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SCREENING FOR NODULATION CHARACTERISTICS IN CHICKPEA AND SUBSEQUENT GENERATION OF SEEDS

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Summary

Chickpea (*Cicer arietinum*) is a self-pollinating species, the cultivars of which differ widely in nodule number, weight, and nitrogenase activity. Studies at ICRISAT have shown a close correlation between these fixation parameters in 61-day-old plants, and have permitted development of a nodulation score highly correlated with both nodule weight and number. This scoring system permits more rapid germplasm evaluation, and should be adjustable to an absolute rating for field-grown plants.

Potted plants can be assayed as intact plants for nitrogenase activity, the nodules removed and weighed, and the plant repotted with 90% survival for plants examined 48 days after planting. This permits seed production from plants of which the genetic potential for nitrogen fixation has already been established, and the use of such plants for hybridization in breeding programs. Chickpeas can also be propagated vegetatively by inducing root development from wounded branches.

INTRODUCTION

Differences in numbers, size and distribution of nodules among cultivars was first observed for soybeans by Voorhees (1915). Since then many reports have appeared on varietal differences in nodulation for various legumes (Johnson & Means, 1960; Nutman, 1961; Gibson, 1962; Döbereiner & Arruda, 1967). More recently the interaction between *Rhizobium* strains and cultivars has been examined in detail (Nutman, 1969; Mytton, El-Sherbeeney & Lawes, 1977; Minchin *et al.*, 1978; Mytton, 1978).

Chickpea is a self-pollinating, herbaceous plant that is normally bushy and semispreading. It has been well described botanically (van der Maesen, 1972).

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In India two types of chickpea are commonly recognized: desi (small, brown seeds that are wrinkled, with a beak at the end) and Kabuli (relatively larger, white, smooth seeds). It is a subtropical crop that is grown mainly on residual moisture in the post-rainy season in India. The *Rhizobium* isolated from chickpea is very specific but will sometimes nodulate *Sesbania bispinosa* and *S. sesban* (Gaur & Sen, 1979).

SCREENING FOR NODULATION

At ICRISAT, nodulation parameters were found to correlate strongly with grain yield under field conditions (Table 1) and to vary widely among 251 germplasm lines examined in the field (see Table 2). We have made crosses between high- and low-nodulating lines to examine the heritability of the parameters, prior to embarking on a breeding program to increase nitrogen (N_2) fixation by chickpea. Unfortunately, observations on nodule number and weight are extremely laborious, and difficult to apply to large-scale germplasm holdings such as those at ICRISAT. Because of this we have developed a visual scoring system, more suitable for evaluating numerous lines. This has proved at least 10 times faster than actual measurement and correlates strongly with nodulation parameters (see Table 3).

TABLE 1: Correlations between N_2 -fixing parameters for 61-day-old chickpea plants and final yield.¹

	Nodule weight	N_2 -ase activity/plant	Grain yield
Nodule number	.788***	.778***	.761***
Nodule weight		.763***	.813***
N_2 -ase activity/plant			.668**

¹ n = 20, based on means for 5 cultivars grown on a Vertisol with 4 reps and a 25-plant sample at 61 days and 12.6 m²/plot not final harvest area.
Significant at 1% *Significant at 0.1%.

SALVAGING FIELD-GROWN PLANTS

To make observations on nodule number and weight it is usually necessary to harvest destructively. Nondestructive acetylene reduction assays can be used only on a limited scale. For breeding purposes it is important that seed be

TABLE 2: Range of symbiotic parameters and yield of chickpea cultivars.¹

Parameter	Harvest (days after planting)	Yield	
		1976-77	1977-78
Nodule no/ plant	25-30	4-48	2-18
	45-50	10-75	1-20
	70-75	1-20	4-28
Nodule dry wt (mg/plant)	25-30	0.3-55	1-18
	45-50	2-105	2-34
	70-75	1-195	3-52
Top wt (g/plant)	25-30	ND ²	0.1-1.7
	45-50	0.7-6.2	1.3-12.0
	70-75	1.8-39.2	19.0-26.8

¹Two-hundred and fifty-one cultivars were grown in the post-rainy season (1976-77) at ICRISAT, without inoculation and replication. Nodulation was observed 25, 45, 50, and 70-75 days after planting. Thirty plants per cultivar were scored at each harvest. In the 1977-78 post-rainy season the same cultivars were again planted at ICRISAT. Cultivars were inoculated throughout with *Rhizobium* strain CC-1192. Observations were made on 30 plants from 3 replicates.

²ND - No data.

TABLE 3: Correlation between N_2 fixation parameters and visual scoring.¹

	Nodule weight	Top weight	N_2 -ase activity/plant	Specific N_2 -ase activity/nodule	Visual score
Nodule number	.69***	.64***	.65***	.3***	.72***
Nodule weight		.63***	.84***	.38***	.85***
Top weight			.48***	.28***	.62***
N_2 -ase activity/plant				.38***	.68***

¹Sixteen cultivars and a check (cultivar K-850, twice) in three replicates were grown in the post-rainy season at ICRISAT. Nodulation was observed 25, 45, 50, and 70-75 days after planting. Nodules were assayed for nitrogenase activity by acetylene reduction at 126 days after planting. After harvest, 30 plants containing two plants each were assayed per replication. After scoring, the plants were scored visually and nodule number and nodule weight then measured.

*Significant at 5%; **Significant at 1%; ***Significant at 0.1%.

produced from selected plants already scored for nodulation. The technique we have developed for this is detailed below. Uprooted field-grown plants are brought from the field under wet gunny (hessian) sacks and are kept as cool as possible until scored. After scoring they are transplanted into pots containing sand:vermiculite:grit (1:2:2), covered with polythene bags (see Figure 1); then kept in the glasshouse at temperatures of approximately 25°C and with 70% relative humidity (RH). One can observe, record, and transplant one plant every two minutes. After four to five days the polythene bags can be removed, but pots must always be kept moist. The success rate for establishing field-grown plants 45-50 days old in pots is about 90%. Plants can subsequently be transferred back into the field with almost 100% success.

Glasshouse-grown plants can generally be scored and repotted more easily than those from the field. In one experiment 270 of 291 plants repotted after nodule scoring survived. In another trial with 20 cultivars, 97% of 200 plants potted survived. When ambient conditions are favorable (ambient temperature less than 30°C, and with high RH) scored plants can even be returned directly to the field.



Figure 1. Potted chickpea plants covered with polythene bags (left). After four to five days the covers are removed (right) when the plants establish.

RAPID MULTIPLICATION OF MATERIALS

We developed a method for taking cuttings of chickpea in order to obtain more seeds of a given genetic stock within a short period. Rooting is induced

by wounding a branch by making a transverse cut halfway through at the fourth or fifth internode. The branch is allowed to grow on in the glasshouse at about 25-28° and about 70% RH. After about seven days, 20-50% of the wounds form rootlets on the wound surface closest to the growing point of the branch, while the others swell at the wounds. Wounded branches, with or without roots, are detached from the plant below the wound and potted in sand plus vermiculite. Root growth is hastened if the original wound is dipped in root hormone powder (Seradex B No. 2, May and Baker, India) while potting up the branch. Pots containing these branches should be kept shaded in the glasshouse for about a week. Almost all branches that have aerial roots, and more than 70% of the branches with swelling at the wound, form roots and grow into plants. Removal of reproductive structures after transplanting stimulates plant growth. When the cuttings are growing vegetatively they can also be transplanted to the field for faster seed production with almost 100% success.

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