CP057

1255

Transgressive segregation was observed for all the characteristics studied among the 16 lines of F_3 plants grown in the field. The ranges in values of the characters studied for the parents and the F plants are shown in Table 1. Most of the segregates flowered 2 days earlier than their parents except line 9, which took 40 days to flower, as did 'Bossier'. Lines 4, 6, 9, and 11 were shorter in stature than 'Bossier', and all of them had lower seed yields than their parents. Among them, lines 9 and 11 had less nodulation (nodule mass between 2 and 3 mg/plant) and low nitrogenase activity (2.2 µ mol C_2H_4 produced/plant per h), while line 6 showed extensive nodulation (191 mg nodule tissue/plant) and moderate to high nitrogenase activity (7.32 µ mol C_2H_4 produced/plant per h). Two lines (Nos. 3 and 16) showed both high yield and good N_2 fixation (see Table 1).

Given the promising nature of these results additional crosses between cvs. Bossier and IH 92 have been made and are being advanced to the F_3 generation for detailed testing of both agronomic traits and ability to fix N_2. With the high cost of fertilizer N to countries such as Tanzania, it is imperative that future soybean breeding programs consider BNF as a prime criterion in the development and selection of new cultivars.

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GENETIC MANIPULATION OF NODULATION IN GROUNDNUT

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Summary

There is large variation among cultivars of groundnut in ability to nodulate and fix nitrogen (N), and in seasonal and diurnal patterns of nitrogenase activity. Total N uptake, or total dry matter production, may be a useful index in ranking cultivars for N_2-fixing ability.

Certain cultivars of *Arachis hypogaea* ssp. *hypogaea* var. *hypogaea* formed up to 13% of their total nodule number on the hypocotyl, and some cultivars even nodulated on the stem above the crown of the plant. In contrast, cultivars from *A. hypogaea* ssp. *fastigiata* var. *fastigiata* and var. *vulgaris* formed few nodules on the hypocotyl. Non-nodulating plants have been observed in 13 crosses. Genetic analysis indicates that two independent recessive genes are involved. Some progeny of these crosses also form a few big nodules, a trait which seems to be controlled by the host plant.

INTRODUCTION

The number of nodules formed by legumes, and their effectiveness, is governed by both plant and *Rhizobium* genes, with large differences in nodulation and nitrogen (N_2) fixation already reported for cultivars of soybean (Vorhees, 1915), peas (Holl & LaRue, 1976) cowpea (Zary, *et al.*, 1978) and groundnut (Wynne, Elkan & Schneeweis, 1980; Nambiar & Dart, 1980). It may be possible to increase N_2 fixation in legumes by genetic manipulation. This paper summarizes our efforts to establish genotypic differences in N_2 fixation among groundnut cultivars and understand the genetics of non-nodulation in groundnut.

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BOTANICAL VARIATION IN *ARACHIS HYPOGAEA* L.

The genus *Arachis* belongs to the tribe Aeschynomeneae (Leguminosae, subtribe, Papilionoideae) with 22 described and possibly 40 undescribed species (Gregory, Krapovickas & Gregory, 1980). There is a great diversity within the genus. For example plant type varies from upright forms to prostrate types with runners, and the growing period required extends from short-duration annual to perennial. The cultivated groundnut, *A. hypogaea,* is an annual tetraploid (4n = 40). Based on morphology, the species is subdivided into subspecies *hypogaea,* which includes var. *hypogaea,* and var. *hirsuta,* and subspecies *fastigiata* which includes var. *fastigiata* and var. *vulgaris* (Krapovickas, 1973). The *hypogaea* ssp. includes long-duration, alternately branched "Virginia types," mostly with runner and spreading bunch growth habits while the *fastigiata* ssp. includes short-duration, sequentially branched types mainly with an upright branch habit, the "Spanish" and "Valencia types."

The cultivated groundnut is grown throughout the tropics and subtropics between latitudes 40° N and 40° S, where rainfall during the growing season exceeds 500 mm. The crop grows best in well-drained, sandy loams, and tolerates air temperatures between 20° C and 40° C. The crop duration varies with the location and season of cultivation — the Spanish and Valencia types normally mature 90 to 110 days after planting, while the Virginia types mature 130 to 150 days after planting.

