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Shoot versus Root Signal Involvement in Nodulation and Vegetative Growth in Wild-Type and Hypernodulating Soybean Genotypes

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Grafting studies involving Williams 82 (normally nodulating) and NOD1-3 (hypernodulating) soybean (*Glycine max* [L.] Merr.) lines and *Lablab purpureus* were used to evaluate the effect of shoot and root on nodulation control and plant growth. A single- or double-wedge graft technique, with superimposed partial defoliation, was used to separate signal control from a photosynthate supply effect. Grafting of hypernodulated soybean shoots to roots of Williams 82 or *L. purpureus* resulted in increased nodule numbers. Grafting of two shoots to one root enhanced root growth in both soybean genotypes, whereas the nodule number was a function of shoot genotype but not of the photosynthetic area. In double-shoot, single-root-grafted plants, removing trifoliolate leaves from either Williams 82 or NOD1-3 shoots decreased root and shoot dry matter, attributable to decreased photosynthetic source. Concurrently, Williams 82 shoot defoliation increased the nodule number, whereas NOD1-3 shoot defoliation decreased the nodule number on both soybean and *L. purpureus* roots. It was concluded that (a) soybean leaves are the dominant site of autoregulatory signal production, which controls the nodule number; (b) soybean and *L. purpureus* have a common, translocatable, autoregulatory control signal; (c) seedling vegetative growth and nodule number are independently controlled; and (d) two signals, inhibitor and promoter, may be involved in controlling legume nodule numbers.

The economic and environmental importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). The extent of nodulation upon successful inoculation is generally restricted by a plant-mediated, feedback-regulated process termed autoregulation. This process involves suppression of nodule emergence from ontogenetically younger root tissues by previously formed nodules on older parts of the root system (Bhuvaneswari et al., 1981; Pierce and Bauer, 1983; Kosslak and Bohlool, 1984). It has been proposed that once a critical number of SCDs in the root cortex are initiated, a precursor molecule from the root is transported to the shoot, where it is converted into the SDI, which, in turn, is transported back to the root and suppresses the later-formed SCDs from developing into emergent nodules (Caetano-Anolles and Gresshoff, 1990, 1991b). However, autoregulation does not appear absolute in that genotypic variability in autoregulatory control of the nodule number has been reported (Heron and Pueppke, 1987). In addition, autoregulation in soybean (*Glycine max* [L.] Merr.) cv Williams 82 and its hypernodulating mutants does not always maintain a constant number of nodules. Rather, the nodule number in Williams 82 can be affected by available infection sites (root size) at the time of inoculation, so that a delayed inoculation will result in more nodules (Francisco and Harper, 1995a). It appears certain that new nodule primordia are arrested during early nodule ontogeny by previously formed SCDs in the root through a shoot-mediated feedback process (Gresshoff and Caetano-Anolles, 1992), and signal communication must occur in the root-shoot interactions (Delves et al., 1986, 1987; reviewed by Gresshoff and Caetano-Anolles, 1992; Francisco and Harper, 1995b).

Our knowledge about the proposed plant-translocatable signals (the putative SDI and its more speculative root-derived precursor) is limited. The biochemical nature, the biosynthetic site(s), and the pathway by which the signals are transported and function are unknown. Wedge-grafting studies with most legume nodulation mutants and their respective wild-type parents have demonstrated that it is the shoot that determines the root nodulation phenotype, which suggests that the shoot is the source of the proposed SDI (Delves et al., 1986, 1987; Gremaud and Harper, 1989; Francisco and Akao, 1993; Hamaguchi et al., 1993). Recently, by wedge- and approach-grafting with different shoots (with or without cotyledons and with or without primary leaves) and by nodulation of rooted leaf cuttings, Francisco and Harper (1995b) clearly demonstrated that the leaf autoregulates the number of nodules in soybean plants, which confirmed a report by Delves et al. (1992). However, root genotypes also have some effects on nodulation patterns of soybean grafts (Hamaguchi et al., 1993; Francisco and Harper, 1995b). The biosynthesis of the putative SDI may not be restricted to the leaf. It is possible

[1] This paper is dedicated to the memory of Chuxing "Chuck" Sheng, who unexpectedly passed away on July 15, 1996, while in the prime of his career. His friendship and scientific expertise will be remembered by those with whom he came into contact during his short scientific career.

