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THE ROLE OF ROOT SYSTEM IN LATERAL BUD GROWTH OF PEA (*PISUM SATIVUM* L., VAR. ALASKA)[1]

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Abstract

The role of root-produced cytokinin in the growth of lateral buds has been studied by using rootless shoots of 7-day-old seedlings of pea (***Pisum sativum*** L. var. Alaska). The presence of the root system is essential for the growth of lateral buds following decapitation. Kinetin applied to the basipetal ends of rootless shoots, at concentrations ranging from 0.1 to 100 μg/ml can partially substitute for the root system. GA_3 alone, supplied to the basipetal end of rootless shoot is not effective in releasing lateral buds from correlative inhibition. GA_3 or IAA, however, promotes the kinetin induced bud growth as shown by sequential application of GA_3 or IAA to the basipetal ends of rootless shoots. The presence of IAA in the shoot greatly promote the uptake and acropetal transport of ^{14}C supplied as ^{14}C-8-kinetin applied at the basipetal end of the rootless shoot. Large portion of ^{14}C taken up has been found moving forward the shoot apex of the rootless shoot and the first true leaf of the decapitated rootless shoot. The possible role of this polarized movement of kinetin as related to apical dominance are discussed.

Introduction

The activation of lateral buds from apical dominance is a well-known physiological action of cytokinin (Hugon, 1962; Sachs and Thimann, 1964; Wickson and Thimann, 1958). It is often assumed that the inhibition of lateral buds is dependent upon an antagonism between the endogenous auxin and cytokinins (Sachs and Thimann, 1967; Wickson and Thimann, 1958). According

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(4) Abbreviations: GA: gibberellins; GA_3: gibberellic acid or gibberellin A-3; IAA: 3-indole-acetic acid; Kinetin: 6-furfurylaminopurine; PPO: 2,5-diphenyloxazole.

to Thimann and Skoog's concept (Thimann and Skoog, 1933; 1934) the inhibitive auxin was polarly transported from the shoot apex. However, the source of endogenous cytokinin as related to lateral bud activation has not been resolved. Sachs and Thimann (1967) assumed that the cytokinins may be synthesized in the inhibited organ itself. Recently, circumstantial evidences for the existence of a root-produced shoot growth substance have come from a variety of sources. Rootless shoots of tomato maintained with water and minerals do not elongate until new root formation (Went and Bonner, 1943). Supplying the rootless shoot with coconut milk, now known to be rich in kinetin-like factors, can partly resumed the shoot elongation (Leoffler and van Overbeek, 1964). Substances retarding chlorophyll degradation are recently found in the root exudate of a number of plants (Carr and Reid, 1968; Kend, 1965; Leoffler and van Overbeek, 1964). Cytokinin activity has been reported in ethanol extracts of three-day-old roots of Alaska pea (Short and Torrey, 1970). Thus, it follows that roots may be the main source of cytokinin for the activation of lateral bud during the release of apical dominance.

Moreover, evidence has accumulated that hormone-directed transport of metabolites may play an important part in correlative bud inhibition (Phillips, 1969). This led to a suggestion that the apically synthesized auxin in the shoot apex caused a shortage of available cytokinins for lateral bud activation (Phillips, 1969).

This paper reports the preliminary investigation on the possible importance of the roots in the control of bud activation by using isolated shoot sections and simulation of the roots by supplementing the basal medium of known growth substances. The IAA[4] effect on kinetin movement as related to apical dominance has also been studied.

Materials and Methods

Pea plants (*Pisum sativum* L. var. Alaska) were grown in a vermiculite and soil mixture at 25°C±1°C in darkness for the first 4 days; then, seedlings were maintained under constant illumination of about 2000 ft-c intensity provided by cool white flourescent lamps and incandescent lighting. Seven-day-old seedlings were used for all the experiments, at which time the first true leaf on the third node was fully expanded. After soil and vermiculite were removed from the root by washing in running tap water, the seedlings were ready for the experiments. In the treatments without roots, roots were cut at 3 cm below the first node. The shoot tips were cut approximately 2 mm below the fourth nodes. All the treated plants were maintained in test tubes with 5 ml half-strength Hoagland's solution with or without growth re-

gulators. The culture medium was changed every two days. The longitudinal growth of the 3 lateral buds was measured every two days using metric millimeter scale calipers. The length of each lateral bud was measured as the distance between the point of attachment at the base of the bud and the tip of stipules enclosing the bud. The results were expressed as the mean combined lateral bud growth at the three nodes in ten plants.

