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CHARACTERIZATION OF CHICKPEA GERMPLASM FOR NODULATION AND EFFECT OF RHIZOBIUM INOCULATION ON NODULES NUMBER AND SEED YIELD

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ABSTRACT

Chickpea (*Cicer arietinum* L.) germplasm, collected from diverse research centers of the world were characterized for nodulation (Nod^+ or Nod^-) and evaluated for response to seed inoculation with *Rhizobium leguminosarum* in terms of number of nodules $plant^{-1}$ and seed yield $plant^{-1}$. Forty three genotypes, procured from Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Gram Research Station (GRS) Karak and International Center for Agricultural Research in Dry Areas (ICARDA), Syria, showed nodulation (Nod^+) while four genotypes, received from International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India failed to produce any nodule (Nod^-). Pooled analysis of variance for both the traits revealed highly significant differences among genotypes, interaction of genotypes with treatment and also between treatments (inoculated and un-inoculated/control). Inoculated genotypes exceeded control in treatment means both for nodules $plant^{-1}$ (10.86 and 7.86) and seed yield $plant^{-1}$ (15.1 g and 10.0 g). High heritability estimates for nodules $plant^{-1}$ (53.2) and seed yield $plant^{-1}$ (77.0) as well as low level of differences among PCV and GCV of both the traits showed that genotypes played more influential role in the expression of these characters. It is concluded that the studied germplasm could be successfully utilized in future breeding programs. Furthermore, Genotypes Karak-3 is recommended for general cultivation and NDC 5-S10 for crop rotation programs.

Key words: chickpea, rhizobium, inoculation, nodules, seed yield.

INTRODUCTION

Nitrogen is an essential nutrient for plant growth and development but is unavailable in its most prevalent form as atmospheric nitrogen. Plants instead depend upon combined, or fixed forms of nitrogen such as ammonia and nitrate. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogenous fertilizers. Use of these fertilizers has led to worldwide, ecological problems, such as the formation of coastal dead zones. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. It is a critical component of many aquatic, as well as terrestrial ecosystems across our biosphere (Wagner, 2011).

Among the leguminous crops, chickpea (*Cicer arietinum* L.) occupies an important position due to its nutritive value. It is an important source of protein in the diets of the poor peoples and vegetarians. Chickpea is considered to sustain cropping system productivity due to its ability to fix atmospheric nitrogen. It has highly specific symbiotic association, with a unique group of rhizobia necessary for formation of nodules and nitrogen fixation. Absence of suitable strains, small population size and poor survival of rhizobia cause problems in nodules formation (Kantar *et al*., 2007).

Presence of appropriate nodule forming bacteria in the soil is essential for management and utilization of atmospheric nitrogen. If nodulating crop has not been sown in recent past and grown for the first time then seed inoculation is essential before sowing. Further, to avoid uncertainty about natural inoculation, the seed should be inoculated every time. Nitrogen fixing potential of chickpea genotypes can be increased significantly by rhizobial inoculation. Grain yield of chickpea increased considerably with rhizobial application (Khattak *et al*., 2006). Romdhane *et al*. (2008) also reported that chickpea yield can be enhanced by inoculation with competitive rhizobia and is especially economical promising to increase chickpea production.

Artificial seed inoculation of chickpea in those soils lacking native effective rhizobia is a very useful practice for improving root nodulation and yield of the crop (Khattak *et al*., 2006, Muhammad *et al*., 2010). Soils of Pakistan are generally deficient in nitrogen; the most important element in the metabolism of plants and protein synthesis. Its deficiency in soil usually results in low crop yield (Romdhane *et al*., 2008). Inoculation increase soil nitrogen along with the increase in root and shoot nitrogen (Ahmed *et al*., 2008). In the light of above and existing situation, the said research work was planned to categories the genotypes for the presence or absence of nodules, to evaluate genetic diversity among genotypes for number of nodules $plant^{-1}$, and to study the influence of seed inoculation with *Rhizobium leguminosarum* on the number of nodules plant^{-1} and seed yield plant^{-1}.

