Effect of exogenous hormones on leaf yellowing in cut flowering branches of *Alstroemeria pelegrina* L.*

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Abstract

Inclusion of IAA in the vase water had little effect on leaf yellowing in cut flowering branches of *Alstroemeria pelegrina* L. while kinetin delayed leaf yellowing at 10^{-4} M (continuous treatment). Chlorophyll was effectively retained by 10^{-7} M gibberellic acid (GA) in the vase water or by a 20 h pulse at 5°C with $10^{-5}/10^{-4}$ M GA. After 16 h of ^{14}C -GA₃ uptake at 20°C relatively high levels of ^{14}C were found in leaves and low levels in stems and flowers. After this treatment about half of the ^{14}C -GA₃ in leaves was metabolized into unknown compounds.

1. Introduction

Detached leaves of many species show a rapid onset of yellowing, especially when held in the dark [15, 16]. In most plant species the net breakdown of chlorophyll in detached leaves is effectively delayed by natural cytokinins or compounds with cytokinin-activity [16]. In a number of species chlorophyll breakdown can be delayed by IAA [11, 15] or GA [2, 9, 11].

Cut flowering branches of *Alstroemeria pelegrina* L., placed in water, show rapid leaf yellowing, even when held in the light. Preliminary experiments showed that in many cultivars visible yellowing started a few days after cutting and preceded the onset of petal wilting and abscission. Preliminary tests also showed that the inclusion of a mixture of IAA, GA and kinetin in the vase solution effectively delayed the onset of leaf yellowing in cut flowering branches of *A. pelegrina* [14].

The present investigation is a more detailed analysis of the effects of exogenous hormones on leaf yellowing in *A. pelegrina*, both in cut flowering

stems and in detached leaves. IAA, kinetin or GA were applied either as a 20 h pulse shortly after harvest, or as a continuous treatment in the solution. The distribution of GA in the cut flowering stem and the stability of GA in the tissue was also assessed, using ^{14}C -GA₃.

2. Materials and methods

2.1 Plant material

Cut flowering branches of *Alstroemeria pelegrina* L. (cvs. Yellow King, Orchid, Flamingo, Marina, Carmen, Ontario) were obtained from commercial growers. Flowering stems with a stem length of about 45 cm were harvested at the commercial cutting stage and transported dry to the laboratory.

Five cm of the stems were cut in air and the leaves were stripped from the basal 10 cm of the stem. Stems were placed in pulse solutions containing appropriate hormone concentrations (at 5°C for 16 or 20 h in the dark). The stems were then placed in individual vases at 20°C, 60% RH and a photosynthetically active quantum flux of $15\ \mu\text{mol m}^{-2}\text{s}^{-1}$ (12 h per day) from fluorescent tubes (Philips TLD 58 W/84). Alternatively, the

* **Corrigendum.** Owing to an error in the proofreading process, the article was published incorrectly. The article as it should have been published is presented here.

vase solutions contained the same concentration of the hormones.

2.2 Chemicals

IAA was obtained from Merck, kinetin (furfuryl-aminopurine) from BDH, commercial preparations containing a mixture of GAs from Serva and from Bendien (Naarden, The Netherlands), and GA₃ from Sigma.

2.3 Chlorophyll analysis

The analysis of chlorophyll was according to Vernon [18], harvesting leaves only from the basal 25 cm of the stems.

2.4 Distribution of ¹⁴C-GA₃

Cut flowers were allowed to take up to 3.7×10^4 Bq [¹⁴C]-GA₃ (Amersham, spec. act. 259 MBq mmol⁻¹). In this experiment the isotope was mixed with SVB-1, a commercial preparation of GAs (Bendien, Naarden, The Netherlands). Total GA concentration in the solution was 20 mg/. Uptake for 16 h at 20°C. Immediately after uptake at various levels of the stem 1 gram stem segments, single leaves and single flowers were cut, placed in polycarbonate cups and dried at 70°C for 24 h. Dried samples were combusted in a L.S. Sample Oxidizer (IN 4101, Inter-technique). Radioactivity was dissolved in 10 ml Carbomax (Lumac) and counted in a liquid scintillation spectrometer (Packard, A 300C).

2.5 Stability of ¹⁴C-GA₃ in the leaves

Cut flowering branches were allowed to take up ¹⁴C-GA₃ for 16 h as described above. Immediately after uptake about 1 gram of leaf mass was blended in 10 ml cold methanol. This mixture was centrifuged at 5000 rpm and supernatant evaporated under reduced pressure. The residue was loaded on a TLC plate [Silicagel F 254, Merck]. Chromatography was carried out with toluene/butanol/acetic acid (70/25/5; v/v). Radioactivity on the plate was measured using a TLC scanner [Bertold, LB 2723].

Table 1. Effects of IAA, kinetin and GA₃ on levels of chlorophyll a + b in the basal leaves of cut flowering branches of *Alstroemeria pelegrina* L. (cv. Yellow King), 16 days after the onset of vase life. The initial concentration of chlorophyll a + b was 17.4 ± 1.5 g per kg fresh wt. Analysis of variance and LSD test showed significant differences at $P < 5\%$ (*) or $P < 1\%$ (**)

