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INTERACTION OF ROOTS, GIBBERELLIC ACID AND LIGHT IN THE PROMOTION OF STEM GROWTH IN PEAS (PISUM SATIVUM L.)

The influence of roots on the growth of stems in certain plant species is a well-know fact, noticed a long time ago. Many authors attempted to explain this influence by the undoubted role of roots in the absorption of water and minerals. In his classic paper Went (1938a) showed that roots were also the site of synthesis of a specific substance, named »caulocaline« and supposed to be of major importance for shoot elongation. The existence of such a substance was subsequently confirmed by several authors (De Ropp, 1946; Howell and Skoog, 1955), but it has never been identified by physiological or biochemical methods. There have been some data published recently, suggesting that the effect of roots might in some way be related to the metabolism of gibberellins in plants. Cajlahjan and Hlopenkova (1960) found that certain long-day plants did not elongate under favourable photoperiods, if their roots had been cut off, though they developed floral buds. The synthesis of gibberellin-like substances can indeed occur in root tissues, as was shown by Butcher (1963) for isolated tomato roots. There are also data showing that bleeding-sap of certain plants, including a pea variety, contained gibberellin-like substances (Carr, Reid and Skene, 1964; Phillips and Jones, 1964). The two groups of authors put forward a hypothesis, that »caulocaline« might be identical with gibberellins. On the other hand, Lockhart (1957) presented the evidence that apical parts of the pea plant were the site of synthesis of gibberellins. He extended later his work (Lockhart 1964) and concluded that young leaves were the source of precursors, while the actual synthesis of gibberellins took place in the elongating zone of young internodes. He concluded further that roots affected the growth of shoots independently of this system.

The present paper contains the results of some experiments, concerning the interaction of root growth factors ,added gibberellic acid and light. Experiments were performed with axillary buds of peas. The results obtained suggest that neither root growth factors, nor the factors produced in darkness were identical with gibberellic acid.

MATERIAL AND METHODS

Young pea seedlings were used for all experiments. The tall variety »Alaska« was used throughout, but some experiments were done, for comparison, with the dwarf variety »Meteor«. Plants were grown in sterile cultures. Pea seeds were sterilized with 5% sodium hypochlorite and germinated on wet filter paper, in darkness, at 25°C. After 72 hours, when the epicotyls and the roots reached 5—10 mm. and 30—40 mm. respectively, plants were divided into four groups and treated as follows:

Group A: Plants were decapitated 2—3 mm. above the cotyledonary node and the seed coat was removed. The fragment of the plant used for further work consisted of cotyledons, root and short lower part of the epicotyl.

Group B: Plants were decapitated ,the root was cut off as well, 2—3 mm. below the cotyledonary node. The fragment consisted of cotyledons and short parts of stem and root near the node.

Group C: Epicotyl and cotyledons were cut off, but care was taken to leave the petioles and axillary buds intact. The fragment consisted of the root and a short part of the epicotyl.

Group D: Epicotyl, root and cotyledons were removed, as described for the previous groups. The fragment consisted of the cotyledonary node with axillary buds and parts of the stem and the root, to which the petioles remained attached.

In groups B and D, with the roots cut off, secondary roots usually grow out 2—3 days after the operation. These cultures were inspected every day and all new roots were immediately removed.

Plant fragments obtained in this way were transferred on a nutrient medium, consisting of mineral solution after H e l l e r (1953), 2% succrose and 0,8% agar. Gibberellic acid (Light & Co.) was added in drops, put on the cotyledons or on the cut surface of the stem. Drops were added by means of a calibrated capillary tube, containing exactly 0,02 ml. (Drummond's microcaps). Gibberellic acid (GA_3) was dissolved in 50% alcohol, the concentration being adjusted in such a way, that a drop of 0.02 ml. contained a desired amount of GA_3, usually 20 μg.

Plants were maintained in an electrically heated room, at 25°C. Usually half of the plants were grown in light of 3000 lux in intensity, obtained from six incandescent bulbs, cooled by water. The daylength was regulated to 16 hours. The other half of the plants was grown in darkness, all necessary manipulations being done in weak light of a yellow-green photographic filter.