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Abbreviations: ABC, auxin burst control; SCD, subepidermal cell division; SDI, shoot-derived inhibitor.

that the putative SDI can be produced in both the root and shoot, with the leaf being the major pool, so that it dominantly controls the autoregulation process over the other organs.

One must keep in mind that much of our knowledge of legume nodule autoregulation is based on the comparative studies of wild-type and hypernodulating or supernodulating mutants (Delves et al., 1986, 1987, 1992; Caetano-Anolles and Gresshoff, 1990, 1991a; Francisco and Akao, 1993; Francisco and Harper, 1995b). It is possible that the hypernodulating or supernodulating mutants have been chemically altered in a biosynthetic step associated with the production of a "common" hormonal compound rather than the proposed nodulation-specific signal. The altered signal may be responsible for regulation of a number of physiological events in plant growth and development, including root and shoot morphology, as well as differentiation of SCDs to emergent nodules. Therefore, nodulation in hypernodulating (and supernodulating) mutants may be indirectly affected through an as-yet-unidentified signal that may be antagonistic to the proposed SDI, resulting in inactivation of the autoregulation mechanism in this phenotype.

Alternatively, the proposed SDI itself may not be an autoregulation-specific signal. Gresshoff (1993) suggested an ABC hypothesis for autoregulation and hypernodulation (or supernodulation) in legume-*Rhizobium* symbiosis. According to the ABC working model, autoregulation is a general morphogenic response mediated by auxin. Supernodulation or hypernodulation and nitrate tolerance may be the result of alteration in ABC. This theory postulates that supernodulating plants contain a lower level of auxin because they are unable to generate the auxin burst in response to the first nodules formed in the root. In other words, the previously proposed SDI (Caetano-Anolles and Gresshoff, 1990, 1991a) is possibly an auxin (Gresshoff, 1993). The ABC hypothesis remains quite speculative because critical supporting evidence has not been presented.

The objectives of this study were to (a) more definitively identify the sites where the putative autoregulation and growth response signal(s) are generated and determine if both the shoot and the root are involved in generation of the signal that affects nodulation and seedling morphogenesis; (b) determine if there is a commonality in the autoregulatory signal between legumes; and (c) identify, using a double-wedge graft technique recently developed in our laboratory, if autoregulation in wild-type soybean is due to the production of a nodule development inhibitor (SDI) and/or if hypernodulation in the mutant is associated with the loss of the SDI or with an as-yet-unknown nodule promoter.

MATERIALS AND METHODS

Bradyrhizobium japonicum USDA 110 (obtained from P. van Berkum, U.S. Department of Agriculture-Agricultural Research Service, Beltsville, MD) was grown in yeast-mannitol broth at 90 rpm and 30°C on a shaker (Orbit Environ-Shaker,[2] Lab-Line Instruments, Melrose Park, IL) until the mid-exponential phase (10^7 to 10^8 cells/mL). Cells were harvested by centrifugation and adjusted to the desired density by adding sterile, distilled water prior to inoculation of soybean (*Glycine max* [L.] Merr.). Soybean cv Williams 82 and its hypernodulating mutant (NOD1-3) were used in all experiments in this study. The hypernodulating mutant was derived from Williams through *N*-nitroso-*N*-methylurea mutagenesis and produces at least two to four times the nodule number as on roots of the wild-type parent under similar growth conditions (Gremaud and Harper, 1989; Francisco and Harper, 1995b). Experiments were repeated and data presented are from representative experiments.

Wedge Grafts of Double-Shoot Plants (Y-Shaped Grafts)

