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IDENTIFICATION OF NON-NODULATING, AND LOW AND HIGH NODULATING PLANTS IN PIGEONPEA

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Summary—A glasshouse screening program was initiated to identify non-nodulating (Nod^-) plants in pigeonpea (*Cajanus cajan* L. Millsp.). A visual rating scale of 1 (minimum nodulation) to 5 (maximum nodulation) was developed that allowed rapid screening of large numbers of plants. The scale correlated significantly ($r = 0.89$–0.94, $P < 0.001$, $n = 24$) with nodule number and nodule mass. No Nod^- plants were identified even after screening 100,000 plants involving five cultivars and advanced breeding lines. However, Nod^- plants were obtained from F_2 populations of six different crosses. Progeny of these selections when selfed to F_3 or to F_5 remained Nod^-. Plants with a low and high capacity for nodulation were also identified within particular cultivars and advanced breeding lines. After two cycles of pure line selection in the glasshouse progenies of selected materials maintained their relative nodulation ratings when planted in a field of low mineral N (about 10 mg kg^{-1} soil).

INTRODUCTION

Non-nodulating (Nod^-) legume plants are of value as a reference base for quantifying biological N_2 fixation (BNF) (Fried *et al.*, 1983) and are available in groundnut (Nigam *et al.*, 1980), soybean (Weber, 1966), chickpea (Davis *et al.*, 1985), alfalfa (Peterson and Barnes, 1981) and pea (Kneen and LaRue, 1984). In all these crops Nod^- plants were identified from segregating materials in crossing programs or after mutagenesis. In chickpea, a self-pollinating legume, Nod^- variants also were observed in landraces and cultivars (Rupela, 1992) and, more recently, a pure line selection procedure was followed in developing high and low nodulating selections from a generally low nodulating landrace of chickpea ICC 4948 (=G 130) (O. P. Rupela and C. Johansen, unpubl.). The high nodulating selection of ICC 4948 fixed more N and yielded 31% more than its low nodulating version in a field trial on a soil of low mineral N. It will be interesting to see if the natural occurrence of nodulation variants is confined to chickpea or is a widespread phenomenon present in other legumes also. We chose pigeonpea [*Cajanus cajan* (L.) Millsp.] for this purpose, as a case study.

Pigeonpea is an important grain legume of the tropics and subtropics and is grown on > 3 million ha annually (Nene and Sheila, 1990). In the field, pigeonpea cultivars are difficult to evaluate for their nodulation and N_2-fixation capacity, mainly because of ready detachment of nodules during digging (Kumar Rao and Dart, 1987). We describe procedures used for identification of plants with grossly different degrees of nodulation at early growth stages in the glasshouse, and for salvaging the identified plants for seed production. A visual rating scale that allows evaluation of large number of plants in a short time, required for such a selection program, is described. Emphasis in this study was on stability or otherwise of the observed relative differences in nodulation capacity within materials.

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MATERIALS AND METHODS

Rhizobial strains and seed material

A mixture of four rhizobial strains IC 3100, IC 3195, IC 4059 and IC 4060 was used to inoculate test plants. Peat-based inoculants of these strains were prepared separately and mixed in equal quantities by weight. Each inoculant contained > 10^8 rhizobia g^{-1} of peat as carrier.

Seeds of different cultivars, advanced breeding lines and segregating material used in these studies were obtained from scientists in the Pigeonpea Breeding and Crop Physiology Units of the Legumes Program at ICRISAT. No special crossing program was carried out for the material reported in this paper. Varying quantities of seeds of 189 crosses at different filial stages were obtained from plant breeders to identify Nod^- segregants. Of these, 83 were at F_2, 17 at F_3, 35 at F_4 and 54 at F_5.

Glasshouse culture

Thirty pigeonpea seeds per pot were sown in 15 cm × 15 cm pots and grown for 20–25 days in a glasshouse (temperatures ranged from 26–34°C day to 20–25°C night) and watered with 500 ml of 1/4 strength N-free Arnon solution (Arnon, 1938) in which the mixed rhizobial inoculant was suspended at a rate of 1 g l^{-1}. Subsequent waterings were done alternately with tap water and 1/4 strength N-free

Arnon solution without rhizobial inoculant. One full batch of plants grown in a glasshouse bay of 32 m^2 bench space accommodated at least 20,000 plants. After nodulation observations individual plants were transplanted in 18 or 20 cm dia pots and grown to seed production. Pots contained a mixture of Alfisol, vermiculite, sand and compost in equal volumes. Regular spraying with 0.2% Endosulfan (20 E.C.) in water kept the glasshouse free of insects and we thus produced selfed seed of the selected plants.

