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GA_4 Does Not Require Conversion into GA_1 to Delay Senescence of *Alstroemeria hybrida* Leaves

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Abstract. The biological activity and metabolism of applied GA_1 and GA_4 were studied in leaves of alstroemeria (*Alstroemeria hybrida*). It appeared that GA_4 was 2 orders of magnitude more active in delaying leaf senescence than GA_1. GA_3-13-OMe, a GA analog that cannot be hydroxylated on the 13-C position, also retarded chlorophyll loss, although less efficiently. Tritiated and deuterated GA_1, GA_4, and GA_9 were applied to leaves, and their metabolites were analyzed. According to high performance liquid chromatography and gas chromatography-mass spectrometry analyses, GA_9 was converted into GA_4 and GA_{34}, and GA_4 was converted into GA_{34} and more polar components. No evidence was found for the conversion of both GA_9 and GA_4 into GA_1, even at the relatively high concentrations that were taken up by the leaf. The results strongly suggest that GA_4 is recognized directly by a receptor involved in regulation of leaf senescence in alstroemeria.

Key Words. Alstroemeria—Gibberellins—Metabolism—Senescence

Gibberellins (GAs) play an important role in the control of growth and development of plants. It has been hypothesized that different types of GAs are involved in different processes; vegetative and generative processes might be regulated by specific GAs (Sponsel 1995).

There is considerable evidence that GA_1 has biological activity per se especially in regulating seed germination and shoot and internode elongation (Graebe 1987, Sponsel 1995). Next to GA_1, a number of studies also indicate GA_4 as biologically active in regulating stem elongation. Metabolism of GA_4 has been studied in various systems, and in seedlings of *Phaseolus* (Turnbull and Crozier 1989), *Zea*, *Oryza*, and *Arabidopsis* (Kobayashi et al. 1991), GA_4 can be metabolized to GA_1. Thus, the biological activity of applied GA_4 may be the result of its conversion to GA_1. However, GA_4 has been suggested to be active per se for stimulation of stem elongation in *Cucumus sativus* (Nakayama et al. 1991), *Arabidopsis thaliana* (Talon et al. 1990), and *Tulipa gesneriana* (Rebers et al. 1995) based on a higher endogenous GA_4 concentration compared with GA_1. In addition, GA_{34} is a common metabolite of GA_4 in vegetative tissues of *Phaseolus coccineus* (Turnbull and Crozier 1989). These references suggest the existence of the non-early-3,13-hydroxylation pathway yielding GA_4 as intrinsically active GA.

Limited information is available as to whether GA_1 is also the most active GA in regulating other plant developmental processes such as senescence. Alstroemeria leaves offer a good model system to study senescence closely related to the in situ situation. It has the advantage of being highly sensitive to GAs, and the time course of the dark-induced senescence of detached leaf tips is very comparable with that of attached leaves (Jordi et al. 1993). In previous studies with alstroemeria, endogenous GA concentrations have been related to leaf senescence (Kappers et al. 1997). It has been shown that several GAs of both the 13-hydroxylation and the non-13-hydroxylation pathways were present in these leaves. In fresh leaves, GA_4 and GA_1 were present in comparable concentrations (approximately 0.7 pmol $(g\ FW)^{-1}$). GAs of the non-13-hydroxylation pathway changed in concentration because of senescence-inducing treatment (darkness), whereas 13-hydroxylated GAs did not. GA_4

Abbreviations: GA(s), gibberellin(s); FW, fresh weight; HPLC, high performance liquid chromatography; GC-MS, gas chromatography-mass spectrometry; SIM, selected ion monitoring.

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especially was influenced; after 3 days of dark treatment no GA_4 could be detected. Under low red light conditions, which delayed leaf senescence, the concentration of GA_4 remained at a much higher level (Kappers et al. 1998). Apparently, the decrease in GA_4 is a very early response of the senescence process. Exogenously applied GA_4 was found to be active at a concentration that was several orders of magnitude lower than that of GA_1 (Jordi et al. 1995, Kappers et al. 1998). This observed difference might be explained in terms of uptake of GAs by the tissue or in terms of metabolism of active GAs. Additionally, applied GA_4 could function as a precursor (slow release form) for GA_1, whereas exogenously applied GA_1 is inactivated rapidly.

