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EFFICIENCY OF NITROGENASE ACTIVITY AND NODULE MASS IN PREDICTING THE RELATIVE PERFORMANCE OF GENOTYPES ASSESSED BY A NUMBER OF CHARACTERS IN GROUNDNUT (*ARACHIS HYPOGAEA*)†

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SUMMARY

Twenty one genotypes belonging to Spanish, Valencia and Virginia groups of groundnut were studied at Patancheru and a further seventeen at Delhi. Their relative performance based on a large number of characters spanning the entire growth phase was assessed. Nitrogenase activity and nodule mass were measured at three stages of crop growth starting from first flowering, and these two characters alone when measured at 30 days after flowering closely matched the measure of relative performance based on the entire set of characters.

Biological nitrogen fixation has an important bearing on the process of yield improvement of legumes. The variation in characters such as nitrogenase activity and nodule mass, which are indicative of nitrogen fixation, is large enough in the leguminous crops so far examined to make breeding for enhanced nitrogen fixation worthwhile (Zary *et al.*, 1978; Sinclair *et al.*, 1980; Nambiar and Dart, 1980; Wynne *et al.*, 1981). However, the acetylene reduction assay for nitrogenase activity measures only current activity which is influenced by environmental factors such as diurnal fluctuations, soil moisture and temperature (Nambiar and Dart, 1980). A stable and significant association of single assays of nitrogenase activity with direct yield components which are also influenced by environmental factors would seem unlikely. Thus identification of plant attributes, selection for which will result in an associated improvement in nitrogenase activity, may not be easy. In this paper we compare the efficiency of two characters, nodule mass and nitrogenase activity, measured at three stages of growth, with other plant phenotypic traits in differentiating groundnut genotypes belonging to various botanical groups: Spanish (SB), *Arachis hypogaea*

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subsp. *fastigiata* var. *vulgaris*; Valencia (VL), *A. hypogaea* subsp. *fastigiata* var *fastigiata*; Virginia Bunch (VB) and Virginia Runner (VR), *A. hypogaea* subsp *hypogaea* var. *hypogaea*.

MATERIALS AND METHODS

Twenty one genotypes, five each from SB, VL and VR and six from VB, were grown at ICRISAT, Patancheru and a further seventeen at the Indian Agricultural Research Institute, Delhi during the rainy season, 1980. They were planted in a completely randomized block design with four replications at Patancheru and two at Delhi. Each replication consisted of a single row 10 m in length, spaced 75 cm apart at Patancheru and five rows each 6 m in length, spaced 60 m apart at Delhi. The inter-plant distance was 20 and 30 cm respectively at Patancheru and Delhi.

The crop was sown without inoculation in a sandy loam during the second week of June, 1980 at Delhi and in an Alfisol during the first week of July, 1980 at Patancheru. A basal dose of 20 kg P_2O_5 per ha was applied as triple superphosphate at Patancheru and 20 kg N as urea and 40 kg P_2O_5 per ha as triple superphosphate at Delhi. The crop received irrigation whenever there was moisture stress and regular plant protection measures as needed.

Three growth stages were identified: S1, first flowering; S2 and S3, 20 and 40 days after S1 for SB and VL, and 30 and 60 days after S1 for VB and VR.

Nitrogenase activity was measured only at Patancheru by the acetylene reduction technique. Destructive sampling of eight representative plants from each replication was done at each physiological stage. The roots were placed in two 800 ml bottles, each containing the roots of four plants, which were then made air-tight. Eighty ml of acetylene were injected into each of them after evacuating an equal volume of air. After half an hour, two samples of 5 ml gas were collected from each bottle in Vacutainers. Nitrogenase activity was estimated by the procedure described by Dart *et al.* (1972).

Data on a number of characters, as shown in Table 1, at each stage were recorded both at Patancheru and Delhi. At Patancheru, these were based on eight plants per replication; yield and its components were recorded only at Delhi. Measurements were made on a random sample of five plants per replicate at the stage S1 and at final harvest and on three plants per replicate at S2 and S3 at Delhi. Physiological characters were recorded following standard procedures described by Kvet *et al.* (1971).

The phenotypic value for each character is the result of quantitative effects of interacting genes controlling the expression of each character in the particular environment. Furthermore, the characters are sequentially dependent. For instance, the seedling phase influences the flowering phase, which in turn influences the post-flowering and reproductive phases. Thus final yield alone cannot reflect the performance of a genotype with respect to characters in the seedling, flowering and post-flowering phases. Hence it was decided to compute

a score for each genotype taking into account the variation for different characters in these phases. On the basis of this score a High (H) or Low (L) status of performance was assigned to each genotype. For each character the following steps were involved in the process: (i) A standard error of the mean (s) was obtained from the error m.s. of the analysis of variance; (ii) The mean value (m) over all the genotypes was calculated and (iii) Genotypes which had values for the character greater than or equal to m + s were allotted a score +1, those which had values less than m + s but greater than or equal to m − s, a score zero, and those which had values below m − s, a score −1. This process thus ensured a score for each character for each genotype.

