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Cytokinin/Auxin Control of Apical Dominance in *Ipomoea nil*

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Although the concept of apical dominance control by the ratio of cytokinin to auxin is not new, recent experimentation with transgenic plants has given this concept renewed attention. In the present study, it has been demonstrated that cytokinin treatments can partially reverse the inhibitory effect of auxin on lateral bud outgrowth in intact shoots of *Ipomoea nil*. Although less conclusive, this also appeared to occur in buds of isolated nodes. Auxin inhibited lateral bud outgrowth when applied either to the top of the stump of the decapitated shoot or directly to the bud itself. However, the fact that cytokinin promotive effects on bud outgrowth are known to occur when cytokinin is applied directly to the bud suggests different transport tissues and/or sites of action for the two hormones. Cytokinin antagonists were shown in some experiments to have a synergistic effect with benzyladenine on the promotion of bud outgrowth. If the ratio of cytokinin to auxin does control apical dominance, then the next critical question is how do these hormones interact in this correlative process? The hypothesis that shoot-derived auxin inhibits lateral bud outgrowth indirectly by depleting cytokinin content in the shoots via inhibition of its production in the roots was not supported in the present study which demonstrated that the repressibility of lateral bud outgrowth by auxin treatments at various positions on the shoot was not correlated with proximity to the roots but rather with proximity to the buds. Results also suggested that auxin in subtending mature leaves as well as that in the shoot apex and adjacent small leaves may contribute to the apical dominance of a shoot.

Key words: Apical dominance — Auxin — Cytokinin — *Ipomoea nil* — Lateral bud.

There has been considerable recent interest in the hypothesis that the ratio of cytokinin to auxin controls apical dominance (Klee and Romano 1994, Li and Bangerth 1992, Stafstrom 1993). Over the years it has been demonstrated in most species that exogenous auxin applied to the stump of a decapitated shoot will repress outgrowth of one or more of the lower axillary buds (Thimann and Skoog 1933, Cline 1996) and that the direct application of cytokinin to the axillary bud of an intact shoot will often promote the initiation of outgrowth of this bud (Pillay and Railton 1983, Semeniuk and Griesbach 1985).

In the classic work of Thimann's laboratory with peas (Wickson and Thimann 1958, 1960, Sachs and Thimann 1967), it was shown both in isolated nodes and in intact plants that the promotive effects of exogenous cytokinin treatments on lateral bud outgrowth could reverse the inhibitory effects of exogenous auxin treatments. To our knowledge data for these effects have not been demonstrated in any other plant systems.

This recent interest in cytokinin/auxin control of apical dominance probably has been sparked in large measure by the dramatic results obtained with transgenic plants which leave little doubt concerning the vital role of these substances. Overproducing cytokinin plants transformed with the *ipt* gene exhibit a vigorous proliferation of branching in their shoots (Li et al. 1992. Medford et al. 1989, Memelink et al. 1987, Smigocki and Owens 1988) whereas overproducing auxin plants transformed with the *iaaH* or *iaaM* genes exhibit little or no branching (Klee et al. 1987, Klee and Romano 1994, Sitbon et al. 1992). Sano et al. (1994) have demonstrated elevated cytokinin levels and reduced apical dominance in tobacco transformed with *rgpl*, a gene coding for small GTP binding proteins.

There have also been a number of recent in-depth studies with transgenic overproducing hormone plants on the interaction between cytokinin and auxin with respect to their content, syntheses, metabolism and transport (Brzobohaty et al. 1994, Hobbie et al. 1994, Li et al. 1992, Sitbon et al. 1992, Song et al. 1995, Zhang et al. 1995). As Palni et al. (1988) point out, auxin and cytokinin interact in a complex manner to control metabolism and content.

What has been lacking is a credible explanation of a mechanism of interaction between cytokinin and auxin in controlling apical dominance both at the cell and the whole-plant level. There have been suggestions. Sachs and Thimann (1967) proposed that when the auxin concentration in the axillary bud is sufficiently decreased by removal of the shoot apex, the presumed auxin source, then cytokinin synthesis in the bud increases to a level where bud outgrowth can be promoted. Many workers believe that auxin from the shoot apex somehow influences the distribution and metabolism of cytokinins from the roots which promote lateral bud outgrowth (Goodwin et al. 1978, Letham

Abbreviations: BA, benzyladenine; CCET, 4-chloro-2-cyclobutylamino-6-ethylamino-s-triazine; AACK, adenylate anticytokinin, 4-(*p*-isoproylphenylamino)-2 methylpyrrolo(2,3-d) pyrimidine, iPA, isopentenyl adenine.

