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Influence of Root and Shoot on N_2 Fixation in Cowpea

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Abstract. **Reciprocal and self-grafts were made using four cowpea [*Vigna unguiculata* (L.) Walp.] genotypes, 'H-Brown Crowder', 'H-Calico Crowder', 'L-Bush Purple Hull', and 'L-TX460', previously identified for their high (H) or low (L) N_2 fixation efficiency. Grafts were made on day 12, and N_2 fixation variables were evaluated 38 days after planting. Highest nitrogenase activity was obtained in the self grafts of high N_2 fixing lines, whereas nitrogenase activity of low N_2-fixing genotypes was generally increased by grafting onto high N_2-fixing stocks. Nodule number was influenced only by rootstock. Whether rootstock or scion is the limiting factor in N_2 fixation efficiency may be cultivar-specific.**

Intraspecific variability for N_2 fixation (15) and the inheritance of N_2 fixation efficiency in cowpea (10) have been reported. However, the role of rootstock and scion is not clearly understood. Intervarietal grafting has been used with soybean [*Glycine max* (L.) Merr.] to determine the effects of rootstock and scion on several physiological characteristics (1, 2, 5–8, 11, 12). Among these effects, N_2 fixation has been studied with cultivars having different fixation potentials. Reciprocal graft experiments have shown that nodulation is controlled by the rootstock through its susceptibility to infection by *Bradyrhizobium* (1, 8). The rootstock also was shown to be dominant in determining specific nodule activity, although the dry weight, developmental stage, and photosynthetic activity of the scion also influenced specific nodule activity. Nodule fresh weight appeared to be controlled by available photosynthate from the scion (8). The shoot may regulate N_2 fixation in the nodules by producing some stimulatory and/or inhibitory substances (13).

Specific nodule activity in soybean was found to vary with the developmental stage of the plant, with high activity soon after nodulation (28 days), and low activity during vegetative growth (38 days). This difference may have been due to a high N demand by seedlings grown in N-free media, which had developed chlorosis before the nodules became active (7, 8). Grafting also was used to study the decline in N_2 fixation during fruit development in soybean (9). Young scions grafted onto fruiting rootstocks that had passed their peak of N_2 fixation produced a second N_2 fixation peak on the same root system. This peak indicated that the decline is reversible (9) and that physiological changes in the shoot, rather than simply nutritional stress due to the fruit load, controls the cessation of N_2 fixation. Graft effects per se on N_2 fixation in soybean (7) and production or distribution of vegetative or reproductive dry matter in cowpea (14) have not been demonstrated. The objective of our investigation was to determine the effects of rootstock and scion on N_2 fixation in cowpea.

Four cowpea genotypes, 'H-Brown Crowder', 'H-Calico Crowder', 'L-Bush Purple Hull', and 'L-TX460', representing a broad range of horticultural types and previously identified for their high (H) or low (L) N_2 fixation potential (10), were grafted using reciprocal hypocotyl approach grafts in all combinations, including self grafts, using the techniques of de Stigter (3). Ungrafted controls were included to observe growth relative to grafted plants and reconfirm N_2 fixation potential. The plants were grown in a greenhouse in the spring in 3.7-liter black plastic pots, with a 12-hr day at a temperature of 25° ± 5°C. Potting medium was 1 sand : 1 vermiculite (v/v). Two grams of peat based cowpea single-strain '32'H' rhizobial inoculum obtained from the Nitragin Company, Milwaukee, Wis., were placed in each seed hole, and pots were watered with N-free nutrient solution.

Grafting was done near sunset on the 12th day after planting, when the first trifoliate leaf was emerging on most seedlings. The complementary reciprocal grafts were performed as one operation to reduce drying of cut stem ends. Amber Tygon tubing slit