VARIATION IN N_2 FIXATION AMONG GROUNDNUT CULTIVARS

N_2 fixation in groundnut is closely related to photosynthesis (Nambiar & Dart, 1980). Figure 1 shows seasonal variation in N_2 fixation among nine groundnut cultivars. In general, the Virginia types ('Florunner', MK 374, 'Florigiant', M 13, and 'Kadiri 71-1') formed more nodules and fixed more N_2 than the Valencia ('Ganpapuri', MH 2, and PI 59747) and Spanish (Ah 8189) types. A similar trend was observed in field experiments in North Carolina by Wynne *et al.* (1980). In all the cultivars, N_2 fixation started at around 25-30 days after planting with significant genotypic differences apparent by 30 days, and with no interaction between sampling date and cultivar ranking until after 50 days, a time that generally corresponds to early pod filling. Cultivar differences in pod filling and partitioning also become evident at this growth stage (McCloud *et al.*, 1980), and in turn affect energy supply to the nodules. This may explain the cultivar x sampling-time interaction for nitrogenase activity per plant that could be observed from the fourth sampling on. In most cultivars nodule senescence started during this stage of plant growth, along with a decline in N_2 fixation per plant.

Fifty-two selected germplasm lines were screened over three seasons (1977-1979) for nodulation and N_2 fixation using the acetylene reduction assay

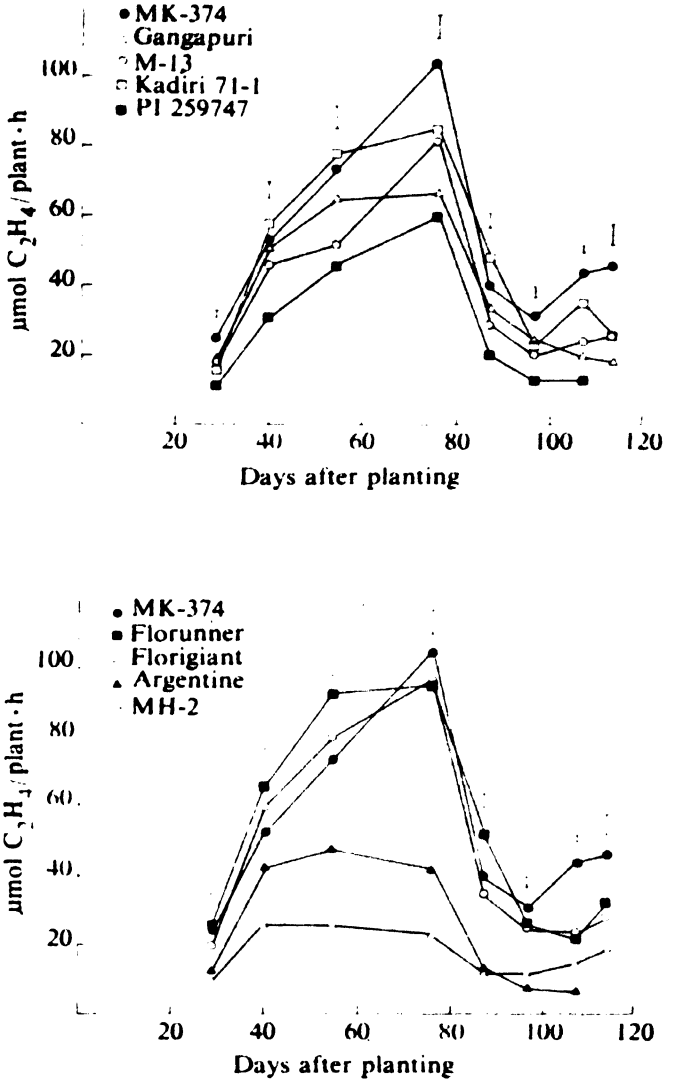

Figure 1.
Nitrogenase activity per plant of selected groundnut cultivars during the post-rainy season, 1980. Virginia cv. MK 374 is presented in both figures for comparison.

