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Grafting and Gibberellin Effects on the Growth of Tall and Dwarf Peas[1]

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ABSTRACT

Tall peas var. Alaska and dwarf peas var. Progress No. 9 were grafted onto their own roots or reciprocally grafted to determine the rootstock effect on the growth of the stem. In all cases the grafted stems grew the same as their ungrafted controls regardless of which rootstock they were grown on. When similarly grafted plants were supplied with gibberellic acid, good graft unions did not inhibit its translocation. This evidence supports the thesis that the mechanism controlling stem growth in peas is located in the stem and that the roots have no direct control over this mechanism.

Since Went (17, 18) in 1938 postulated the existence of the caline growth substance which he suggested was produced in the roots, it has been assumed that the root system and cotyledons controlled stem and leaf growth. Recent investigations on the organ of synthesis and the movement of gibberellins have resulted in different opinions on this subject.

Gibberellin-like growth substances have been found in the bleeding sap collected from the cut stump of various plants (2, 14, 15), indicating that gibberellins are synthesized in the roots. Bioassay experiments with extracts from root tips have also demonstrated the presence of gibberellin in this organ (1, 6). Gibberellins have, however, been found in shoot tips of many plants, notably in peas; and diffusion and excision experiments have indicated that gibberellins are synthesized in the shoot tips as well as in the roots (6, 12, 16). Lockhart (10) attempted to demonstrate the controlling aspect of shoot tip-syhthesized gibberellin by grafting a stem from a tall pea onto the root of a dwarf pea. No increased elongation occurred in the stem section of the dwarf rootstock, contrary to expectation, and he concluded that his experiments failed to provide evidence for or against the production of gibberellin in roots or cotyledons.

Lockhart (9, 11) suggested that light regulates stem elongation in dwarf pea through some effect, or effects, on the metabolism of gibberellin. He considered three possible mechanisms: (*a*) light may inhibit the synthesis of endogenous gibberellin, (*b*) light may cause a destruction or diversion of endogenous gibberellin, or (*c*) light may make the tissue less responsive to a given amount of gibberellin; and he concluded that visible radiation probably inhibited stem elongation in pea through an effect on the level of endogenous gibberellin (11). However, Kende and Lang (7) rejected the first two possibilities since they found equal amounts of gibberellin in dark- and light-grown dwarf peas. They favored the third mechanism, that light makes the tissue less responsive. Köhler and Lang (8) produced evidence for substances in higher plants (lima beans) which interfered with the response of dwarf peas to gibberellin, and they suggested that inhibitors may participate in the growth regulation of plants, particularly by an interplay with gibberellins.

Our experiments were aimed at finding whether stem growth due to gibberellin is controlled by the root system or stem and/or if an inhibitor is produced in the roots which may be translocated to the stem of the pea plant and in this way regulate stem growth.

MATERIALS AND METHODS

Two varieties of peas (*Pisum sativum* L.) were used: Alaska, a tall pea, and Progress No. 9, a dwarf pea. The Alaska seeds were obtained from a local seed house and the Progress No. 9 seeds from Northrup King, Minneapolis, Minnesota.

The seeds were soaked for 5 hr in water and then planted in vermiculite in 3.8-cm pots. The plants were grown in a growth chamber at 20° under continuous illumination. For the first 3 days the plants were watered with distilled water, and after the 3rd day they were supplied daily with Hoagland's nutrient solution. Generally plants were grafted at 9 days from seeding when the dwarf peas were about 5 cm high and the tall plants were 10 cm high. At this stage the pea plants had two immature internodes above the cotyledons. The grafts were done similarly to those of Paton and Barber (13). The stem was cut about 1.5 cm above the cotyledons, the cotyledons remaining intact (this portion of the plant, roots plus cut stem, will be referred to as "rootstock" in this paper). A cleft graft was made by splitting the rootstock stem radially for 1 cm. The scion base was then cut into the shape of a wedge to fit the split in the rootstock. The graft was held firmly together by a small piece of crepe rubber (Sealtex, recommended by Burdean Struckmeyer). The grafted plants were then placed in a plastic-covered humidity chamber which was kept at 97 to 100% relative humidity and under continuous illumination at 20°. After 5 days in the humidity chamber the pea plants were returned to the growth chamber. Final measurements were usually made 15 to 16 days after grafting.

RESULTS

The results of two representative grafting experiments are presented in Table I. Stem length measurements in experiments 1 and 2 were taken 15 and 16 days after grafting, respectively. The important aspect of these data is that the stem grew about the same amount whether the tall stem was grafted on a tall or dwarf rootstock. However, the tall ungrafted control grew slightly more than the grafted plants. Similarily, dwarf pea stems grew

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Table I. *Mean Stem Length of Grafted and Ungrafted Pea Plants*

Final measurements of experiments 1 and 2 were taken 15 and 16 days after grafting, respectively.