The kinetin solutions were prepared by dissolving kinetin in a few drops of 0.01 N HCl. After the kinetin was dissolved, the final solutions of desired concentrations were made with distilled water and the pH of the solution was adjusted to 6.0 with dilute KOH solution. The 1% (w/w) IAA lanolin paste was prepared by dissolving crystalline IAA in a few drops of absolute ethanol and mixing throughly with melted lanolin.

About 0.1 ml of paste was applied to the cut surface of decapitated stump in treatments without the shoot apex. Reapplication was made at 24 hr intervals on the recut stem surface resulting in removal of 2 mm of stem and the old lanolin.

In case of ^{14}C-8-kinetin experiment, all the rootless sections were maintained in 1 ml half-strength Hoagland's solution containing 0.25 μc ^{14}C-8-kinetin (sp. act. 10 mC$_i$/m Mole) at approximately 5 μg/ml in concentration. After 24 hr incubation in constant illumination of 2000 ft-c at 25 C, all plants were divided into parts and were placed in separate counting vials containing 1 ml methanol. After 3 days extraction, 10 ml of liquid scintillation solution was added to each vial and radioassayed in a Beckmann (Model LS 100) scintillation counter. The liquid scintillation cocktail contained 8 g PPO and 100 g naphthalene in one liter dioxane. Quenching by chlorophyll was corrected by an external standard. Results were expressed as percentage of distribution of translocated ^{14}C in each portion of the plant.

Chemicals: IAA and GA_3 were purchased from CaliBiochem, Los Angles. Kinetin was obtained from Sigma Chemical Co., St. Louis. ^{14}C-8-kinetin with a sp. act. 15.0 mC$_i$/m Mole was purchased from The Radiochemical Center, Amersham, UK. All reagents were of the highest grade available and were used without further purification.

Results and Discussion

The Role of Root on Lateral Bud Growth

The experimental arrangements and results of the demonstration that exogenous kinetin could substitute for the function of root system in controlling the lateral bud growth following decapitation were shown in Fig. 1. It is apparent that if shoot apex of the derooted plants were removed only very