MATERIALS AND METHODS

The experiment was carried out in pots in the net house facility of Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, during chickpea growing season 2006-07. A total of forty seven genotypes (Table A) collected from different research centers of the world [International Center for Agricultural Research in Areas (ICARDA), Syria, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, Nuclear Institute for Food and Agriculture (NIFA) Peshawar, and Gram Research Station (GRS) Karak] were evaluated. The experiment comprised of two treatments (un-inoculated/control and inoculated with *Rhizobium leguminoserum*). Pots were arranged in three replications using Completely Randomized Design (CRD) with factorial arrangement. Seeds of each genotype were sown in two pots replication^{-1} (one control and one treated); per pot six seeds were sown, so each replication consisted of 94 pots. Pots having a diameter of 22 cm, filled with 4.5 kg soil (50% clay and 50% sand) were used in the experiment.

The collected data was analyzed by using statistical software, SAS (statistical analysis system) version 9 appropriate for two factorial design. Out of forty seven genotypes, four were non-nodulating while forty three genotypes were nodulating, therefore, number and seed yield data of only forty three nodulating genotypes were analyzed. The differences among the treatments' means, genotypes' means and interactions of treatment were compared, using least significant difference (LSD) test at 5% probability level (Steel and Torrie, 1980). Genotypic and phenotypic variances, their coefficient of variation and heritability (broad-sense) were estimated as suggested by Burton (1952) and Hanson *et al.* (1956), respectively

Percent change (increase or decrease) in number of nodules plant^{-1} and seed yield plant^{-1} after rhizobium inoculation of each genotype was obtained by the following formula

$$\%\ \text{change} = \frac{MI - MU}{MU} \times 100$$

Where, MI = Mean of inoculated, and MU = Mean of un-inoculated

Inoculants preparation and use: Chickpea inoculation slurry was prepared by adding 40 g of inoculant in 300 ml of 5% sugar solution. The contents were stirred well. Sugar solution improves the adhesion of inoculant to the seed. Slurry was then poured on seed, and mixed in a clean vessel or on a plastic sheet until all the seeds were uniformly coated. The whole inoculation procedure was completed in shade as sunlight damages the bacteria. Seed was sown immediately after inoculation. All genotypes were evaluated for number of nodules plant^{-1} and seed yield plant^{-1} in both treatments.

RESULTS

Characterization of genotypes for the presence or absence of nodules: Genotypes were examined without rhizobium inoculation as well as with rhizobium inoculation for the presence or absence of nodules (Table 1). Results showed that genotypes procured from NIFA Peshawar and GRS Ahmedwala Karak were nodulating (produce nodules) while genotypes received from ICRISAT India failed to produce any nodule (non-nodulating). The nodulating group consisted of forty three genotypes i.e., from NDC 122 to Lawaghar (Table 1) while non-nodulating group contained four genotypes (ICC4993, ICC19183, ICC4918 and ICC19181).

Characterization of germplasm for nodules and seed yield plant^{-1} (un-inoculated condition): Analysis of variance revealed highly significant differences for nodules number as well as for seed yield plant^{-1} among genotypes (un-inoculated /control) shown in Table 2. Mean values of the genotypes for both the parameters are given in Table 1, while components of variance and heritability percentages are presented in Table 3.

The highest and lowest values for nodules in addition with seed yield plant^{-1} under un-inoculated (control) condition revealed wide range of variation and showed highly significant differences among the genotypes (Table 1). Maximum numbers of nodules plant^{-1} (14.5) were produced by NDC-5-S10 followed by NDC 4-20-4 (12.5) whereas Lawaghar showed minimum number of nodules plant^{-1} (2.4). Average seed yield plant^{-1} of Karak 3 was the maximum (28.9 g) while NDC-15-2 was the genotype with lowest (4.1 g) seed yield plant^{-1}.

Components of variance for number of nodules plant^{-1} (Table 3) revealed moderate phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) i.e. 32.85 and 17.48 respectively, while heritability percentage was high i.e. 53.2% whereas, seed yield plant^{-1} showed high heritability (77%), with larger amount of PCV (49.9) and GCV (43.6).

