

THE DIFFERENTIAL EFFECTIVENESS OF TWO SYNTHETIC AUXINS IN DELAYING FLORET ABSCISSION IN RED CESTRUM CUT FLOWERS DEPENDS ON THEIR TRANSPORT AND METABOLISM

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ABSTRACT

Floret abscission in Red Cestrum (*Cestrum elegans* Schlecht) cut flower shoots was significantly delayed by a pulse treatment of 2,4-dichlorophenoxyacetic acid (2,4-D) while 1-naphthaleneacetic acid (NAA) was less effective. This phenomenon is attributed to the findings showing that significant amount of 2,4-D moved acropetally and accumulated in florets, leaves, and upper parts of the stem, while NAA remained in the lower parts of the stem. In addition, a significant amount of the accumulated 2,4-D remained in the active free form for a relatively longer period of time during vase life, while NAA was quickly metabolized. 2,4-D induced higher rates of ethylene evolution and increased expression levels of *Aux/IAA* homologous genes, cloned from the floret abscission zone, compared to those observed in response to NAA. This suggests that ethylene evolution and expression of *Aux/IAA* homologous genes may serve as markers for the activity of these two synthetic auxins.

INTRODUCTION

It is well accepted that both the natural auxin, indole-3-acetic acid (IAA), and the synthetic auxins exhibit polar transport (Lomax et al., 1995). This is also true for the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), which has transport characteristics similar to those of IAA, except for a slower transport rate (McCready, 1963; McCready and Jacobs, 1963). When 2,4-D was combined with silver thiosulfate (STS) for pulsing 'Red Cestrum' (*Cestrum elegans* Schlecht) cut flowers, floret abscission was significantly reduced compared with a similar treatment with 1-naphthaleneacetic acid (NAA) (Meir et al., 1999; Abebie et al., 2005). This suggests that 2,4-D moved acropetally in a significant amount, which was sufficient to reduce floret abscission, while NAA failed to do so. However, when NAA was conjugated to glycine to form 1-naphthylacetyl glycine (NAGly), it was as effective as 2,4-D in reducing floret abscission (Meir et al., Unpublished data). This may be related to the ability of the conjugated form to move non-polarly, as NAGly is not an active auxin. Thus, our data suggest that the ability of 2,4-D to move acropetally is the major factor in its efficient inhibition of floret abscission. However, this assumption does not rule out the possibility that differences in the metabolism of these two auxins may also contribute to their differential effects on floret abscission. The objective of this work was to compare the nature of acropetal transport and metabolism of 2,4-D and NAA in cut 'Red Cestrum' flowering shoots, in order to study the reasons for the observed differences between the two auxins in reducing floret abscission.

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MATERIALS AND METHODS

Acropetal Transport and Distribution of NAA and 2,4-D in Shoots. Experiments were performed with Red Cestrum (*Cestrum elegans* Schlecht.) cut flowers obtained from local growers. Auxin transport and metabolism were studied in whole vegetative or flowering shoots. Commercial size shoots were desiccated for 2 h at 20°C to increase uptake of the treatment solutions and then 30-cm-long shoots were pulsed with aqueous solution that contained 0.2% 8-hydroxyquinoline citrate (HQC), either 200 µM NAA or 2,4-D, and [³H-4]NAA (specific activity of 345 MBq mmol⁻¹) or [¹⁴C-1]2,4-D (specific activity of 592 MBq mmol⁻¹) as tracers, respectively. The transport experiments were carried out for 24 h at 20°C under a 16-h photoperiod. After 24 h of transport the lower part of the stem was washed, 2 cm of the stem base were trimmed off, and the shoots were transferred to an antimicrobial aqueous solution of 50 µl l⁻¹ sodium dichloroisocyanurate (TOG-6, Milchan Bros Ltd, Israel). Samples of stem segments, leaves and florets were extracted in 20% ethanol, and aliquots were taken for measuring radioactivity in a scintillation counter.