(Dart, Day & Harris, 1972). Plants were sampled 20-25 days after flowering. The data was analyzed by the Scott-Knot method (Gates & Bilbro, 1978) and the clusters formed were grouped as high, medium, or low in BNF traits (see Table 1). Three- to five-fold differences in nodulation and N_2 (C_2H_2) reduction were observed among these groundnut genotypes. A Virginia type, NC Ac 2821, ranked high over three tests and a Valencia type, NC Ac 2654, ranked high during two tests, while a Spanish type Ah 3275, was clustered as low during four tests. Crosses have been made between the cultivars of the high and low groups in order to estimate the heritability of N_2 fixation in groundnuts. Wyne *et al.* (1980) reported high heritability for such traits in 30 F_4 lines derived from a cross between cv. 922 (Spanish) and cv. NC 6 (Virginia) in fields at Raleigh. However they observed that correlations between parental and general combining ability effects for N_2 fixation were nonsignificant for progenies of a diallel cross grown in the glasshouse, indicating that simple evaluation of lines for capacity to fix N_2 may not identify superior parents for use in breeding programs (Isleib *et al.*, 1980).

TABLE 1: Variation in the nodulation and N_2 (C_2H_2) fixation of groundnut cultivars over three seasons of testing.

Cultivar	ICG No	Botanical type	1977-1978				1978		1978-1979[1]	
			1st sampling		2nd sampling					
			Nodulation	N_2ase	Nodulation	N_2ase	Nodulation	N_2ase	Nodulation	N_2ase
Ah 3277	1218	Spanish	L[2]	L	L	L	L	L	M	M
Ah 3275	1216	Spanish	L	L	L	L	L	L	L	L
No. 421	3158	Valencia	L	L	L	L	–	–	–	–
9	1161	Spanish	L	L	M	H	L	L	–	–
144	1235	Spanish	L	L	M	M	M	–	M	M
NC Ac 888	359	Spanish	L	L	L	L	M	L	L	L
Ah 61	1173	Spanish	L	L	L	M	L	M	–	–
Ah 3272	1213	Spanish	L	L	L	M	L	M	–	–
No. 3527	1524	Spanish	L	–	L	M	L	–	–	–
Faizpur-1-5	1102	Spanish	L	M	L	L	M	M	–	–
No. 418	1500,2202	Spanish	L	L	L	M	–	–	–	–
NC Ac 1337	358	Valencia	L	L	M	M	M	L	–	–
NC Ac 516	279	Valencia	L	–	M	M	L	–	L	L
NC Ac 945	366	Valencia	L	L	M	M	M	–	–	–
NC Ac 699	1630	Spanish	L	L	L	M	L	M	–	–
148-7-4-3-12-B	1573	Spanish	L	–	L	M	–	–	–	–
No. 1780	1508	Spanish	L	L	M	L	–	–	–	–
NC Ac 738	331	Valencia	L	–	M	M	M	–	–	–
TG 17	2976	Spanish	L	L	L	M	L	L	L	L
No. 3270	1489	Spanish	L	L	L	L	–	–	L	M
NC Ac 51	263	Valencia	L	M	L	L	L	–	–	–
TG 8	95	Valencia	L	L	M	M	L	L	–	–
Ah 42	1163	Valencia	L	–	M	L	M	–	–	–
NC Ac 2651	402	Spanish	L	L	L	M	M	–	–	–
NC Ac 1002	380	Valencia	L	–	M	M	M	–	–	–
NC Ac 524	283	Valencia	L	M	M	M	M	M	–	–
GAUG 1	-	Spanish	L	-	M	M	L	L	–	–
NC Ac 2734	420	Valencia	L	L	M	M	M	L	–	–
NC Ac 495	1623	Spanish	M	M	L	M	L	L	L	L
Spancross	3472	Spanish	M	–	M	M	M	L	–	–
NC Ac 1286	389	Valencia	M	L	M	M	M	L	–	–
NC Ac 17149	475	Valencia	M	L	M	M	M	M	–	–
Ah 1069	1196	Spanish	M	M	M	M	L	L	–	–
Kadiri 71-1		Virginia	M	M	M	M	L	L	–	–
79	2983	Spanish	M	–	M	M	–	–	–	–
2600	400	Virginia	M	L	M	M	L	M	L	L
	154	Spanish	M	M	M	M	L	L	–	–
	3375	Spanish	M	M	M	M	L	L	L	M
NC Ac 1303	393	Spanish	M	L	M	M	M	M	M	L
NC Ac 975	376	Valencia	M	M	M	M	M	M	–	–
Sm-S	2956	Spanish	M	M	M	M	L	L	–	–
Argentina	3150	Spanish	M	M	M	L	L	L	L	L
Tifspan	3495	Spanish	M	M	M	L	L	L	–	–
Robut 33-1	799	Virginia	M	M	M	M	M	–	L	M
Pollachi 1	127	Spanish	M	L	M	M	M	M	L	M
NC Ac 17113	1699	Spanish	M	M	M	M	M	M	–	–
Ah 8254	2962	Spanish	M	M	M	M	M	L	M	M
Ah 7436	1547	Spanish	M	M	M	M	M	–	–	–
NC Ac 490	274	Valencia	M	M	M	M	M	M	H	M
X-14-4-B-19-B	1561	Spanish	H	M	M	M	M	–	–	–
NC Ac 2821	2405	Virginia	H	M	H	M	M	M	H	H
NC Ac 2654	404	Valencia	H	M	M	H	M	–	M	M