Effect of rhizobial inoculation on number of nodules plant^{-1}: Among forty seven genotypes forty three were nodulating while four genotypes were recorded as non-nodulating. The non-nodulating genotypes were excluded from the analysis. According to analysis of variance (Table 2) the interaction of genotypes with treatments (un-inoculated and inoculated) were highly significant. The genotype means as well as treatment means also showed significant differences. In genotypes by treatment interaction (Table 4), the maximum nodules plant^{-1} was exhibited by inoculated genotypes i.e. NDC 4-20-1 (16.6) followed by NDC 4-20-7 and NDC 15-4 with 16.5 and 16.4 number of nodules plant^{-1} respectively. While, the minimum number of nodules plant^{-1} was produced by un-inoculated (control) genotypes i.e. Lawaghar (2.4) followed by genotype NKC 5-S-18 (3.1). In general most of the studied genotypes showed increase in number of nodules plant^{-1} as compare to that when they were un-inoculated (control). In genotype over treatment (inoculated and un-inoculated) means, (Table 4) which shows the average performance of a genotype over treatments, the genotype NDC 5-S10 showed best performance followed by NDC 4-20-4 with 14.8 and 13.6 nodules plant^{-1} respectively. Genotype Lawaghar showed poor performance with 4.8 nodules plant^{-1} in the means of genotype. The treatment means were greater for inoculated treatment (10.86 nodules palnt^{-1}) than that of un-inoculated treatment (7.86 nodules palnt^{-1}) (Table 4).

Among genotypes different levels of percent increase (Table 4) were observed for number of nodules plant^{-1}.With rhizobium application, above 100% increase in nodules plant^{-1} was noticed in 4 genotypes (NKC 5-S20, NKC 5-S23, NKC 5-S18 and Lawaghar), 50 – 100% in 10 genotypes (NDC 727, NDC 15-1,NDC 15-2, NDC 15-4, NDC4-15-2, NDC 4-20-7, NIFA 88, NKC 10-99, Karak 3 and Sheenghar) while less then 50% was recorded in 28 genotypes (NDC 122, NDC 728-5, NDC-730-2, NDC-15-3, NDC-4-15-1, NDC-4-15-3, NDC-4-20-1 to NDC 4-20-6, NDC-5-S10, NDC 5-S11, NIFA 95, NIFA 2005, NKC-5-S112 to NKC-5-S17, NKC-5-S21, Hassan 2K to Karak 2). Two genotypes (NKC-5-S22, NKC-5-S24) did not show any positive response to rhizobium inoculation, rather a decrease was observed in their number of nodules plant^{-1}. The greatest response to seed inoculation was recorded for genotype Lawaghir (196%) whilst lowest by genotype NIFA 2005 (2.1%). The treatment means showed that the inoculated genotypes produced 38.1% more nodules plant^{-1} over the un-inoculated.

Effect of rhizobium inoculation on seed yield plant^{-1}: Rhizobium inoculation also had significant effect on seed yield plant^{1} (Table 2). Highly significant differences were also recorded in interaction of genotypes with treatments (control and inoculated) (Table 5). In the interactive means with treatments the inoculated genotypes showed highest seed yield plant^{-1} i.e. genotype Karak-3 (33.0) followed by Sheenghar (30.2) and NKC 5-S24 (29.1). Whereas, minimum seed yield plant^{-1} in the interaction means was revealed by un-inoculated genotypes i.e. NDC 15-2 with 3.6 g and NDC 4-20-4 with 4.3 g of seed yield plant^{-1}.In the genotype over treatment means, Karak 3 was reported as genotype with highest seed yield plant^{-1} i.e. 30.4 g. whilst, Sheenghar stood second to Karak 3 with 21.8 g of seed yield plant^{-1}. NDC 15-2 remained a poor yielder in genotype over treatment means with 3.8 g seed yield plant^{-1}. Treatment mean for seed yield plant^{-1} was 10.0 g for un-inoculated and 15.1 g for inoculated treatments.

Like nodulation, varied increase for seed yield plant^{-1} amongst genotypes was also observed after seed inoculation with rhizobium (Table 5). Seven genotypes (NDC-727, NDC-15-4, NDC 4-15-1, NDC-5-S11, NKC-5-S13, NKC-5-S21, NKC-5-S24, and Sheenghar) were in a position to have more than 100% increase in seed yield plant^{-1}, Six genotypes (NDC 4-20-3, NDC-4-20-5, NIFA 95, NKC-5-S15, NKC-5-S20 and NKC-5-S23) provided 50 to 100% greater seed yield plant^{-1}, twenty five genotypes (NDC 122, NDC 728-5 to NDC 15-3, NDC 4-15-2 to NDC 4-20-1, NDC 4-20-4, NDC 4-20-6 to NDC 5-S10, NIFA 88 to NKC 5-S12, NKC 5-S16, NKC 5-S17, NKC 5-S19, NKC 5-S22, Hassan 2K, Karak 2, karak 3 and Lawaghar) produced less than 50% seed yield plant^{-1} after inoculation. Four genotypes (NDC-4-20-2, NKC-5-S14, NKC-5-S18, and Karak 1) were observed with no response to seed inoculation rather there was reduction in their seed yield plant^{-1}. Maximum response of 195.3% increase in seed yield plant^{-1} to inoculation was recorded for genotype NKC 5-S13 while genotype NDC 15-1 revealed minimum percent increase (4.0) for the said trait. The overall increase in seed yield plant^{-1} of inoculated treatment over the un-inoculated treatment was 50.5%.