Seeds of Williams 82 and NOD1-3 were surface-sterilized with 70% ethanol for 1 min and then 1% sodium hypochlorite for 5 min, followed by a thorough rinse (six times) with sterile, distilled water. The sterilized seeds were germinated and grown in autoclaved (121°C, 30 min) sand beds in a growth chamber that was programmed for 14-h photoperiods with mixed sodium vapor (Bulbtronics, Farmingdale, NY) and cool-white fluorescent lamps (General Electric) at 450 μmol photons m^{-2} s^{-1} of PAR. Temperatures and RHs in the chamber were, respectively, 28°C and 65% for the day, and 20°C and 70% for the night. Six days after planting, vigorous seedlings with visible; unifoliolate leaves were selected for grafting, either single- or double-wedge grafts. Hypocotyls were severed with a razor blade at the mid-point between cotyledons and the sand surface. The upper hypocotyl section with cotyledons was then cut at an angle on opposite sides to form a V-shaped stem base. The lower hypocotyl section with roots was vertically split with a razor blade about 1 cm deep. Either one or two V-shaped stems were inserted into one root slit (see Fig. 1A for double-wedge graft technique). Self-grafts and reciprocal grafts were made with two soybean lines (Williams 82 and NOD1-3) as shoots on either Williams 82 or NOD1-3 roots, plus ungrafted control seedlings. After the graft joints were secured with tape, plants were covered with transparent plastic trays for 4 d to maintain a high RH. Five days after grafting, the graft union was sufficiently strong to allow transfer of plants. Plants were then transplanted to 2-L polystyrene pots containing a modified nutrient solution (Gremaud and Harper, 1989) supplemented with 1 mM urea-nitrogen. The nutrient solution was changed weekly and pH was maintained at 6.8 ± 0.5 with Amberlite IRC-50 resin in a recirculating ion-exchange column, which also served to aerate the solution (Nicholas and Harper, 1976). Growth conditions in the growth chamber were as for germination. One week after the transplant, the plants in

[2] Trade and manufacturer names are necessary to report factually on available data; however, the U.S. Department of Agriculture (USDA) neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

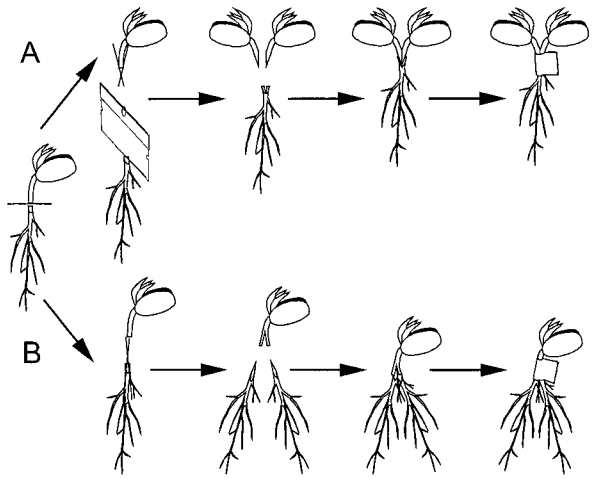

Figure 1. Diagrammatic representation of double-shoot (A) and double-root (B) grafting techniques. Grafts were made at 5 to 6 d after planting when the hook was straightened and the unifoliolate leaves were just emerging from between the cotyledons. Grafts were secured in place with tape.

hydroponic pots were inoculated with *B. japonicum* USDA 110 at 10^7 cells per root. At the same time, one-half of the Y-shaped plants received defoliation treatment, which involved continuous removal of all existing and newly formed trifoliolate leaves from one of the two shoots. Plants were harvested 28 d after inoculation for examination of nodulation and vegetative growth.

Y-shaped soybean shoots were also grafted onto *Lablab purpureus* root stocks using the same procedure as described above (Fig. 1A). The single- and double-wedge grafts of soybean shoots to *L. purpureus* roots were inoculated with *B. japonicum* USDA 4785 (10^7 cells per root) 7 d after being transplanted to hydroponic pots. The same defoliation treatment was also applied to this group of Y-shaped shoots as noted above for the grafted soybean plants. The plants were harvested 28 d after inoculation.

Wedge Grafts of Double-Root Plants

Wedge-grafted double-root plants (see Fig. 1B) were created from 6-d-old soybean seedlings that were germinated and grown in sand beds in a growth chamber. Combinations involved grafting of two roots (Williams 82 and NOD1-3) to a single shoot, either Williams 82 or NOD1-3. After severing at the midpoint of the hypocotyls, the lower half of the hypocotyl with roots was cut at an angle on opposite sides to form inverted V-shaped stem inserts. The upper half of the hypocotyl was split vertically, about 1 cm deep, to form a stem base for root attachment (see Fig. 1B). After the graft joint was secured with tape, each grafted plant was transferred to paired 500-mL culture pots, with the two roots grown separately in individual pots. The pots were filled with well-watered vermiculite that was inoculated with *B. japonicum* USDA 110 at 1.5×10^7 cells per root. Pots were covered with transparent bags for the first 4 d to increase the RH and facilitate a graft union formation. To further decrease transpiration, the growth chamber was programmed to be dark at 20°C and 80% RH for 12 h followed by half-light intensity for 12 h, and then returned to the normal growth conditions. The plants were watered with a modified nutrient solution (Gremaud and Harper, 1989) supplemented with 1 mM urea-nitrogen as needed. Plants were harvested 21 d after inoculation and nodulation and growth were examined.

