J Plant Growth Regul (2002) 20:346–353
DOI: 10.1007/s003440010034

J P G R
Journal of Plant Growth Regulation

Auxin-Gibberellin Interactions and Their Role in Plant Growth

John J. Ross,* Damian P. O'Neill, Carla M. Wolbang, Gregory M. Symons, and James B. Reid

School of Plant Science, University of Tasmania, GPO Box 252-55, Hobart, Tasmania 7001, Australia

Abstract

Recently it was discovered that auxin promotes gibberellin (GA) biosynthesis in decapitated stems of pea (*Pisum sativum* L.) and tobacco (*Nicotiana tabacum* L.), and here we review the evidence for this interaction. We also discuss the possible relationship between auxin and the mechanisms by which bioactive GAs (such as GA_1) regulate their own levels, and the implications of the auxin-GA interaction for the control of plant growth. It is now possible to envisage auxin as a messenger linking the apical bud with the biosynthesis of active GAs in the expanding internodes. Finally, new evidence is presented that the promotion of growth by GA_1 does not depend on GA_1-induced increases in auxin content.

Key words: Auxin; Feed-back regulation; Gibberellin; Hormone interactions; Internode elongation; *Pisum sativum*

Introduction

Auxin is one of the five ''classical'' plant growth hormones (Kende and Zeevaart 1997) and was the first to be isolated. The discovery of a second growth hormone, gibberellin (GA), brought with it much speculation about how auxin and GA might interact (Brian and Hemming 1957, 1958; Vlitos and Meudt 1957; Galston and Warburg 1959). This question was, and still is, of paramount importance for understanding the hormonal regulation of plant growth.

Nevertheless, much of our knowledge on auxin and GAs has come from studying them independently. In GA studies Mendel's *le* mutant (now termed *le-1*) has played a pivotal role. Early research demonstrated the spectacular growth-promoting effects of applying GA_3 to intact *le-1* plants (Brian and Hemming 1955). In the 1980s it was shown that *le-1* blocks the crucial activation step GA_{20} to GA_1 (Figure 1), reducing the content of bioactive GA_1 and thereby resulting in dwarfism (Ingram and others 1984). More recently, *LE* was cloned and shown to encode the enzyme for the step GA_{20} to GA_1 (Lester and others 1997; Martin and others 1997). It was also shown that *LE* (now termed *PsGA3ox1*) is down-regulated by GA_1 (Martin and others 1997; Ross and others 1999), as part of a ''feed-back'' mechanism (Hedden and Croker 1992).

Auxin research has also made great progress. For example, there have been major breakthroughs in isolating key elements of the polar auxin transport system (reviewed by Jones 1998). Furthermore, genes that are up- or down-regulated by auxin have been extensively characterized (Abel and Theologis 1996; Guilfoyle and others 1998). Some of these genes are up-regulated within a few minutes of auxin treatment. These ''early'' or ''primary'' genes are thought in turn to regulate secondary or ''late'' auxin response genes (Abel and Theologis 1996).

Recently, Ross and O'Neill (2001) proposed a new model that brings together the auxin and GA fields.

Online publication: 18 March 2002
*Corresponding author; e-mail: John.Ross@utas.edu.au

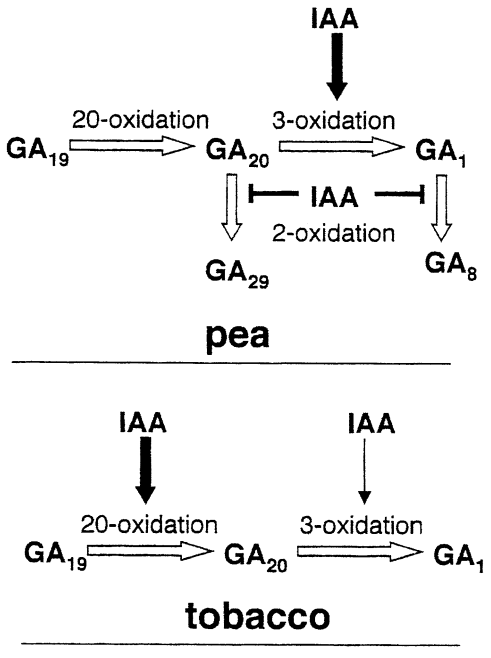

Figure 1. Relevant parts of the GA pathway in pea and tobacco. Solid arrows or bars indicate the steps known to be affected by IAA, on the basis of metabolism experiments. The stronger the effect, the broader the arrow or bar.

The model is based on the discovery that *LE* (*PsGA3ox1*) is an auxin-regulated gene (Ross and others 2000). In pea shoots the main auxin is indole-3-acetic acid (IAA), which is transported from the apical bud into the elongating internodes (Lomax and others 1995). Decapitation (removing the apical bud) reduces the IAA content of the remaining stem tissue (Beveridge and others 1994; Ross and others 2000). In decapitated stems *PsGA3ox1* transcript levels, and consequently GA_1 biosynthesis, are also dramatically reduced (Ross and others 2000). In contrast, expression of the GA deactivation gene *SLN (PsGA2ox1),* which encodes the enzyme for the steps GA_{20} to GA_{29} and (at least *in vitro*) GA_1 to GA_8 (Lester and others 1999; Martin and others 1999), is increased by decapitation (Ross and others 2000).