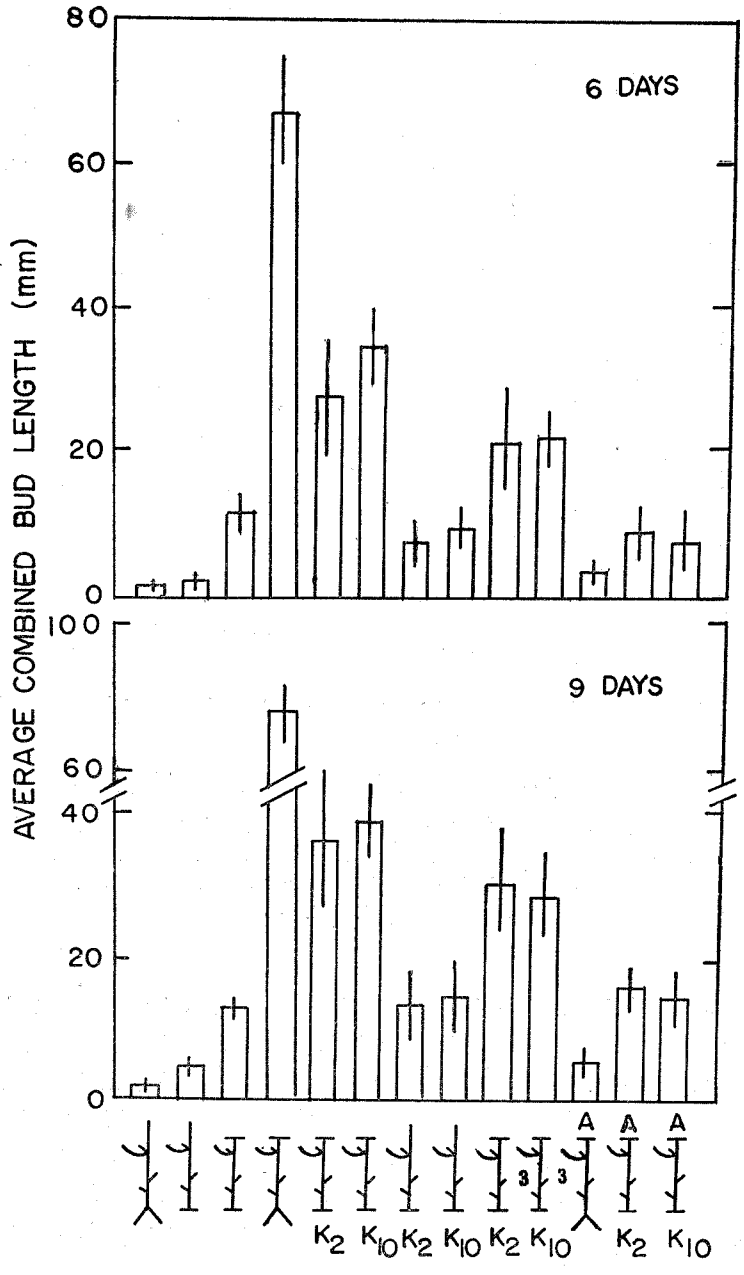

Fig. 1. Substitution for the root system by kinetin. All plants or shoot sections were maintained in tissue culture tubes containing 5 ml half-strength Hoagland's solution as basic medium. Kinetin at 2 or 10 μg/ml was supplied to some treatments as shown in the diagram. The number "3" indicates the date that kinetin was first supplied. Each treatment had ten replicates. The vertical lines were standard deviation.

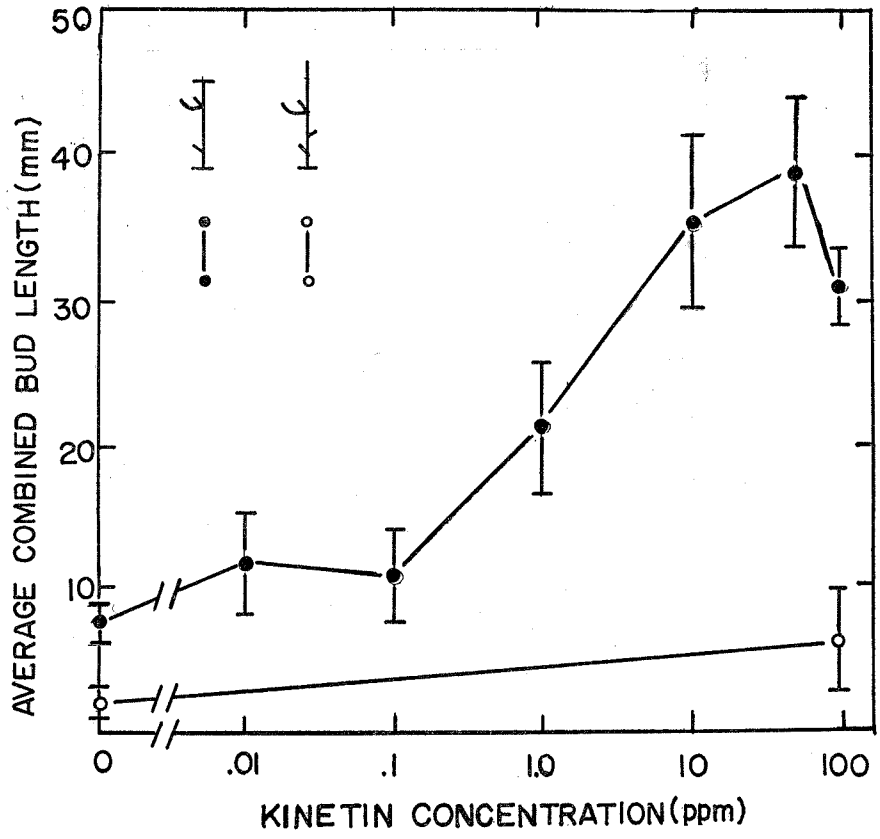

Fig. 2. Effect of kinetin concentration on the growth of lateral buds of rootleess seedlings decapitated at the top of the fourth internode. The vertical line represented standard deviation of the mean of ten replicates. Basic medium was 5 ml half-strength Hoagland's solution.

limited growth of lateral buds occurred. These results suggested that in decapitated plants the essential factor(s) for lateral bud growth was supplied from the root system. A supplied of a synthetic cytokinin, kinetin, to the culture medium resulted in rapid elongation of the lateral bud in the decapitated shoot. This finding supported the views of earlier investigators that the cytokinins were the factors involved in the primary action of lateral buds in releasing from apical dominance (Sachs and Thimann, 1967; Scott and Pritchard, 1968).