The focal point of this study with alstroemeria leaves is to compare the biological activity and metabolism of exogenously added GA_1 and GA_4 to elucidate whether the involved putative receptor has the ability to recognize GA_4. Dose-response experiments were performed with GA_1, GA_4, and GA_3-13-OMe, a GA_3-analog that is unlikely to be converted back into GA_3, as in GA_1 hydroxylated at 13-C. In metabolic studies with $^2H_2$- and $^3H_3$-labeled GA_1, GA_4, and GA_9, GA metabolites were determined with HPLC and GC-MS. Our results suggest that in delaying senescence in alstroemeria leaves GA_4 is biologically active per se and is probably recognized by the specific receptor involved.

Materials and Methods

Chemicals

GA_1, GA_4, GA_9 (purity > 99%), [17-$^2H_2$]GA_1, [17-$^2H_2$]GA_4, and [17-$^2H_2$]GA_9 were obtained from Prof. L. N. Mander (Canberra, Australia); GA_3-13-OMe was a gift from Dr. M. H. Beale (Bristol, UK). GC-MS control experiments demonstrated that GA_3-13-OMe contains ≤0.01% of other GAs such as GA_1 and GA_4. [1,2-$^3H_2$]GA_1 and [1,2-$^3H_2$]GA_4 were obtained from Amersham (Buckinghamshire, UK) and [2,3-$^3H_2$]GA_9 from Dr. A. Crozier (Glasgow, UK). The specific activity of tritiated GAs was about 50,000 Ci mol^{-1}. Stock solutions in ethanol were prepared at 10^{-2} M for the various GAs. In all experiments the final ethanol concentration was ≤1% v/v. Control experiments demonstrated that this ethanol concentration did not affect the rate of chlorophyll loss (data not shown).

Plant Material and Dose-Response Experiments

The five uppermost leaves of *Alstroemeria hybrida* cv. Cinderella, positioned in a whorl directly below the flowers and therefore of the same physiological age, were used for the experiments. Leaf tips were excised with a sharp knife. The weight of the leaf tips was determined, and the leaf tips (FW approximately 500 mg, leaf area approximately 7 cm^2) were incubated in various concentrations of GAs (total volume 3 mL) in darkness. Logistic dose-response curves were calculated from the individual data points, using a nonlinear regression method described by the formula $(R = R_{min} + (R_{max} - R_{min})/(1 + ([GA]/[GA]_{50})^p)$, where R is the response, R_{min} is the response in the absence of exogenous GA, R_{max} is the maximum response, [GA] is the applied concentration, $[GA]_{50}$ is the concentration required for half-maximum response, and p is the Hill coefficient representing the abruptness of the curve (Weyers et al. 1987).

Chlorophyll Analysis

The chlorophyll ($a + b$) content of the leaves was calculated using the molar extinction coefficient in dimethylformamide (Inskeep and Bloom 1985). Data are presented as the percentage of the initial amount of chlorophyll in order to correct for small differences between experiments.

Uptake and Metabolism of Deuterated and Tritiated GAs

Tritiated GAs were first purified by HPLC before feeding. Each leaf was placed in 3 mL of a 10^{-7} M GA solution enriched with 50,000 dpm of tritiated labeled GA. In experiments in which combinations of deuterated and tritiated GAs were fed, GAs were diluted in 0.1 M citrate phosphate buffer, pH 5.0. Leaf tips were incubated in darkness at 20°C. After incubation, leaves were frozen in liquid N_2 and stored at −80°C until extraction. Experiments were performed in duplicate with four replications in each.

Extraction and Purification of GAs

Leaves were homogenized in ice-cold 100% MeOH, 5 mL g FW^{-1}, containing 0.1% ascorbic acid. When leaves were fed both deuterated and tritiated GAs, three leaves were combined, making one sample. After extraction for 2 h at 4°C and continuous shaking, the extract was centrifuged (5 min at 5,000 ×g), and the pellet was washed twice with 80% MeOH. The pooled MeOH extracts were evaporated to the water phase under reduced pressure at 35°C. The aqueous phase (±2 mL) was adjusted to pH 2.5 with 1 N HCl and partitioned against ethyl acetate (2 mL three times). The combined ethyl acetate fractions were evaporated to dryness, dissolved in 2 mL of water, and adjusted to pH 8.0 with 1 N NaOH. The extract was then purified further through a QAE-Sephadex A-25 (Pharmacia, Uppsala, Sweden) column (4 cm × 0.25 cm, inner diameter) equilibrated with sodium formate (1% w/v) and fed directly through a preequilibrated C_{18} column (Waters, Millipore Corporation, Milford, MA, USA). GAs were eluted with 80% MeOH and evaporated to dryness under reduced pressure before HPLC and GC-MS analysis.