RESULTS

In all plant fragments put on the nutrient medium, axillary buds start growing and after 48 hours two small buds were visible, one in the axil of each cotyledon. One of the buds grows more quickly and overtakes the function of the main stem. The response of these buds to the

root factors, GA_3 and light was further investigated. It was found in several experiments that light exerted a great influence on the elongation of stems, but that this effect varied, depending on the presence of roots and cotyledons. Table 1 shows the results of a typical experiment, performed with both tall and dwarf pea varieties. Half of the plants in each group was grown in light, the other half in darkness. Plants were measured after 14 days.

Table 1

Influence of light on the stem length in »Alaska« and »Meteor« peas

	Groups							
	A		B		C		D	
	Stem length							
	mm.	%	mm.	%	mm.	%	mm.	%
»Alaska«								
Light	122,0	49	16,6	98	71,0	125	3,5	129
Darkness	247,0	100	18,9	100	56,8	100	2,7	100
»Meteor«								
Light	27,0	11	20,0	110	9,0	34	3,0	60
Darkness	228,0	100	18,0	100	26,0	100	5,0	100

As it was expected, plants which had roots and cotyledons left intact (group A) had the longest stems. In the variety »Alaska« plants with roots, but without cotyledons (group C) had shorter stems, but still much longer than plants without roots and with cotyledons (group B). Stems in the group D (without both roots and cotyledons) were very small. In this respect, our results were very similar to those published by W e n t (1938a).

The Table 1 also shows that the effect of light was different in the four groups of plants. In the dark-grown »Alaska« peas, only plants in the group A showed the excessive stem elongation, characteristic for etiolated plants. Plants in the group B were not affected by light and had approximately the same length in light and darkness. Group C not only was unaffected by light, but on the contrary, it was stimulated. Light-grown plants in the group D were also longer than the dark-grown ones.

Plants of the variety »Meteor« differed in some respects from »Alaska«. Light inhibition in the group A was very strong. Light almost levelled the difference between groups A and B, i. e. the difference due to the presence of roots. Group B was not affected by light, and the stimulation in the groups C and D did not take place. Plants in these two groups were rather inhibited by light. Nevertheless, the relationship bet-

ween the groups in dark-grown plants was quite similar to that found in »Alaska« plants. So, the principal difference between »Alaska« and »Meteor« was observed in light-grown plants, in the groups A and C, which had their roots left intact. While the presence of roots in »Alaska« reversed partially (A) or completely (C) the light inhibition, roots of the dwarf »Meteor« had no such effect.

Experiments in which GA_3 was applicated to the light and dark-grown plants, were performed in the same time as the previous ones. Half of the plants in each group received a drop with 20 μg of GA_3, while the other half received a drop of 50% alcohol only. GA_3 was added on the 2nd, 4th, 6th, 8th and 10th day, so that each plant received in whole 100 μg GA_3 up to the end of experiments. Fig. 1 represents typical re-

