

EARLY CONTROL OF SEEDLING GROWTH BY TREATING SEEDS WITH GROWTH REGULATORS

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ABSTRACT

Control of seedling growth is a challenge in plug production. Efficacy of seed treatments with plant growth regulators for early plant height control, as well as accumulation of growth regulators in fruits were evaluated. Verbena, salvia, pansy, dill, and cucumber seeds soaked in 50 mg·L⁻¹ paclobutrazol solutions during 5 min produced seedlings that were up to 43, 18, 30, 22, and 44% shorter than controls, respectively. For most crops, increasing triazole concentrations during seed soaking were associated with decreases in seedling emergence. Soaking verbena and marigold seeds in paclobutrazol solutions was associated with reduced metabolic heat and respiration rates. No presence of paclobutrazol was detected in cucumber fruits harvested from plants grown from seeds soaked in 1000 mg·L⁻¹ paclobutrazol solution.

INTRODUCTION

Plant stretching is an unwanted effect in plug production, making it difficult to perform mechanized transplanting. Traditional root and foliar applications of plant growth regulators (PGR) have practical disadvantages including losses and drift of PGR (Holcomb and Rose, 1991; Barrett and Nell, 1992) and associated increases in application costs. Presowing seed soaking in PGR solutions is an alternative approach in plant growth control (Fletcher et al., 2000; Pasian and Bennett, 2001; Pill and Gunter, 2001). Advantages of seed treatments with PGR include less usage and drift of active ingredient, and simplicity of application (Pasian and Bennett, 1999; Fletcher et al., 2000). The goal of the present study was to evaluate the effect of seed soaking in several PGR solutions during different soaking durations on seedling height and emergence of ornamental (verbena, salvia, pansy, marigold, celosia) and horticultural (cucumber, dill) crops and accumulation of growth regulators in cucumber fruits.

MATERIALS AND METHODS

Seed treatments. The studied species included verbena (*Verbena x hybrida* Voss., cv. Quartz White), celosia (*Celosia cristata*, cv. New Look), marigold (*Tagetes patula* L., cv. Bonanza Gold), pansy (*Viola wittrockiana*, cv. Bingo Yellow Blotch), salvia (*Salvia splendens*, cv. Vista Red), dill (*Anathemum graveolens*, cv. Fernleaf), and cucumber (*Cucumis sativus*, cv. Poinsett 76SR). One hundred seeds in each treatment were placed in a glass beaker with 50 ml (verbena, marigold, celosia, pansy, salvia, and dill) or 100 ml (cucumber) water only or solutions of PGR of different concentrations ranging according to the crop from 50 to 1000 mg·L⁻¹ for paclobutrazol (Bonzi®), 1 to 10 mg·L⁻¹ for uniconazole (Sumagic®), 10 to 200 mg·L⁻¹ for ancymidol (A-Rest®), 100 to 5000 mg·L⁻¹ for chlormequat (Cycocel®). After soaking, seeds were transferred to a sieve and dried for 24 h on an open bench at 20°C.

Plant growth. Within 2-4 d after the treatment, seeds were sown one seed per cell in plastic 288 or 164 (cucumber) cell plug trays filled with plug growth mix Sunshine LP5. Plugs were covered with a small portion of growth mix, placed under intermittent mist at 25°C during 1 d (verbena, celosia, marigold, pansy, and salvia), 2 d (cucumber), or 3 d (dill), and moved to a

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25°C greenhouse on a mat-covered bench. Seedling height and emergence percentage were determined for each crop. Thirty-three days after soaking (DAS), cucumber plants were transplanted into 3.8 L plastic containers (one plant per pot) filled with Metro-Mix 360. Fruits from cucumber plants grown from seeds soaked in water or 1000 mg·L⁻¹ paclobutrazol solution during 180 minutes were collected.

Calorimetric analysis. Verbena and marigold seeds were soaked in 10 or 500 mg·L⁻¹ paclobutrazol solutions during 5 or 180 min. Immediately after soaking, seeds were dried on a filter paper for 24 h on an open bench at 20°C (non-washed seeds) or transferred to a sieve, rinsed with water (28-30°C) during 30 seconds and dried (washed seeds). Ten DAS, seeds were germinated on moistened filter paper during 48 (marigold) or 72 h (verbena) at 25°C. Rates of heat production and CO₂ evolution were simultaneously measured on a CSC Model 4100 differential scanning calorimeter operating in the isothermal mode.