HPLC and GC-MS

The HPLC system and operating conditions are described in Kappers et al. (1997). The column was eluted at a flow rate of 1 mL min^{-1} with 30% methanol for 15 min followed by a linear gradient to 75% methanol over 35 min and subsequently to 90% methanol over 5 min (solvents contained 0.01% acetic acid).

To analyze metabolic conversion products, successive 0.5-mL fractions were collected, and the radioactivity in an aliquot of each fraction was assayed by liquid scintillation counting. In experiments with both tritiated and deuterated labeling, fractions containing radioactivity were analyzed by GC-SIM as described by Kappers et al. (1997). For the detection of [$^2H_2$]GAs, their characteristic ions were monitored together with the characteristic ion of the endogenous GA. The labeled metabolites were identified based on co-chromatography with the en-

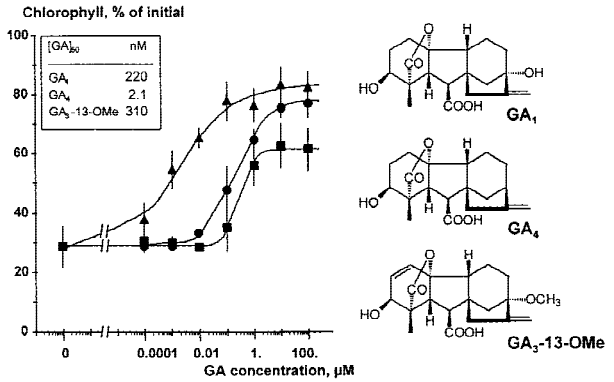

Fig. 1. Effect of various concentrations of GA_1 (●), GA_4 (▲), and GA_3-13-OMe (■) on chlorophyll retention in alstroemeria leaf tips placed in darkness for 10 days. Data are the means of two experiments with five replications each. *Vertical bars* represent the standard error. The *inset* shows the doses needed for the half-maximum response of the tested gibberellins ($[GA]_{50}$).

dogenous GAs, which had been identified previously using Kováts retention indices and full-scan mass spectra compared with those of authentic standards (Kappers et al. 1997) except for GA_8 and GA_{34} catabolites, which were identified tentatively by comparison with data from Gaskin and MacMillan (1991).

Results and Discussion

GAs Delay Senescence

There was no significant loss of chlorophyll during the first 5 days in all treatments (data not shown). Thereafter, chlorophyll decreased rapidly from day 6 onward in leaves that were placed in darkness, and leaves were uniformly yellow after 10 days. Application of a number of GAs delayed the loss of chlorophyll (Jordi et al. 1995). We determined the effect of the various treatments after 10 days of incubation, when dark-treated leaves without GA retained only 20% of the initial amount of chlorophyll.

Fig. 1 shows the effects of various concentrations of GA_1, GA_4, and GA_3-13-OMe chlorophyll loss in leaf tips of alstroemeria placed in darkness for 10 days. Both GA_1 and GA_4 showed a concentration-dependent effect on the retention of chlorophyll with a maximum effect of about 80% of the initial amount of chlorophyll left (Fig. 1). However, the efficacy of GA_4 is 2 orders of magnitude higher than GA_1 as can be seen from the calculated concentrations needed for the half-maximum effect ($[GA]_{50}$) (2.1 and 220 nM, respectively). These data indicate that the receptor involved will recognize exogenously applied GA_4. However, a number of arguments can be proposed which argue against the simplicity of this conclusion. Therefore, we investigated the relation between GA_1 and GA_4 further.

Table 1. Uptake of $[^3H_2]GA_1$ and $[^3H_2]GA_4$ by alstroemeria leaf tips during 48 h in darkness.