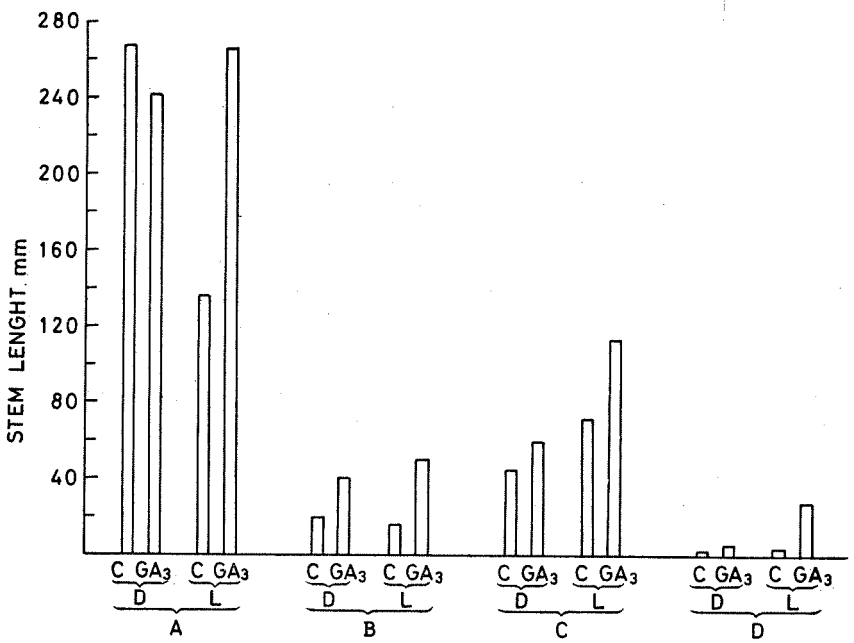

Fig. 1. Influence of light and GA_3 on the stem length in the variety »Alaska«

C = controls; GA_3 — 10 μg GA_3 added per plant; D = plants grown in in darkness; L = plants grown in light; A = with roots, with cotyledons; B = without roots, with cotyledons; C = with roots, without cotyledons; D = without roots, without cotyledons. All plants measured after 14 days.

sults obtained with »Alaska« peas. GA_3 stimulated the elongation of all light-grown plants. In the group A, plants treated with GA_3 reached the same length as the etiolated ones. Plants in groups B, C and D were stimulated relatively to the same extent. Dark-grown plants in the group

A were not significantly affected by GA_3, while in all other groups the stimulation in darkness was approximately the same as in light.

Fig. 2 represents parallel experiments with »Meteor« peas. The treatment was the same as for »Alaska« (Fig. 1), except that the amount of GA_3 added was 2 µg per plant. The differences between the two varieties in the final stem length in the groups with roots (A and C) were already

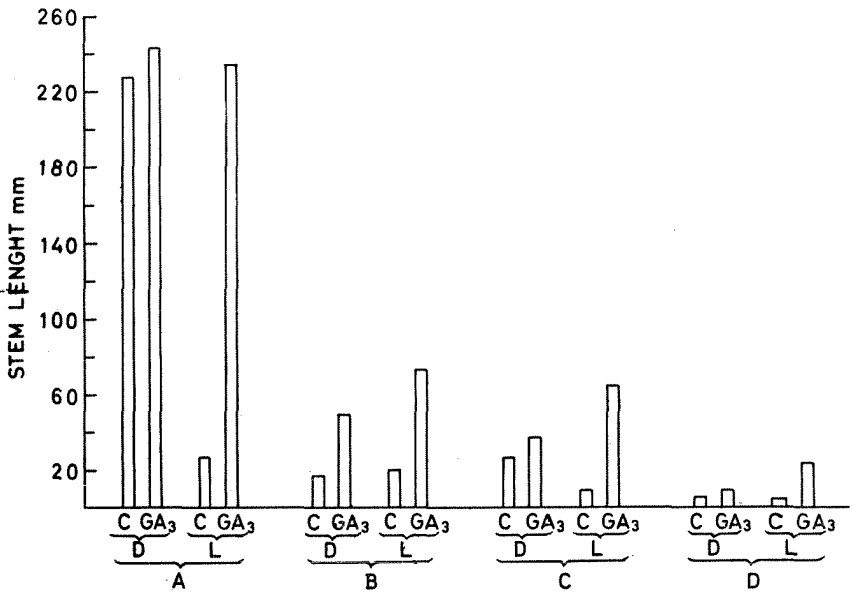

Fig. 2. Influence of light and GA_3 on the stem length in the var. »Meteor«

C = controls; GA_3 — 10 µu GA_3 added per plant; D = plants grown in darkness; L = plants grown in light; A = with roots, with cotyledons; B = without roots, with cotyledons; C = with roots, without cotyledons; D = without roots, without cotyledons. All plants measured after 14 days.

mentionned. In all other respects, »Meteor« peas responded to the treatments in a way similar to »Alaska«.