RESULTS AND DISCUSSION

Leaf Production of the Autoregulatory Signal That Determines the Root Nodulating Phenotype

With the newly developed double-wedge graft technique used in this study, additional evidence was obtained that the leaf is in primary control of the final number of nodules that can be formed on a successfully inoculated soybean root. Plants with two Williams 82 shoots did not increase the nodule number on either Williams 82 (Fig. 2A) or NOD1-3 (Fig. 2B) roots when compared with those with only one Williams 82 shoot. This indicated that photosynthate production was not limiting the nodule number. In contrast, two NOD1-3 shoots promoted nodule formation on both Williams 82 (Fig. 2A) and NOD1-3 (Fig. 2B) roots relative to the respective grafted plants with a single NOD1-3 shoot. Plants with one Williams 82 shoot and one NOD1-3 shoot produced significantly fewer nodule numbers than those with two NOD1-3 shoots, regardless of root genotype (Fig. 2). Promotion of nodulation on plants with

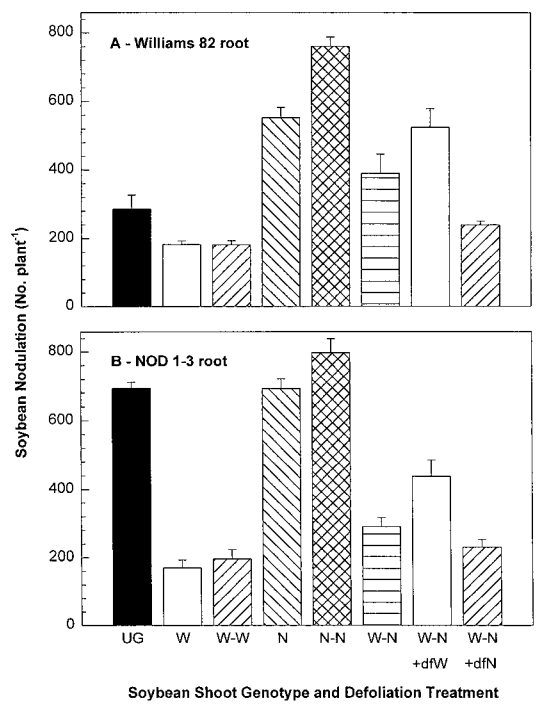

Figure 2. Effect of soybean shoot genotype and defoliation treatment on nodulation of single- and double-shoot grafts with Williams 82 (A) and NOD1-3 (B) soybean root stocks. Data represent the means plus SE of six replicate plants. UG, Ungrafted soybean seedlings; W, Williams 82 shoot; N, NOD1-3 shoot; +dfW, defoliation of Williams 82; +dfN, defoliation of NOD1-3.

one Williams 82 shoot and one NOD1-3 shoot was significant on Williams 82 roots relative to the plants with two Williams 82 shoots (Fig. 2A). A positive but smaller promotion of nodulation resulted when a Williams 82 shoot and a NOD1-3 shoot were grafted to a NOD1-3 root (Fig. 2B) relative to the graft with two Williams 82 shoots. These results (Fig. 2) are consistent with previous reports involving soybean grafts (Delves et al., 1992; Francisco and Harper, 1995b). The putative leaf signal from soybean also exercised control over the nodule number on *L. purpureus* roots grafted to soybean shoots (Fig. 3) and provided support for the concept that autoregulatory control of nodule number is common among certain legume species, including between soybean and *L. purpureus* (Fig. 3) and soybean and mung bean (*Vigna radiata*) (Harper et al., 1997).