Correlation of nodules plant^{-1} with seed yield plant^{-1}: Correlation analysis showed non-significant negative association of nodules plant^{-1} with seed yield under both un-inoculated (r = -0.26) and inoculated conditions (r = -0.20) (Table 6). Negative non significant association of nodules plant^{-1} with seed yield indicated no association between these two traits, and it could be due to rhizobium strain used in the study, which may be effective in enhancing yield of some chickpea but not in others.

Table A. Pedigree and origin of genotypes/accessions used in the study.

Genotype name	Parentage	Origin	Genotype name	Parentage	Origin
NDC-122	C-44 x ILC-195	NIFA, Pakistan	NKC-10-99	Flip98-138c x Sel99th15039	ICARDA/,Syria
NDC-727	C-44/M	NIFA, Pakistan	NKC-5-S12	BAHODIR x SEL99TER85530	ICARDA, Syria
NDC-728-5	C-44/M	NIFA, Pakistan	NKC-5-S13	SEL99TH15039 x S98008	ICARDA, Syria

NDC-730-2	C-44/M	NIFA, Pakistan	NKC-5-S14	SEL99TH15039 x S98008	ICARDA, Syria
NDC-15-1	Pb-91/M	NIFA, Pakistan	NKC-5-S15	FLIP98-15C x S98033	ICARDA, Syria
NDC-15-2	Pb-91/M	NIFA, Pakistan	NKC-5-S16	S99456 x SEL99TER85314	ICARDA, Syria
NDC-15-3	Pb-91/M	NIFA, Pakistan	NKC-5-S17	S99456 x SEL99TER85314	ICARDA, Syria
NDC-15-4	Pb-91/M	NIFA, Pakistan	NKC-5-S18	(ILC4291xFLIP98-129C) x S98008	ICARDA, Syria
NDC-4-15-1	C-44/M	NIFA, Pakistan	NKC-5-S19	(ILC4291xFLIP98-129C) x S98008	ICARDA, Syria
NDC-4-15-2	C-44/M	NIFA, Pakistan	NKC-5-S20	(FLIP98-138C x SEL99TH15039)	ICARDA, Syria
NDC-4-15-3	C-44/M	NIFA, Pakistan	NKC-5-S21	GLK95069 x SEL99TER85530	ICARDA, Syria
NDC-4-20-1	C-44/M	NIFA, Pakistan	NKC-5-S22	CA9783007 x SEL99TER85534	ICARDA, Syria
NDC-4-20-2	C-44/M	NIFA, Pakistan	NKC-5-S23	CA9783007 x SEL99TER85534	ICARDA, Syria
NDC-4-20-3	C-44/M	NIFA, Pakistan	NKC-5-S24	CA9783007 x SEL99TER85534	ICARDA, Syria
NDC-4-20-4	C-44/M	NIFA, Pakistan	HASSAN-2K	ILC-195/M	NIFA, Pakistan
NDC-4-20-5	C-44/M	NIFA, Pakistan	Karak 1	Local selection	Karak, Pakistan
NDC-4-20-6	C-44/M	NIFA, Pakistan	Karak 2	Local selection	Karak, Pakistan
NDC-4-20-7	C-44/M	NIFA, Pakistan	Karak 3	Local selection	Karak, Pakistan
NDC-5-S10	JG74xICC12071	ICRISAT, India	Sheenghar	Local selection	Karak, Pakistan
NDC-5-S11	JG74xICC12071	ICRISAT, India	Lawaaghar	Local selection	Karak, Pakistan
NIFA-88	6153/M	NIFA, Pakistan	ICC 4993	Rabat	Karnataka, India
NIFA-95	6153/M	NIFA, Pakistan	ICC 19183	ICC 4993	ICRISAT
NIFA-2005	PB-91/M	NIFA Pakistan	ICC 4918	Annigeri	Morocco
			ICC 19181	ICC 435	ICRISAT

