

# A New Gene that Controls Root Nodulation in Chickpea

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## ABSTRACT

Nonnodulating ( $Nod^-$ ) genotypes are an important genetic resource for studies of the legume-rhizobium symbiosis. Investigations were carried out to determine the host genetic control of nodulation in two newly identified  $Nod^-$  chickpea (*Cicer arietinum* L.) genotypes, Annigeri NN and Rabat NN, and the genetic relationship between these and other known  $Nod^-$  genotypes. Annigeri NN and Rabat NN were crossed with each other, with their respective wild-type parents 'Annigeri' (Desi) and 'Rabat' (Kabuli), and with already known  $Nod^-$  mutants P319-1 NN and PM 233. The parents,  $F_1$ ,  $F_2$ , and backcross progenies of the crosses were tested for nodulation by mean of a mixture of rhizobial strains in the greenhouse. Chi-square tests were conducted on the crosses to examine genetic hypotheses of monogenic and digenic inheritance. Genetic analyses of  $Nod^- \times Nod^+$  crosses showed a monogenic recessive gene action for nonnodulation in both Annigeri NN and Rabat NN. The studies of  $Nod^- \times Nod^-$  crosses indicated that the same gene controls root nodulation in Annigeri NN and P319-1 NN, and that this gene is different from the ones controlling the  $Nod^-$  trait in Rabat NN and PM 233. These results implied that the recessive gene controlling nonnodulation of roots in Rabat NN is different from the ones earlier identified in PM 233, P319-1 NN, and Annigeri NN. A new gene symbol, *rn8*, is proposed to be assigned to the locus producing the  $Nod^-$  phenotype in Kabuli chickpea genotype Rabat NN. This is the first report of a nonnodulating gene in Kabuli chickpea.

NONNODULATING genotypes of legumes are useful as nonfixing references in studies quantifying biological  $N_2$  fixation (BNF). Davis et al. (1985) first reported gamma ray-induced nonnodulating mutants PM 233, PM 665, and PM 679 in chickpea. Later, they assigned gene symbols *rn1*, *rn2*, and *rn3* (*rn* = root nodule) to single recessive genes controlling nonnodulating trait in these mutants (Davis et al., 1986). In another genetic study by Davis (1988), symbols *rn4* and *rn5* were assigned to two recessive genes responsible for ineffective nodulation in induced mutants PM 405 and PM 796. The genetics of nonnodulation in a spontaneous mutant ICC 435M (hereafter renamed as P319-1 NN), reported by Rupela and Sudarshana (1986), was studied by Singh et al. (1992) and the single recessive gene controlling its  $Nod^-$  trait was assigned the gene symbol *rn6*. Rupela (1992) reported three additional nonnodulating genotypes that were isolated from Annigeri (ICC 4918), Rabat (ICC 4993), and 'K850' (ICC 5003) through pure line selection. These were named ICC 4918M, ICC

4993M, and ICC 5003 M (hereafter renamed as Annigeri NN, Rabat NN, and K850 NN). K850 NN was later found to form root nodules in around 10% of the plants when grown in the field.

Root temperature is known to influence nodulation of chickpea (Dart et al., 1975). Pea (*Pisum sativum* L.) cultivar Chi-Un-To has been reported by Lie et al. (1976) to nodulate well at 20°C but not at 26°C while 'Iran' formed nodules at 26°C in combination with certain *Rhizobium* strains, but not at 20°C. In the same experiments, 'Rando' formed effective nodules at both temperatures. Davis et al. (1986) reported a chickpea mutant PM 679 which formed effective nodules at 24°C but not at 29°C. To avoid such complications, the studies by Rupela (1992) characterizing nonnodulating selections and those reported in this paper were conducted in the field and in the greenhouse in the cool season in soil having native rhizobia nodulating chickpea. It was hoped that this would allow favorable soil temperatures for nodulation of chickpea lines by native and/or inoculant rhizobia. The objectives of this study were to determine the inheritance of nonnodulation in Annigeri NN and Rabat NN, and to determine the allelic relationship among these and previously reported nonnodulating genotypes.