Nodulation observations

From 22 to 25 days after sowing (DAS), plant roots were visually rated for nodulation after removal of sand by washing. A visual rating scale of 1 (minimum nodulation) to 5 (maximum nodulation) of the type described for chickpea (Rupela, 1990) was developed and used. Representative plants of each of the five nodulation ratings were selected from more than 20,000 plants involving three cultivars ICPL 88037, ICPL 87 and ICPL 227 and photographed (Fig. 1). This depiction served as a standard for subsequent studies. For verification of the rating scale, 1559 F_3 plants of cross ICPX 870130 B (Parents: ICPL 131 and Kenya 823/11) were grown for 22 days as explained above, and observed for nodulation and plant growth. Only 5–10 plants matching closest to each rating in Fig. 1 were selected and their nodule number, fresh nodule mass, root mass and shoot mass was observed.

Verification of nodulation status

Glasshouse studies. Ten selfed seeds of each plant (single plant progeny seeds) from the initial selection stage (S1) were grown as described earlier and ranked for nodulation. This selection stage was designated S2 (= F_3 for crosses) when at least 30 single plant progenies (SPP) each of low and high nodulation capacity (Table 1) within materials were examined. Plants with a nodulation rating similar to the previous evaluation (S1) were kept for seed production and others were discarded. A pure line selection procedure was thus followed. Randomly selected SPP of Nod^- plants from two crosses IPH 1067 and ICPX 890011 were evaluated for nodulation into F_5.

Field studies. S2 seed of selected material of different nodulation ratings was used in a field trial. The soil was a Vertisol (Typic Pellustert). The selected material included high and low nodulating selections of ICPL 87, ICPL 227, ICPL 83015; and Nod^- (previously advanced to F_5 in the glasshouse) low and high nodulating selections of crosses IPH 1067 and ICPX 890011 as sub-plot treatments. Application of 0 (N1) and 100 kg N ha^{-1} (N2) to a previous cover crop of sorghum in the rainy season of 1992 allowed creation of two distinctly different soil profiles of mineral N, which served as the two main plots of a split plot design. At the time of sowing pigeonpea on 1 November 1992, N1 plots had 13 mg N kg^{-1} soil and N2 had 27 mg N kg^{-1} soil of plant available N in the top 15 cm of the profile. The field had 2.3×10^4 native pigeonpea rhizobia g^{-1} soil and at sowing each seed was inoculated with about 10^5 rhizobia of the mixture of four strains used in pot culture. One 4 m long row of each of the selected materials was grown at the two different soil-N concentrations in three replications. The crop was sown on 60 cm ridges at a 20 cm intra-row spacing, and was irrigated soon after sowing and thrice subsequently at 35, 53 and 73 DAS. Three

Table 1. Nodulation ratings in different pigeonpea materials

Material	Maturity[a] group	No. of plants evaluated	No. of Nod^- plants	Percentage of plants at different nodulation ratings: 1	2	3	4	5	Mean[b] rating
Cultivars									
ICPL 87	S	27697	0	ND	ND	ND	ND	ND	ND
					39 low and 30 high nodulating plants selected				
ICPL 227	M	31977	0	ND	ND	ND	ND	ND	ND
					83 low and 36 high nodulating plants selected				
Advanced breeding line									
ICPL 83015	ES	41830	0	ND	ND	ND	ND	ND	ND
					36 low and 35 high nodulating plants selected				
Crosses where Nod^- plants were identified at F_2									
IPH 1067	S	469	3	< 1	2	54	33	11	4.6
ICPX890011	ES	477	10	15	36	58	6	1	2.9
ICPX860137	L	40	1	88	10	0	0	0	1.1
ICPX860148	L	180	6	40	57	0	0	0	1.6
ICPX860149	L	73	10	51	34	0	0	0	1.2
ICPX860155	L	226	4	38	60	0	0	0	1.6
Parents of crosses from where Nod^- plants were identified									
Cross IPH 1067									
IMS 1	S	1787	0	24	42	30	4	< 1	2.1
ICPL 89031	S	5905	0	11	34	36	17	2	2.7
*Cross ICPX*890011									
ICPL 81	ES	22155	0	32	61	7	0	0	1.8
ICPL 88037	ES	486	0	14	37	43	4	2	2.4

ND, Not determined.

[a]Maturity groups: ES = extra short; S = short; M = medium; L = long.