Table 1. Presence or absence of nodules, number of nodules plant^{-1} and seed yield plant^{-1} (un- inoculation/control) in chickpea genotypes

Genotype name	Nodules Present/absent	No of Nodules plant^{-1} (uninoculated)	Seed yield plant^{-1} (uninoculated)
NDC-122	Present	8.0	9.4
NDC-727	Present	7.8	6.4
NDC-728-5	Present	7.2	6.8
NDC-730-2	Present	7.9	6.8
NDC-15-1	Present	8.1	14.8
NDC-15-2	Present	7.3	3.6
NDC-15-3	Present	9.3	4.7
NDC-15-4	Present	9.9	8.8
NDC-4-15-1	Present	8.1	4.9
NDC-4-15-2	Present	8.8	6.8
NDC-4-15-3	Present	8.7	4.5
NDC-4-20-1	Present	11.0	17.9
NDC-4-20-2	Present	12.0	5.6
NDC-4-20-3	Present	10.4	4.5
NDC-4-20-4	Present	12.5	4.3
NDC-4-20-5	Present	9.2	5.3
NDC-4-20-6	Present	8.8	14.8
NDC-4-20-7	Present	9.0	4.4
NDC-5-S10	Present	14.5	8.3
NDC-5-S11	Present	9.6	8.6

NIFA-88	Present	6.4	6.7
NIFA-95	Present	6.7	6.0
NIFA-2005	Present	9.1	13.8
NKC-10-99	Present	7.2	6.8
NKC-5-S12	Present	6.6	12.7
NKC-5-S13	Present	6.9	6.5
NKC-5-S14	Present	6.9	13.7
NKC-5-S15	Present	7.7	12.3
NKC-5-S16	Present	4.7	11.4
NKC-5-S17	Present	9.6	12.5
NKC-5-S18	Present	3.1	12.6
NKC-5-S19	Present	6.8	9.3
NKC-5-S20	Present	4.0	12.4
NKC-5-S21	Present	5.1	11.1
NKC-5-S22	Present	7.4	9.5
NKC-5-S23	Present	4.9	11.3
NKC-5-S24	Present	4.9	14.1
HASSAN-2K	Present	7.8	12.1
Karak 1	Present	8.1	17.1
Karak 2	Present	7.9	15.5
Karak 3	Present	6.7	27.8
Sheenghar	Present	5.8	13.4
Lawaghar	Present	2.4	12.6
ICC 4993 (Rabat)	Absent	0.0	7.0
ICC 19183	Absent	0.0	6.0
ICC 4918 (Annigeri)	Absent	0.0	8.4
ICC19181	Absent	0.0	5.9
LSD		2.8	4.16

Table 2. Mean square for inoculation effect on nodules plant^{-1} and seed yield plant^{-1}

Source of variance	Degrees of freedom	Mean squares	
		Nodules plant^{-1}	Seed yield plant^{-1}
Treatment	1	648.3**	1104.01**
Genotype	42	28.6**	201.59**
Treatment X Genotype	42	8.0**	28.72**
Error	172	2.19	11.86

Table 3. Estimates of variability parameters of number of nodules and seed yield plant^{-1}

Variability parameters	Number of nodules plant^{-1}	Seed yield plant^{-1}
Genotypic variance	3.51	21.3
Phenotypic variance	6.62	27.9
Genotypic coefficient of variation	17.48	43.6
Phenotypic coefficient of variation	32.85	49.9
Heritability (broad sense)%	53.2	77

Table 4. Effect of rhizobial inoculation on nodules plant^{-1} in chickpea genotypes

NDC-15-2	7.3 K-N	12.6 CD	10.0 F-K	72.6
NDC-15-3	9.3 E-I	12.3 C-F	10.8 E-H	32.2
NDC-15-4	9.9 D-F	16.4 AB	13.2 A-C	65.6
NDC-4-15-1	8.1 G-L	11.2 D-H	9.6 G-L	38.2
NDC-4-15-2	8.8 E-K	14.9 AB	11.9 C-E	69.3
NDC-4-15-3	8.7 F-K	10.6 G-J	9.6 G-L	21.8
NDC-4-20-1	11.0 B-D	16.6 A	13.5 A-C	45.4