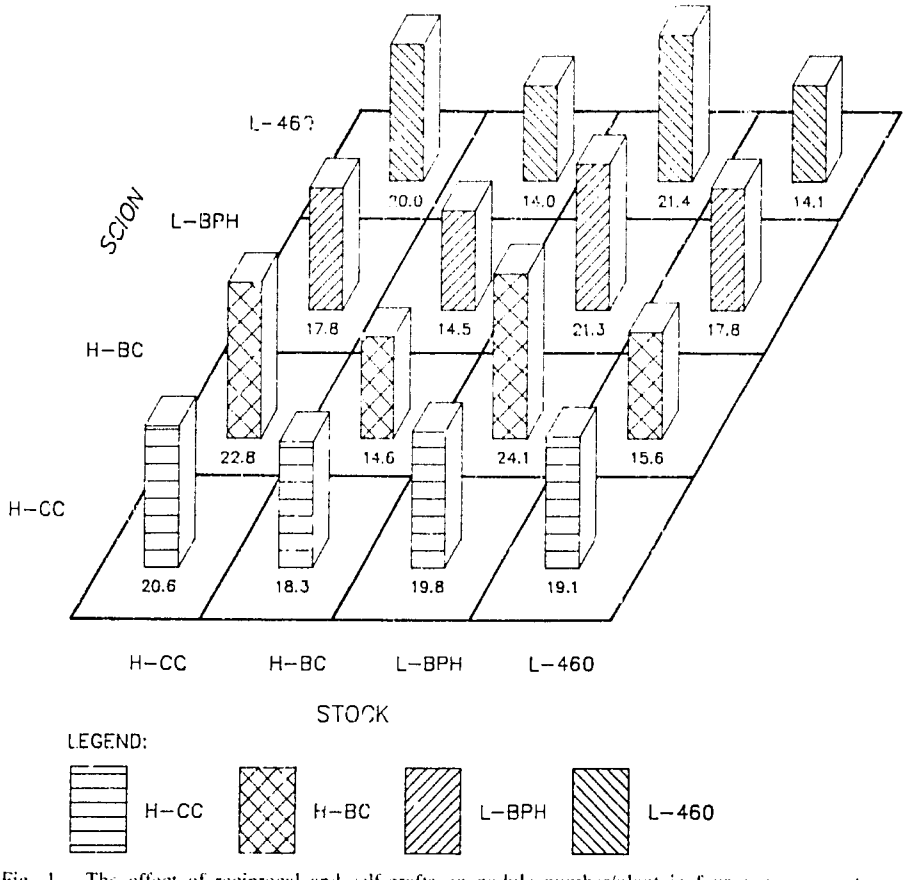

Fig. 1.—The effect of reciprocal and self-grafts on nodule number/plant in four cowpea genotypes differing in N_2 fixation efficiency, where H = high and L = low. H-CC = 'Calico Crowder', H-BC = 'Brown Crowder', L-BPH = 'Bush Purple Hull', L-460 = 'TX460'. LSD (0.05) = 5.51 for comparison of any two treatment means.

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Table 1. The effects of root stock, scion, and root stock × scion on N_2 fixation variables in cowpea.

Effects	df	Nodule (no./plant) Type I SS[y]	PR > F	Nodule (wt./plant) Type I SS	PR > F	Nitrogenase activity[z] Type I SS	PR > F	Top dry wt. Type I SS	PR > F
Root stock	3	908.41	0.001	0.00976	0.13	24.66	0.0001	0.0075	0.93
Scion	3	121.90	0.37	0.0117	0.08	10.79	0.02	0.101	0.15
Root stock × scion	9	257.82	0.66	0.0177	0.33	17.04	0.07	0.28	0.10

[z]μmol C_2H_4/plant per hr.
[y]Type I sums of squares.