[b]Mean rating = (No. of plants of rating 1 × 1) + (No. of plants of rating 2 × 2) + ··· + (No. of plants of rating 5 × 5) / Total No. of plants observed)

Table 4. Field performance of progenies selected for different nodulation ratings at 72 days

Material	Single plant progeny No.	Nodulation rating[a]		Nodule No. plant^{-1} ± SE		Nodule dry mass (mg plant^{-1}) ± SE		Shoot dry mass (g plant^{-1}) ± SE	
		Glasshouse	Field at N1	N1[b]	N2[b]	N1	N2	N1	N2
IPH 1067	91234	0	0	0 ± 0	0 ± 0.0	0 ± 0.0	0 ± 0.0	1 ± 0.1	2 ± 0.3
	91235	0	0	0 ± 0	0 ± 0.2	0 ± 0.0	3 ± 3.1	2 ± 0.7	3 ± 0.7
	91236	0	0	0 ± 0	0 ± 0	0 ± 0.0	0 ± 0.0	1 ± 0.3	3 ± 0.1
	91237	1	0	0 ± 0.1	0 ± 0.2	0 ± 0.4	1 ± 0.7	1 ± 0.0	2 ± 0.3
	91275	1	0	0 ± 0.1	0 ± 0	0 ± 0.4	0 ± 0.0	1 ± 0.0	3 ± 0.6
	91239	3	2	4 ± 0.9	0 ± 2.1	35 ± 4.2	42 ± 10.2	2 ± 0.2	3 ± 0.5
	91240	3	2	6 ± 1.6	3 ± 1.1	37 ± 9.9	24 ± 12.3	2 ± 0.1	2 ± 0.9
ICPX 89001	91243	0	0	0 ± 0	0 ± 0	0 ± 0.0	0 ± 0.0	1 ± 0.3	1 ± 0.2
	91278	0	0	0 ± 0	0 ± 0	0 ± 0.0	0 ± 0.0	0.3 ± 0.3	1 ± 0.3
	91280	0	0	ND	0 ± 0	ND	ND	0 ± 0.0	1 ± 0.4
	91282	2	2	6 ± 0.3	5 ± 0.9	60 ± 4.0	46 ± 10.1	2 ± 0.1	4 ± 0.7
	91317	2	2	6 ± 2.4	4 ± 0.4	59 ± 15.9	49 ± 9.5	4 ± 0.2	5 ± 0.8
	91312	3	3	9 ± 0.4	13 ± 5.1	68 ± 20.9	68 ± 20.9	4 ± 0.5	6 ± 1.9
	91313	3	2	6 ± 2.6	9 ± 1.5	47 ± 21.4	66 ± 15.1	3 ± 0.6	5 ± 1.7
ICPL 227	91182	3	2	6 ± 1.1	9 ± 2.3	50 ± 14.7	140 ± 46.7	3 ± 0.6	5 ± 0.7
	91204	5	4	13 ± 3.1	16 ± 4.5	99 ± 31.3	95 ± 22.0	4 ± 0.8	5 ± 0.5
	91208	5	4	14 ± 1.8	18 ± 4.1	97 ± 6.2	130 ± 25.1	3 ± 0.4	4 ± 0.6
ICPL 87	91070	2	2	7 ± 2.7	0 ± 0.2	60 ± 23.4	1 ± 1.0	3 ± 0.7	3 ± 0.7
	91084	1	2	3 ± 1.1	1 ± 0.5	32 ± 12.8	8 ± 8.0	2 ± 0.9	1 ± 0.4
	91116	4	1	5 ± 1.5	3 ± 0.5	29 ± 10.7	25 ± 5.7	3 ± 0.4	4 ± 0.8
	91118	4	2	7 ± 1.5	9 ± 2.4	39 ± 7.4	45 ± 5.3	3 ± 0.4	3 ± 1.0
ICPL 83015	91010	2	2	4 ± 3.1	ND	41 ± 3.1	ND	10 ±	ND
	91013	2	2	5 ± 0.2	1 ± 0.1	49 ± 4.3	15 ± 3.4	5 ± 0.4	2 ± 0.1
	91046	4	4	7 ± 0.8	5 ± 1.6	91 ± 39.6	43 ± 8.6	4 ± 0.0	2 ± 0.1

ND, Not determined.

[a] Visual rating of field-grown plants was difficult due to detachment of nodules during digging. To compare nodulation evaluation of glasshouse and field-grown plants a rating system based on nodule mass was developed for field plants with mean fresh nodule mass of < 30 mg plant^{-1} rated as 1, 31–60 mg as 2, 61–90 mg as 3, 91–120 mg as 4 and > 121 mg plant^{-1} rated as 5.