Graft Treatment	Experiment 1		Experiment 2	
	No. of plants	Stem length	No. of plants	Stem length
		cm		*cm*
Tall ungrafted control	6	39.8	8	52.5
Tall stem on tall root[1]	3	41.3	10	30.2
Tall stem on dwarf root	6	40.0	16	35.6
Dwarf ungrafted control	6	13.5	8	10.9
Dwarf stem on dwarf root[1]	6	12.0	10	8.1
Dwarf stem on tall root	3	13.9	16	7.6
Standard error of treatment mean		1.87		1.36

[1] Stems grafted back onto their own roots.

Table II. *Mean Stem Length of Grafted and Ungrafted Pea Plants with and without 5 ml of Gibberellic Acid (10 μg/ml) Added to the Plant Roots*

Standard error of treatment means = 2.68.

Graft Treatment	Minus GA		Plus GA		Increase due to GA_3
	No. of plants	Stem length	No. of plants	Stem length	
		cm		*cm*	%
Tall ungrafted control	6	38.0	6	41.6	9.5
Tall stem on tall root[1]	5	34.5	4	33.3	−3.5
Tall stem on dwarf root	6	32.8	6	36.0	9.8
Dwarf ungrafted control	6	13.5	6	29.7	139.5
Dwarf stem on dwarf root[1]	5	9.2	5	17.7	92.4
Dwarf stem on tall root	6	7.7	5	16.5	114.3

[1] Stems grafted back onto their own roots.

about the same whether they were grafted on a tall or dwarf rootstock.

The amount of growth did not appear to be dependent on the roots or cotyledons. The control of growth seems to reside in the stem because the root system did not modify the amount of growth, although it did alter some of the morphological features of the plants in some cases. The tall stems on dwarf roots had thicker stems and larger leaves than the tall control plants.

In order to verify that gibberellins can cross the graft union, 50 μg of GA_3[2] were applied to the plant roots of grafted and ungrafted pea plants 3 days after the grafts were made. The tall ungrafted plants showed only a 10% response to the applied GA_3, but all dwarf stems, whether grafted onto tall or dwarf roots, showed a very marked response (Table II). These data show that GA_3 can pass the graft union and that lack of stem growth following grafting is not controlled by restriction of GA translocation.

Variation in the results in Table II is probably due to the rate of formation of the graft union and to the fact that the GA was applied 3 days after grafting while the plants were still in the humidity chamber. Grafted plants normally cannot be removed successfully from 97 to 100% relative humidity until 5 days after grafting.

[2] Abbreviation: GA: gibberellin.

DISCUSSION

There is evidence that GA is synthesized in plant roots (8) and that some of this root-synthesized GA is translocated from the root to the stem (2, 14). The experiments described here indicate that the root-synthesized GA has no direct controlling effect on pea stem growth (Table I). The mechanism controlling pea stem growth must be located in the stems, presumably near the apex, and the stem-synthesized GA (6, 12, 16) is presumed to be a factor in this mechanism (7, 11). Based on our grafting experiments, the stem-synthesized GA must be the dominant controlling factor because dwarf pea stems grew the same amount whether they were grafted onto tall or dwarf rootstocks (Table I). Apparently the rootstock had no effect on the growth of tall pea stems, again indicating that the growth-controlling mechanism is in the stem. This does not, however, preclude the translocation of GA precursors from the root to the stem, but the conversion to active growth factor must be controlled by the the stem.

Köhler and Lang (8) isolated substances which had no activity when applied to a particular gibberellin-sensitive test system alone, but they decreased the response of the system to gibberellin and Kende and Lang (7) suggested that light may induce the formation of an inhibitor. Based on the grafting experiments described here, if an inhibitor is involved the inhibitor must be produced by the stem apex since the dwarf rootstock did not inhibit stem growth of tall peas, and dwarf stems remained dwarf even when growing on tall roots (Table I). However, GA_3 did pass the graft union (Table II), and it may be assumed that growth inhibitors could also pass the graft union. If this is a correct assumption, then the expression of the dwarfing characteristics in dwarf pea plants must not be controlled by a root-synthesized inhibitor as tall stems were not reduced in growth when grafted onto a dwarf rootstock.

The difference in growth between dwarf and tall peas when grown in the light initially seemed to be related to gibberellin concentration in the stem tips (4, 11). The logical conclusion was that light affected the concentration or the effectiveness of the naturally synthesized gibberellin. Kende and Lang (7) have shown that there are two gibberellins in pea plants, probably GA_1 and GA_5. The authors (unpublished data) have also found only two zones of gibberellin-like activity in tall pea extracts using the lettuce seed hypoctoyl test (3). Jones and Lang (5) also showed that dwarf and tall peas contain the same amount of these two gibberellins regardless of whether they were grown in the dark or in the light. These results indicate that the light effect on the growth of dwarf peas is not due to a change in gibberellin concentration *per se*. They explained the light effect on the basis that it lowers the sensitivity of the cells to endogenous gibberellin (probably GA_5) and suggested that light may interfere with one of the reactions leading from gibberellin to the growth response proper. Our results agree with this theory.

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