The application of IAA (in lanolin paste) to the 'stump' of decapitated peas restores both *PsGA3ox1* transcript levels and GA_1 biosynthesis in the stems. Furthermore, IAA down-regulates *PsGA2ox1* transcript levels, compared with untreated decapitated plants (Ross and others 2000). In a subsequent study, auxin was shown to exert these effects within 2 h of application (O'Neill and Ross unpublished).

Further evidence for the new model comes from applying auxin transport inhibitors to intact plants (Ross 1998). When applied in a ring of lanolin, these compounds reduce both the IAA and GA_1 content of the stem, below the application site, while GA_{29} accumulates (Ross 1998). Results from the *bushy* mutant of pea, which is characterized by profuse branching and short internodes (Symons and others 1999), are also consistent with the model. Free IAA levels in intact *bushy* shoots are markedly reduced compared with wild-type plants (G,M. Symons and others unpublished). After feeds of [^{14}C]GA_{20}, the pattern of metabolism in *bushy* plants was very similar to that observed in decapitated wild-type plants, with the step GA_{20} to GA_{29} predominating over GA_{20} to GA_1 (G.M. Symons and others unpublished). As a result, *bushy* plants contain less endogenous GA_1 than the wild-type. Two other pea mutants, *gi* and *veg1*, also contain reduced levels of both IAA and GA_1 (Beveridge and others 2001).

Therefore there is clear evidence for a link between IAA and GA in pea, but how widespread is the relationship? We chose tobacco as a second species in which to investigate the auxin-GA interaction. Tobacco is not closely related to pea, and any similarity in the interaction might indicate that it is widespread.

In tobacco, decapitation again inhibited the step GA_{20} to GA_1, and this was reversed by IAA, but the effects were less dramatic than in pea (Wolbang and Ross 2001). The most intriguing outcome was that the conversion of GA_{19} to GA_{20} (Figure 1) was strongly inhibited by decapitation. This step, termed GA 20-oxidation, is an important, regulated step in a number of species (Hedden and Kamiya 1997; Hedden 1999). Once again, IAA counteracted the effect of decapitation, increasing GA_{20} levels to three times that observed in intact plants. Hence, it was concluded that in tobacco, as in pea, IAA from the apical bud is required for GA biosynthesis in stems. However, the main steps promoted appear to differ between the two species: 20-oxidation in tobacco, and 3-oxidation in pea (Figure 1).

In tobacco, GA_{19} is known to accumulate to a greater extent than other GA intermediates (Nilsson and others 1993), indicating that GA_{19} to GA_{20} might be the rate-limiting step. In contrast, GA_{19} to GA_{20} proceeds rapidly in pea, where the rate-limiting step is thought to be 3-oxidation (Martin and others 1996). Therefore, the GA biosynthetic step most affected by IAA in tobacco and pea stems appears to correspond with the rate-limiting step.

In pea stems there was no evidence from endogenous GA levels for a strong effect of IAA on the conversion of GA_{19} to GA_{20}. However, in pea pods this step does appear to be auxin-regulated, but by a different auxin, 4-chloro-IAA (van Huizen and others 1997). It was suggested that 4-Cl-IAA might move from young seeds into the pods, promoting the

step GA_{19} to GA_{20}, and therefore pod elongation (van Huizen and others 1997). However, while the importance of GA_1 for internode elongation is beyond dispute, its role in pod elongation is not so clear (MacKenzie-Hose and others 1998). The main reason for this is that the pods of certain dwarf mutants (such as *le-1*) elongate to the same extent as wild-type pods, despite being GA_1-deficient (Santes and others 1993; MacKenzie-Hose and others 1998).

Critical Assessment of the Evidence: the "Pesigs Rules"

Does the evidence discussed above actually establish that endogenous IAA is necessary for GA_1 biosynthesis in stems? Certainly it seems to meet the requirements of the "PESIGS Rules" (Jacobs 1979; Davies 1995), established for determining whether or not a hormone performs a particular role. The first of these rules ("P") concerns the *presence* of the hormone and *parallel* variation between the level of the hormone (IAA in this case) and the response in question (GA_1 biosynthesis). IAA is definitely present in pea stems, and there were parallel reductions in levels of the two hormones after treatment with auxin transport inhibitors (Ross 1998), and in the *bushy* mutant. The second PESIGS requirement is also satisfied, since *excision* of the apical bud, thought to be the main source of IAA for stems, reduces stem GA_1 content (Sherriff and others 1994; Ross and others 2000). The third requirement, *substitution,* is clearly met by the ability of applied IAA to promote GA_1 biosynthesis in decapitated plants (Ross and others 2000; Wolbang and Ross 2001). In *isolated* stem sections of pea, IAA also promotes GA_1 production (D.P. O'Neill and J.J. Ross unpublished). The *generality* of the auxin-GA interaction is indicated by the results from tobacco, but we aim to further extend our studies, especially to monocotyledonous species. The final PESIGS requirement concerns the *specificity* of the response. We have not yet tested a wide range of compounds, but it is clear that GA_1 itself does not promote GA_1 biosynthesis in the same way as IAA. Indeed, GA_1 is known to inhibit its own biosynthesis (see following section).