The fact that removal of newly formed trifoliolate leaves from the Williams 82 shoot of the double-shoot (Williams 82 and NOD1-3) single-root grafts promoted nodule formation (Figs. 2 and 3) has provided further evidence that the leaf is the most important source of the putative autoregulation signal (nodule inhibitor) in soybean plants. Defoliation of the Williams 82 shoot left the NOD1-3 shoot intact but increased the final nodule numbers by 35% on Williams 82 roots (Fig. 2A), by 62% on NOD1-3 roots (Fig. 2B), and by 29% on *L. purpureus* roots (Fig. 3) relative to plants in which both Williams 82 and NOD1-3 shoots were intact. In contrast, removing trifoliolate leaves from the NOD1-3 shoot and leaving the Williams 82 shoot intact decreased nodule numbers by 39% on Williams 82 roots (Fig. 2A), by 14% on NOD1-3 roots (Fig. 2B), and by 22% on *L. purpureus* roots (Fig. 3), again relative to plants in which both Williams 82 and NOD1-3 shoots were intact. It is still risky, however, to conclude that the leaf is the only tissue in which the proposed autoregulation signal is formed. It is widely recognized that, unlike animal hormones, the biosynthesis of almost all known plant hormones and related signal compounds is not tightly restricted to a specific tissue (organ). Instead, biosynthesis of translocatable hormonal signals in higher plants can occur in more than one tissue, but usually there is a major site during specific developmental stages. Therefore, it is highly possible that the proposed autoregulation signal in legumes may also be produced in a relatively small amount by tissues other than the leaf, such as apices, shoot meristems, and even roots, but clearly the leaf is the dominant source of the autoregulatory signal.

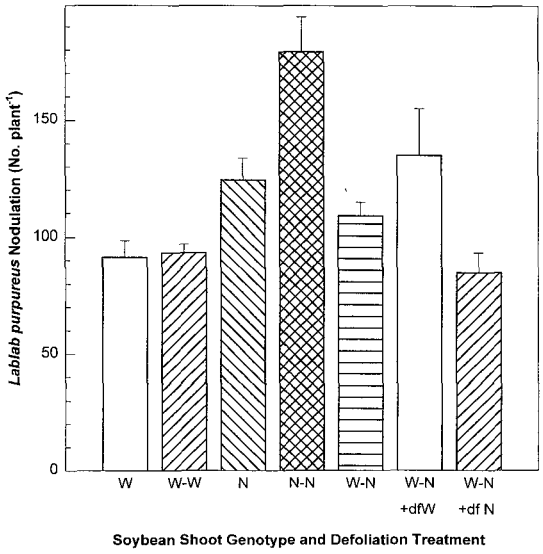

Figure 3. Effect of soybean shoot genotype and defoliation treatment on nodulation of single- and double-shoot grafts with *L. purpureus* roots. Data represent the means plus SE of five to six replicate plants. Symbols and abbreviations are as in Figure 2.

The Role of the Root in Autoregulation and Hypernodulation

No clear role for the root in regulation of nodule numbers has been identified so far. It was reported that the NOD1-3 root stock produced more nodules than did the Williams 82 root stock when each was grafted to a Williams 82 shoot (Francisco and Harper, 1995b), indicating that the root may produce a signal involved in autoregulation and/or hypernodulation. In the present study the NOD1-3 root stock had a slightly greater number of nodules than the Williams 82 root when single-wedge grafted to a NOD1-3 shoot, but not when single-wedge grafted to a Williams 82 shoot (Fig. 2). There was no difference in nodulation of NOD1-3 and Williams 82 roots when single roots were double-wedge grafted to two NOD1-3 shoots or when single roots were single- or double-wedge grafted to Williams 82 shoots (Fig. 2). Thus, it appears that the impact of root genotype on nodulation expression is relatively weak. Expression of nodulation on a per root (Fig. 4A) or per root dry matter (Fig. 4B) basis gave different results due to the smaller root size of NOD1-3 compared with Williams 82. The weak effect of the root on nodulation was noted in double-root (Williams 82 and NOD1-3) wedge-grafted plants, in which the NOD1-3 root produced significantly more nodules on a root dry mass basis than did the Williams 82 root when both roots were grafted to a Williams 82 shoot (Fig. 4B). On the basis of total nodules per root, however, the NOD1-3 root produced fewer nodules than its partner Williams 82 root (Fig. 4A). The size of the root likely affects infectable sites, which can partially offset the normally observed autoregulatory control of the nodule number.