Incubation in dark (h)	$[^3H_2]GA_1$ (dpm)	% of applied label	$[^3H_2]GA_4$ (dpm)	% of applied label
5	123	0.2	178	0.4
10	355	0.7	366	0.7
24	560	1.1	467	0.9
48	802	1.6	970	1.9

Uptake of $[^3H]GA_1$ and $[^3H]GA_4$

First, differences in the uptake of GA_1 and GA_4 might explain the differences found in efficacy. Leaves were incubated with a 10^{-7} M GA solution enriched with tritium-labeled GA. Leaves were shown to have a comparable uptake during the experimental period of 48 h (Table 1). Thus, differences in GA uptake cannot explain the differences in the efficacy of GA_1 and GA_4.

13-Hydroxylation Necessity

It is thought that active GAs have two characteristics in common: a 10-C–19-C lactone bridge below the plane of the A-ring and a hydroxyl group at the 3-C position (Pearce et al. 1994). According to Stoddart (1986) hydroxylation of the 13-C position enhances efficacy further. The presence of hydroxyl groups cannot simply explain the activity in alstroemeria leaves because the less hydroxylated GA_4 was much more active than GA_1.

To find out whether conversion of GA_4 into GA_1 and hence a 13-OH group is a prerequisite for activity we used a GA analog. This modified GA has an O-CH_3 group on the 13-C position and is unlikely to be converted back to its basis GA (Dr. M. H. Beale, personal communication). From previous work it is known that an order of efficacy exists in GA acitivity from $GA_4 = GA_7 > GA_9 > GA_1 > GA_3$ (Jordi et al. 1995). We used GA_3-13-OMe to test whether a 13-OH group is necessary for biological activity. From the results in Fig. 1 it is clear that GA_3-13-OMe showed a concentration-dependent effect on the delay of chlorophyll loss. No GA_1, GA_3, or GA_4 was detected in an aliquot of 100 ng of GA_3-13-OMe analyzed by GC-MS. Although the calculated concentration needed for the half-maximum response is 310 nM for GA_3-13-OMe compared with 2.1 nM for GA_4, it is clear that GA_3-13-OMe delays leaf senescence. The observed difference in maximum activity could be partly a result of the difference found between the maximum activity of GA_4 and GA_1. We conclude that hydroxylation on the C-13 position seems not essential for delaying chlorophyll loss.

Metabolism of GA_1, GA_4, and GA_9

Our results show that uptake of GAs cannot explain the differences found in efficacy. Furthermore, 13-hydroxylation of GAs is not a prerequisite for biological activity

Table 2. GC-SIM data on metabolites from $[^2H_2]GA_1$, $[^2H_2]GA_4$, and $[^2H_2]GA_9$ incubation of alstroemeria leaves for 48 h. Data are means of four independent experiments ± S.D. Enrichment is calculated by ([*m/z* + 2]/[*m/z*] of sample)/([*m/z* + 2]/[*m/z*] of standard).

GA fed to the leaves	Deuterated GAs[a]		Relative peak area ratio		Enrichment
	KRI	GA_n	[*m/z* + 2]/[*m/z*][a]		
GA_1 standard	2,689		0.150	(508/506)	
GA_4 standard	2,522		0.053	(286/284)	
GA_8 standard	2,837		0.254	(596/594)	
GA_9 standard	2,319		0.034	(300/298)	
GA_{34} standard	2,675		0.169	(508/506)	
GA_8-catabolite[b]	2,723		0.182	(536/534)	
GA_{34}-catabolite[b]	2,569		0.294	(373/371)	
$[^2H_2]GA_1$ fed	2,689	GA_1	2.257 ± 0.12	(508/506)	15.05
	2,522	GA_4	0.059 ± 0.003	(286/284)	1.11
	2,837	GA_8	1.056 ± 1.89	(596/594)	4.22
	2,319	GA_9	0.039 ± 0.004	(300/298)	1.15
	2,675	GA_{34}	0.153 ± 0.009	(508/506)	0.91
	2,723	GA_8-cat	0.319 ± 0.18	(536/534)	1.75
	2,569	GA_{34}-cat	0.291 ± 0.06	(373/371)	0.99
$[^2H_2]GA_4$ fed	2,689	GA_1	0.138 ± 0.01	(508/506)	0.92
	2,522	GA_4	1.864 ± 0.09	(286/284)	35.17
	2,837	GA_8	0.272 ± 0.04	(596/594)	1.07
	2,319	GA_9	0.040 ± 0.002	(300/298)	1.18
	2,675	GA_{34}	17.601 ± 1.27	(508/506)	104.15
	2,723	GA_8-cat	0.178 ± 0.04	(536/534)	0.98
	2,569	GA_{34}-cat	0.641 ± 0.12	(373/371)	2.18
$[^2H_2]GA_9$ fed	2,689	GA_1	0.162 ± 0.005	(508/506)	1.08
	2,522	GA_4	1.532 ± 0.08	(286/284)	28.91
	2,837	GA_8	0.292 ± 0.02	(596/594)	1.15
	2,319	GA_9	3.029 ± 0.13	(300/298)	89.08
	2,675	GA_{34}	16.431 ± 0.88	(508/506)	97.22
	2,723	GA_8-cat	0.180 ± 0.05	(536/534)	0.99
	2,569	GA_{34}-cat	0.451 ± 0.04	(373/371)	1.53