Thus, the PESIGS rules seem to be satisfied with regard to demonstrating that IAA is *required* for normal GA_1 biosynthesis in stems. However, this does not mean that GA_1 levels are actually *regulated* by IAA. It is possible that a threshold level of IAA is required, above which GA_1 biosynthesis is normal, and below which it is essentially prevented. We are presently studying the quantitative relationship between IAA content and GA_1 content. It is interesting to note that in Ross and others (2000), decapitated plants treated with IAA contained 3-fold more GA_1 than did intact plants, indicating that in some circumstances at least, the stem IAA content might be limiting for GA_1 biosynthesis.

Is there A Relationship Between Auxin and the Feed-Back Regulation of GA Biosynthesis?

The most widely studied factor influencing GA biosynthesis is the "feed-back" mechanism, whereby bioactive GAs negatively regulate their own production (Scott 1990; Croker and others 1990; Hedden and Kamiya 1997). Recently it has been shown that bioactive GAs can also up-regulate expression of GA deactivation genes (Thomas and others 1999) and this "feed-forward" mechanism, along with feed-back, tends to maintain constant levels of bioactive GAs such as GA_1. How auxin relates to the feed-back and feed-forward mechanisms is not yet known. Because of feed-back regulation, wild-type pea plants contain lower levels of *PsGA3ox1* transcript than do GA_1-deficient mutants (Martin and others 1997; Ross and others 1999). In contrast, in decapitated wild-type internodes, a low *PsGA3ox1* transcript level is associated with a low GA_1 content (Ross and others 2000). This indicates that the auxin deficiency of decapitated internodes might override the feed-back mechanism.

At this stage, however, we cannot be sure that feed-back regulation of *PsGA3ox1* occurs in the internodes themselves, rather than being confined to the leaves and/or apical bud (Elliott and others 2001). On the other hand, feed-forward regulation of *PsGA2ox1* transcript levels is known to occur in internodes (Elliott and others 2001), but is still overridden by auxin deficiency: decapitated internodes contain high, not low, levels of *PsGA2ox1* mRNA (Ross and others 2000).

It might be suggested that the up-regulation of *PsGA3ox1* transcript level in GA_1-deficient mutants is mediated by an increase in IAA level. However, this is not supported by evidence that GA_1-deficient mutants contain less, not more, IAA than the wild type (Law and Davies 1990; McKay and others 1994).

Implications of the Auxin-GA Relationship for Growth

According to the Ross and O'Neill (2001) model, IAA is a messenger compound from the apical bud,

activating GA biosynthesis in the elongating internodes. Indeed, the main growth-regulating function of endogenous IAA might be to maintain GA_1 levels in the internodes, although our model by no means precludes a direct effect of auxin on growth. Certainly, it is clear that IAA can affect GA_1 biosynthesis in very young internodes (less than 25% of their final length) (J.J. Ross and C.M. Wolbang unpublished), and internodes at this stage of expansion are GA-responsive (Ross 1998). It is therefore highly likely that in these internodes auxin-induced GA_1 is important for elongation.

It is interesting to reconsider previous studies on the interaction between auxin and GA in view of our recent evidence. For example, Davies and Ozbay (1975) and Haga and Iino (1998) decapitated tall pea plants, applied IAA in lanolin paste, measured the growth response, and ascribed that response to IAA. It now appears possible that at least part of the growth response was in fact mediated by an increase in GA_1 content. Certainly the decapitated plants studied by Davies and Ozbay (1975) were GA-responsive.

Our results also raise the possibility that if an environmental factor affects auxin content, GA_1 levels might also be altered as a consequence. For example, a conventional wisdom is that pea stems bending towards the light should contain more auxin on the shaded side than on their irradiated side (the Cholodny-Went theory) (Kaufman and others 1995). If this is the case, does the shaded side also contain more GA_1 than the irradiated side? Physico-chemical analyses are required to answer this question. At present it is not even clear that auxin itself is asymmetrically distributed across phototropically bending pea stems.

It is clear, however, that there is a gradient of IAA from the tip of the pea shoot to its base (Beveridge and others 1994). Gibberellin A_1 levels are also lower in mature tissue than in immature tissue (Smith and others 1992; Ross 1998), and it might be speculated that this is due to the low IAA content of the former. However, this appears not to be the case. When decapitated mature internodes were treated with IAA, GA_{20} 3-oxidation was certainly enhanced, compared with untreated decapitated controls, but the GA_1 content was not restored to that of immature tissue (J.J. Ross and others unpublished). The reason for this appears to be that in mature pea tissue, GA 2-oxidation activity is very strong, and although mature tissue synthesizes GA_1, it is rapidly deactivated to GA_8. IAA application appears incapable of substantially reducing the 2-oxidase activity of mature tissue, and it is likely, therefore, that the 2-oxidase responsible is not *PsGA2ox1* (which is IAA-responsive) (Ross and others 2000).