Shoot Growth and Root Growth Are Locally Regulated

With the double-shoot wedge-grafted soybean plants, shoot growth appeared to be a function of the shoot genotype. The Williams 82 shoot generally accumulated greater dry matter than did the NOD1-3 shoot, regardless of the graft combination (Table I). This relationship was more clear in the wedge-grafted double-root (NOD1-3 and Williams 82) plants, in which the NOD1-3 shoot had inferior growth to that of the Williams 82 shoot (Fig. 5A). Therefore, the shoot genotype appeared to control shoot growth. Likewise, the root genotype primarily determined the root growth when grafted to two shoots (NOD1-3 and Williams 82), although the shoot had some limited effect on the root

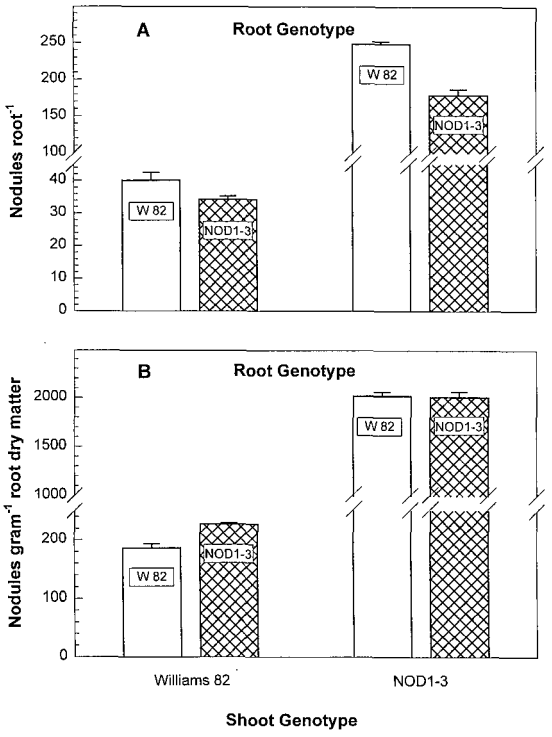

Figure 4. Effect of soybean root and shoot genotype on nodule number/root (A) and nodule number/g root dry matter (B) of double-root, single-shoot-grafted plants. Data represent the means plus SE of 8 to 10 grafted plants.

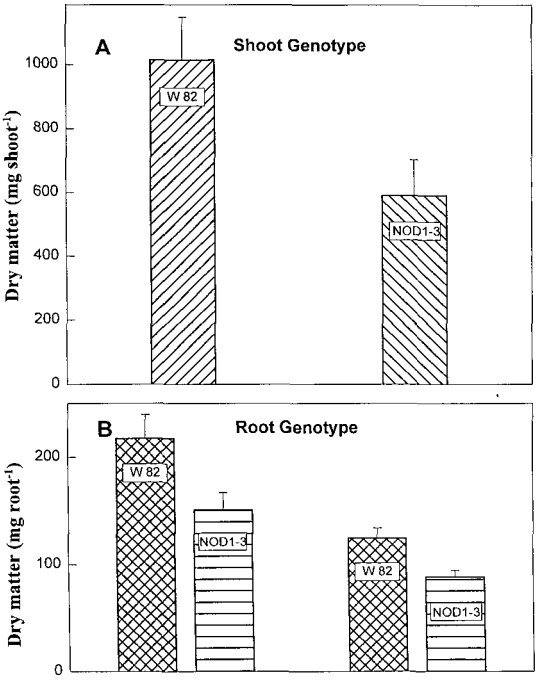

Figure 5. Shoot (A) and root (B) dry matter of single-shoot, double-root-grafted soybean plants. Shoots of Williams 82 or NOD1-3 were grafted to double roots of Williams 82 and NOD1-3 as depicted below their respective shoots. Data represent the means plus SE of 8 to 10 replicate grafts.

growth (Table I). In the one-shoot, double-root (NOD1-3 and Williams 82) system, the Williams 82 root grew significantly faster than did the NOD1-3 root, regardless of the shoot genotype (Fig. 4B). This indicated that root growth in soybean was primarily controlled by local gene expression. From results of this study it was concluded that the inferior shoot growth in hypernodulating mutants was not solely due to a slower root growth, but rather resided in an inherent genetic response within the shoot.