1994, Woolley and Wareing 1972). Sachs (1972) has proposed a positive feedback relationship between shoot-derived auxin and root-derived cytokinin. Brown et al. (1979) have suggested an auxin-established metabolic sink in the shoot apex which diverts nutrients and cytokinins from the roots away from lateral buds. Alternatively, shoot-derived auxin may cause this diversion by direct control of phloem transport (Patrick 1987). Since so little is known about these transport processes, such hypotheses have been difficult to test.

Bangerth has recently proposed several innovative hypotheses (1989, 1995, Li and Bangerth 1992) focusing on the role of auxin transport in apical dominance. One of these involves a homeostatic system where shoot-derived auxin moves to the roots and inhibits cytokinin production, thus reducing its availability in the shoot xylem to promote lateral bud outgrowth. His evidence for this is that when decapitation of the shoot apex occurs, the cytokinin concentration in the xylem greatly increases but when exogenous auxin is applied to the decapitated stump, the cytokinin content decreases (Bangerth 1994, Zhao et al. 1995). Furthermore, if cytokinin is added to the shoot apex then auxin production and transport out of the shoot apex are enhanced. This hypothesis is conceptionally appealing and lends itself to more direct testing than the others mentioned above.

It may be that cytokinin directly enters the bud and initiates outgrowth whereas auxin's influence is indirect via its effect on cytokinin production and/or transport. That being the case, it would seem likely that cytokinin antagonists would repress bud outgrowth. However, little is known concerning the effects of these compounds on bud growth. Skoog and Ghani (1981) found that the antagonists, pyrrolo (2,3-d) pyrimidines, promoted bud outgrowth in peas whereas Suge and Iwamura (1993) reported the anti-cytokinin, CCET to retard tillering of barley.

The objectives of the present study were: (1) to determine whether the cytokinin reversal of exogenous auxin repression of lateral bud outgrowth as found in pea could also be demonstrated in other systems (e.g., *Ipomoea nil*) both in isolated nodes and in intact plants; (2) to determine whether the location of hormone application (i.e., the stem stump vs. the bud) following shoot decapitation would have any significant effect on bud outgrowth; (3) to test the effects of cytokinin antagonists on bud growth in *Ipomoea*; and (4) to test the auxin inhibition of root cytokinin-production hypothesis. This latter test was carried out by determining whether the inhibiting effects on lateral bud outgrowth of exogenous auxin application at various positions on the shoot were consistent with the hypothesis.

Ipomoea nil is an ideal plant system for this study. It grows vigorously with moderately strong apical dominance (Cline 1996) under our light room conditions as described. The inhibited lateral buds (2–3 mm in length) of the intact plant are separated by large internodes and are easily observed and measured. When released from apical dominance by decapitation of the main shoot apex, the highest lateral bud below the point of decapitation will begin to grow out within 4 to 8 h and after a week will be elongating 8–12 cm day^{-1}. This outgrowth can be inhibited by the application of auxin on the stump of the shoot immediately following decapitation.

Materials and Methods

Seeds of *Ipomoea nil* L. Roth, strain violet (syn *Pharbitis nil*) (Japanese Morning Glory) were scarified for 35 min in concentrated sulfuric acid, soaked overnight in running water, germinated in Petri dishes and grown in Promix, a general purpose peat-vermiculite growing medium in growth rooms (27–33°C) under continuous light (General Electric, Power Groove cool white fluorescent and incandescent sources, 25–450 μmol m^{-2} s^{-1}). The age of the plants at the time shoot decapitation ranged from 15 d in the 2-node plants (10–12 cm in height) up to 34 d in the 8-node plants (70–90 cm in height). Indoleacetic acid (IAA) was applied in lanolin at a concentration of 1% or in aqueous solution at 10^{-6}–10^{-4} M as indicated. 6-benzylamino adenine (BA) was applied in aqueous solution at 10^{-6}–10^{-4} M as indicated. In the experiments with the intact plants, 15–20 μl of BA (10^{-4} M) were applied directly to the lateral buds (at either nodes 4, 5, 6, or 7) daily as indicated. In the Petri dish experiments, nodal sections 4, 5, and 6, extending about 2 cm above and 2.5 cm below the lateral bud were excised from the shoots and cultured in a 20-ml 0.05% sucrose solution together with IAA or BA or anti-cytokinins for 6–8 d in the dark. In five replications where the effects of BA (10^{-5} M) mixed with IAA were tested, the concentration of IAA was 10^{-5} M in two trials and 5×10^{-5} M in three trials. In the three experiments to test the effects of the location of hormone application on bud growth, auxin (0.5% NAA) or cytokinin (0.5% BA) or an equal mixture of the two was added in lanolin to the top of the shoot stump immediately after decapitation about 1 cm above the second node from the base of the plant. Alternately, 10 μl auxin (10^{-4} M NAA) or cytokinin (10^{-4} M BA) or a mixture was added daily in aqueous solution with 0.05% Tween 20 directly to the lateral bud. Measurements were made of elongation of the second lateral bud after one week. Two anti-cytokinins were used, CCET, (Shimizu et al. 1989) and AACK, (Iwamura et al. 1983) at 10^{-5} M. With both compounds there was some difficulty in eliminating all turbidity in the solution when dissolving in water after dissolving in DMSO. Most experiments (with four to eight plants in each treatment) were repeated at least two or three times with essentially similar results.