In contrast to our findings with pea, Collett and others (2000) concluded that there is no interaction between auxin and GA in controlling the growth of *Arabidopsis* hypocotyls. However, it should be borne in mind that there are significant differences between pea stems and *Arabidopsis* hypocotyls. For example, auxin was inhibitory to elongation in *Arabidopsis* hypocotyls (Collett and others 2000) but stimulatory in decapitated peas (Ross and others 2000). Furthermore, GA affects both cell length and cell number in pea internodes (Reid and others 1983), but only the former in *Arabidopsis* hypocotyls (Gendreau and others 1997).

Evidence that GA_1 Promotes Elongation *Per Se*

The suggestion by Ross and O'Neill (2001) that auxin might promote elongation (at least in part) by increasing GA_1 levels contrasts with an earlier theory (Lantican and Muir 1969; Kuraishi and Muir 1962) that part of the GA response is mediated by an increase in auxin level. Further evidence for the early theory is the apparent correlation between IAA content and stem elongation across a range of GA-related mutants (Law and Davies 1990). McKay and others (1994) also found that the internodes of a dwarf mutant with reduced GA_1 levels, *le*^{5839} (since re-named *le-3*), contained less IAA than wild-type internodes. Furthermore, GA application was reported to increase IAA levels in intact plants (Law and Hamilton 1984) and in isolated stem sections (Barratt and Davies 1997). Thus GA_1-induced elongation is usually accompanied by an increase in IAA content, and it has always been difficult to exclude the possibility that these increases, although typically only about 2-fold, actually cause the growth response. Clearly, it is important to ask, ''Can GA_1 promote elongation independently of its effect on IAA level?''

To address that question, we designed experiments to gauge the significance for growth of the GA_1-induced changes in IAA content, using Mendel's *le-1* dwarf mutant. The *le-1* line was 205-, which is isogenic with 205+, the main tall line used in previous auxin-GA experiments (Ross and others 2000). In a series of experiments *le-1* plants were decapitated and treated with auxin or GA_1 and their growth responses were monitored. A previous paper reported the strong GA response of decapitated *le-1* internodes (Ross 1998).

The data in Figure 2 show that the GA_1 response in decapitated *le-1* internodes was 70% of that

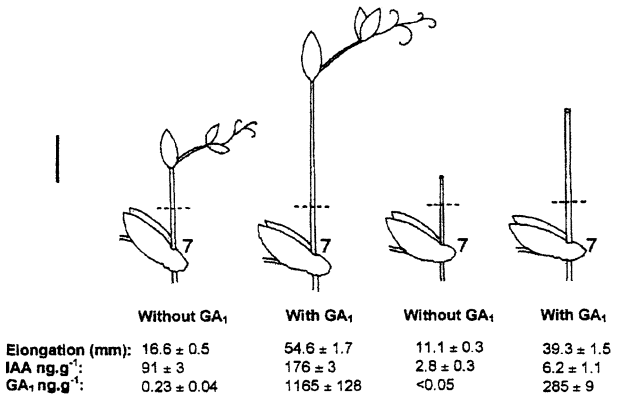

Figure 2. Effects of applied GA_1 on elongation and hormone content of decapitated *le-1* plants. Plants were grown in a heated glasshouse with an 18 h photoperiod, as described previously (Ross and others 2000). When the plants had 7 fully expanded leaves (node 7 is indicated), uniform plants were either left intact (2 plants on the left) or decapitated immediately below node 8. GA_1 was then applied (10 μg in 10 μl of ethanol) to leaf 6. Internode 7-8 was measured at decapitation (initial length indicated by dashed lines) and, 2 days later, measured again and harvested for hormone analysis. Hormone levels were measured using stable isotope-labeled internal standards and GC-MS, as before (Ross 1998). The internodes are drawn to scale (scale bar = 20 mm). Shown are means and standard errors from 9 replicates for elongation data and from 2 replicate harvests (each consisting of internodes from 4 or 5 plants) for hormone levels.

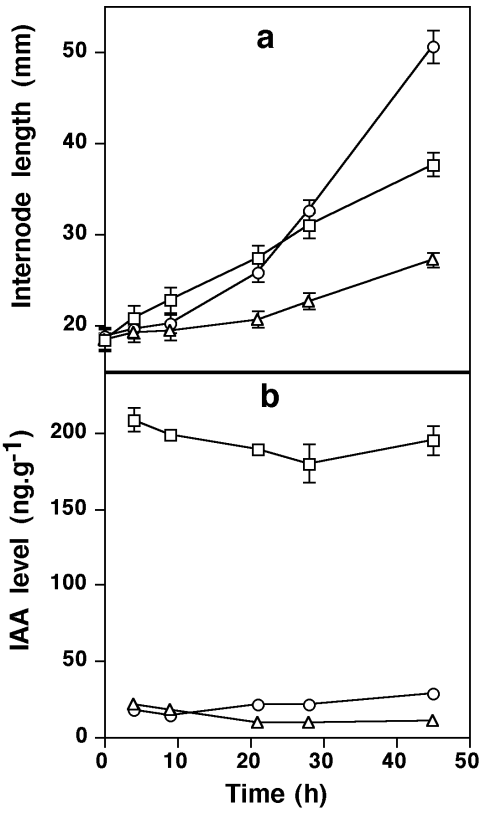

Figure 3. Length (**a**) and IAA content (**b**) of *le-1* internodes at several time points after decapitation and GA_1 treatment, performed as described for Figure 2. Intact, □; decapitated, △ or ◇; decapitated + GA_1, O. Shown are means from 10 replicates for length data and from 2 replicate harvests (each consisting of internodes from 5 to 7 plants) for IAA levels. Standard errors are shown where they exceed the symbol.