[a] Ions representing [*m/z* + 2] and [*m/z*] for the different GAs are given in parentheses.
[b] Obtained by interpolation of data of Gaskin and MacMillan (1991).

in the delay of leaf senescence. We have studied further the metabolism of deuterated GA_4 and GA_1 to elucidate the possible conversion pathways in vivo of these GAs.

In alstroemeria leaves, endogenous amounts of GA_1 and GA_4 in mature (fresh) leaves are almost identical: 0.66 ± 0.05 pmol $(g\ FW)^{-1}$ and 0.72 ± 0.02 pmol $(g\ FW)^{-1}$, respectively (Kappers et al. 1998). To get insight in their metabolism, we followed the fate of deuterated and tritiated labeled compounds. Although to date it is nearly impossible to estimate exact turnover rates, we can compare relative turnover rates of both GAs. To ensure a high uptake of deuterated GAs into the leaf, the GAs were dissolved in 0.1 M citrate phosphate buffer at pH 5.0. Previous experiments showed that uptake was maximal at this pH (data not shown). The amount of deuterated GA taken up by the leaf was calculated from the tritiated GAs. Assuming no discrimination in the uptake of deuterated and tritiated GA, the uptake of deuterated GA was estimated to be 65 pmol of GA_1, 59 pmol of GA_4, and 182 pmol of $GA_9/(qFW)^{-1}$, resulting in an increase of the endogenous concentration of GA_1, GA_4, and GA_9 with a factor 98, 82, and 152, respectively. When leaves were incubated with $[^2H_2]GA_4$ the ratio *m/z* 286/284, an estimation of the ratio deuterated vs non-deuterated GA_4, found after 48 h of labeling was 35 times the ratio found in nonlabeled leaves, which confirmed an increased endogenous concentration of GA_4 (Table 2). The discrepancy between the total uptake of 82 times the endogenous concentration and the enrichment of the ratio between deuterated and endogenous GA (35 times) is caused by the metabolism of the applied GA. The increased ratios of 508/506 and 373/371 found at the retention times of GA_{34} and GA_{34} catabolite suggested the presence of deuterated GA_{34} and possibly GA_{34} catabolite, demonstrating the conversion of GA_4 into GA_{34}. This step involves a 2β-hydroxylation, known to inactivate GAs (Graebe 1987). GA_{34} was found in alstroemeria leaves at a low concentration of 0.04 pmol $(g\ FW)^{-1}$ (Kappers et al. 1997). After incubation with GA_4, endogenous GA_1 was detected, but the *m/z* 508/506 ratio observed corresponded to the natural abundance of the isotope. This indicated that even at a very high en-

dogenous GA_4 concentration no significant extra GA_1 was formed (i.e. less than 3%). To exclude as much as possible experimental errors in demonstrating GA_4 metabolism, we also incubated leaves with labeled GA_9 (Table 2). It can be seen that the ratios of GA_9, GA_4, and GA_{34} differed from the endogenous values. Also in this case no increase in endogenous GA_1 could be found. Incubation of leaves with deuterated GA_1 led to increased ratios for GA_1 and GA_8 and possibly GA_8 catabolite (Table 2).

