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## Genetics of Nonnodulation in the New Nonnodulating Selections of Chickpea

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### Abstract

Nonnodulating ( $Nod^-$ ) legumes are valuable reference plants for studies on quantification of biological nitrogen fixation. Several such  $Nod^-$  chickpea genotypes have been identified at ICRISAT and elsewhere, and the inheritance of the  $Nod^-$  trait worked out in some of them. The present study was undertaken to determine the genetics of nonnodulation in the newly identified  $Nod^-$  mutants, and the genetic relationship among these and the ones reported earlier. Five new  $Nod^-$  mutants were crossed with their  $Nod^+$  parents. These, and two previously reported  $Nod^-$  mutants were also crossed among themselves. Parents and the  $F_1$ ,  $F_2$ , and backcross progenies of the above crosses were inoculated with a mixture of rhizobial strains, and tested for the presence or absence of root nodules in the greenhouse. A single recessive gene was found to control nonnodulation in all but two mutants. Similarly, genetic complementation to produce nodules was observed in most mutant x mutant crosses. Accordingly, six parents were classified into four allelic groups, and two new nonnodulation genes were identified. Further studies have been proposed to resolve some of the anomalies in segregation ratios. This paper also reports breakdown of the nonnodulation trait in two of the seven  $Nod^-$  lines, PM 233 and ICC 5003 M.

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## Introduction

Nonnodulating genotypes of legumes are important for use as nonfixing references in quantifying fixed nitrogen. Davis et al. (1985) first reported gamma ray-induced nonnodulating (Nod<sup>-</sup>) mutant chickpea genotypes, followed by Rupela and Sudarshana (1986) who selected nonnodulating plants from a landrace, highly likely spontaneous nonnodulating mutant. In their genetic studies, Davis et al. (1986) assigned the gene symbol *m1* to the single recessive gene controlling the Nod<sup>-</sup> trait in the gamma ray-induced mutant PM 233, and similarly, the symbol *m2* to PM 665, and *m3* to PM 679. Symbols *m4* and *m5* were later assigned to genes controlling ineffective nodulation in gamma ray-induced mutants PM 405 and PM 796 by Davis (1988). Another NN mutant P 319-1 NN (ICC 435M) reported by Rupela and Sudarshana (1986), was studied by Singh et al. (1992), and the symbol *m6* was assigned to the gene responsible for its Nod<sup>-</sup> trait. Gene *RN7* responsible for the Nod<sup>+</sup> but Fix<sup>-</sup> phenotype has been proposed by T. M. Davis (personal communication 1996).

Rupela (1992) reported three more Nod<sup>-</sup> spontaneous mutants that were isolated from cultivars Annigeri (= ICC 4918), Rabat (= ICC 4993), and K 850 (= ICC 5003) through pure line selection. These are: Annigeri NN (= ICC 4918M), Rabat NN (= ICC 4993), and K 850 NN (= ICC 5003M). Since then, two more Nod<sup>-</sup> mutants, ICCV 2 NN and ICCV 6 NN, have been identified at ICRIASAT Asia Center (IAC). Of the two, ICCV 2 NN was a spontaneous mutant while the ICCV 6 NN was a gamma ray-induced mutant (O P Rupela, unpublished). The present study was undertaken to determine the mode of inheritance of the Nod<sup>-</sup> trait in each mutant, and to work out the genetic relationships among these and other NN mutants reported earlier.

## Materials and Methods

The study was conducted at IAC, Patancheru, India. The Nod<sup>-</sup> mutant selections, Annigeri NN, Rabat NN, K 850 NN, ICCV 2 NN, and ICCV 6 NN were grown in the field and crossed with their respective nodulating parents. These five, and two previously reported Nod<sup>-</sup> mutants, PM 233 and P 319-1 NN, were also crossed among themselves in a half-diallele fashion. The resulting F<sub>1</sub>s were advanced to F<sub>2</sub> generation, and also backcrossed to both of their respective parents. The naturally self-fertilized F<sub>2</sub> seeds and backcrossed F<sub>1</sub> seeds were harvested from F<sub>1</sub> plants.

The parents, F<sub>1</sub>, F<sub>2</sub>, and backcross progenies of all the crosses mentioned above were tested for nodulation in the greenhouse during 1995. Temperature during the test ranged from 12–18°C to 24–31°C, and relative humidity from 65 to 80%. Around 30 plants in the case of the F<sub>1</sub>, 340–400 in F<sub>2</sub>, and up to 100 in the case of

parents and backcross generations were examined in the nodulation studies. Thirty seeds per pot were sown in 20-cm diameter plastic pots filled with 5 kg coarse river sand. Inoculation with a mixture of rhizobial strains IC 59, IC 76, and IC 2002 was done at the time of sowing by applying 10 mL suspension of the peat inoculant in water having > 10<sup>8</sup> rhizobia mL<sup>-1</sup>. A nitrogen-free Arnon nutrient solution was applied to all pots whenever the surface 2 cm top was dry. All plants were uprooted at 33–35 days after sowing (DAS), washed, and examined for root nodules by spreading the roots in trays filled with water. All plants were scored for the presence or absence of root nodules. The data thus obtained were subjected to Chi-square tests with genetic hypotheses based on one-gene ratios for the mutant × parent and two-gene ratios for the mutant × mutant crosses.