[1]The three seasons for testing were post-rainy season, 1977-1978; rainy season 1978, and post-rainy season, 1978-1979.
[2]L = low; M = medium; and H = high.

Groundnut shows a marked diurnal periodicity in C_2H_2 reduction (Nambiar & Dart, 1980). A preliminary survey of nitrogenase activity in 14 groundnut lines selected for differences in foliage production, showed a significant interaction between lines and time of measurement of nitrogenase activity. This suggests that, if cultivars with less diurnal variability in N_2 fixation can be found, they may have larger overall daily fixation.

There are difficulties in relating the nodulation and N_2 fixation scores of groundnut lines obtained from sampling at a particular stage of the growth cycle to their overall seasonal activity. Moreover, such methods are destructive and, hence, not useful for examining early generation populations in a breeding program. An alternate method is to use the total N uptake of the crop at harvest as an indication of N_2 fixation. Nitrogenase activity through the season and total N_2 uptake by two cultivars grown at ICRISAT are shown in Figure 2 and Table 2. Cv. Kadiri 71-1, a Virginia runner, nodulated better and fixed more N_2 than the dwarf Valencia-type cv. MH 2. The differences in N_2 fixation rates are not reflected in the pod yield, but are evident in the total dry matter produced, and total N harvested.

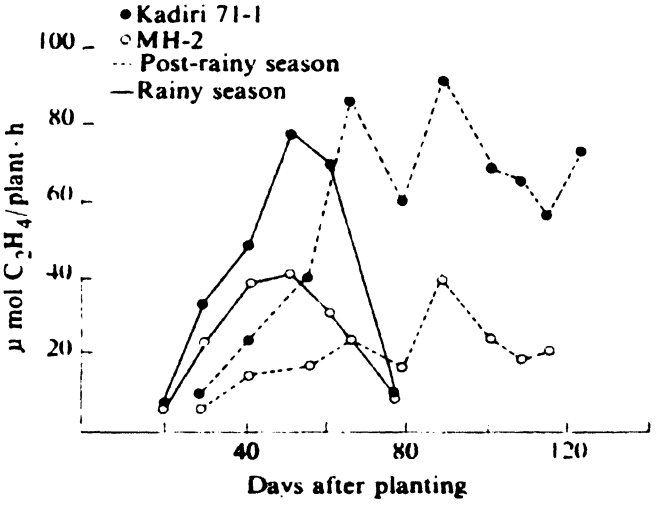

Figure 2. Nitrogenase activity per plant of the cv. Kadiri 71-1 and cv. MH 2 during the post-rainy and the 1980 rainy seasons.

TABLE 2: Dry matter production by cvs. Kadiri 71-1 and MH 2 during post-rainy season.