NDC-4-20-2	12.0 BC	15.0 AB	13.5 A-C	25.0
NDC-4-20-3	10.4 C-E	12.4 C-F	11.4 D-F	19.2
NDC-4-20-4	12.5 B	14.8 B	13.6 AB	18.4
NDC-4-20-5	9.2 E-I	11.2 D-H	10.2 F-J	21.7
NDC-4-20-6	8.8 E-K	12.4 C-F	10.6 E-I	40.9
NDC-4-20-7	9.0 E-J	16.5 A	12.7 B-D	83.3
NDC-5-S10	14.5 A	15.1 AB	14.8 A	4.1
NDC-5-S11	9.4 D-H	12.9 C	11.1 D-G	37.7
NIFA-88	6.4 M-P	9.9 H-L	8.1 L-P	54.6
NIFA-95	6.7 L-O	9.9 H-L	8.3 L-P	47.7
NIFA-2005	9.2 E-I	9.4 I-M	9.3 H-M	2.1
NKC-10-99	7.2 K-N	10.8 F-I	9.0 I-N	50.0
NKC-5-S12	6.6 L-O	9.0 J-N	7.8 M-Q	36.3
NKC-5-S13	6.9 L-N	9.6 H-M	8.3 L-P	39.1
NKC-5-S14	6.9 L-N	7.2 OP	7.1 O-R	4.3
NKC-5-S15	7.7 I-M	10.9 E-I	9.3 H-M	41.5
NKC-5-S16	4.7 Q-S	6.9 O-Q	5.8 RS	46.8
NKC-5-S17	9.6 D-G	11.7 C-G	10.6 E-I	21.8
NKC-5-S18	3.1 ST	7.5 NO	5.3 S	141.9
NKC-5-S19	6.8 L-N	8.4 K-O	7.6 N-Q	23.5
NKC-5-S20	4.0 R-T	10.0 H-K	7.0 P-R	150
NKC-5-S21	5.1 O-R	5.8 PQ	5.5 RS	13.7
NKC-5-S22	7.4 J-N	5.4 Q	6.4 Q-S	-27.0
NKC-5-S23	4.9 P-R	12.5 C-E	8.7 J-O	155.1
NKC-5-S24	8.2 G-L	7.5 NO	7.8 M-Q	-8.5
HASSAN-2K	7.8 H-M	8.4 K-O	8.1 L-P	7.7
Karak 1	8.1 G-L	8.3 L-O	8.2 L-P	2.4
Karak 2	7.9 H-M	9.0 J-N	8.5 K-P	13.9
Karak 3	6.7 L-O	10.5 G-J	8.6 J-P	56.7
Sheenghar	5.8 N-Q	11.0 D-I	8.4 K-P	89.6
Lawaghar	2.4 T	7.1 OP	4.8 S	196.0
ICC 4993	0.0	0.0	0.0	0.0
ICC 19183	0.0	0.0	0.0	0.0
ICC 4918	0.0	0.0	0.0	0.0
ICC19181	0.0	0.0	0.0	0.0
Overall mean	7.86 B	10.86 A	8.6	38.1

LSD(0.05) values:
Interaction LSD = 1.81; Genotype means = 1.34; Treatment means = 0.36

Table 5. Effect of rhizobial inoculation on seed yield plant^{-1} in chickpea genotypes

Genotype Name	Un-inoculated	Inoculated	Genotype means	% change
NDC-122	9.4 G-J	14.0 I-M	11.7 G-L	48.0
NDC-727	6.4 J-N	15.0 F-L	10.7 H-M	134.0
NDC-728-5	6.8 J-N	8.0 P-T	7.4 M-P	17.6
NDC-730-2	6.8 J-N	10.0 N-S	8.4 L-O	47.0
NDC-15-1	14.8 B-E	14.2 G-L	14.5 D-H	4.0
NDC-15-2	3.6 N	4.1 T	3.8 P	13.8
NDC-15-3	4.7 L-N	7.0 R-T	5.8 N-P	48.9
NDC-15-4	8.8 G-K	18.2 EF	13.5 E-I	106.8
NDC-4-15-1	4.9 K-N	14.1 H-L	12.0 G-L	187.7
NDC-4-15-2	6.8 J-N	12.0 L-O	9.4 J-N	76.4
NDC-4-15-3	4.5 MN	8.0 P-T	6.2 N-P	77.7
NDC-4-20-1	17.9 B	22.2 B-D	20.0 BC	24.0
NDC-4-20-2	5.6 J-N	5.0 T	5.3 OP	-10.7
NDC-4-20-3	4.5 MN	7.2 Q-T	5.8 N-P	60.0