Although the estimated concentration of the biologically active hormones was the same for GA_1 and GA_4, the turnover rate, that is, the number of molecules/time unit formed and subsequently turned over, seemed to be different. From the calculated enrichment (Table 2) it can be concluded that the non-13-hydroxylated GA_{34} and GA_{34} metabolite were formed relatively more than their corresponding 13-hydroxylated counterparts, GA_8 and GA_8 catabolite. Although the uptake of both GA_1 and GA_4 is comparable, the enrichment of the leaf with GA_1 is lower after 48 h. This indicates a higher turnover rate for GA_1 than for GA_4. However, it is obvious that even at high concentrations of GA_4 in the leaf no conversion into GA_1 occurs. Combined with the greater biological efficacy of applied GA_4 toward delay of senescence this makes it very likely that the non-13-hydroxylation pathway yielding GA_4 is a part of early processes regulating senescence in alstroemeria leaves. As no conversion into GA_1 is needed, GA_4 will be recognized directly by the receptor involved in the regulation of leaf senescence.

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References

Gaskin P, MacMillan J (1991) GC-MS of the gibberellins and related compounds: Methodology and a library of spectra. 1st Ed. Cantocks Enterprises, Bristol

Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll *a* and *b* in *N,N*,-dimethylformamide and 80% acetone. Plant Physiol 77:483–485

Jordi W, Dekhuijzen HM, Stoopen GM, Overbeek JHM (1993) Role of other plant organs in gibberellic acid-induced delay of leaf senescence in *Alstroemeria* cut flowering stems. Physiol Plant 87:426–432

Jordi W, Stoopen GM, Kelepouris K, Krieken WM (1995) Gibberellin-induced delay of leaf senescence of *Alstroemeria* cut flowering stems is *not* caused by an increase in the endogenous cytokinin content. J Plant Growth Regul 14:121–127

Kappers IF, Jordi W, Maas FM, Stoopen GM, Van der Plas LHW (1998) Gibberellin and phytochrome control senescence in alstroemeria leaves independently. Physiol Plant, in press

Kappers IF, Jordi W, Maas FM, Van der Plas LHW (1997) Gibberellins in leaves of *Alstroemeria hybrida:* Identification and quantification in relation to leaf age. J Plant Growth Regul 16:219–225

Kobayashi M, Suzuli Y, Spray C, Phinney B, Gaskin P, MacMillan J (1991) GA_4 is metabolized to GA_1 (not GA_7/GA_3) in maize, rice, and *Arabidopsis.* In: Karssen CM, van Loon LC, Vreuqdenhil O, (eds) Abstracts of the 14th International Conference on Plant Growth Substances. Kluwer Academic Publishers, Amsterdam, p 63

Nakayama M, Yamane H, Murofushi N, Takahashi N, Mander LN, Seto H (1991) Gibberellin biosynthetic pathway and the physiologically active gibberellin in the shoot of *Cucumis sativus.* J Plant Growth Regul 10:115–119

Pearce DW, Koshioka M, Pharis RP (1994) Chromatography of gibberellins. J Chromatogr A 658:91–122

Rebers M, Vermeer E, Knegt E, Shelton CJ, Van der Plas LHW (1995) Gibberellin levels and floral stalk elongation in *Tulipa gesneriana* Physiol Plant 99:687–691

Sponsel VM (1995) Gibberellin biosynthesis and metabolism. In: Davies PJ (ed) Plant hormones, physiology, biochemistry and molecular biology. Kluwer Academic Publishers, Dordrecht, pp 66–97

Stoddart JL (1986) Gibberellin receptors. In: Chadwick CM, Garrod DR (eds) Hormones, receptors and cellular interactions in plants. Cambridge University Press, Cambridge, pp 91–114

Talon M, Koornneef M, Zeevaart JAD (1990) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc Natl Acad Sci USA 87:7983–7987

Turnbull CGN, Crozier A (1989) Metabolism of [1,2-$^3H_2$]gibberellin A_4 by epicotyls and cell-free preparations from *Phaseolus coccineus* L. seedlings. Planta 178:267–274

Weyers JDB, Paterson NW, A'Brook R (1987) Towards a quantitative definition of plant hormone sensitivity. Plant Cell Environ 10: 1–10