Growth and Nodulation in Soybean Are Relatively Independent

The fact that hypernodulating mutants have inferior growth, but can form greater numbers of nodules than do

Table I. *Effect of shoot genotype (Williams 82 and/or hypernodulating mutant NOD1-3) and defoliation treatment on dry matter production of shoots, roots, and nodules of wedge-grafted single- or double-shoot plants*

Root genotype was either NOD1-3 or Williams 82 soybean or *L. purpureus*. Data represent the means ± SE of six replicate plants.

Root Genotype	Plant Part	Dry Matter Shoot Genotype and Defoliation Treatment[a]							
		UG	1W	2W	1N	2N	WN	WN-dfW	WN-dfN
		g plant⁻¹ part ± SE							
NOD1-3	Shoot	4.65 ± 0.22	5.20 ± 0.28	7.37 ± 0.17	4.60 ± 0.28	6.06 ± 0.14	6.40 ± 0.22	4.89 ± 0.24	5.37 ± 0.21
	Root	1.28 ± 0.08	1.00 ± 0.09	1.32 ± 0.11	0.99 ± 0.05	1.21 ± 0.07	1.36 ± 0.12	1.06 ± 0.07	1.10 ± 0.07
	Nodule	0.57 ± 0.04	0.17 ± 0.01	0.20 ± 0.01	0.59 ± 0.04	0.61 ± 0.04	0.28 ± 0.02	0.35 ± 0.07	0.25 ± 0.02
Williams 82	Shoot	5.80 ± 0.76	5.05 ± 0.09	6.94 ± 0.13	4.10 ± 0.40	5.44 ± 0.60	6.74 ± 0.25	4.33 ± 0.20	5.68 ± 0.12
	Root	1.88 ± 0.11	1.66 ± 0.09	1.92 ± 0.12	1.55 ± 0.09	1.82 ± 0.08	1.96 ± 0.07	1.25 ± 0.08	1.61 ± 0.05
	Nodule	0.35 ± 0.04	0.31 ± 0.04	0.30 ± 0.01	0.49 ± 0.02	0.56 ± 0.03	0.54 ± 0.05	0.47 ± 0.06	0.28 ± 0.01
L. purpureus	Shoot	–[b]	4.17 ± 0.20	4.62 ± 0.11	3.15 ± 0.08	3.99 ± 0.10	4.21 ± 0.18	2.83 ± 0.09	3.65 ± 0.20
	Root	–	1.11 ± 0.08	0.96 ± 0.05	1.08 ± 0.10	1.03 ± 0.08	0.97 ± 0.02	0.84 ± 0.07	0.93 ± 0.06
	Nodule	–	0.29 ± 0.03	0.34 ± 0.06	0.40 ± 0.01	0.50 ± 0.05	0.41 ± 0.03	0.36 ± 0.08	0.28 ± 0.05

[a] Grafting and defoliation treatments as follows: UG, ungrafted; 1W, one Williams 82 shoot; 2W, two Williams 82 shoots; 1N, one NOD1-3 shoot; 2N, two NOD1-3 shoots; WN, one Williams 82 and one NOD1-3 shoot; WN-dfW, one Williams 82 and one NOD1-3 shoot with defoliation of the Williams 82 shoot; WN-dfN, one Williams 82 and one NOD1-3 shoot with defoliation of the NOD1-3 shoot. [b] –, Ungrafted treatment not feasible between two species.

their wild-type parents, has indicated that vegetative growth and nodulation in legume-rhizobia symbiosis are likely to be regulated by independent mechanisms. It has not been established if photosynthate supply plays any role in autoregulation of the final nodule number in a particular genotype. In double-shoot-grafted plants with two Williams 82 shoots, significantly greater root dry matter was observed than with single-shoot-grafted plants (Table I), whereas there was no associated difference in nodulation between the two types of grafts (Fig. 1). From this we suggest that an increase in photosynthate supply affects root growth, but not the nodule initiation process. One may argue, however, that an increase in photosynthate translocation from the Williams 82 shoot may also result in the simultaneous translocation of more autoregulatory inhibitor molecules to the root, which in turn could offset the effect of extra photosynthate supply on promotion of root nodulation. This alternative should be evaluated further. The results of the Williams 82 defoliation treatment involving double-shoot-grafted (Williams 82 and NOD1-3) plants provide some insight (Table I; Fig. 1). The removal of trifoliolate leaves from the Williams 82 shoot, although leaving the NOD1-3 shoot unaltered, significantly decreased root dry mass of both genotypes relative to the plants with undefoliated Williams 82 and NOD1-3 shoots (Table I). In contrast, the final nodule number on the roots of both genotypes was greater when the Williams 82 shoot was defoliated than when it was intact (Fig. 1). This strongly implies that (a) the leaf of Williams 82 is the source of the proposed autoregulation signal that can dominantly control (inhibit) the nodulation process in the root, and (b) photosynthate supply can affect root vegetative growth without affecting the autoregulation process, e.g. root growth and nodulation are independent events in the legume-rhizobia symbiosis.