## Results

In the genetic studies of Nod<sup>-</sup> mutant genotypes and their parents, all plant-nodulating parents nodulated normally (Nod<sup>+</sup> phenotype), and all mutants lacked nodules (Nod<sup>-</sup> phenotype), except K 850 NN (21% nodulated plants) and PM 233 (44% nodulated plants) (Table 1). In all parent (Nod<sup>+</sup>) × mutant (Nod<sup>-</sup>) crosses, F<sub>1</sub> plants showed normal nodulation, indicating a recessive mode of gene action in the control of the nonnodulation trait. The segregation in F<sub>2</sub> and backcrosses was in good agreement with the phenotypic ratios of 3:1 and 1:1, expected for recessive, single gene inheritance. However, this was not true for ICCV 6 NN and K 850 NN mutants. The segregation ratio in neither case appeared to follow a particular genetic pattern.

In the NN × NN crosses, all the nonnodulating genotypes showed absence of root nodules. However, all the F<sub>1</sub>s, except P 319-1 NN × Annigeri NN, and Rabat NN × ICCV 2 NN, showed a normal nodulation, suggesting nonallelism of the nodulation genes among mutants involved in crosses that showed nodulation (Table 2). There was no nodulation in the F<sub>1</sub> plants of the other two crosses, P 319-1 NN × Annigeri NN, and Rabat NN × ICCV 2 NN, indicating an allelic relationship between the nodulation genes of these selections. Segregation in the F<sub>2</sub> progenies of all crosses, except the ones involving ICCV 6 NN, K 850 NN, and PM 233 were in a proportion of 9 Nod<sup>+</sup> : 7 Nod<sup>-</sup> phenotypes (data not shown) as expected for independent assortment of two recessive genes for this trait. Segregation in the backcross progenies of their crosses were in 1 Nod<sup>+</sup> : 1 Nod<sup>-</sup> ratio, as expected. The remaining crosses, however, did not follow a particular pattern of segregation.

## Discussion

In chickpea, seven Nod<sup>-</sup> genotypes have been identified to date. These are: two gamma ray-induced mutants PM 233 (Davis et al. 1986) and ICCV 6 NN; P 319-1 NN

**Table 1. Nodulation response of Nod<sup>+</sup> and Nod<sup>-</sup> parents F<sub>1</sub>, F<sub>2</sub>, and backcross progenies to rhizobial inoculation in chickpea.**

Generation	Parent or Cross	No. of plants		Expected segregation ratio	X <sup>2</sup>
		Nod <sup>+</sup>	Nod <sup>-</sup>		
Parent	Annigeri (P <sub>1</sub> )	98	00		
Parent	Annigeri (P <sub>2</sub> )	00	107		
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	30	00		
F <sub>2</sub>	P <sub>1</sub> × P <sub>2</sub>	339	111	3:1	0.026
BC <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	99	00		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	59	40	1:1	3.646
Parent	Rabat (P <sub>1</sub> )	114	00		
Parent	Rabat (NN) (P <sub>2</sub> )	00	86		
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	26	00		
F <sub>2</sub>	P <sub>1</sub> × P <sub>2</sub>	329	95	3:1	1.522
BC <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	67	00		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	50	41	1:1	0.890
Parent	ICCV 2 (P <sub>1</sub> )	78	00		
Parent	ICCV 2 (NN) (P <sub>2</sub> )	00	93		
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	30	00		
F <sub>2</sub>	P <sub>1</sub> × P <sub>2</sub>	259	81	3:1	0.251
BC <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	61	00		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	23	37	1:1	3.266
Parent	ICCV 6 (P <sub>1</sub> )	106	00		
Parent	ICCV 6 (NN) (P <sub>2</sub> )	00	101		
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	30	00		
F <sub>2</sub>	P <sub>1</sub> × P <sub>2</sub>	384	85	3:1	11.827 <sup>1</sup>
BC <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	96	00		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	63	36	1:1	11.000 <sup>1</sup>
Parent	K 850 (P <sub>1</sub> )	110	00		
Parent	K 850 (NN) (P <sub>2</sub> )	21	88		
F <sub>1</sub>	P <sub>1</sub> × P <sub>2</sub>	29	00		
F <sub>2</sub>	P <sub>1</sub> × P <sub>2</sub>	02	357		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	91	00		
BC <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	62	22		

1. Significant at 1% level of probability.

**Table 2. Nodulation response of Nod parents and their F<sub>1</sub>, F<sub>2</sub> and backcross progenies to rhizobial inoculation in chickpea.**