Results

Interaction of BA and IAA in lateral buds of isolated nodes—Lateral buds on isolated nodes were incubated in Petri dishes in BA solutions of various concentrations in 0.05% sucrose for 6 to 8 d in the dark (Fig. 1). Maximum promotion of bud outgrowth occurred at 10^{-5} M as is shown in the dose response curve (Fig. 2A). Although the BA promotion here was not statistically significant, it clearly was in the data shown in Fig. 3. Incubation of the nodes

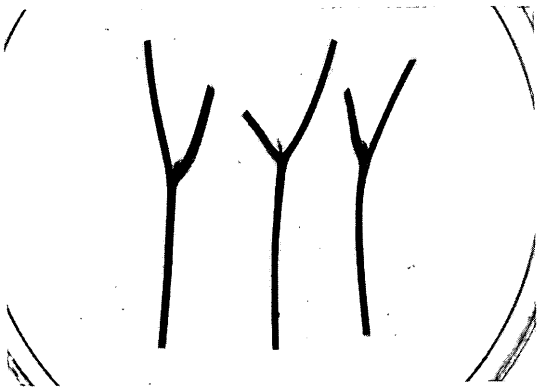

Fig. 1 Isolated nodes with lateral buds immersed in 20 ml 0.05% sucrose solution in Petri dish.

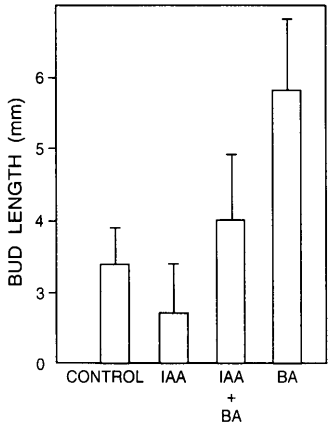

Fig. 3 Growth of lateral buds in isolated nodes in Petri dish solutions after 6–8 d in dark. IAA (5×10^{-6} M). BA (10^{-5} M). SD±mm.

in 10^{-4} M BA did cause an inhibition of growth (Fig. 2). When the isolated nodes were immersed in solutions (in 0.05% sucrose) of IAA at various concentrations, growth of the buds was strongly inhibited at 10^{-5} and 10^{-4} M (Fig. 2B). When the nodes containing the buds were immersed in mixed solutions containing both BA (10^{-5} M) and IAA (5×10^{-6} M), the promotive effect of BA appeared to partially reverse the inhibitory effect of the IAA (Fig. 3). Although the BA promotive effect in the latter experiment was statistically significant in only one (data not shown) of the five trials, there appeared to be a promotive effect in all the five experiments.

Interaction of BA and IAA in lateral buds of decapitated shoots—When the shoot apex of an intact plant was decapitated and 1% IAA in lanolin was applied to the top of the stump, the lateral bud outgrowth of the highest lateral bud (below the point of decapitation) was inhibited (Fig. 4). However, when BA in aqueous solution (10^{-4} M) was applied directly to the bud on a daily basis, the promotive effect of the BA partially reversed the inhibitory effect of the IAA. It can be observed that the same concentration of BA (10^{-4} M) which inhibited lateral bud outgrowth in the isolated nodes during the constant exposure to the BA in the Petri dish solution (Fig. 2A), promoted outgrowth when applied only once a day in a small quantity (15–20 μl) to the bud on the intact or decapitated plant (Fig. 4).

Effects of location of hormone application—When auxin was added either to the top of the stump of the decapitated shoot (about 1 cm above the second node) as 0.5% NAA in lanolin or directly to the lateral bud in aqueous 10^{-4} M NAA, outgrowth of the lateral bud at the second node was strongly inhibited (Table 1). When 0.5% BA in lanolin was added to the top of the decapitated shoot stump, there was no discernable effect on lateral bud growth. However, when 10^{-4} M BA in aqueous solution was added directly to the lateral bud, there did appear to be a synergistic effect with decapitation in promoting elongation, although it was not statistically significant (Table 1). The possibility that the BA in the lanolin did not move into