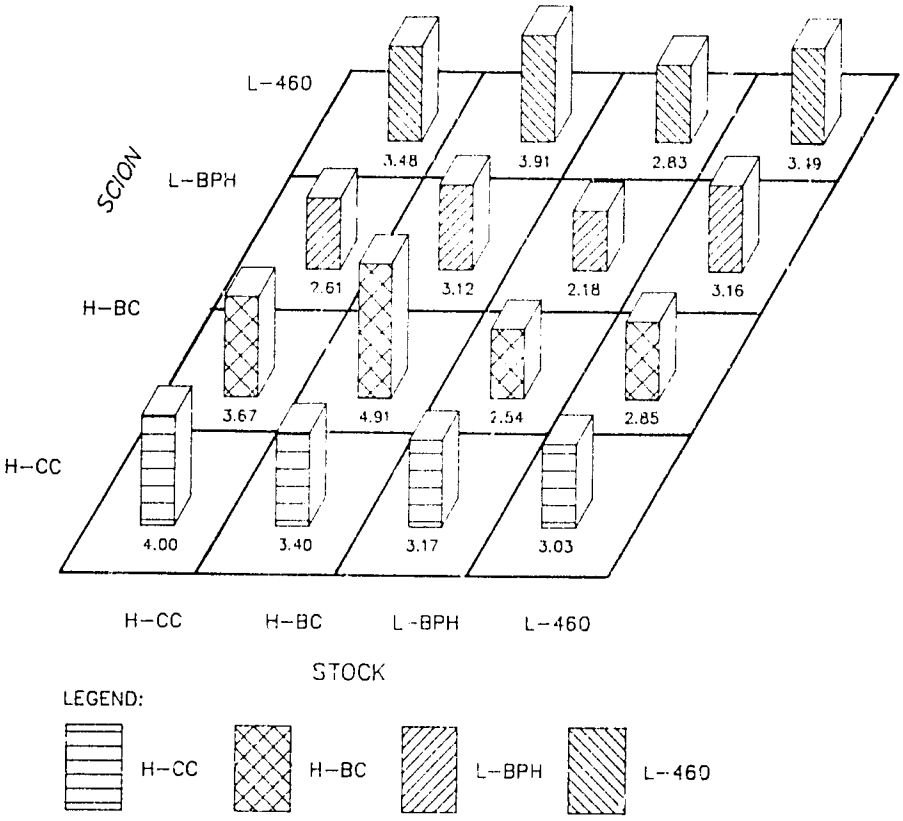

Fig. 2.—The effect of reciprocal and self-grafts on nitrogenase activity (μmol C_2H_4/plant per hr) in four cowpea genotypes differing in N_2 fixation efficiency, where H = high and L = low. H-CC = 'Calico Crowder', H-BC = 'Brwon Crowder', L-BPH = 'Bush Purple Hull', L-460 = 'TX460'. LSD (0.05) = 0.98 for comparison of any two treatment means.

lengthwise was used to hold graft unions in place. All leaves except one simple leaf were removed from each stem. Following grafting, the pots were placed in a polyethylene humidity tent until the graft unions had healed and tubing was removed, 13 days after grafting. Because preliminary studies had indicated that grafting retarded plant growth, the ungrafted controls were planted 10 days later.

Ten replications were arranged in a completely randomized design and analyzed as a 4 × 4 factorial. Grafts in all combinations and self-grafts were the four treatments of the second factor. After 38 days, plants were decapitated and the roots removed carefully from the growing medium. The following N_2 fixation variables were measured: nitrogenase activity (μmol C_2H_4/plant per hr) of the nodules by C_2H_2–C_2H_4 reduction assay (15), nodule number/plant, nodule weight/plant, and shoot dry weight/plant. The significant levels of rootstock, scion, and the interaction effects from the analysis of variance were used to determine whether rootstock, scion, or the specific combination was the major factor in controlling N_2 fixation variables.

Relative N_2 fixation efficiency of the four genotypes was confirmed based on nitrogenase activity of the self-grafts and the ungrafted controls, which did not differ. Results of the statistical analysis are presented in Table 1. Nodule number/plant was influenced by the rootstock only. The effects of scion or rootstock on nodule weight and top dry weight were not significant at the 5% level. However, nitrogenase activity was influenced greatly by both stock and scion and, to a lesser extent, by the interaction between stock and scion.

The effect of rootstock and scion on nodule number/plant is illustrated in Fig. 1. 'H-Calico Crowder' and 'L-Bush Purple Hull' produced more nodules than 'H-Brown Crowder' and 'L-TX460' in the self-grafts. Thus, the number of nodules per plant was increased in 'H-Brown Crowder' and 'L-TX460' by grafting them to rootstocks of 'H-Calico Crowder' or 'L-Bush Purple Hull'. However, these combinations reduced nitrogenase activity (Fig. 2), indicating that nodule number and nitrogenase activity might be negatively correlated or simply not related.

The self grafts of 'H-Calico Crowder' and 'H-Brown Crowder' expressed more nitrogenase activity than did the self-grafts of 'L-Bush Purple Hull' and 'L-TX460' or any other combination of grafts (Fig. 2). The nitrogenase activity of 'L-Bush Purple Hull' was improved by grafting with the other three lines as either scion or stock, suggesting that use of rootstocks with high N_2 fixation genes can increase the N_2 fixing potential of the low-fixing 'L-Bush Purple Hull'. Stock or scion did not affect top dry weight (Table 1); however, top dry weight per plant harvested at full flower is not a good indicator of N_2 fixation potential (4).

In conclusion, rootstock was found to be more important than scion in influencing N_2 fixation variables in cowpea, as has been indicated in soybean (1, 7). However, whether rootstock or scion is the limiting factor may be cultivar-specific. We have shown that nitrogenase activity of low-fixing lines can be increased through grafting. Field studies are now needed to determine whether yield of low-fixing lines can be increased by grafting onto high-fixing rootstocks; i.e., will the use of rootstocks with high-fixing genes per se improve yield?

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