shown by accompanying intact plants. In both intact and decapitated plants, GA_1 application resulted in a higher IAA content, confirming previous results (see, for example, Barratt and Davies 1997). However, it is improbable that these small (approximately 2-fold) changes in IAA mediated the growth response to GA_1 because decapitated internodes treated with GA_1 still contained very little IAA: about 7% of the level present in the intact internodes without GA_1 (measured 2 days after decapitation). Despite their low IAA content, the elongation of decapitated GA_1-treated internodes was more than twice that of intact internodes (Figure 2). These results clearly break the correlation between rapid elongation growth and high IAA levels, and indicate that GA_1 promotes elongation in its own right, rather than by increasing IAA content.

It was possible, however, that GA_1 caused a large but transient increase in IAA, which had dissipated by 2 days after application. We therefore conducted a time-course experiment with intact, decapitated, and decapitated + GA_1 treatments. Internode length and endogenous IAA levels were measured 4, 9, 21, 28, and 45 h after decapitation/GA_1 application. There was no evidence for a large early increase in IAA content in GA_1-treated plants. Decapitation rapidly reduced IAA content, by approximately 10-fold after 4 h, and this was the case with and without applied GA_1 (Figure 3). By 21 h, both the IAA content and elongation of decapitated plants were increased by GA_1, but the level of IAA was still only about 11% of the level in intact internodes.

It was also possible that decapitated plants were so sensitive to auxin that even the small GA_1-induced increase in IAA content (Figures 2 and 3) could account for the growth response to GA_1. To investigate this possibility we applied varying dosages of IAA to decapitated plants and monitored the elongation response compared with decapitated plants treated with GA_1. Internodes given the lowest IAA dose contained a similar level of IAA to GA_1-treated plants, but their elongation was not promoted compared with decapitated controls (Table 1).

Table 1. Effects of Applied GA_1 and IAA on Elongation and IAA Content of Decapitated *le-1* Internodes, 48 h after Decapitation

Treatment	Elongation (mm)	IAA level (ng.g FW^{-1})
Intact	15.9 ± 0.6 (b)	108 ± 7.0 (b)
Decap.	9.5 ± 0.7 (d)	6.0 ± 1.0 (e)
Decap. + 10 μg GA_1	27.8 ± 2.5 (a)	9.4 ± 0.6 (d,e)
Decap. + IAA/lanolin at 0.006 $mg.g^{-1}$	9.6 ± 0.8 (d)	10.0 ± 0.5 (d)
Decap. + IAA/lanolin at 0.02 $mg.g^{-1}$	11.7 ± 0.7 (b,c,d)	17.0 ± 0.7 (c)
Decap. + IAA/lanolin at 0.1 $mg.g^{-1}$	11.6 ± 1.1 (c,d)	72 ± 4 (b)
Decap. + IAA/lanolin at 1.0 $mg.g^{-1}$	14.7 ± 0.4 (b,c)	312 ± 11 (a)

IAA was applied in lanolin paste to the top of the decapitated internode every 12 h, as before (Ross and others 2000). GA_1 was applied as described in Figure 2. Data are means ± s.e. of 8 replicates for elongation and 2 replicates (each comprising 4 plants) for IAA levels. Within a column, means with the same letter, are not significantly different ($P < 0.05$).

Again, these data indicate that GA_1 did not promote elongation by increasing IAA level. Furthermore, there was no evidence that decapitated plants were more sensitive to IAA than intact plants, consistent with a previous finding (Haga and Iino 1998).

In summary, it appears that the slight effect of GA_1 on IAA content, although consistently observed, is too small to contribute markedly to GA_1-induced elongation. It should be noted also that the effect of IAA on GA_1 biosynthesis is much stronger than the reverse effect. For example, in a previous study, decapitation reduced IAA content by 6-fold, and virtually eliminated the ability to accumulate labeled GA_1 after feeds of labeled GA_{20} (Ross and others 2000). In contrast, the data in Figure 2 show that changes in GA_1 of more than 1000-fold caused only 2-fold changes in IAA content.

The GA_1-induced elongation of decapitated internodes is also significant because it has been suggested that, at least in some circumstances, a growth response to GA depends on the presence of auxin (Brian and Hemming 1958; Barratt and Davies 1997). It now appears that under our conditions GA can in fact strongly promote growth even when auxin levels are low (Figures 2 and 3; Table 1). Nevertheless, the lower elongation of GA_1-treated decapitated plants, compared with GA_1-treated intact plants (Figure 2), might indicate that IAA is required for the *maximal* GA response. The growth difference between GA-treated decapitated and intact plants was most likely not due to a difference in GA_1 level, since this level was very high in both cases (Figure 2).