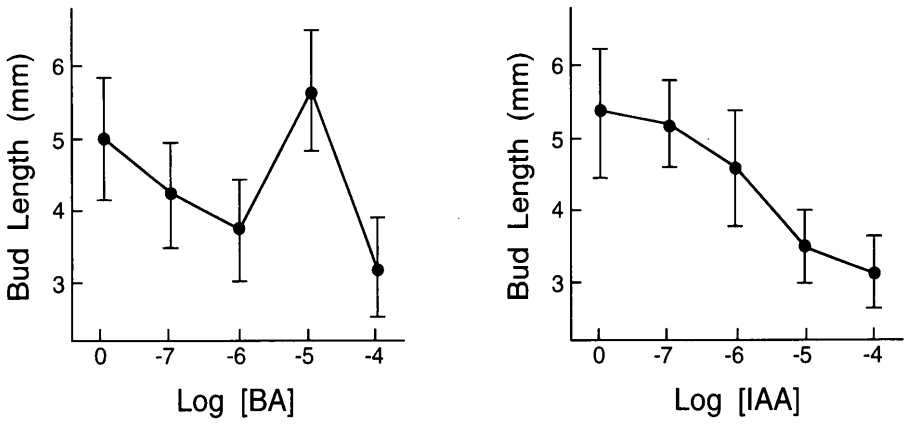

Fig. 2 Dose response curves for growth of lateral buds in isolated nodes in Petri dish solutions after 6–8 d in dark. Left, 6-benzylamino adenine (BA). Right, indoleacetic acid (IAA). SD±mm.

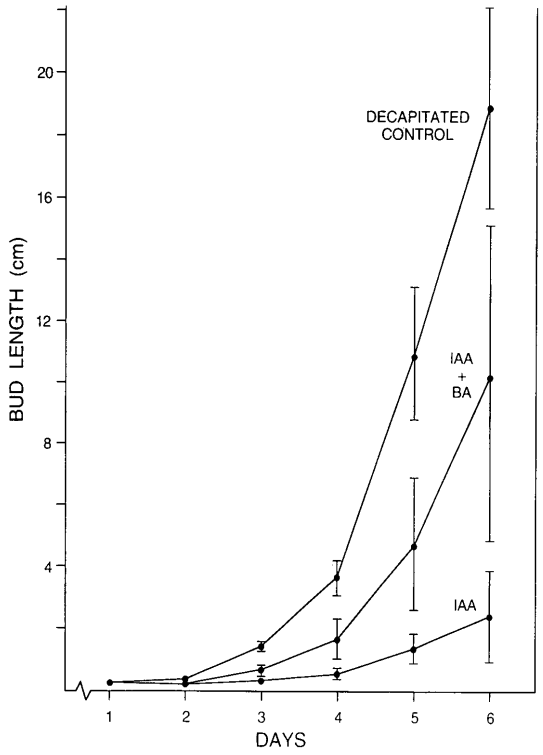

Fig. 4 Lateral bud length in shoots of potted plants 6 d following decapitation about 1.5 cm above the 6th node. IAA (1% in lanolin) applied to the top of the stumps. BA (10^{-4} M) in aqueous solution with 0.05% Tween 20 was applied directly to the buds on a daily basis. SD±cm.

the tissue was virtually eliminated by other tests showing tissue responses to BA application in lanolin (data not shown). The application of a mixture of NAA and BA in lanolin to the buds strongly inhibited their growth.

Effects of cytokinin antagonists—When either of the cytokinin antagonists, CCET or AACK, were added alone to buds on isolated nodes in the Petri dish experiments or to the lateral buds of intact plants, no consistent effects were observed (Table 2 and Fig. 5). However, synergistic effects were observed in some experiments with the intact

Table 2 Effects of the cytokinin antagonist, CCET and BA, on bud elongation of isolated nodes immersed in 0.05% sucrose in Petri dishes after 8 days in dark

Control (H_2O)	3.1±2.5 mm
BA (10^{-5} M)	4.6±0.9 mm
CCET (10^{-5} M)	3.0±1.4 mm
BA (10^{-5} M)+CCET (10^{-5} M)	4.9±2.4 mm

SD±mm.

plants when these antagonists were applied with BA. Although the majority of experiments exhibited this synergistic effect, the usual method of application involved the sequential addition of the antagonists 4 to 6 h following BA treatment on the same lateral buds. There was some evidence that this sequential treatment itself might have had some promotive anomalous effects. This problem was avoided in the final experiment in which the antagonists were added simultaneously with the BA mixed in the same solution (Fig. 5). When compared with their effects alone, the antagonists showed a strong synergistic effect with BA.

Positional auxin treatments with respect to buds and roots—In the results given for the following four experiments, the distances between the roots, the lateral buds and the site of auxin (1% IAA in lanolin) application to the shoot were varied in different combinations.