Mass spectrometry. Cucumber seeds were soaked in 1000 mg·L⁻¹ paclobutrazol solution, dried and manually separated into seed coats and the rest of the seed (embryo plus cotyledons). Paclobutrazol was extracted according to the protocol developed by Minghong (2001). The limit of paclobutrazol detection by mass spectrometry (MS) was 0.025 µg·g⁻¹ DM. Experiments were performed on a Micromass LC-ToFTM II MS equipped with an orthogonal electrospray source (Z-spray) operated in positive ion mode. Optimal ESI conditions were: capillary voltage 3000 V, source temperature 110°C and a cone voltage of 55 V. The ESI gas was nitrogen. All ions transmitted into the pusher region of the TOF analyzer were scanned over m/z (your range) with a 1 s integration time and data was acquired in continuum mode during the liquid chromatography (LC) run. The LC/autosampler system consisted of a Waters Alliance 2690 Separations Module. A 1.0 x 250 mm C18 column was used. The mobile phase was 65/35 water/acetonitrile with 0.1% formic acid; the flow rate was 0.05 mL/min.

Statistical analysis. Plant height and percentage seedling emergence as a response to growth regulator and soaking time were analyzed using the general linear model procedure in SAS (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Seeds of verbena and cucumber soaked during 5 min in 50 mg·L⁻¹ paclobutrazol solutions produced seedlings that were up to 43 and 22% shorter than the controls, respectively (Tables 1, 2). Seedling emergence of verbena seeds soaked during 5 min in 50 mg·L⁻¹ paclobutrazol solution was up to 87% lower than in controls (Table 3).

Increasing concentrations of paclobutrazol solutions significantly reduced seedling emergence in verbena (Table 3), as well as in pansy, salvia and dill (data not shown). Seedling emergence from cucumber seeds soaked in the highest paclobutrazol concentrations (1000 mg·L⁻¹) was only slightly reduced compared with the controls, while no effect of increasing soaking time on seedling emergence was found (Table 4).

Table 1. Height (cm) of verbena (*Verbena x hybrida* Voss., cv. Quartz White) seedlings from seeds soaked in 0-500 mg·L⁻¹ paclobutrazol solutions during 5, 45 or 180 min. Seedling height measurements were taken 25 and 39 d after sowing (DAS).

Treatment	25 DAS			L ¹	39 DAS			L ¹
	5 min	45 min	180 min		5 min	45 min	180 min	
0 mg·L ⁻¹	2.7	2.8	3.0	NS	5.0	5.0	4.4	**
50 mg·L ⁻¹	1.8	2.0	1.6	NS	3.4	3.9	2.8	**
200 mg·L ⁻¹	2.2	1.5	1.3	*	3.9	2.8	2.7	**
500 mg·L ⁻¹	1.4	0.8	0.4	NS	3.0	1.5	-	NS
L ²	***	***	***		***	***	***	

Table 2. Height (cm) of cucumber (*Cucumis sativus*, cv. Poinsett 76SR) seedlings from seeds soaked in 0-1000 mg·L⁻¹ paclobutrazol solutions during 5, 45 or 180 min. Seedling height measurements were taken 7 and 23 d after sowing (DAS).

Treatment	7 DAS			L ¹	23 DAS			L ¹
	5 min	45 min	180 min		5 min	45 min	180 min	
0 mg·L ⁻¹	2.7	3.0	2.8	NS	6.6	7.7	7.2	NS
50 mg·L ⁻¹	2.0	2.1	2.2	**	4.2	3.7	4.0	NS
200 mg·L ⁻¹	1.6	1.7	2.0	***	3.3	3.4	4.0	***
500 mg·L ⁻¹	1.5	1.5	1.5	NS	2.8	2.8	3.0	NS
1000 mg·L ⁻¹	1.3	1.5	1.4	NS	3.1	3.1	2.8	**
L ²	***	***	***		***	***	***	

Table 3. Seedling emergence (%) of verbena (*Verbena x hybrida* Voss., cv. Quartz White) from seeds soaked in 0-500 mg·L⁻¹ paclobutrazol solutions during 5, 45 or 180 min. Seedling emergence measurements were taken 6 and 20 d after sowing (DAS).

Treatment	6 DAS			L ¹	20 DAS			L ¹
	5 min	45 min	180 min		5 min	45 min	180 min	
0 mg·L ⁻¹	40	51	55	NS	73	63	70	NS
50 mg·L ⁻¹	2	0	0	NS	23	8	8	*
200 mg·L ⁻¹	0	0	0	-	24	7	0	***
500 mg·L ⁻¹	1	0	0	NS	11	0	0	**
L ²	***	***	***		***	***	***	

***, **, *, NS: significant at P = 0.001, = 0.01, = 0.05 and nonsignificant, respectively
L¹, L²: Linear models for the soaking time and paclobutrazol concentration effects, respectively.