Cultivars	Pod weight (kg/ha)	Top weight (kg/ha)	Total dry matter (kg/ha·day)	Harvest N index (kg seed/kg tops)
Kadiri 71-1	2426	4103	43	0.37
MH 2	1833	1041	24	0.64

HYPOCOTYL AND STEM NODULATION

Host genotype and *Rhizobium* strain have been shown to influence the distribution of nodules in legumes (Caldwell, 1966). In groundnut, cultivars belonging to the botanical variety *hypogaea* form more nodules in the hypocotyl region than do those from var. *fastigiata* and var. *vulgaris*. Nodules on the hypocotyl of var. *hypogaea* accounted for 13.4% of the total number of nodules per plant while in the other botanical groups they only accounted for 0.5-1.0%. These hypocotyl nodules form 40-60 days after planting and only develop when the soil around the hypocotyl is moist. Some cultivars such as cv. MK 374 (var. *hypogaea*) also nodulate on the stem.

ing the pod filling stage the nodules on the hypocotyl and stem remained pink while many nodules on the roots turned green, indicating senescence. Selecting for these traits in breeding material might increase N_2 fixation during the pod filling and maturation stages.

GENETICS OF NODULATION

Host plants unable to form nodules have been observed in soybeans and peas (Williams & Lynch, 1954; Holl & LaRue, 1976). Recently Gorbert & Burton (1979) reported non-nodulating lines of *Arachis hypogaea* in the progenies of a cross 487A-1-1-2 x PI 262090. Non-nodulating groundnut lines have also been reported from Georgia (R.O. Hammons, personal communication). During the 1978 rainy season, F_2 progenies from three crosses in the rust screening nursery at ICRISAT segregated for non-nodulation. All the parents of the crosses nodulated normally. Later, during the rainy season 1979, non-nodulating lines were found in 10 additional crosses (see Table 3).

Genetic analysis for nodulation vs. non-nodulation showed that a pair of independent, recessive genes control non-nodulation (Nigam *et al.*, 1980). It

nteresting to note that one of the parents in most of the crosses is a rust-resistant Valencia cultivar — either cv. PI 259747, cv. NC Ac 17090, or cv. EC 76446 (292). Any of these parents crossed with cultivars NC 17, Shantung Ku No. 203, or NC Ac 2731, always segregated for non-nodulation, but cv. PI 259747 crossed with cv. NC Ac 17090 or cv. EC 76446 (292) did not produce non-nodulating plants in the F_2 generation, nor did cv. NC Ac 2731 when crossed with cv. Shantung Ku No. 203. This indicates that one set of genes for non-nodulation is present in cvs. PI 259747, NC Ac 17090, and EC 76447 (292) and another set in cvs. NC 17, Santung Ku No. 203 and NC Ac 2731.

Some nodulating segregants formed only a few nodules, which were much larger than those formed by either parents or the normally nodulating F_2 plants. This character is not stable genetically. For example, a plant with three big nodules in the F_5 generation segregated in the F_6 generation into normal nodulating, non-nodulating and "big nodule" types. *Rhizobium* isolates from

TABLE 3: Crosses producing progeny that fail to nodulate.

1.	Shantung Ku No. 203 x NC Ac 17142
2.	NC Ac 2731 x NC Ac 17090
3.	NC Ac 2731 x EC 76446 (292)
4.	NC Ac 2768 x NC Ac 17090
5.	NC 17 x NC Ac 17090
6.	Shantung Ku No. 203 x NC Ac 17090
7.	Shantung Ku No. 203 x EC 76446 (292)
8.	Shantung Ku No. 203 x PI 259747
9.	NC 17 x EC 76446 (292)
10.	NC-Fla-14 x NC Ac 17090
11.	RS-114 x NC Ac 17090
12.	NC 17 x PI 259747
13.	NC Ac 2731 x PI 259747

the big nodule type formed normal nodules on the parent plants under sterile conditions. Moreover, big nodule segregants were observed in F_2 populations grown in controlled conditions and inoculated with a single strain that forms normal nodules on the parents. These observations indicate that the big nodule trait is essentially a plant character.

We are using the non-nodulating groundnut lines in experiments to measure N fixation by groundnut, with the N uptake by the non-nodulated plants providing an estimate of the mineral N uptake by the nodulated plants.

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