Measurement of Floret Abscission and Ethylene Evolution. Individual inflorescences of whole flowering shoots were covered with polyethylene bags, tapped gently and abscised florets at different stages of maturity were collected and counted. At the end of the experiment, florets that did not abscise were also counted and summed up to the number of abscised ones to determine the percentage of accumulated floret abscission during and at the end of the experiment. For ethylene measurements, excised leaves or inflorescences were enclosed in sealed 1.70 liter plastic containers for 1.5 h at 20°C. At the end of the incubation period, 1-ml gas samples were withdrawn with a hypodermic syringe and injected into a gas chromatograph.

Metabolism Studies. The metabolism of the two synthetic auxins was compared following procedures described by Centeno et al. (1999). Samples were taken from stems, leaves and florets after shoot pulsing and during vase life, and stored at -20°C until analysis. The samples were then extracted and analyzed on silica gel TLC plates with authentic standards for quantitative determination of free and metabolized auxins.

Molecular Studies. *Aux/IAA* homologous genes were cloned from the floret abscission zone (AZ) following procedures described by Sambrook et al. (1989), and their temporal and spatial expression in response to 2,4-D and NAA were studied as markers for auxin activity.

RESULTS AND DISCUSSION

Acropetal Transport of NAA and 2,4-D. After 24 h of transport the distribution of NAA in the various parts of the shoots was completely different from that of 2,4-D (Fig. 1). While a significant amount of NAA remained in the lower parts of the shoot (Fig. 1A), 2,4-D was translocated upward and a significant amount was accumulated in the upper parts of the shoot (Fig. 1B). The accumulation of 2,4-D in the first 7 or 15 cm of the stem was similarly high, and it decreased towards the top of the stem (Fig. 1B). Similarly, a higher amount of 2,4-D was accumulated in leaves and florets of flowering shoots compared to that of NAA (data not shown). Although there are controversial reports on the acropetal translocation of auxins, there are some previous reports which claimed that IAA is transported acropetally in the phloem and xylem (Taiz and Zeiger, 1998). The authors reported that most of the IAA synthesized in mature

leaves appeared to be transported non-polarly to the rest of the plant via the phloem. We have recently provided evidence showing the presence of both polar and non-polar transport pathways for 2,4-D in *Cestrum* stem sections, whereas NAA exhibited a polar transport with a negligible acropetal transport (Abebie et al., 2005). Similarly, a faster acropetal than basipetal transport of auxins in the transpiration stream of mature *Arabidopsis* stem sections was also reported (Ludwig-Muller et al., 1995).

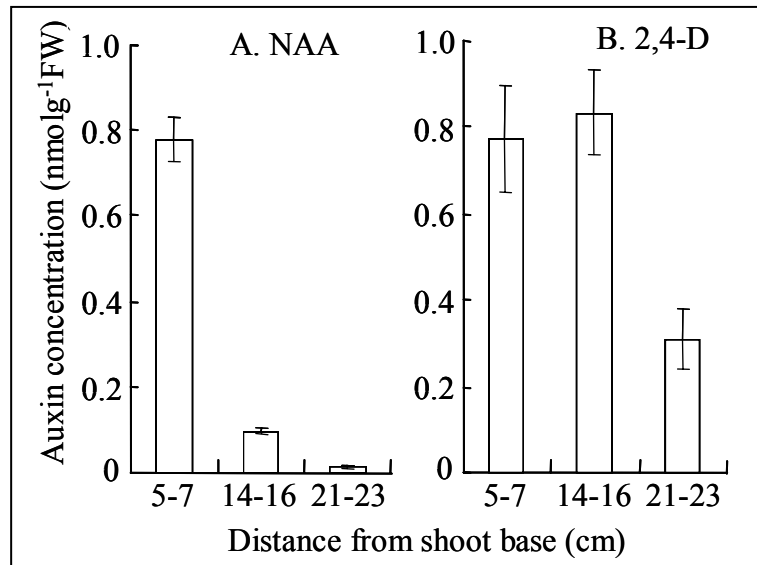


Fig.1. Distribution of NAA (A) and 2,4-D (B) in vegetative shoots of 'Red Cestrum' after 24 h of pulsing with the synthetic auxins. Auxin concentration in the tissue was extrapolated from the label extracted from the samples.



Fig. 2. Effect of NAA and 2,4-D on floret abscission in 'Red Cestrum' shoots after 7 days of vase life.