NDC-4-20-4	4.3 N	6.2 R-T	5.2 OP	44.1
NDC-4-20-5	5.3 K-N	10.1 M-R	7.7 M-P	90.5
NDC-4-20-6	14.8 B-E	24.0 BC	19.4 BC	62.1
NDC-4-20-7	4.4 MN	6.1 ST	5.2 OP	40.0
NDC-5-S10	8.3 I-M	11.2 L-P	9.7 I-N	34.9
NDC-5-S11	8.6 H-L	18.1 E-G	13.3 E-J	110.4
NIFA-88	6.7 J-N	6.2 R-T	6.4 N-P	7.4
NIFA-95	6.0 J-N	9.0 O-S	7.5 M-P	51.0
NIFA-2005	13.8 C-E	25.0 B	19.4 BC	81.1
NKC-10-99	6.8 J-N	11.1 L-Q	8.9 K-O	63.2
NKC-5-S12	12.7 D-G	20.2 C-E	16.4 C-F	59.0
NKC-5-S13	6.5 J-N	19.2 D-E	12.8 F-K	195.3
NKC-5-S14	13.7 C-E	13.1 J-N	13.4 E-I	-4.3
NKC-5-S15	12.3 D-H	23.0 B-D	17.6 CD	86.9
NKC-5-S16	11.4 E-I	12 L-O	11.7 G-L	5.2
NKC-5-S17	12.5 D-H	18.2 EF	15.3 D-G	45.6
NKC-5-S18	12.6 D-G	12.1 L-O	12.3 G-L	-3.9
NKC-5-S19	9.3 G-J	13.0 K-N	11.1 H-M	39.7
NKC-5-S20	12.4 D-H	24.0 BC	18.2 B-D	93.5
NKC-5-S21	11.1 E-I	22.2 B-D	16.6 C-F	100.0
NKC-5-S22	9.5 F-J	13.1 J-N	11.3 H-M	37.8
NKC-5-S23	11.3 E-I	18.0 E-H	14.6 D-H	59.2
NKC-5-S24	14.1 B-E	29.1 A	20.1 BC	106.0
HASSAN-2K	12.1 D-I	14 I-M	13.0 F-J	15.7
Karak 1	17.1 B-C	17 E-J	17.0 C-E	0.0
Karak 2	15.5 D-D	17.1 E-I	16.3 C-F	10.3
Karak 3	27.8 A	33 A	30.4 A	18.7
Sheenghar	13.4 C-F	30.2 A	21.8 B	125.3
Lawaghar	12.6 D-G	16.3 E-K	14.4D-H	29.3
Overall means	10.0 B	15.1 A	12.6	50.5

LSD (0.05) values :
Interaction = 1.11; Genotypes mean = 3.93; Treatment mean = 0.84

Table 6. Correlation coefficients of nodules plant^{-1} with seed yield plant^{-1} under both inoculated and non inoculated levels

	Nodules plant^{-1}	
	Uninoculated	**Inoculated**
Seed yield plant^{-1}	-0.26^{NS}	-0.20^{NS}

DISCUSSION

In the present study significant differences were recorded in the number of nodules and seed yield plant^{-1} among the genotypes. This confirmed that these genotypes showed different response to rhizobia. The reason of this difference is the interaction between bacteria (*Rhizobium leguminosarum*) and genotype of plant. The variation in nodulation among chickpea genotypes have also been reported by Tellawi *et al.* (2007), Mensah and Olukoya, (2007) and Gallani *et al*. (2005). Similarly, Muhammad and Khalil (2013) recorded significant variation in the seed yield of soybean genotypes. Values of both phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV) were moderate which showed that, adequate amount of variability exists for the said trait. Moderate to high heritability indicated that genetic improvement of the under study traits in chickpea can be achieved successfully. As moderate to high heritability shows that genotype has a prominent role in the phenotypic expression. This means that for character under study genotypic variance in the phenotype is more as compare to environmental variance, so selection for this trait is reliable and can bring permanent improvement in the genotypes.