In these experiments the length of the plants was measured every second day. Fig. 3 represents the elongation of plants in groups B and C, plotted against time. Plants in the group C had a steady rate of growth in light. In darkness, their growth somewhat declined after 8 days. In the group B, plants grew very slowly in light and in darkness as well. However, in plants treated with GA_3, stem elongation was very stimulated up to the 8th day. After that time their growth rate also declined to the

level of control plants. Their growth seemed to be limited by the exhaustion of a substance not replaceable by GA_3.

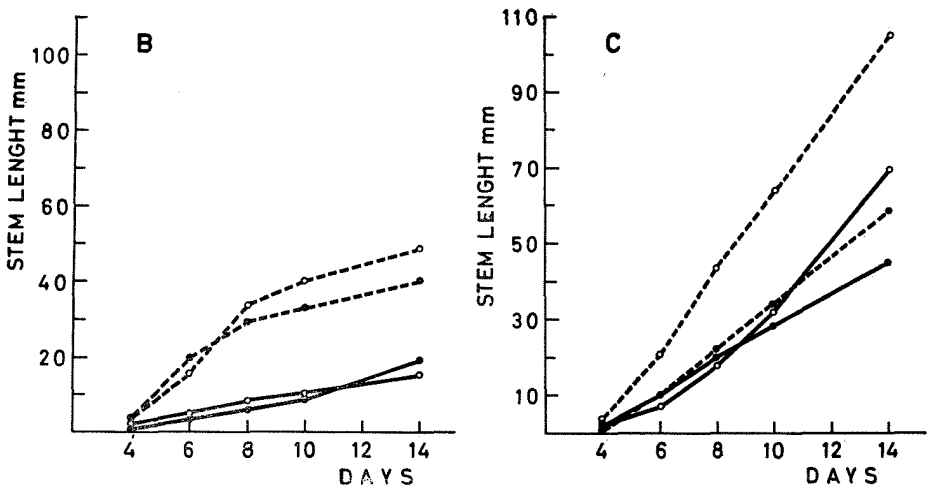

Fig. 3. Rate of stem elongation in the var. »Alaska«, plotted against time

Full lines (———) = controls; dotted lines (— — —) = 100 µg GA_3 per plant; open circles (—o—) = plants grown in light; closed circles (— • —) = plants grown in darkness; B = without roots, with cotyledons; C = with roots, without cotylendos.

DISCUSSION

Results obtained in the experiments concerning the influence of roots on the growth of stems, support the hypothesis of W e n t (1938a) that specific factors, necessary for stem growth, are synthesized in roots. Preliminary experiments had shown (N e š k o v i ć, 1965) that the addition of several different substances (aminoacids, adenin, kinetin, synthetic auxins, etc). had no significant effect on the growth of stems. GA_3 was the only exogenous substance tested, which exerted a marked influence on stem elongation. The growth of stems was also greatly dependent on root factors and on light conditions. The question was therefore formulated, whether GA_3 were identical with substances supplied by the roots, or with substances produced in darkness. Results presented in Figs. 1 and 2 do not lend support to the hypothesis of the identity of »caulocaline« with gibberellins. GA_3 stimulated to the same extent plants in all four groups, regardless of the presence or absence of roots. Fig. 3 clearly shows that stem growth in rootless plants is limited by factors other than gibberellin, since their growth ceased after certain time, in spite of continued addition of GA_3. Of course, this does not prove that gibberellins are not synthesized in roots. This only points to the existence of still other factors, acting probably in conjunction with gibberellins and being supplied exclusivelly by the roots.