[b] Low N (N1) plots had 13 mg mineral N kg^{-1} soil and high N (N2) plots had 27 mg mineral N kg^{-1} soil sowing.

with brown spots. There was considerable variation for nodule formation at the F_2 stage. For the cross IPH 1067, 0.6% of the plants were non-nodulating and 11% had a nodulation ranking of 5.

In conclusion, by screening large numbers of plants at early growth stages we identified large and stable variation for nodulation within cultivars, advanced breeding lines and segregating populations of pigeonpea. A genetic study on inheritance of these traits will be required for better understanding of BNF in pigeonpea. The selection of high nodulating lines from agronomically accepted cultivars seems possible. This may obviate the need for specific projects on breeding for high nodulation and high N_2 fixation. F_2

Table 5. Field performance of progenies selected for different nodulation ratings at final harvest

Material	Single plant progeny No.	Dry matter ± SE (g plant^{-1})		Seed mass ± SE (g plant^{-1})	
		N1[a]	N2[a]	N1	N2
IPH 1067	91234	2.4 ± 0.49	8.8 ± 4.63	0.3 ± 0.09	2.5 ± 1.57
	91235	4.0 ± 0.47	10.0 ± 1.80	1.1 ± 0.17	4.3 ± 1.41
	91236	4.2 ± 0.97	8.9 ± 1.00	1.1 ± 0.38	2.0 ± 0.44
	91237	3.4 ± 2.08	9.9 ± 0.12	0.4 ± 0.01	4.2 ± 0.10
	91275	2.5 ± 0.93	5.5 ± 2.23	0.4 ± 0.01	2.5 ± 1.11
	91239	6.3 ± 0.41	8.1 ± 0.69	2.4 ± 0.40	2.9 ± 0.34
	91240	8.1 ± 1.74	9.8 ± 0.80	3.0 ± 0.34	3.5 ± 0.64
ICPX 89001	91243	4.4 ± 0.00	3.7 ± 1.96	1.4 ± 0.66	1.8 ± 0.70
	91278	1.3 ± 0.02	6.1 ± 3.67	0.2 ± 0.00	1.4 ± 0.74
	91280	2.4 ± 1.26	4.9 ± 1.16	0.2 ± 0.07	2.1 ± 0.18
	91282	14.6 ± 1.02	13.1 ± 2.29	7.1 ± 0.22	7.0 ± 1.22
	91317	11.3 ± 1.39	14.8 ± 3.83	5.3 ± 1.02	7.6 ± 1.81
	91312	16.1 ± 1.46	19.0 ± 3.89	8.3 ± 0.86	8.4 ± 1.35
	91313	11.8 ± 0.92	12.5 ± 5.43	6.1 ± 0.67	9.2 ± 0.69
ICPL 227	91182	76.3 ± 8.68	87.6 ± 10.12	14.2 ± 1.63	23.2 ± 3.43
	91204	40.6 ± 8.93	47.0 ± 12.4	13.9 ± 3.22	16.3 ± 5.44
	91208	19.7 ± 1.25	39.8 ± 9.48	8.9 ± 0.56	15.7 ± 5.2
ICPL 87	91070	11.8 ± 3.3	18.0 ± 0.85	4.6 ± 1.46	6.7 ± 1.29
	91084	4.3 ± 0.81	5.4 ± 0.69	1.3 ± 0.21	1.9 ± 0.24
	91116	12.1 ± 2.80	17.8 ± 2.49	4.5 ± 1.19	7.4 ± 0.81
	91118	9.3 ± 1.63	16.3 ± 3.3	4.6 ± 0.41	5.5 ± 1.29
ICPL 83015	91010	4.1 ± 1.78	6.5 ± 0.12	1.6 ± 0.12	4.2 ± 0.20
	91013	6.5 ± 0.81	7.1 ± 1.85	2.9 ± 0.31	3.4 ± 0.80
	91046	24.9 ± 5.16	14.5 ± 2.2	8.1 ± 1.14	9.6 ± 0.79

[a] Low N (N1) plots had 13 mg mineral N kg^{-1} soil and high N (N2) plots had 27 mg mineral N kg^{-1} soil sowing.

materials may be ranked for nodulation and the required selections be advanced further for evaluation of BNF, yield and other traits of interest. The Nod$^-$ lines need to be evaluated for suitability as reference base in BNF quantification studies particularly in those using ^{15}N based methods.

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