Rhizobial strain, *Rhizobium leguminosarum* was used to test the 47 genotypes for their nodulation expression. All genotypes developed normal nitrogen fixing nodules except four, procured from ICRISAT India, which didn't produce any nodule in control as well as inoculated treatments. In un-inoculated pots majority of genotypes showed nodulation confirming the presence of existing indigenous rhizobia in the soil. The nitrogen fertilizer was not applied to the pots; however the plants didn't show any nitrogen deficiency symptoms which confirmed the role of indigenous rhizobia appeared to fix atmospheric nitrogen at reasonable levels. Furthermore, the level of indigenous rhizobia might be less than 300 rhizobia g^{-1} of soil because rhizobium inoculation significantly increased the nodules number and seed yield $plant^{-1}$, as reported by Dilworth *et al.* (2008) that 300 rhizobia g^{-1} is the threshold value to differentiate between soils that would not respond to inoculation. The seed inoculation with rhizobium significantly increased the nodules and seed yield $plant^{-1}$. After inoculation, the nodules $plant^{-1}$ and seed yield $plant^{-1}$ increased at the rate of 38.1% and 50.5 % respectively, over the un-inoculated chickpea genotypes. The possible reason for significant increase in the nodules $plant^{-1}$ might be the increased rhizobial population per gram of soil. Due to increased rhizobia in the soil, the nodules $plant^{-1}$ increased which may possibly result in greater nitrogen fixation and eventually the yield components might be influenced positively which resulted in significant increase in the seed yield $plant^{-1}$. A similar promotive effect of inoculation on nodules and seed yield $plant^{-1}$ in chickpea was also observed by Akhter *et al*. (2013); Bhuiyan *et al.* (2008); Romdhane *et al.* (2008); Tellawi *et al*. (2007); Khattak *et al*. (2006). Better effect of rhizobium inoculation on seed yield and yield components of common Vetch (*Vicia sativa* L.) were also shown by Albayrak *et al*, (2006)

The effect of inoculation on nodules number and seed yield $plant^{-1}$ was different in different genotypes; in some genotypes it was very high (more than 100%). Many genotypes were recorded with medium to high (50 to 100%) response. A large number of genotypes showed low to medium (less than 49%) increase and at the same time a small number of genotypes were observed with no response or somewhat decrease in nodules and seed yield $plant^{-1}$. The possible explanation of differences in response of miscellaneous genotypes to rhizobium inoculation could be the compatibility and interaction between rhizobium and genotypes. Romdhane *et al*. (2007) also found variable response to inoculation in different chickpea cultivars. Moreover, Katy (2009) reported that the benefits of symbiosis with rhizobia for *Madicago truncatula* ranged from uncooperative to highly mutualistic. He also reported significant genotype by rhizobial strain interaction for yield and yield components. They concluded that, the performance of rhizobia strain is significantly modified by the plant genotype, the strain which was highly effective in one cultivar could be rated moderately or less efficient in other cultivar. Saideh *et al.* (2004), also detected significant rhizobia × plant cultivar interaction for symbiotic parameters and plant growth in annual medics. Reports of Otieno *et al.* (2009), also indicated the variable influence of inoculation on grain legumes that depends on specie, parameter being measured and other environmental factors. The above mentioned observations identified the importance of considering both symbiotic partners while attempting to improve different plant parameters.

The result of genotype means for nodules $plant^{-1}$ and seed yield $plant^{-1}$ (Tables 4 and 5) showed non significant negative association between these two parameters. One possible explanation may be the complexity of yield character which is the product of several other traits. Our results are in line with those of Bhuiyan *et al*. (2008) who didn't observe any significant correlation between seed yield $plant^{-1}$ and nodules $plant^{-1}$ in chickpea. Moreover, Vieira *et al*. (2001) also reported that the increase in number of nodules was not directly reflected in soybean yield. They suggested that the reason for such response of yield to nodules number might be that induction of more nodules may provoke a diversion of carbohydrate to maintain the metabolic activities of a larger mass of nodules.

Conclusion: From the present study it was concluded that nodulation and seed yield of chickpea can be improved by inoculation with compatible rhizobia which could be economically feasible to increase chickpea production. Furthermore, breeding efforts are needed to develop chickpea genotypes (having positive and highly significant association with rhizobium strains that found in our soils or with which these genotypes are inoculated) that produce nodules consistently across a wide range of chickpea growing regions in Pakistan to overcome problem of nitrogen deficiency. Among the studied genotypes Karak-3 and Sheenghar showed best performance across environments (inoculated and un-inoculated), therefore, it is recommended for general cultivation. While, NDC 5-S10 was recorded as the highest nodulating genotype and so it could be used successfully in crop rotation programs

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