Table 4. Seedling emergence (%) of cucumber (*Cucumis sativus*, cv. Poinsett 76SR) from seeds soaked in 0-500 mg·L⁻¹ paclobutrazol solutions during 5, 45 or 180 min. Seedling emergence measurements were taken 7 and 14 d after sowing (DAS).

Treatment	7 DAS			L ¹	14 DAS			L ¹
	5 min	45 min	180 min		5 min	45 min	180 min	
0 mg·L ⁻¹	89	85	87	NS	89	85	87	NS
50 mg·L ⁻¹	85	87	87	NS	85	87	87	NS
200 mg·L ⁻¹	83	86	87	NS	83	87	87	NS
500 mg·L ⁻¹	81	82	80	NS	81	82	80	NS
1000 mg·L ⁻¹	75	76	76	NS	78	76	77	NS
L ²	**	*	**		**	*	**	

***, **, *, NS: significant at P = 0.001, = 0.01, = 0.05 and nonsignificant, respectively
L¹, L²: Linear models for the soaking time and paclobutrazol concentration effects, respectively.

Growth of marigold plugs from seeds soaked in 5 mg·L⁻¹ uniconazole or 60 mg·L⁻¹ ancymidol solutions during 45 min was associated with 23% or 6% plant height reduction, respectively (Table 5). Soaking marigold seeds in chlormequat solutions did not significantly affect seedling growth. Increasing time of seed soaking in PGR solutions did not influence emergence of marigold seedlings. Soaking celosia seeds in the solutions of all studied PGR significantly reduced seedling height, although the reductions were very small (data not shown).

Increased PGR concentrations and soaking time generally corresponded to a greater reduction of plant height, as well as delays and reduction in seedling emergence of all crops, except marigold and cucumber. A reduction in seedling emergence with an increase in PGR concentration suggests more PGR penetrated the seeds during soaking. Soaking seeds in PGR solutions for short times (0.15 min for marigold, or 5 min for pansy, verbena, salvia, and cucumber) was also associated with a seedling height reduction. It may be that, for short soaking times, PGR are absorbed attached to seed coats rather than penetrating into the seeds (Pasian and Bennett, 1999). After sowing in plugs, PGR might diffuse into growth media and then be absorbed by the root (Pasian and Bennett, 2001). Seedling growth reduction observed after soaking seeds in PGR solutions during 0.15-5 min suggests that the seed coats performed a carrier role for PGR.

Seeds of verbena (Table 6) and marigold (data not shown) soaked in paclobutrazol solutions had lower respiration and heat production rates than the controls. Lowering seedling emergence in verbena with increasing paclobutrazol concentration and soaking time (Table 3) was associated with inhibition of respiration and heat production rates (Table 6). We can speculate that seeds soaked in increasing paclobutrazol concentrations acquired deeper levels of dormancy, which was reflected by decreasing respiration rates. This was not found in washed seeds (Table 7) suggesting that paclobutrazol was washed out from the seeds.

Table 5. Height (cm) of marigold (*Tagetes patula* L., cv. Bonanza Gold) seedlings from seeds soaked in 0-5 mg·L⁻¹ uniconazole, 0-60 mg·L⁻¹ ancymidol, or 0-5000 mg·L⁻¹ chlormequat solutions during 0.15, 5 or 45 min. Seedling height measurements were taken 10 and 30 d after sowing (DAS).

Treatment	10 DAS			L ¹	30 DAS			L ¹
	0.15 min	5 min	45 min		0.15 min	5 min	45 min	
Uniconazole								
0 mg L ⁻¹	2.5	2.6	2.5	NS	7.2	7.3	7.3	NS
1 mg L ⁻¹	2.4	2.3	2.0	***	6.0	5.6	5.7	*
2 mg L ⁻¹	2.4	2.3	2.0	***	5.7	5.6	5.6	NS
5 mg L ⁻¹	2.2	2.0	1.9	***	5.6	5.5	5.6	NS
L ²	***	***	***		***	***	***	
Ancymidol								
0 mg L ⁻¹	2.5	2.6	2.5	NS	7.2	7.2	7.3	NS
10 mg L ⁻¹	2.5	2.6	2.7	**	7.0	7.1	7.0	NS
20 mg L ⁻¹	2.7	2.6	2.6	NS	7.0	7.0	7.1	NS
60 mg L ⁻¹	2.6	2.6	2.6	NS	7.1	6.8	6.9	NS
L ²	NS	NS	NS		NS	***	***	
Chlormequat								
0 mg L ⁻¹	2.5	2.6	2.5	NS	7.2	7.3	7.3	NS
1000 mg L ⁻¹	2.6	2.6	2.6	NS	7.1	7.3	7.2	NS
3000 mg L ⁻¹	2.7	2.6	2.7	NS	7.2	7.0	7.1	NS
5000 mg L ⁻¹	2.6	2.7	2.7	NS	7.0	7.2	7.2	NS
L ²	*	***	***		NS	*	NS	