Conclusions

In pea and tobacco, and possibly in other species, it now appears that auxin from the apical bud is required for normal GA_1 biosynthesis in stems. It is surprising that there is such a simple and direct relationship between the two classical growth hormones. Interestingly, as far as stem elongation is concerned, it is now possible to view GA_1 as a component of the auxin signalling pathway. A different viewpoint is that auxin is important for growth primarily because it increases the level of bioactive GA. Regardless of perspective, our results provide further evidence that internode elongation is strongly influenced by the apical bud. When the bud is damaged or removed (for example, by grazing), the levels of both auxin and GA_1 decrease in the internodes, in turn reducing elongation growth. Therefore, potentially wasteful extension of the decapitated internodes can be avoided, and future growth concentrated instead in the side shoots that grow out after decapitation.

Our present results indicate that in some circumstances at least, GA_1 can stimulate growth *per se*, rather than by increasing auxin content. The idea of GA_1 as the final hormonal regulator of stem growth is consistent with conclusions from studies on deep-water rice. Although ethylene, abscisic acid, and GA are all thought to play a role in regulating the elongation of deep-water rice, the actual effector is thought to be GA_1 (Kende and others 1998). In the present paper we have referred to the growth-*promoting* effects of GA_1, because the effects are dramatic after applying the hormone. However, it should be borne in mind that GA_1 is probably an inhibitor of a growth inhibitor (Brian and Hemming 1958; Richards and others 2001). Significantly, recent models based on this concept (Richards and others 2001) do not appear to involve other hormones as mediators of the GA message.

Future studies will address the question of whether or not other hormones affect the GA pathway. It is well known that auxin stimulates the production of ethylene. However, it is unlikely that

ethylene mediates the promotion of GA biosynthesis by IAA, because in terrestrial plants, ethylene is actually a growth inhibitor (Kende and Zeevaart 1997; Fuchs and Lieberman 1968). This contrasts markedly with its role in submerged plants, where the increased ethylene level plays a major role in *promoting* elongation by enhancing GA levels and/or GA sensitivity (Kende and others 1998).

In view of our recent results from pea and tobacco, it is interesting to speculate on the auxin-GA relationship in the classical auxin model system, the grass coleoptile. Is auxin required for GA biosynthesis in coleoptiles? The history of GA research in maize, as sketched by Phinney (1983), is relevant to that question. Phinney quotes the early studies of Harris (1953), who reported that after decapitation, *dwarf*-1 maize coleoptiles responded less to auxin application than did wild-type coleoptiles (and the same was true for isolated coleoptile segments). Subsequently it was shown that the *dwarf*-1 mutant is GA_1-deficient, with (as in *le-1* peas) a block in the step GA_{20} to GA_1 (Spray and others 1984). Although other explanations are possible, Harris's data are consistent with the theory that in wild-type maize, auxin promotes coleoptile elongation (at least in part) by up-regulating the step GA_{20} to GA_1. This step is blocked in the *dwarf*-1 mutant, which cannot, therefore, respond normally to auxin. However, more detailed study is required before it can be concluded that auxin acts *via* GAs in coleoptiles or in other parts of the grass plant.

In fact, even for pea, it has not yet been established that auxin is required for GA biosynthesis throughout the whole plant. Therefore, in the future we will extend our studies from internodes to the roots, seeds, leaves, and apical bud. The auxin-GA relationship in roots, for example, might be different, because there the main 3-oxidase gene might not be *PsGA3ox1* (*LE*), but rather a second, unidentified gene. The evidence for this is that the *le-1* mutation, and even the null *le-2* mutation, do not reduce root GA_1 levels, and their root phenotypes are normal (Yaxley and others 2001). Gibberellin A_1 is, however, important for root elongation, as shown by the short roots and low GA_1 content of another GA mutant, *na* (Yaxley and others 2001). *PsGA3ox1* does not seem to be the main gene for GA activation in young seeds either, and a further complication in these organs is that GA_{20} might not be the GA_1 precursor (Rodrigo and others 1997; MacKenzie-Hose and others 1998). The effects of auxin on GAs in roots and young seeds are presently unknown. Indeed, despite the recent progress, there is still much to learn about the auxin-GA relationship.

ACKNOWLEDGMENTS

We thank Dean Williams for statistical analyses, Emma Bayly-Stark for photography, Noel Davies, Ian Cummings, Tracey Jackson, and Robert Wiltshire for technical assistance, Jane Burrell and Andre Phillips for artwork, and the Australian Research Council for financial support.

REFERENCES

Abel S, Theologis A. 1996. Early genes and auxin action. Plant Physiol 111:9–17.

Barratt NM, Davies PJ. 1997. Developmental changes in the gibberellin-induced growth response in stem segments of light-grown pea genotypes. Plant Growth Regul 21:127–134.

Beveridge CA, Batge SL, Ross JJ, Murfet IC. 2001. Hormone physiology of pea mutants prevented from flowering by mutations *gi or veg1*. Physiol Plant 113:285–291.