## MATERIALS AND METHODS

The study was conducted at ICRISAT Asia Center, Patancheru, India. Annigeri NN and Rabat NN were grown in the field and crossed with their respective wild-type parents Annigeri (Desi) and Rabat (Kabuli) during the post-rainy season 1992–1993. These two and previously reported nonnodulating mutants PM 233 (Davis et al., 1985) and P319-1 NN (Rupela and Sudarshana, 1986) were crossed with each other in the same season. Because genetic relationships for the nodulation trait between P319-1 NN and PM 233 and between P319-1 NN and Annigeri were already known (Singh and Rupela, 1991; Singh et al., 1992), these two cross combinations were not attempted. Part of the  $F_1$  seeds obtained from all crosses were sown in the field with their parents during the 1993–1994 season. All  $F_1$ s were backcrossed to their respective parents. The  $BC_1$   $F_1$  seeds and  $F_2$  seed from naturally self-fertilized  $F_1$  plants (outcrossing in chickpea is negligible) were harvested separately.

The parents,  $F_1$ ,  $F_2$ , and backcross progenies of all crosses were tested for nodulation in the greenhouse during 1995. A total of 30  $F_1$  plants, 400  $F_2$  plants, and 100 parental and backcross generation plants were used in the nodulation studies. Thirty seeds were sown per 20-cm-diam. plastic pot filled with about 5 kg sterilized coarse river sand. Inoculation was done at the time of sowing with a mixture of rhizobial strains IC 59, IC 76, and IC 2002. A 10-mL suspension of peat inoculant in water having  $>10^6$  rhizobia  $mL^{-1}$  was applied to each pot. Pots were irrigated with a nitrogen-free Arnon nutrient

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solution (Arnon 1938) whenever the surface 2 cm was dry. Temperature in the greenhouse ranged from 12 to 18°C (daily minimum) to 24 to 31°C (daily maximum) with relative humidity of 65 to 80%. All plants were uprooted at 33 to 35 d of age, washed, and examined for root nodules by spreading the roots in enamel trays filled with water. All plants were scored for presence or absence of root nodules. Data were analyzed by Chi-square tests for hypotheses of one-gene and two-gene ratios for  $Nod^- \times$  parent and  $Nod^- \times Nod^-$  crosses, respectively.

## RESULTS AND DISCUSSION

The parents Annigeri and Rabat nodulated normally, while those of variant genotypes lacked nodules (Table 1). All the  $F_1$  plants in Annigeri NN  $\times$  Annigeri and Rabat NN  $\times$  Rabat showed normal nodulation indicating a recessive mode of gene action controlling the non-nodulation trait in both  $Nod^-$  variants. The  $F_2$  segregation into  $Nod^+$  and  $Nod^-$  phenotypes in a ratio of 3:1 also indicated a monogenic inheritance of the trait. This was further corroborated by the distribution of  $Nod^+$  and  $Nod^-$  phenotypes in the backcross progenies which showed a good fit to the ratio of 1  $Nod^+$ : 1  $Nod^-$  as expected for recessive single gene inheritance.

In the  $Nod^- \times Nod^-$  crosses, all plants of  $Nod^-$  genotypes (Annigeri NN, P319-1 NN, and Rabat NN) showed absence of root nodules. All plants in the  $F_1$ ,  $F_2$ , and backcross generations of Annigeri NN  $\times$  P319 NN failed to produce nodules indicating that the same allele of the  $Nod^-$  gene controls root nodulation in the two  $Nod^-$  variants (Table 2). However, nodulation patterns in the  $F_1$ ,  $F_2$ , and backcross progenies of crosses Annigeri NN  $\times$  Rabat NN, Rabat NN  $\times$  P319-1 NN, and Rabat NN  $\times$  PM 233 were different from the earlier cross. The  $F_1$  plants in the three crosses were well nodulated, indicating that different genes control the  $Nod^-$  phenotype of variants Annigeri NN, Rabat NN, and PM 233. The  $F_2$  progenies of the three crosses showed a good fit to a segregation ratio of 9  $Nod^+$ : 7  $Nod^-$ , as expected for independent assortment of two complementary re-