1. The repressive effects of applied auxin were compared on the lateral bud located relatively close to the roots (i.e., near the third node, about 10 cm above ground level) with that located relatively far from the roots (i.e., near the seventh node, about 68 cm above ground level) (Fig. 6). In each case, the auxin was applied either 12 cm above the bud (A, on the top of the decapitated stump of the stem) or 1-2 cm above the bud (B, in a ring around the stem). Since the third node was much closer to the roots than the seventh node, to be consistent with the hypothesis, the increase in repression from auxin application in the vicinity of node three over that in the vicinity of node seven should have

Table 1 The effect of location (top of stem stump vs. lateral bud) of hormone application, following decapitation of the shoot apex, on the outgrowth of the lateral bud at the second node up from the base of the plant

	Control	NAA	BA	BA+NAA
Applied to top of stem stump in lanolin	38.4±20.4	1.1±0.6	30.6±6.0	1.3±0.5
Applied directly to lateral bud in solution	29.3± 4.8	0	39.5±7.8	1.8±1.0

Total bud elongation (S.D.±mm) was measured for 1 week following decapitation about 1 cm above the second node. NAA and BA, 0.5% in lanolin (added to the stump on first day) or 10^{-4} M in aqueous solution with 0.05% Tween 20 (approximately 10 μl added daily to the bud). N=4.

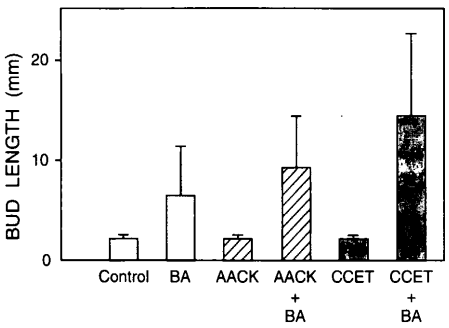

Fig. 5 Lateral bud length at the 4th node in shoots of potted plants after 6 d with daily treatments (15–20 μl) of BA (10^{-4} M) and/or antagonists, CCET or AACK (10^{-4} M) applied directly to the buds. SD±mm.

been much greater than the increase in repression of the application at B over that at A. However, such was not the case. The increase in auxin repression of bud outgrowth was relatively greater when the auxin treatment site was moved closer to the bud (from A to B) than when moved closer to the roots (from node 7 to node 3). Sachs (1991) has suggested that the number of nodes in a shoot is a rough measure of the "physiological distance" of the apices from the roots.

2. Auxin was applied in a ring around the stem at two locations on the stem (8–10 cm apart) between the fifth and the sixth nodes (Fig. 7). Hence, the relative distance between the sites of auxin application and the roots did not greatly differ but the relative distance to the buds did greatly differ. The unique aspect of this experiment was that the auxin treatment sites were located at two different distances below (and in the opposite direction from the roots) the bud of interest at the sixth node. The repressive effect of IAA on the bud at the sixth node appeared greater, although not statisitically significant, when applied at A (closer to the bud) than at B (further from the bud and a little closer to the roots). Hence, the results appeared to show

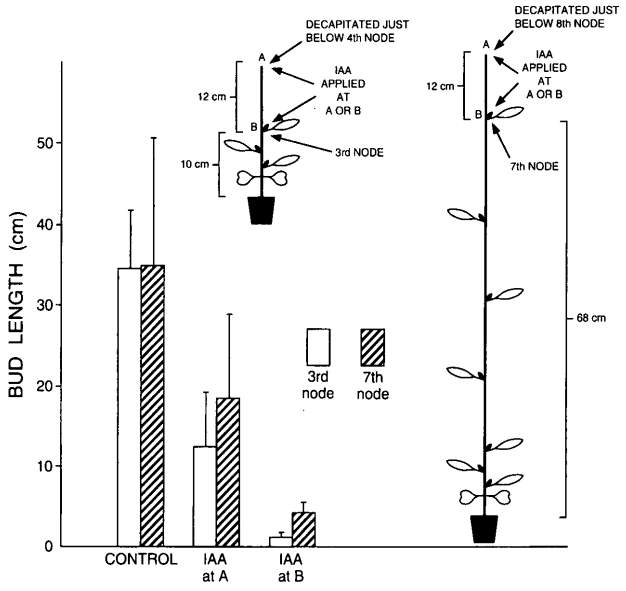

Fig. 6 A comparison of repressive effects of auxin (1% in lanolin) treatments after one week when applied on the top of the stumps (at A) of the decapitated shoots or in a ring around the stem at B near the 3rd and 7th nodes on the outgrowth of lateral buds following decapitation just below the 4th or 8th nodes, respectively. Approximate heights of shoots are indicated. SD±cm.