The scores obtained by a genotype for every character were assumed to be of equal importance and were added up to provide its total score. The mean (M) of the total scores obtained by the genotypes was found. Those genotypes which had a total score greater than or equal to M were allotted a high and the rest a low status of performance as assessed by these characters. The performance thus measured is only a 'relative performance' - relative to the specific genotypes and characters chosen. For brevity, the term relative performance is used with this meaning in the rest of the paper.

It may be noted that the method is general enough to permit assignment of a high or low status of relative performance based on a subset of the total number of characters measured. For example, a status can be assigned on the values of nitrogenase activity in the three stages, S1, S2 and S3 alone. For this purpose, the scores given to this character at each stage S1, S2 and S3 would be added up to provide a final score for each genotype. The mean value (M) of these scores would then be computed. Those genotypes whose scores were greater than or equal to M would be allotted a high and the rest a low status of relative performance based on these characters. It is easy to extend this approach to assign a status based on the values of nodule mass at S1, S2 and S3 or for that matter, based on six characters, nitrogenase activity measured at S1, S2, S3 and nodule mass measured at S1, S2, S3.

The relative performance of each genotype based on a chosen subset of characters was then compared with the relative performance based on all the characters. The proportion of genotypes in which the relative performance given by the subset of characters agreed with that given by the total number of characters was then computed for each of the four groups, SB, VL, VB, VR and over all the groups. This proportion was taken to indicate the efficiency of the subset of characters in predicting the relative performance of genotypes as assessed by all the characters.

RESULTS

The analysis of variance indicated significant differences ($P < 0.05$) among the genotypes for all characters including nitrogenase activity and nodule mass. The range of variability for the characters measured at various stages (Table 1) was

Table 1. *Mean (m) and standard error (s.e.) observed for various characters of groundnut measured at Patancheru and New Delhi*

		Patancheru		New Delhi	
	Character†	m	s.e.	m	s.e.
Stage 1‡	Seedling height (cm)	11.0	0.65	15.4	2.17
	Shoot weight (g)	24.5	2.22	10.5	2.79
	Root weight (g)	1.6	0.09	0.4	0.08
	Nodule weight (mg)	426.0	59.00	89.0	19.00
	Shoot:root weight ratio	15.5	0.97	20.7	1.80
	Nitrogenase activity (μmoles C_2H_4 plant^{-1} h^{-1})	48.4	4.74	–	–
Stage 2	Number of primary branches	5.9	0.32	8.8	1.13
	Number of secondary branches	7.1	1.31	17.7	3.43
	Shoot weight (g)	62.4	8.91	85.8	11.60
	Root weight (g)	3.5	0.39	1.9	0.26
	Nodule weight (mg)	941.0	114.00	350.0	72.00
	RGR (Shoot weight)	0.04	0.004	0.07	0.01
	Nitrogenase activity (μmoles C_2H_4 plant^{-1} h^{-1})	31.9	6.16	–	–
Stage 3	Shoot weight (g)	79.3	10.30	176.2	24.58
	Root weight (g)	3.2	0.26	2.4	0.20
	Nodule weight (mg)	719.0	94.00	360.0	67.00
	Nitrogenase activity (μmoles C_2H_4 plant^{-1} h^{-1})	8.6	1.71	–	–
Harvest	Number of mature pods	–	–	61.6	7.68
	Mature pod weight (g)	–	–	50.8	9.61
	Kernel weight (g)	–	–	25.9	6.96
	100-kernel weight (g)	–	–	38.3	4.64
	Shelling (%)	–	–	50.4	6.92

† All values given per plant.
‡ Stage 1 = first flowering; Stage 2 = 20 days after first flowering for SB and VL, 30 days for VB and VR and Stage 3 = 40 days after first flowering for SB and VL, 60 days for VB and VR.

enough to permit grouping of cultivars into those of high and low relative performance at both the locations. The relative performance of the cultivars of SB and VL groups was low while that of VB and VR was high (Table 2).

At Patancheru, the relative performance judged by 17 characters agreed with that given by nitrogenase activity over the three stages for 18 out of 21 genotypes (Table 3). With nodule mass over the three stages, the agreement was 19 out of 21. The relative performance based on nitrogenase activity and nodule mass measured at each of the stages S1, S2 and S3 agreed with the performance given by 17 characters for 19 out of 21 genotypes. This was slightly higher than the agreement based on nitrogenase activity alone. The performance indicated by nitrogenase activity alone at stages S1, S2 and S3 agreed with the performance given by 17 characters at Patancheru for 12, 17 and 15 out of 21 genotypes, respectively. The performance indicated by nitrogenase activity or nodule mass at stage S2 was close to that indicated by them over all the three stages (Table 3), but nitrogenase activity measured at stage S1 only agreed in 12 of the 21 comparisons.