Autoregulation and Hypernodulation Signals

The above observation on the relationship of root growth and nodulation extends our understanding of the hypernodulation (or supernodulation) phenotype and its responsible signal. The removal of trifoliolate leaves from the NOD1-3 shoot of wedge-grafted, double-shoot (Williams 82 and NOD1-3) plants significantly decreased the nodule number on Williams 82 roots (239/plant) (Fig. 1A) compared with the undefoliated plants (390 nodules/plant) and the plants in which the Williams 82 shoot was defoliated (525 nodules/plant). In contrast, Williams 82 root growth was minimal with the defoliation treatment of the Williams 82 shoot (1.25 g/plant) and considerably greater with the defoliation treatment of the NOD1-3 shoot (1.61 g/plant) (Table I). It does not appear, therefore, that the nodulation response to defoliation treatment of NOD1-3 was related to decreased photosynthate supply. Furthermore, it does not appear that increased SDI above some threshold level suppresses nodule development in soybean roots, since grafts with two Williams 82 shoots produced as many nodules as those with one Williams 82 shoot on both root genotypes (Fig. 1), even though the former accumulated a significantly greater root dry mass (Table I). It was expected that the double-shoot treatment (NOD1-3 and Williams 82) would produce a similar amount of SDI as the single Williams 82 shoot grafts. Given the above results, it is speculated that the reason the NOD1-3 shoot defoliation treatment inhibited nodulation was due to removal of a source that supplies a nodule promoter. This speculation of a nodule promoter was indirectly supported by the fact that a Williams 82 root grafted to two NOD1-3 shoots produced significantly more nodules (761/plant) than it did with one NOD1-3 shoot (553/plant) (Fig. 2A). When the root stock was NOD1-3, the effect of the trifoliolate leaf-removal treatment on nodulation was similar to that on the Williams 82 root stock. However, the nodule promotion by the NOD1-3 leaf was less significant. This trend was also true for the wedge-grafted, double-root plants, in which the NOD1-3 shoot promoted nodulation more on the Williams 82 root than on the NOD1-3 root relative to grafts with the Williams 82 shoot (Fig. 4A).

It is proposed that the putative hypernodulating signal (a nodule promoter) is not produced only in the leaf (dominant amount) but also in the root (limited amount) of the hypernodulating mutant. Since it has been shown that the NOD1-3 hypernodulating line is a single-gene mutant (rj_7) (Vuong et al., 1996), one might conclude that the signal altered in this genotype is responsible for both its inferior growth and its hypernodulation (supernodulation) phenotype. It is possible that the low levels of an as-yet-unknown hypernodulating signal can inhibit seedling vegetative growth so that the growth of hypernodulating mutants is always inferior to that of their respective parents, with or without rhizobial symbiosis. The root itself may be capable of forming the signal at a level inhibitory to root development but at a level insufficient to interfere with the nodulation process. It is the leaf that produces the signal in a large enough amount to promote nodule formation or to offset the effect of SDI on autoregulation.

Therefore, autoregulation in Williams 82 plants and enhanced nodulation in the hypernodulating mutant may be separate events in the legume-rhizobia symbiosis and regulated by two different kinds of signals. There are likely interactions between these possible control mechanisms through which nodule development is manipulated and the final number of nodules on the root is determined. Auxins are still considered to be potentially involved in the hypernodulation (supernodulation) process; nevertheless, it is risky to draw any conclusions on autoregulation and/or hypernodulation until the proposed signal(s) is isolated and identified from legume plants.

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