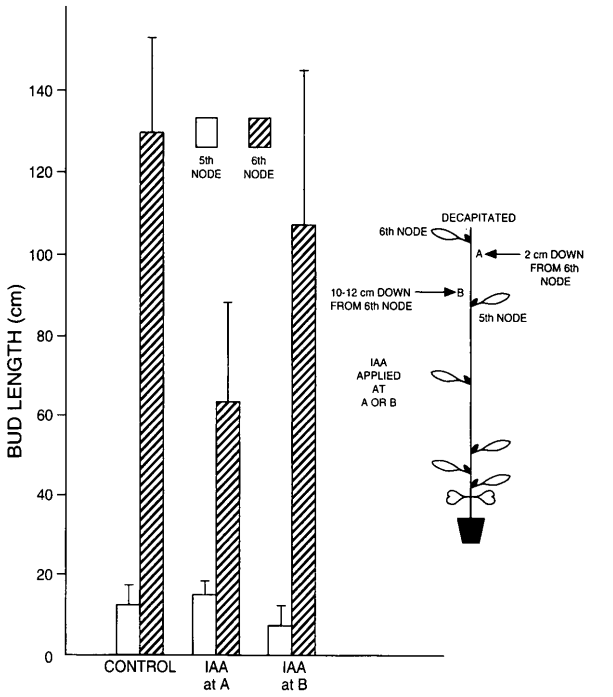

Fig. 7 A comparison of repressive effects of auxin (1% in lanolin) treatment after one week when applied in a ring around the stem at A or B on the outgrowth of lateral buds near the 5th and 6th nodes after decapitation. SD±cm.

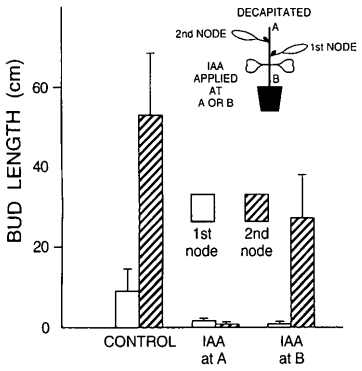

Fig. 8 A comparison of repressive effects of auxin (1% in lanolin) treatments, a week after decapitation, when applied on the top of the stumps (at A) of the shoots about 1 cm above the second node or in a ring around the stem at B (near the ground level close to the roots) on the outgrowth of lateral buds. SD±cm.

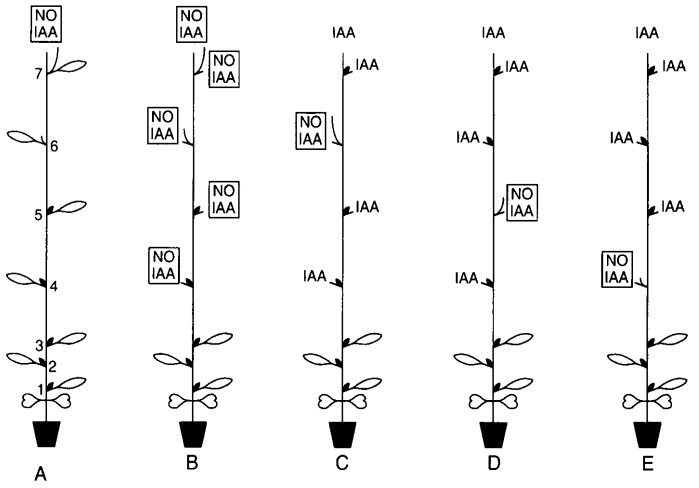

Fig. 9 A comparison of repressive effects of auxin (1% in lanolin) treatments on both shoot stump and debladed petioles on outgrowth of lateral buds at the 4th, 5th and 6th nodes following decapitation above the 7th node and defoliation of the leaf blades as shown. See data in Table 3.

the repressive effect to be correlated with the distance to the nearest lateral buds and not with the distance to the roots.

3. An analysis was made on repressibility of lateral bud outgrowth at the second node when auxin was applied on the top of the stump of the decapitated stem 2 cm above the second node and when auxin was added in a ring around the stem at the base of the shoot just above ground level adjacent to the roots (Fig. 8). The inhibitory effect of the auxin was much stronger in the former case when it was applied close to the lateral bud rather than in the latter case when it was applied close to the roots.

4. Auxin was applied on the ends of certain debladed petioles of decapitated shoots rather than to the stems (Fig. 9 and Table 3). This method of imposing apical dominance on decapitated shoots has been described (Burgess 1985) and suggests that auxin produced in the subtending mature leaves can partially contribute to apical dominance of a shoot as well as can the apex and its young surrounding leaves. In the two decapitated control groups of plants, A (with blades) and B (without blades), the highest lateral bud grew out to the greatest extent whereas the second bud grew out partially (Fig. 9). In C, D and E, the stumps of the decapitated shoots and all the petioles except one were treated with auxin. In C and D, the only lateral buds which grew out were those whose adjacent petioles were devoid of exogenous auxin application. If auxin was present, the buds were repressed. In E, the response for the third and fourth buds down was not so clear cut. But overall, the pattern was clear, the proximity of auxin application to the lateral bud was the key determinate with respect to its repressibility and not the proximity to the roots.