In »Meteor« peas, the effect of the roots was evident only in dark-grown plants. Assuming the synthesis of specific substances in roots, two explanations of the later fact may be possible. Roots in the dwarf variety perhaps synthesize a substance which is light sensitive and which causes stem elongation only in darkness. Alternatively, roots in this variety do not produce substances which are able to reduce light inhibition of the stem, as is the case in the tall variety »Alaska«. The second explanation seems to be more plausible, since W e n t (1938b) also noticed a less »caulocaline« content in dwarf varieties of peas. On the other hand, it is known that the dwarfness in peas is light — dependent and reversible by gibberellins (G o r t e r, 1961). B r i a n (1959) has put forward a hypothesis on the existence of a growth inhibitor in dwarf peas, whose action were counteracted by gibberellins. Extending this hypothesis on the relationship between light- and dark-grown plants, S i m p s o n and W a i n (1961) suggested, that the supposed inhibitor were synthesized in light and responsible for the light inhibition of peas. However, there seems to be no general agreement about the existence of such an inhibitor. Whatever the mechanism of light inhibition and gibberellin action may be, one could suggest, on the basis of the results obtained in this paper, that »caulocaline« were the factor, which in addition to gibberellin were necessary to counteract the effect of light. The inability of roots to produce enough »caulocaline« in dwarf peas may be one of the causes of their dwarfness.

As it has been shown in Fig. 1, GA_3-treated light-grown »Alaska« plants had approximately the same stem length as plants grown in darkness. Nevertheless, some data suggest that this effect can not be explained by a simple reversible interaction between light and gibberellic acid, as postulated by L o c k h a r t (1961). Light inhibited only the elongation of plants with intact roots and cotyledons. It seems, therefore, that both organs were necessary for the synthesis of a substance (or substances) causing the elongation of etiolated plants. GA_3 added to the plants grown in light causes the doubling of their stem length, but it does the same in groups B, C and D, which were not inhibited in light. So, it seems that GA_3 causes the same relative stimulation in all plants, no matter if the light inhibition occurred or not. Dark-grown plants in groups B, C and D were also stimulated by GA_3, approximately to the same extent as the light-grown ones. The only exception in these experiments were the dark-grown plants in group A, which seemed to be unaffected by GA_3, or even slightly inhibited. A more detailed study on the interaction of light and GA_3 has already been done and the results will be published elsewhere. These results will give the evidence, that the dark-grown plants in the group A can also be stimulated by GA_3 under certain special conditions.

It is interesting to note, that plants with roots, but without cotyledons (group C) were significantly stimulated by light. It is possible, that their growth is partly limited by some substances stored in the cotyledons, and that the plants are able to synthesize these substances in light. This effect of light was not further investigated.

SUMMARY

It has been shown, that the elongation of cotyledonary buds in decapitated peas is dependent on at least three different factors: light, substances produced in roots and gibberellic acid.

GA_3 stimulated the elongation of all plants relatively to the same extent, including those which were not inhibited by light. GA_3 does not seem to be identical with the substances causing the elongation of etiolated plants.

GA_3 also seems to be different from substances produced in roots, which are necessary for stem elongation. GA_3 can not substitute for the presence of roots. Substances produced in roots seem to play a role in reversing the light inhibition of stems. This effect is much more pronounced in the tall variety »Alaska«, than in the dwarf »Meteor«.

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Rezime

MIRJANA NEŠKOVIĆ

INTERAKCIJA KORENA, GIBERELNE KISELINE I SVETLOSTI U RASTENJU STABLA GRAŠKA

(*PISUM SATIVUM* L.)

Izduživanje kotiledonarnih pupoljaka kod dekapitiranog graška zavisi najmanje od tri različita faktora: od svetlosti, od supstanci koje proizvodi koren i od giberelne kiseline.

GA_3 stimulira izduživanje svih biljaka relativno u istoj meri, uključujući i one koje nisu inhibirane na svetlosti. Verovatno je da GA_3 nije identična sa supstancama koje izazivaju izduživanje etioliranih biljaka.

GA_3 se takođe razlikuje od supstanci koje proizvodi koren i koje su neophodne za izduživanje stabla. GA_3 ne može da zameni prisustvo korena. Supstance koje proizvodi koren imaju izvesnu ulogu u otklanjanju inhibitornog dejstva svetlosti na izduživanje stabla. Ovaj efekat je mnogo više izražen kod visokog varijeteta »Aljaska«, nego kod patuljastog mutanta »Meteor«.