**Table 1. Nodulation response of  $Nod^-$  variants, their  $Nod^+$  parents, and  $F_1$ ,  $F_2$ , and backcross progenies of  $Nod^+ \times Nod^-$  crosses to rhizobial inoculation in chickpea.**

Generation	Parent or cross	Plants		Expected ratio	$\chi^2$	<P<
		$Nod^+$	$Nod^-$			
<b>Annigeri <math>\times</math> Annigeri NN</b>						
Parent (P1)	Annigeri	98	0			
Parent (P2)	NN	0	107			
$F_1$	$P_1 \times P_2$	30	0			
$F_2$	$P_1 \times P_2$	339	111	3:1	0.027	0.90-0.80
$BC_1$	$F_1 \times P_1$	99	0			
$BC_1$	$F_1 \times P_2$	59	40	1:1	3.646	0.10-0.05
<b>Rabat <math>\times</math> Rabat NN</b>						
Parent (P1)	Rabat	114	0			
Parent (P2)	Rabat NN	0	86			
$F_1$	$P_1 \times P_2$	26	0			
$F_2$	$P_1 \times P_2$	329	95	3:1	1.522	0.25-0.20
$BC_1$	$F_1 \times P_1$	67	0			
$BC_1$	$F_1 \times P_2$	50	41	1:1	0.890	0.50-0.35

cessive genes. Segregation in the backcross progenies of the three crosses also showed a good fit to 1  $Nod^+$ : 1  $Nod^-$  ratio.

The studies of crosses involving  $Nod^-$  variants Annigeri NN and Rabat NN, and their nodulating parents indicate that the nonnodulation trait in both variants is controlled by single recessive genes. All mutations from  $Nod^+$  to  $Nod^-$  trait in chickpea to date, whether spontaneous or induced, appear to be recessive.

Since the  $Nod^-$  trait in this study was governed by single recessive genes, the  $Nod^- \times Nod^-$  crosses were used to determine the genetic complementation of the  $Nod^-$  trait among these variants. Genetic complementation occurred between Annigeri NN and Rabat NN, Rabat NN and P319-1 NN, and Rabat NN and PM 233, as suggested by the presence of root nodules in the  $F_1$  and two-gene segregation in the  $F_2$  and backcross progenies in ratios of 9:7 and 1:1 respectively (Table 2). These results confirm a nonallelic relationship among the three mutants. However, failure of nodulation in all the  $F_1$ ,  $F_2$ , and backcross progenies of cross Annigeri NN  $\times$  P319-1 NN suggested an allelic relationship between Annigeri NN and P319-1 NN. Earlier studies on the nonnodulating chickpea lines had also indicated an allelic relationship between P319-1 NN (ICC 435M) and Annigeri NN (Singh and Rupela, 1991) and the absence of allelism between PM 233 and P319-1 NN (Singh et al., 1992). However, the first study was limited to the  $F_1$  generation. These results imply that the Rabat NN

**Table 2. Nodulation response of  $Nod^-$  chickpea genotypes and their  $F_1$ ,  $F_2$ , and backcross progenies to rhizobium inoculation.**