The effect of kinetin concentration in the culture medium on lateral bud growth in derooted seedlings was shown in Fig. 2. The effective concentration range was wide, from 0.1 to 100 μg/ml. At higher concentration (50 and 100 μg/ml) the shoot sections appeared wilted and senescent by the end of the experimental period (8 days). This might have been due to

phytotoxicity of high dosage of kinetin. In general, there was no difference in the growth habit of lateral buds activated by different kinetin concentrations.

The presence of apex or IAA application to the cut surface of decapitated plants greatly reduced the effect of both root and kinetin on lateral bud growth. Thus it was clear that cytokinin exported from the root system could play an important role in the lateral bud growth. Thus, the release of lateral buds by kinetin, might simulate an natural process. Significant acropetal movement of some cytokinins, benzylademine (chvojka, *et al.*, 1961; Guern and Sadorge, 1967; Hovola and Veres, 1963; Pilet, 1968; Pilet, *et al.*, 1967) and kinetin (Kaminek, 1965; Pieniazak, 1964) had been demonstrated in a variety of plants. When an aqueous solution of 6-benzyladenine was injected into the epicotyl of young seedlings of *Cicer arietinum* the buds below the application site remained inhibited while the buds above started growing (Guern, and Sadorge, 1967). It was apparent that there was a good agreement between the acropetal movement of cytokinins and the release of the buds from apical dominance. All these studies suggested that the release of lateral buds from apical dominance was dependent upon availability of sufficient amount of root-produced cytokinins. It was interesting to note that removal of roots or root apices upseted the inflorescence initiation and branching in *Carex flacca*, and only benzyladenine can partially simulate the role of the roots (Smith, 1969).

In comparing the total growth of the control buds on decapitated plants with these released by kinetin in derooted plants, the diffenence was not in the number of internode or leaves, but in the length of new shoots. The control buds on decapitated plants grew much longer than those of derooted plants maintained in kinetin containing basic medium. It was possible that some essential factor other than cytokinins was missing, and cytokinin only might be the factor for releasing the lateral buds from apical dominance. But other hormonal factors might be needed for normal growth of the kinetin-activated buds. The role of gibberellins and auxins on the growth of kinetin-induced lateral buds were next investigated.

Effect of Sequential Application of GA_3 or IAA on Kinetin-induced Lateral Bud Growth

IAA applied to the maintaining medium could not bring about bud release from correlative inhibition (Fig. 3). When applied together with kinetin, IAA did not significantly retard the kinetin induced bud growth. This was very likely due to poor acropetal transport of IAA in the stem tissue in high humidity condition (Chang, 1970, unpublished). However, IAA added to the

