
<tr>
<td>Pacific Pearl</td>
<td>59.22 (1.88) ab</td>
<td>20.10 (1.60) ab</td>
<td>0.98 (0.03) ab</td>
<td>1.13 (0.07) ab</td>
<td>87.30 (2.90) ns</td>
</tr>
<tr>
<td>Evergreen Hardy White</td>
<td>51.08 (2.04) b</td>
<td>16.17 (2.79) b</td>
<td>0.96 (0.12) b</td>
<td>1.14 (0.16) ab</td>
<td>84.47 (1.07) ns</td>
</tr>
<tr>
<td>Kinka</td>
<td>49.20 (1.23) b</td>
<td>17.30 (2.65) ab</td>
<td>0.97 (0.03) ab</td>
<td>1.10 (0.02) ab</td>
<td>87.85 (0.61) ns</td>
</tr>
<tr>
<td>Kruncho</td>
<td>52.73 (1.00) b</td>
<td>17.16 (1.05) ab</td>
<td>0.90 (0.03) b</td>
<td>1.04 (0.02) ab</td>
<td>86.97 (1.43) ns</td>
</tr>
<tr>
<td>Choetsu</td>
<td>47.46 (0.69) b</td>
<td>16.23 (1.84) b</td>
<td>0.93 (0.06) b</td>
<td>1.03 (0.07) b</td>
<td>89.67 (0.52) ns</td>
</tr>
<tr>
<td>Choho</td>
<td>52.79 (0.89) b</td>
<td>16.49 (1.13) b</td>
<td>0.90 (0.01) b</td>
<td>1.03 (0.01) b</td>
<td>87.97 (0.12) ns</td>
</tr>
</tbody>
</table>

No differences observed for all cultivars between lamp treatments, thus means were combined for ANOVA analysis. Values in ( ) represent S.E. (n=6). Values followed by a different letter are significantly different as determined by ANOVA and Tukey’s Multiple Comparison test at P<0.05.
Figure 1. Total dry mass comparison between eight onion cultivars grown under high-pressure sodium (HPS) or cool-white fluorescent (CWF) lamps during the course of the 42 day tests.