Effect of Pulsing Solutions on Floret Abscission and Ethylene Evolution. Abscission of floret buds started 68 h in vase life after pulsing, and continued at an increasing rate in the subsequent days. After 7 days of vase life the highest percentage of floret bud abscission was observed in the control (HQC) (Fig. 2A) or NAA-treated (Fig. 2B) shoots, while the lowest abscission percentage was observed in 2,4-D-treated shoots (Fig. 2C). Small floret buds abscised first, followed by abscission of open florets. While 95% of the florets in NAA-treated shoots abscised after 96 h, no florets abscised during this period in 2,4-D-treated shoots. The open florets either abscised late or remained intact (data not shown). These results are in agreement with previous reports showing that auxin is able to decrease the sensitivity of floral parts to ethylene (van Doorn and Stead, 1997; Bunya-atichart et al., 2006). For example, treatment with IAA reduced abscission in rose pedicels and even reversed the enhanced abscission observed after treating stems with the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Goszczyńska and Zieslin, 1993). The amount of ethylene evolved from 2,4-D-treated flowering shoots of *Cestrum* was significantly higher than the amount evolved from NAA-treated shoots. This elevated levels of ethylene induced by 2,4-D indicate the presence of higher levels of free 2,4-D in the tissue as compared with NAA.

Metabolism of NAA and 2,4-D. A higher amount of NAA was conjugated in the leaves and florets compared with 2,4-D (data not shown). Generally, NAA was conjugated quickly in all parts of the *Cestrum* shoot. These results are in agreement with previous reports showing a slow conjugation of 2,4-D and a rapid conjugation of NAA (Ribnicky et al., 1996; Centeno et al., 1999, Abebie et al., 2005).

Molecular Studies. Homologues of the early auxin responsive (*Aux/IAA*) genes were cloned from *Cestrum* floret AZ and were used as molecular markers to study auxin activity and the relationship between *Aux/IAA* gene expression and floret abscission. Northern hybridization and quantitative real time PCR analyses of six different *Aux/IAA* cDNAs (designated as *CeIAA1-6*), cloned from the floret AZ, were performed. Temporal and spatial differences in the expression level of the six encoding genes were observed, with 2,4-D inducing a higher level of expression compared to NAA (data not shown). The expression level of all of the six *CeIAA* genes increased up to 2 days of treatment and declined thereafter. Generally, *CeIAA* gene expression was inversely related to the increased level of floret abscission during this period.

CONCLUSIONS

It is well accepted that auxin retards abscission by decreasing the sensitivity of the AZ cells to ethylene. A model developed for mature leaf abscission by Morgan (1984) and Osborne (1989) implies that IAA produced by the leaf blade is translocated down to the petiole and retards abscission by decreasing the sensitivity of the AZ cells to ethylene. When the leaf begins to senesce it produces an elevated level of ethylene, which reduces in turn auxin content and transport, resulting in increased sensitivity of the AZ to ethylene. Ethylene then acts directly on the AZ to induce the activity of hydrolytic enzymes, which lead to cell separation. There is evidence showing that the above model also applies to reproductive organ abscission (Huberman et al., 1997 and literature cited therein). Based on the above model, any factor that affects the supply and the level of auxin in the AZ will affect its sensitivity to ethylene (Taylor and Whitelaw, 2001). In the *Cestrum* system, 2,4-D treatment induced a higher amount of ethylene evolution but delayed floret abscission, which apparently might be due to the reduced sensitivity

of the AZ cells to ethylene as a result of a higher level of free 2,4-D accumulation. Thus, ethylene evolution can serve as a useful indicator of auxin activity. The differences in activation of the *CeIAA* genes, in response to the two synthetic auxins, and their negative correlation with floret abscission indicates that they can also serve as molecular markers to study the changes in auxin activity during the abscission process.

Generally, our data indicate that the differences in the effectiveness of the two synthetic auxins in delaying floret abscission can be attributed to the differences in their transport and metabolism in the target tissues. A higher acropetal transport and a lower rate of metabolism of 2,4-D lead to increased levels of free 2,4-D in the AZ cells as well as in other parts of the floret. This seems to be the major reason for reduced floret abscission in shoots treated with 2,4-D.

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Please note:

Dr. Goren's presentation was dedicated to the memory of Daphne Joan Osborne