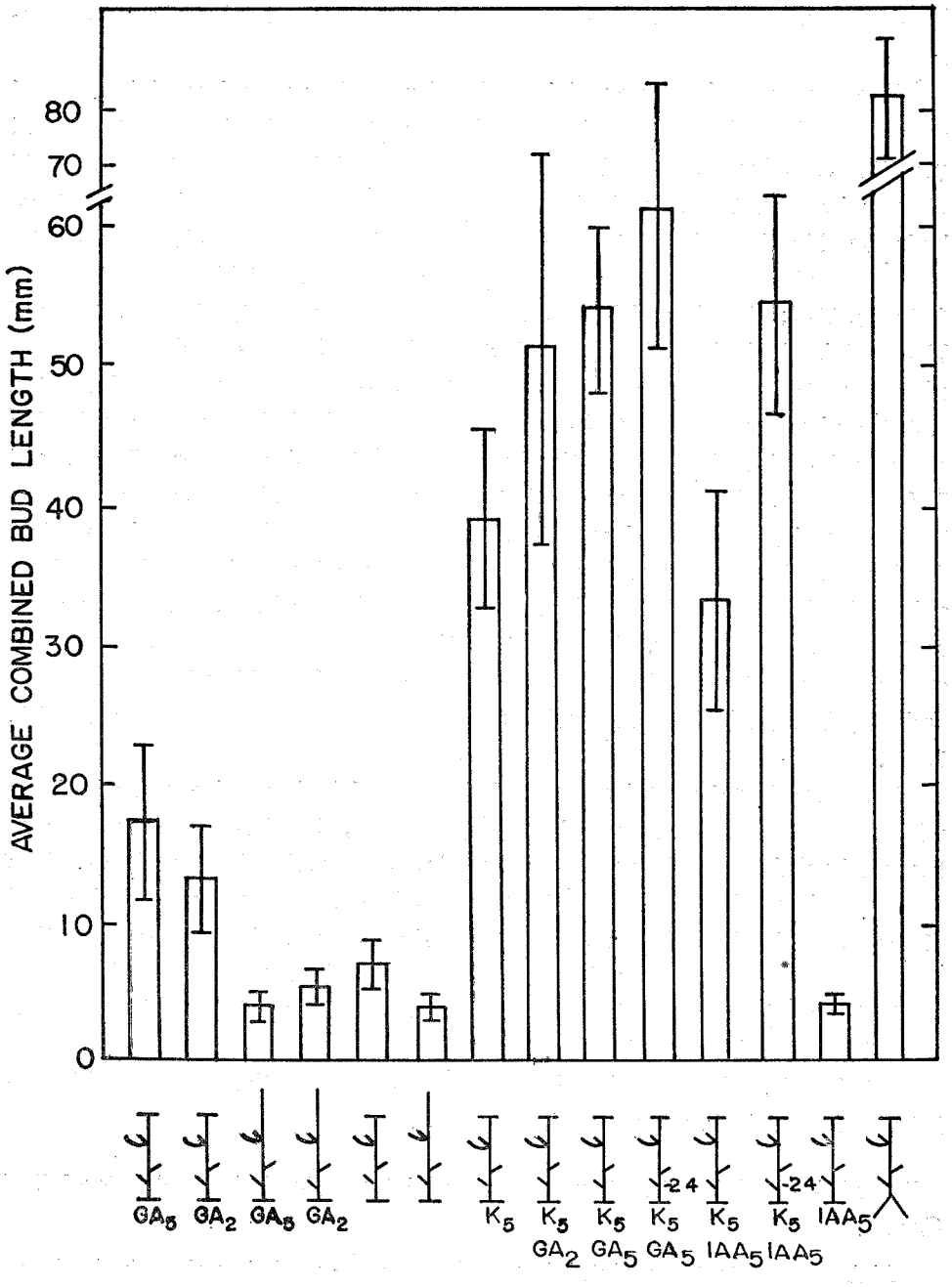

Fig. 3. Effects of IAA (5μg/ml) and GA_3 (1 μg/ml and 5μg/ml) applied sequentially or simultaneously to the basic medium on kinetin-induced lateral bud growth. Bud lengths were recorded 8 days after decapitation. The number below the name of the hormone represented the concentration in μg/ml. The kinetin concentration was 5 μg/ml. Basic medium was half-strength Hoaglad's solution.

medium at 24 hr after starting of the experiment did show an additive effect to kinetin on inducing bud growth. IAA alone might not be sufficient for normal growth of kinetin-induced bud. In the treatment in which IAA was applied 24 hr later to the medium kineitn containing, none of the bud was as large as those on decapitated controls with a root system. The average length of the buds only reached about one-half of that decapitated control. Furthermore, the leaves of buds treated with kinetin and then with auxin plus kinetin remained small.

GA_3 (5 μg/ml) applied to the medium caused a limited release of the bud of derooted plants, though much shorter as compared with those on the kinetin treated plants (Fig. 3). The leaves of GA_3-treated rootless plants were small and chloretic in appearance, and only the first internode of the released lateral bud elongated. These data were very similar to those of decapitated plants in which GA_3 had been applied to the stem stump (Scott and Prittchard, 1968). Thus, GA might not be the factor antagonizing IAA in releasing the buds from apical dominance. But it might promote lateral bud growth only when correlative inhibition had already been overcome by some other promotors, e.g. cytokinin (Fig. 3). The GA effect only represented the usual elongation response of a growing shoot to exogenous GA. A 24 hr delay in GA_3 application to the kinetin-treated derooted plants caused more bud growth than that of kinetin treated alone (Fig. 3). It was apparent that the bud might respond to kinetin and GA sequentially. This observation resembled those of an excised bud in sucrose solution containing kinetin (Wickson and Thimann, 1958) and of intact plants (Panigrahi and Audus, 1964; Sachs and Thimann, 1967).