Beveridge CA, Ross JJ, Murfet IC. 1994. Branching mutant *rms-2* in *Pisum sativum* - grafting studies and endogenous indole-3-acetic acid levels. Plant Physiol 104:953–959.

Brian PW, Hemming HG. 1955. The effects of gibberellic acid on shoot growth of pea seedlings. Physiol Plant 8:669–681.

Brian PW, Hemming HG. 1957. A relation between the effects of gibberellic acid and indolylacetic acid on plant cell extension. Nature 179:417.

Brian PW, Hemming HG. 1958. Complementary action of gibberellic acid and auxins in pea internode extension. Ann Bot 22:1–17.

Collett CE, Harberd NP, Leyser O. 2000. Hormonal interactions in the control of *Arabidopsis* hypocotyl elongation. Plant Physiol 124:553–561.

Croker SJ, Hedden P, Lenton JR, Stoddart JL. 1990. Comparison of gibberellins in normal and slender barley seedlings. Plant Physiol 94:194–200.

Davies E, Ozbay O. 1975. Comparative effects of indoleacetic acid and gibberellic acid on growth of decapitated etiolated epicotyls of *Pisum sativum* cv. Alaska. Physiol Plant 35:279–285.

Davies PJ. 1995. The plant hormone concept: concentration, sensitivity and transport. In: Davies PJ, editor. Plant Hormones. Dordrecht: Kluwer Academic Publishers, p 13–38.

Elliott RC, Ross JJ, Smith JJ, Lester DR, Reid JB. 2001. Feed-forward regulation of gibberellin deactivation in pea. J Plant Growth Regul 20:87–94.

Fuchs Y, Lieberman M. 1968. Effects of kinetin, IAA and gibberellin on ethylene production and their interaction in growth of seedlings. Plant Physiol 43:2029–2036.

Galston AW, Warburg H. 1959. An analysis of auxin-gibberellin interactions in pea stem tissue. Plant Physiol 34:16–22.

Gendreau E, Traas J, Desnos T, Grandjean O, Caboche M, Hofte H. 1997. Cellular basis of hypocotyl growth in *Arabidopsis thaliana*. Plant Physiol 114:295–305.

Guilfoyle T, Hagen G, Ulmasov T, Murfett J. 1998. How does auxin turn on genes? Plant Physiol 118:341–347.

Haga K, Iino M. 1998. Auxin-growth relationships in maize coleoptiles and pea internodes and control by auxin of the tissue sensitivity to auxin. Plant Physiol 117:1473–1486.

Harris RM. 1953. Auxin relations in a dwarf-1 allele of *Zea mays* L. Ph. D. Thesis, University of California, Los Angeles.

Hedden P. 1999. Regulation of gibberellin biosynthesis. In: Hooykaas PJJ, Hall MA, Libbenga KR, editors. Biochemistry

and Molecular Biology of Plant Hormones. Bristol: Elsevier Science. p 161–188.

Hedden P, Croker SJ. 1992. Regulation of gibberellin biosynthesis in maize seedlings. In: Karssen CM, van Loon LC, Vreugdenhil D, editors. Progress in Plant Growth Regulation. Dordrecht: Kluwer Academic Publishers. p 534–544.

Hedden P, Kamiya Y. 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. Ann Rev of Plant Physiol Plant Mol Biol 48:431–460.

Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillian J. 1984. Internode length in *Pisum*. Planta 160:455–463.

Jacobs WP. 1979. Plant hormones and plant development. Cambridge: University Press Cambridge. 339 pp

Jones AM. 1998. Auxin transport: down and out and up again. Science 282:2201–2202.

Kaufman PB, Wu L-L, Brock TG, Donghern K. 1995. Hormones and the orientation of growth. In: Davies PJ, editor. Plant Hormones. Dordrecht: Kluwer Academic Publishers. p 547–571.

Kende H, van der Knaap E, Cho H-T. 1998. Deepwater rice: a model plant to study stem elongation. Plant Physiol 118:1105–1110.

Kende H, Zeevaart JAD. 1997. The five ''classical'' plant hormones. Plant Cell 9:1197–1210.

Kuraishi S, Muir RM. 1962. Increase in diffusible auxin after treatment with gibberellin. Science 137:760–761.

Lantican BP, Muir RM. 1969. Auxin physiology of dwarfism in *Pisum sativum*. Physiol Plant 22:412–423.

Law DM, Davies PJ. 1990. Comparative indole-3-acetic acid levels in the slender pea and other pea phenotypes. Plant Physiol 93:1539–1543.

Law DM, Hamilton RH. 1984. Effects of gibberellic acid on endogenous indole-3-acetic acid and indolacetyl aspartic acid levels in a dwarf pea. Plant Physiol 75:255–256.

Lester DR, Ross JJ, Smith JJ, Elliott RC, Reid JB. 1999. Gibberellin 2-oxidation and the *SLN* gene of *Pisum sativum*. plant J 19: 65–73

Lester DR, Ross JJ, Davies PJ, Reid JB. 1997. Mendel's stem length gene (*Le*) encodes a gibberellin 3β-hydroxylase. Plant Cell 9:1435–1443.