Table 6. Unwashed seeds. Metabolic heat rate (q) and respiration rate (R_{CO2}) 72 hours after verbena seeds were soaked in 0-500 mg·L⁻¹ paclobutrazol solutions during 5 or 180 min.

Treatment	q, μJ·s ⁻¹ ·mg ⁻¹ DM		L ¹	R _{CO2} , nmol·s ⁻¹ ·mg ⁻¹ DM		L ¹
	5 min	180 min		5 min	180 min	
0 mg·L ⁻¹	2.0	3.3	NS	3.4	4.1	NS
10 mg·L ⁻¹	1.1	1.0	NS	2.1	2.4	NS
500 mg·L ⁻¹	1.6	1.0	NS	2.5	1.4	**
L ²	NS	**		*	***	

***, **, *, NS: significant at P = 0.001, = 0.01, = 0.05 and nonsignificant, respectively
L¹, L²: Linear models for the soaking time and paclobutrazol concentration effects, respectively.

Table 7. Washed seeds. Metabolic heat rate (q) and respiration rate (R_{CO_2}) 72 hours after verbena seeds were soaked in 0-500 mg·L⁻¹ paclobutrazol solutions during 5 or 180 min, and washed with water during 30 sec.

Treatment	q, $\mu\text{J}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ DM		L ¹	R_{CO_2} , $\text{nmol}\cdot\text{s}^{-1}\cdot\text{mg}^{-1}$ DM		L ¹
	5 min	180 min		5 min	180 min	
0 mg·L ⁻¹	1.6	1.6	NS	2.6	2.9	NS
10 mg·L ⁻¹	1.8	1.6	NS	3.0	2.5	NS
500 mg·L ⁻¹	1.2	1.4	NS	3.5	2.2	*
L ²	NS	NS		NS	*	

***, **, *, NS: significant at P = 0.001, = 0.01, = 0.05 and nonsignificant, respectively
L¹, L²: Linear models for the soaking time and paclobutrazol concentration effects, respectively.

Cucumber seeds soaked in 1000 mg·L⁻¹ paclobutrazol solution were characterized by higher paclobutrazol concentration in the seed coats than in the rest of the seed (Table 8). Soaking cucumber seeds in paclobutrazol solutions affected not only seedling growth, but also the latter growth stages. Fruit yield was reduced on 53% when cucumber plants were grown from seeds soaked in 1000 mg·L⁻¹ paclobutrazol solution during 180 minutes, while no paclobutrazol presence was detected in fruits (data not shown).

Table 8. Paclobutrazol concentration ($\mu\text{g}/\text{g}$) in cucumber seed parts. Seeds were soaked in 0-4000 mg·L⁻¹ paclobutrazol solutions during 5 or 180 minutes.

Treatment	Seed coats		L ¹	Rest of the seed		L ¹
	5 min	180 min		5 min	180 min	
0 mg·L ⁻¹	ND	ND		ND	ND	
1000 mg·L ⁻¹	0.106	0.208	***	0.002	0.012	***
4000 mg·L ⁻¹	0.419	0.716	**	0.006	0.042	***
L ²	***	***		**	***	

ND: not detected

***, **, *, NS: significant at P = 0.001, = 0.01, = 0.05 and nonsignificant, respectively
L¹, L²: Linear models for the soaking time and paclobutrazol concentration effects, respectively.

Results indicate that seed treatments with growth regulators may be useful in controlling growth of selected plugs. An optimal protocol for treating seeds with PGR should deal with a controlled repression of GA biosynthesis, whereby GA-mediated growth is reduced, but seedling emergence and vigor are not affected. Soaking cucumber seeds in 50-1000 mg·L⁻¹ paclobutrazol solutions could be employed to produce small plugs without accumulation of paclobutrazol residue in fruits.

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