The Effect of Shoot Apex or Auxin on the Distribution of ^{14}C-8-kinetin

In this preliminary experiment, the possibility that the control of lateral bud growth by the shoot apex might involve a hormonal-directed root-producted cytokinin transport was investigated. There was considerable acropetal translocation in all of the three treatments under relatively higher humidity condition (Table 1). The uptake and the acropetal translocation of ^{14}C-8-kinetin were enhanced by the presence of a terminal bud or IAA applied to the decapitated stump. After 24 hr translocation period a greater percentage of translocated ^{14}C moved into the first true leaf and released lateral buds of decapitated rootless plants, while a large portion of ^{14}C moved into apical regions of derooted plants with shoot apex. However, there was only a slightly greater percentage of ^{14}C-kinetin accumulated in the IAA treated apical regions of decapiataed rootless plants than those of decapitated rootless plants without IAA treatment. It was apparent that the high auxin concent-

Table 1. *The 14C distribution in rootless seedlings 24 hr after treatment and application of 14C-kinetin solution to the 1st internodes.*

Plant parts	% distribution		
			
Scale leaves	4.09±1.28	3.76±0.53	2.84±0.55
Lateral buds	2.96±0.48	1.39±0.45	1.09±0.35
2nd internode	18.66±1.40	34.20±4.10	32.34±3.29
1st true leaf	39.01±7.61	17.44±3.36	14.77±3.58
3rd internode	26.12±1.83	29.50±1.79	26.78±3.89
Apex and 2nd true leaf	—	—	16.73±6.87
4th internode	9.09±6.03	13.66±7.17	5.45±1.59
Total translocated ^{14}C, dpm	* 7,102±1,469	16,999±1,937	17,18±02,669

* One ml half-strength Hoagland's solution containing 0.25 μc ^{14}C-8-Kinetin (sp. act. 10 mc/mMole) at approximately 5 μg/ml in concentration.

ration at the apex might prevent kinetin from moving into lateral buds and growing young leaves, but direct cytokinin to move into the shoot apex and first true leaf. Many studies had demonstrated that both minerals and organic metabolites were indeed translocated predominatly to the region of active growth or the site of auxin accumulation. Seth *et al.* (1966) observed that significantly greater amounts of kinetin moved basipetally in the stem of *Phaseolus vulgaris* in the presence of IAA than in its absence. Osborne and Black (1964) have reported that Basipetal tansport of benzyladeinine-^{14}C in bean stem tissue was enhanced in the presence of IAA. Pilet (1968) also observed that the movement of benzylaminopurine was directly related to the presence of the termial bud. Furthermore, IAA-directed metabolite translocation in plants had been reported (Booth *et al.*, 1962; Davis and Wareing, 1965; Nakamura, 1965; Sebanek, 1965a, 1965b, 1967). Therefore, the IAA-controlled kinetin polar transport might be involved as a major component in the mechnism of apical diminance. More studies are needed to verify this concept.

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豌豆根羣和側芽生長的關係

張 唯 勤 J.R. GOODIN

本實驗用七天大的豌豆苗 (*Pisum Sativum* L. Var. Alaska) 來探討根羣在頂芽優勢的重要性。切除頂芽，側芽需要根羣才能生長。用切除根羣的根株作實驗，我們發現 kinetin 可以代替根羣能使切除頂芽的植株的側芽生長。而 gibberellic acid 單獨使用則沒有這種效果。但是 gibberellic acid 可幫助 kinetin 促使生長的側芽伸長。用 ^{14}C-8-kinetin 供給切除根羣的植株的基部，我們發現頂芽可促使向上運移的 ^{14}C-8-kinetin 多向生長中的葉片和頂芽本身運移。文中並討論到這種頂芽或 auxin 所控制的 kinetin 運移是頂芽優勢機程的一部份之可能性。