		Chlorophyll a + b (g per kg fresh wt)	
		20 h pulse treatment	Continuous treatment
IAA	0 M	5.8 ± 1.0	5.8 ± 1.0
	10^{-7}	6.4 ± 1.2	5.7 ± 1.4
	10^{-6}	5.8 ± 1.8	3.9 ± 1.4
	10^{-5}	6.1 ± 2.1	4.5 ± 1.5
	10^{-4}	6.5 ± 1.5	3.8 ± 1.3
Kinetin	0	6.4 ± 1.2	6.4 ± 1.2
	10^{-7}	6.0 ± 1.8	6.9 ± 1.7
	10^{-6}	6.8 ± 1.1	6.1 ± 1.4
	10^{-5}	6.5 ± 1.1	6.8 ± 1.3
	10^{-4}	8.0 ± 1.3	$10.2 \pm 1.8^*$
GA ₃	0	5.7 ± 1.3	5.7 ± 1.3
	10^{-7}	5.4 ± 1.8	$15.3 \pm 1.5^{**}$
	10^{-6}	$9.8 \pm 1.4^{**}$	$15.9 \pm 1.0^{**}$
	10^{-5}	$14.3 \pm 1.7^{**}$	$14.8 \pm 1.1^{**}$
	10^{-4}	$15.5 \pm 1.9^{**}$	$15.6 \pm 1.4^{**}$

3. Results

3.1 Effects of hormones

When flowering stems of *A. pelegrina* (cv. Yellow King) were held in water at a photon flux density of $15 \mu\text{mol m}^{-2} \text{sec}^{-1}$ (12 h per day) visible leaf yellowing occurred within 4–6 days after the onset of vase life. Leaf yellowing started in the basal leaves and was correlated with a decrease in chlorophyll levels. IAA had no positive effect at all tested concentrations, either in continuous treatment or after a 20 h pulse (Table 1). When given as a continuous treatment kinetin delayed chlorophyll breakdown at 10^{-4} M (Table 1).

Including a commercial mixture of GAs or GA₃ in the vase water was very effective in retaining chlorophyll. In 20 h pulse treatments the optimum concentration was 10^{-4} M, whereas in continuous treatment the same effect was found using a concentration of 10^{-7} M (Table 1). The positive effect of GAs and GA₃ was also observed in other tested cultivars (Orchid, Flamengo, Marina, Carmen, Ontario).

Chlorophyll breakdown in detached leaves from flowering *A. pelegrina* branches was not affected by IAA (10^{-7} – 10^{-4} M), but was effectively delayed by

Fig. 1. Levels of radioactivity from ^{14}C -GA₃ in stems (■), basal leaves (○), top leaves (●), and flowers (▲), in cut flowering branches of *Alstroemeria pelegrina* L. (cv. Ontario) as dependent on the distance from the stem base. Treatment period was 16 h.

a continuous treatment with 10^{-7} M kinetin or 10^{-7} M GA₃. The onset of yellowing in detached leaves occurred after the same period of time as in leaves attached to the cut flowering branches, and the rate of yellowing was similar in detached and attached leaves (results not shown).

3.2 Distribution of ^{14}C -GA, after pretreatment

After a 16 h pulse treatment of *A. pelegrina* cv. Ontario with ^{14}C -GA₃ a relatively low level of radioactivity was found in stems and flowers, and a high level in leaves, especially those at the top of the flowering branch (Figure 1).

3.3 Determination of reaction products from ^{14}C -GA₃

After a 16 h pulse with ^{14}C -GA₃ about 50% of the label was found to cochromatograph with GA₃, while about 50% was present in one (or more) unknown compound(s) (Figure 2).

4. Discussion

Of the investigated hormones, a low concentration

Fig. 2. Location of radioactivity in expressed sap from leaves of cut flowering branches of *Alstroemeria pelegrina* L. (cv. Ontario), after 16 h uptake of ^{14}C -GA₃. The extract was loaded on a TLC plate (solvent toluene/n-butano/acetic acid). The peak at B corresponds to GA₃.

of mixed GAs or GA₃ effectively delayed the onset of visible leaf yellowing and chlorophyll breakdown, both in cut leaves and in leaves attached to cut flowering stems of *A. pelegrina*. GA₃ from Sigma may contain up to 10% GA₁ and GA₁ may be the effective species in preparations of GA₃ (Knegt, pers. comm.). Future research, therefore, has to establish which of the various GA molecules is actually causing the physiological response against leaf yellowing.

Effects of GA against leaf yellowing have been reported in detached leaves of a number of other species including *Tropaeolum majus* [2, 3, 4] *Rumex obtusifolius*, [7, 8] *Pharbitis* (= *Ipomoea*) *nil*, *Hydrocotyle volgare*, *Triticum aestivum*, *Secale cereale*, *Avena sativa* and *Hordeum vulgare* [9].

Yellowing in detached leaves in *Tropaeolum majus* [4] and *Taraxacum officinale* [8] was associated with a decrease in endogenous levels of GA. Yellowing of attached leaves in lettuce [1] and in cotyledons of intact cotton plants [5] was also associated with a marked decrease in free GA. These temporal correlations may suggest a role for endogenous GA in leaf yellowing.

We found that the onset of chlorophyll breakdown occurred after the same period in detached leaves and in leaves attached to the cut flowering stems, which may indicate that the signal(s) for the onset of senescence reside in the leaves.

In detached leaves of many plant species kinetin was also found to inhibit yellowing [10, 15]. GA

was as effective as kinetin in delaying leaf senescence in detached leaves of *Taraxacum officinale* [8], *Rumex obtusifolius* [7, 19] and in leaf discs of *Tropaeolum majus* [3]. In cut flowering branches of *A. pelegria* we found only an effect of high concentrations of kinetin against leaf yellowing. Kinetin, however, delayed chlorophyll breakdown in detached leaves at relatively low concentrations.

A similar effect of kinetin was found with respect to the onset of the rise in ethylene production (and petal wilting) in cut carnation flowers. Kinetin was relatively ineffective in delaying petal wilting in flowers with a long stem, but effective in isolated petals or in flowers with a short stem [6, 12, 17].

A pulse treatment with preparations containing GA has become mandatory for all flowering stems of *A. pelegria* sold at the flower auctions in The Netherlands. The present paper confirms this practice and shows its importance for the cut flower trade.

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