Cross	Nodulation response							
	P <sub>1</sub>	P <sub>2</sub>	F <sub>1</sub>	F <sub>1</sub>	F <sub>1</sub> × P <sub>1</sub>	F <sub>1</sub> × P <sub>2</sub>	F <sub>2</sub>	F <sub>2</sub> × P <sub>2</sub>
P 319-1 NN (P <sub>1</sub> ) × Annigeri NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
P 319-1 NN (P <sub>1</sub> ) × Rabat NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
P 319-1 NN (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
Annigeri NN (P <sub>1</sub> ) × Rabat NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
Annigeri NN (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
Rabat NN (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × P 319-1 NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × Annigeri NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × Rabat NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × PM 233 (P <sub>2</sub> )	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
ICCV 6 NN (P <sub>1</sub> ) × K 850 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
PM 233 (P <sub>1</sub> ) × P 319-1 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
PM 233 (P <sub>1</sub> ) × Annigeri NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
PM 233 (P <sub>1</sub> ) × Rabat NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
PM 233 (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
PM 233 (P <sub>1</sub> ) × K 850 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
K 850 NN (P <sub>1</sub> ) × P 319-1 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
K 850 NN (P <sub>1</sub> ) × Annigeri NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
K 850 NN (P <sub>1</sub> ) × Rabat NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod
K 850 NN (P <sub>1</sub> ) × ICCV 2 NN (P <sub>2</sub> )	Nod <sup>+</sup>	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod	All Nod

X<sup>2</sup> showed a good fit to the ratios.

(reported as ICC 435M by Rupela and Sudarshana 1986); Annigeri NN, Rabat NN, and K 850 NN (Rupela 1992); and ICCV 2 NN. The other gamma ray-induced mutants, namely PM 665 and PM 679 (Davis et al. 1986), and PM 405 and PM 796 (Davis 1988) have been reported to form ineffective nodules. A breakdown in the genetic control of the Nod<sup>-</sup> trait in mutant PM 233 has been observed in this study, where 44% plants were nodulated. The selection K 850 NN, when initially reported (Rupela 1992), was found nonnodulating against rhizobial strain IC 59, but in subsequent studies in the field, 5-12% plants were always found nodulated. It seems that K 850 NN either forms symbioses with a restricted population of native rhizobia or is a case of extremely low nodulation such that any slight adverse change in environment at the time of sowing results in absence of nodules in most plants. This needs to be investigated further.

The Nod<sup>-</sup> trait in PM 233 and P 319-1 NN has been reported to be under the control of single recessive genes that are not allelic to each other (Singh et al. 1992). In the present study also, single recessive genes seem to control the Nod<sup>-</sup> trait in Annigeri NN, Rabat NN, and ICCV 2 NN. The genetic control of nonnodulation in cases of ICCV 6 NN and K 850 NN, however, is not clear from the results. Nodulation observed in the expected Nod<sup>-</sup> K 850 NN could have caused such aberration in the results in this case. Studies on ICCV 6 NN are being repeated to determine whether the deviation from single gene ratios is due to the effect of selfing or because of the involvement of two genes. Seeds of the ICCV 6 NN are unlike those of their parent ICCV 6 in that they are small and more shrivelled. Such differences between seed of mutants and their parents in all other Nod<sup>-</sup> lines, be they spontaneous or gamma ray-induced mutants, are absent.

The results from NN×NN crosses have indicated a genetic complementation in the F<sub>1</sub> of all crosses, except those involving K 850 NN, and crosses P 319-1 NN × Annigeri NN, and Rabat NN × ICCV 2 NN. Although segregation ratios in F<sub>2</sub> and backcross progenies in some of these crosses will have to be studied further, all Nod<sup>-</sup> genotypes can be classified into the following four allelic groups: PM 233; P 319-1 NN and Annigeri NN; Rabat NN and ICCV 2 NN; and ICCV 6 NN. It means that Nod<sup>-</sup> genotypes in a group are allelic to each other, i.e., P 319-1 NN has the same gene as Annigeri NN, and Rabat NN has a gene similar to ICCV 2 NN. The genetic relationship of K 850 NN with other NN genotypes is yet to be established. Singh et al. (1992) also found that the Nod<sup>-</sup> trait of PM 233 and P 319-1 NN was under the control of two different recessive genes *rn1* and *rn6*. Singh and Rupela (1991) had earlier indicated that P 319-1 NN and Annigeri NN had similar NN genes which were dissimilar to those in PM 233 and Rabat NN. So, we have discovered two new nonnodulation genes, one in Rabat NN and ICCV 2 NN, and the other in ICCV 6 NN, and will propose new gene symbols in due course. Further studies to resolve some of the anomalies observed in the segregation ratios, and

to reconfirm the observed genetic relationships among various nonnodulating mutants are in progress at IAC.

Most spontaneous mutations are recessive in nature. All the nonnodulating selections discovered so far are due to a recessive gene. All the six cultivars studied by Rupela (1992) had at least three Nod<sup>-</sup> plants. Such a high rate of occurrence of spontaneous mutant in chickpea is surprising. Rupela and Johansen (1995) failed to identify natural occurrence of Nod<sup>-</sup> in 14 pigeonpea cultivars. Also, we are not aware of natural occurrence of Nod<sup>-</sup> plants in any other legumes. Rupela (1992) suggested that in chickpea, the Nod<sup>-</sup> plants may have arisen due to a strong tendency of plants to depend on soil nitrogen. A deliberate development of nodulation mutants along with the available ones should help in a better understanding of the nodulation process (see Thomas et al. these proceedings, for information).

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