Discussion

The fact that alterations in auxin and cytokinin con-

Table 3 A comparison of lateral bud outgrowth at various nodes IAA treatments on debladed petioles 6 days after decapitation of shoot apex

Node number	4	5	6	7
A. Decapitated controls, all leaves intact without IAA	5.4±0.2	14.6± 5	38.6±10.2	174.6 ±46.1
B. Decapitated controls without leaves without IAA	16.5±8.2	19.4± 3.5	72.1±32	161.9 ±23.7
C. Decapitated without leaves; IAA on stump and on all petioles except #6.	1 ±0.6	6.4±12.6	103.6±31.6	0.1 ± 0.2
D. Decapitated without leaves; IAA on stump and on all petioles except #5.	6.1±6.3	65.3±23.3	16.6±17.8	0.25± 0.4
E. Decapitated without leaves; IAA on stump and on all petioles except #4.	26.9±7.5	25.8± 7	3.6± 5.9	0.5 ± 0.4

The stump of the decapitated shoot was also treated with IAA. SD±mm. See Fig. 9.

tent in shoots (either by exogenous treatments or by overproduction in transgenic plants) can significantly affect lateral bud outgrowth together with the fact that these hormones are naturally present in plant tissue are suggestive that apical dominance may be strongly influenced by the interaction between these two growth substances.

In *Ipomoea nil* we were able to provide supportive evidence for Thimann's results with peas that (1) IAA inhibits lateral bud outgrowth whether added to isolated nodes in Petri dish culture solution or to decapitated stumps of otherwise intact plants growing in pots, (2) BA often promotes lateral bud outgrowth whether added to isolated nodes in a Petri dish culture solution or directly to lateral buds of intact plants and (3) the BA promotive effect on bud outgrowth partially reverses the IAA inhibitory effect both in isolated nodes in solution and in decapitated otherwise intact shoots. The isolated nodes with our Petri dish experiments included about 2 cm of stem tissue in both directions from the node. Hence, the possibility for the need of some additional stem factor for bud outgrowth cannot be ignored (Peterson and Fletcher 1975).

When auxin was applied to the top of the stump of the decapitated shoot, the outgrowth of the lower lateral buds on the main stem was repressed as in the classical Thimann-Skoog experiment (1933). This repression was largely reversed by application of BA directly to the lateral bud (Fig. 4) but not to the stump (Table 2). Presumably, auxin moves down by polar transport from the stump and BA does not. Bangerth (personal communication) points out that applied cytokinin only moves acropetally in intact plants. It is also possible that BA applied to the stump strengthens apical dominance via enhancement of IAA synthesis (Li and Bangerth 1992). Davies et al. (1966) found that simultaneous application of cytokinins and auxin to decapitated plants enhanced the inhibitory effect of auxin on axillary bud growth. Our data were consistent with this result when the mix in lanolin was applied to the stump (Table 2). Alternatively, the lack of a BA promotive effect might be due to the lack of stump meristematic tissue which can synthesize IAA (Bangerth, personal communication). Hence, it appears that auxin and cytokinins may have different sites of action and/or are transported in different tissues.

The cytokinin antagonists, CCET and AACK, had no effect when added alone to the buds of isolated nodes in solution or directly to lateral buds of intact shoots but suggest a synergistic effect when added with BA to the intact shoots. This is contrary to what might have been expected. CCET and to a lesser extent, AACK, may have acted as inhibitors of BA degradation. Anti-cytokinins are structural analogues of cytokinin-active compounds. They fit the cytokinin receptor but do not trigger the successive action leading to cell division and growth, and thus they are antagonists in the tobacco callus assay. They sometimes behave as agonists, e.g. in betacyanin synthesis in Amaranthus (Iwamura et al. 1979) and in seed germination in lettuce (Iwamura et al. 1979). In this case, they are thought to fit the receptor in a fashion as do cytokinins. In the present case, a possibility is that CCET fits the active site of a cytokinin or BA degrading enzyme as a structural analogue of BA.

Skoog and Ghani (1981) found pyrrolo (2,3-d) pyrimidine to inhibit cytokinin-induced growth in tobacco callus but to promote bud outgrowth in peas. They concluded that their opposite actions of retarding and promoting growth here occurred at different metabolic sites.

Although the precise mechanism of action of these antagonists is not fully understood (Iwamura 1994), the evidence of various workers demonstrating the important role of cytokinins in the promotion of bud formation and its outgrowth is convincing (Cline 1991, 1994, Kaminek 1992, Mok 1994, Sebanek et al. 1991, Tamas 1995).