Generation	Parent or cross	Plants		Expected ratio	$\chi^2$	<P<
		$Nod^+$	$Nod^-$			
<b>Annigeri NN <math>\times</math> P319-1 NN</b>						
Parent (P1)	Annigeri NN	0	107	All $Nod^-$		
Parent (P2)	P319-1 NN	0	117	All $Nod^-$		
$F_1$	$P_1 \times P_2$	0	24	All $Nod^-$		
$F_2$	$P_1 \times P_2$	0	467	All $Nod^-$		
$BC_1$	$F_1 \times P_1$	0	92	All $Nod^-$		
$BC_1$	$F_1 \times P_2$	0	97	All $Nod^-$		
<b>Annigeri NN <math>\times</math> Rabat NN</b>						
Parent (P1)	Annigeri NN	0	107			
Parent (P2)	Rabat NN	0	86			
$F_1$	$P_1 \times P_2$	31	0	All $Nod^+$		
$F_2$	$P_1 \times P_2$	262	224	9:7	1.082	0.30-0.25
$BC_1$	$F_1 \times P_1$	53	47	1:1	0.360	0.70-0.50
$BC_1$	$F_1 \times P_2$	46	53	1:1	0.495	0.50-0.40
<b>Rabat NN <math>\times</math> P319-1</b>						
Parent (P1)	Rabat NN	0	86			
Parent (P2)	PM233	0	46			
$F_1$	$P_1 \times P_2$	27	0	All $Nod^+$		
$F_2$	$P_1 \times P_2$	274	205	9:7	0.177	0.70-0.50
$BC_1$	$F_1 \times P_1$	55	44	1:1	1.222	0.30-0.20
$BC_1$	$F_1 \times P_2$	54	45	1:1	0.818	0.50-0.30
<b>Rabat NN <math>\times</math> PM 233</b>						
Parent (P1)	Rabat NN	0	86			
Parent (P2)	PM233	0	46			
$F_1$	$P_1 \times P_2$	29	0	All $Nod^+$		
$F_2$	$P_1 \times P_2$	244	194	9:7	0.052	0.90-0.80
$BC_1$	$F_1 \times P_1$	45	50	1:1	0.263	0.70-0.50
$BC_1$	$F_1 \times P_2$	51	34	1:1	3.400	0.10-0.05

genotype has a recessive  $Nod^-$  gene which is different from the ones identified in PM 233, P319-1 NN, and Annigeri NN.

Gene symbols *rn1* to *rn6* have been assigned to the seven genes controlling the nodulation trait in chickpea, the latest being *RN7* proposed to be assigned to a  $Nod^+$  but  $Fix^-$  (nonfixing) chickpea genotype (T.M. Davis, 1996, personal communication). Since not all allelic relationships have been investigated, some *rn* numbers may be redundant and should be verified. We propose a new gene symbol *rn8* for the recessive gene that controls nonnodulation in Kabuli chickpea genotype Rabat NN. The genotype of the mutant and its parent Rabat with respect to this locus can be represented as *rn8/rn8* and *RN8/RN8*, respectively.

It can be concluded from this study that (i)  $Nod^-$  trait of Rabat NN and Annigeri NN is under a single recessive gene control, (ii) the  $Nod^-$  gene identified in Rabat NN is different from the genes that were earlier identified in other  $Nod^-$  genotypes, and (iii) the  $Nod^-$  genes in Annigeri NN and P319-1 NN are allelic to each other. This is the first nonnodulating gene identified in a Kabuli chickpea genotype. Phenotypically, Rabat NN looks very similar to its parent and thus together constitute an excellent  $Nod^+$ - $Nod^-$  near-isogenic pair, suitable for use in studies of quantification of biological  $N_2$  fixation. These also constitute an important genetic resource for physiological and molecular studies of the legume-rhizobium symbiosis. Such genotypes can also be used to develop host plants with restricted *Rhizobium* specificity as discussed by Devine and Weber (1977). Assuming the nonnodulating selection and only a few rhizobial strains are symbiotic, identification of

such strains will be valuable because this will be a case of restricted host-rhizobia specificity. In such a host-rhizobial combination, all the nodules will be formed by a selected efficient inoculant strain, thus bypassing competition from the native inefficient or less efficient rhizobia.

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