Table 2 *Groundnut cultivars tested at Patancheru, relative performance based on all 17 characters and on nitrogenase activity and nodule mass*

Cultivar	Relative performance† based on		
	Nodule mass	Nitrogenase activity	17 characters
SB			
Argentine‡	L	L	L
TG 16	L	L	L
Gaug 1	L	L	L
Chico	L	L	L
Pol. 2	L	L	L
VL			
NC Ac 17090	H	H	L
EC 76446 (292)	L	L	L
PI 259747	L	L	L
Gangapuri	L	L	L
MH 2	L	L	L
VB			
NC Ac 1107	L	L	H
PI 298115	H	H	H
Makulu Red‡	H	L	H
Robut 33 1	H	H	H
28-206	H	H	H
MK 374	H	H	H
VR			
M13	L	H	L
Chalimbana‡	H	H	H
Kadiri 71 1‡	H	H	H
NC Ac 2731	H	H	H
Florunner	H	H	H

† H denotes high and L low relative performance
‡ Cultivars not grown at Delhi.

Table 3 *Number of groundnut genotypes in which performance as indicated by nitrogenase activity or nodule mass agreed with relative performance as determined by 17 characters at Patancheru*

Groundnut group	No of genotypes	Nitrogenase activity				Nodule mass				Nitrogenase activity and nodule mass
		S1	S2	S3	Over all stages	S1	S2	S3	Over all stages	
SB	5	3	4	5	5	5	5	5	5	5
VL	5	2	4	4	4	5	5	4	4	4
VB	6	2	5	3	4	4	5	5	5	5
VR	5	5	4	3	5	5	4	3	5	5
Total	21	12	17	15	18	19	19	17	19	19

Table 4. *Number of groundnut genotypes in which performance indicated by nitrogenase activity or nodule mass agreed with relative performance as determined by 19 characters at Delhi*

Ground-nut group	No. of geno-types	Nitrogenase activity				Nodule mass				Nitrogenase activity and nodule mass
		S1	S2	S3	Over all stages	S1	S2	S3	Over all stages	
SB	4	1	2	3	3	3	3	3	3	3
VL	5	2	4	4	4	5	5	4	4	4
VB	5	2	4	3	4	3	4	4	4	4
VR	3	3	3	2	3	2	3	2	2	3
Total	17	8	13	12	14	13	15	13	13	14

At Delhi, four of the genotypes evaluated at Patancheru were not included, and nitrogenase activity was not measured. The performance of 14 of the 17 genotypes based on nitrogenase activity and of 13 based on nodule mass measured at Patancheru agreed with that based on the range of characters measured at Delhi, while the agreement was 14 out of 17 when both characters were considered (Table 4). This agreement was as good as that obtained at Patancheru. Furthermore, the performance based on nitrogenase activity and nodule mass together at any one stage agreed well with the relative performance based on all 17 characters. Nodule mass measured at stage S2 provided as good an agreement with overall performance at Delhi as either the nitrogenase activity or nodule mass observed over the three stages. In addition, the performance based on shoot weights measured at stages S1, S2 and S3 agreed well with the performance based on all 17 characters (16 out of 17 at Delhi and 21 out of 21 at Patancheru).

DISCUSSION

The results showed that the relative performance of genotypes based on nitrogenase activity and nodule mass alone agreed closely with that based on a number of characters measured from seedling to harvest stage. Moreover, the performance of genotypes based on measurements of these characters at Patancheru agreed well with that based on measurements of 19 characters at Delhi. There is thus a clear indication that nitrogenase activity and nodule mass have good predictive value for the relative performance of genotypes based on a whole range of plant characters associated with the growth and yield of groundnuts.

Genetic variation in nitrogenase activity and nodulation within and between sub-groups has been reported before for groundnut (Burton, 1976; Elkan *et al.*, 1980; Nambiar and Dart, 1980). The observation from this study that Spanish and Valencia genotypes had low and Virginia high performance based on nodule mass and nitrogenase activity agrees with the earlier studies of Wynne *et al.* (1978) and Elkan *et al.* (1980), though they used a different set of groundnut genotypes.

The relative performance based on nitrogenase activity measured at stage S1

alone agreed with that based on 17 characters for only 12 out of 21 genotypes; however, the performance based on both nitrogenase activity and nodule mass at stage S1 improved the agreement to 17 out of 21. But measurements of these two characters at stage S2, 20-30 days after flowering, gave the best agreement of 19 out of 21, showing they would be adequate for ranking the performance of groundnut genotypes at this stage. The fresh weight of nodules in groundnut is reported to increase up to 72 days after sowing (Ayala, 1977) - which broadly corresponds with stage S2 of this study - and then to decrease. This is the period when competition for available photosynthate by developing pods in soyabean begins, resulting in a gradual decrease of nitrogenase activity from then on (Lawn and Brun, 1974; Hardy and Havelka, 1976). These observations suggest that nitrogenase activity and nodule mass, along with shoot dry weight measured at stage S2, would accurately predict the relative performance of groundnut genotypes as defined by a large number of characters from seedling to harvest.

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