Lomax TL, Muday GK, Rubery PH. 1995. Auxin transport. In: Davies PJ, editor. Plant Hormones. Dordrecht: Kluwer Academic Publishers. p 509–530.

MacKenzie-Hose AK, Ross JJ, Davies NW, Swain SM. 1998. Expression of gibberellin mutations in fruits of *Pisum sativum* L. Planta 204:397–403.

Martin DN, Proebsting WM, Hedden P. 1997. Mendel's dwarfing gene: cDNAs from the *le* alleles and function of the expressed proteins. Proc Natl Acad Sci 94:8907–8911.

Martin DN, Proebsting WM, Hedden P. 1999. The *SLENDER* gene of pea encodes a gibberellin 2-oxidase. Plant Physiol 121:775–781.

Martin DN, Proebsting WM, Parks TD, Dougherty WG, Lange T, Lewis MJ, Gaskin P, Hedden P. 1996. Feed-back regulation of gibberellin biosynthesis and gene expression in *Pisum sativum* L. Planta 200:159–166.

McKay MJ, Ross JJ, Lawrence NL, Cramp RE, Beveridge CA, Reid JB. 1994. Control of internode length in *Pisum sativum* - further evidence for the involvement of indole-3-acetic acid. Plant Physiol 106:1521–1526.

Nilsson O, Moritz T, Imbault N, Sandberg G, Olsson O. 1993. Hormonal characterization of transgenic tobacco plants expressing the *rolC* gene of *Agrobacterium rhizogenes* T_L-DNA.' Plant Physiol 102:363–371.

Phinney BO. 1983. The history of gibberellins. In: Crozier A, editor. The Biochemistry and Physiology of Gibberellins, vol 1. New York: Praeger. p 19–52.

Reid JB, Murfet IC, Potts WC. 1983. Internode length in *Pisum*. II. Additional information on the relationship and action of loci *Le, La, Cry, Na,* and *Lm*. J Exp Bot 34:349–364.

Richards DE, King KE, Ait-Ali T, Harberd NP. 2001. How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. Ann Rev Plant Physiol Plant Mol Biol 52:67–88.

Rodrigo MJ, Garcia-Martinez JL, Santes CM, Gaskin P, Hedden P. 1997. The role of gibberellins A_1 and A_3 in fruit growth of *Pisum sativum* L. and the identification of gibberellins A_4 and A_7 in young seeds. Planta 201:446–455.

Ross JJ. 1998. Effects of auxin transport inhibitors on gibberellins in pea. J Plant Growth Reg 17:141–146.

Ross JJ, MacKenzie-Hose AK, Davies PJ, Lester DR, Twitchin B, Reid JB. 1999. Further evidence for feedback regulation of gibberellin biosynthesis in pea. Physiol Plant 105:532–538.

Ross JJ, O'Neill DP. 2001. New interactions between classical plant hormones. Trends Plant Sci 6:2–4.

Ross JJ, O'Neill DP, Smith JJ, Kerckhoffs LHJ, Elliott RC. 2000. Evidence that auxin promotes gibberellin A_1 biosynthesis in pea. Plant J 21:547–552.

Santes CM, Hedden P, Sponsel VM, Reid JB, Garcia-Martinez JL. 1993. Expression of the *le* mutant in young ovaries of *Pisum sativum* and its effect on fruit development. Plant Physiol 101:759–764.

Scott IM. 1990. Plant hormone response mutants. Physiol Plant 78:147–152.

Sherriff LJ, McKay MJ, Ross JJ, Reid JB, Willis CL. 1994. Decapitation reduces the metabolism of gibberellin A_{20} to A_1 in *Pisum sativum* L., decreasing the *Le/le* difference. Plant Physiol 104:277–280.

Smith VA, Knatt CJ, Gaskin P, Reid JB. 1992. The distribution of gibberellins in vegetative tissue of *Pisum sativum* L. Plant Physiol 99:368–371.

Spray C, Phinney BO, Gaskin P, Gilmour SJ, MacMillan J. 1984. Internode length in *Zea mays* L. The dwarf-1 mutation controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta 160: 464–468.

Symons GM, Murfet IC, Ross JJ, Sherriff LJ, Warkentin TD. 1999. *bushy*, a dominant pea mutant characterised by short, thin stems, tiny leaves and a major reduction in apical dominance. Physiol Plant 107:346–352.

Thomas SG, Phillips AL, Hedden P. 1999. Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation. Proc Natl Acad Sci 96:4698–4703.

van Huizen R, Ozga JA, Reinecke DM. 1997. Seed and hormonal regulation of gibberellin 20-oxidase expression in pea pericarp. Plant Physiol 115:123–128.

Vlitos AJ, Meudt W. 1957. Relationship between shoot apex and effect of gibberellic acid on elongation of pea stems. Nature 180:284.

Wolbang CM, Ross JJ. 2001. Auxin promotes gibberellin biosynthesis in decapitated tobacco plants. Planta 214:153–157.

Yaxley JR, Ross JJ, Sherriff LJ, Reid JB. 2001. Gibberellin biosynthesis mutations and root development in pea. Plant Physiol 125:627–633.