To fully evaluate the hypothesis of auxin control of apical dominance via inhibition of cytokinin production in the roots, a number of determinations would need to be made including those of cytokinin and auxin syntheses, metabolism, content, and transport in various tissues. Part of the difficulty in carrying out such determinations is that the hypothesis is not sufficiently explicit as to the exact processes. The present study represents a preliminary attempt to test one aspect of the hypothesis.

If auxin interacts with cytokinin in the control of apical dominance by moving from its point of origin in the shoot apex down to the roots where it inhibits cytokinin production (or promotes its breakdown, Li et al. 1992) which in turn results in the depletion of shoot cytokinin and the subsequent lack of lateral bud outgrowth, then it should be possible to test this hypothesis by applying auxin at key locations on the decapitated shoot with reference to the roots and observing the repressibility of particular nearby lateral buds. In decapitated *Ipomoea* the axillary bud which is most repressible by auxin application to the stump is the highest lateral bud below the point of decapitation and which is the only bud which grows out to a major extent.

If the assumptions of the aforementioned hypothesis are carried to their logical conclusion, it could be concluded that auxin has no direct inhibitory effect on bud outgrowth. The presence of cytokinin in the lateral bud would be required for its outgrowth. The major inhibitory influence of auxin on bud outgrowth would be indirect via inhibition of cytokinin production in the roots. If auxin must move to the roots before it can inhibit cytokinin production, then it could be presumed that the closer are the auxin treatments to the roots (assuming auxin penetration into the stem and movement to the roots), the more effective would be their inhibition of cytokinin production and the more complete would be their repression of bud out-

growth. Hence, the critical factor would not be the distance between the location of the auxin treatment on the stem to the repressible lateral bud but rather it would be the distance to the roots. This line of thinking is supported by the findings of Wareing and Nasr (1961) that the development of lateral buds in many woody plants is dependent upon the proximity to the roots which may produce a cytokinin-like root factor. Therefore, varying the distance between the site of the auxin treatment and the lateral bud of interest should have little or no effect on bud outgrowth. This obviously assumes an over-simplified view of the complex metabolic and transport processes involved.

In all four experiments, the pattern was clear, the determining factor as to whether an auxin treatment of the shoot resulted in repression of lateral bud outgrowth was the proximity to the lateral bud and not the proximity to the roots. The distance between the site of auxin treatment and the roots appeared irrelevant. This appeared to be the case even when the bud of interest was located above the site of the auxin application and in the opposite direction from the roots (Fig. 7).

Hence, these foregoing results did not support the hypothesis of auxin inhibition of cytokinin production in the roots for the control of apical dominance. That exogenous auxin treatments of the shoot did result in penetration of the auxin into the stem tissue was clearly demonstrated by the repression of outgrowth of nearby buds which otherwise did not occur.

If auxin and cytokinin do not interact in controlling apical dominance by auxin inhibition of cytokinin production in the roots, then how do they interact? There is an interesting dichotomy here in that the source of auxin which is necessary for the repression of axillary bud growth is located at the top of the shoot along with the repressible lateral bud (in *Ipomoea*), whereas the source of cytokinins which are necessary for lateral bud outgrowth is presumably located in the roots. Hence, signals are being transmitted up and down the shoot between shoot and root apices. The situation seems reminiscent of Sachs' (1991) conceptual model of auxin and cytokinin as major correlative signals of the shoot and root, respectively, with each one "being the source of its characteristic hormone and a...sink for the signal of the complimentary apices". Grafting studies with the *rms*4 branching mutant and wildtype plants show that the shoot controls the import of cytokinins from the roots (Beveridge et al. 1997). Bollmark et al. (1995) have proposed that polarly transported auxin may control bud outgrowth by regulating cytokinin metabolic changes. Auxin might act by controlling the level of active cytokinins (e.g. via the prevention of iPA conversion to the more active zeatin, King and van Staden 1990 or by inactivation via the promotion of glucosylation, Crouch and van Staden 1995).

That lateral buds may be inhibited by IAA transport autoinhibition is another hypothesis which justifies attention (Bangerth 1989; personal communication). Accordingly, lateral bud inhibition is not due directly to the shortage of cytokinin in the bud but is due rather to the inhibition of IAA transport out of the lateral bud by the greater IAA transport down from the main shoot apex. If IAA transport down from the main shoot weakens, then IAA transport out of the lateral bud can occur and then with the involvement of cytokinin, the lateral bud will grow out. Perhaps IAA synthesis and metabolism in the bud are altered by IAA transport and these alterations may be reversed by cytokinin.

A continuation of the fruitful progress being made on auxin effects on cytokinin synthesis, metabolism and transport at the cellular level should be strongly encouraged. In addition, cytokinin transport in the xylem/phloem of roots and shoots needs precise elucidation as does the kinetics and induction of lateral bud outgrowth.

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