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Research Article

Effect of rhizobium inoculation on morphological and yield contributing traits in chickpea (*Cicer arietinum* L.)

Muhammad Adil Younis[1*], Rozina Gul[2], Muhammad Adil[1], Saboor Naeem[1], Muhammad Sajid[1], Ayesha Riaz[1] and Sajid Ali[3]
1. Department of Agriculture, Hazara University, Dhodial, Mansehra-Pakistan
2. Department of Plant Breeding and Genetics, The University of Agriculture Peshawar-Pakistan
3. Department of Agronomy, The University of Agriculture Peshawar-Pakistan
**Corresponding author's email:* muhammadadil@aup.edu.pk

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Abstract

To evaluate the effect of rhizobium inoculation on morphological and yield contributing traits in chickpea (*Cicer arietinum*L.) cultivars under inoculated and non-inoculated production systems during 2011-12 at The University of Agriculture Peshawar, Pakistan. Randomized complete block design were used with three replications. The Genotype × Environment interaction values for seed yield plant^{-1}ranged from 5.73 g to 15.86 g attained by genotypes NKC-5-S-17 and NDC-5-S-11, respectively. Genotype NDC-5-S-11 displayed highest values for seeds plant^{-1} (63.67), and harvest index (39.59%) in inoculated conditions. The same genotype (NDC-5-S-11) outshined for yield (14.67 g) and yield associated traits under non-inoculated production system as well as it exhibited maximum seed yield plant^{-1} (15.27 g) and largest harvest index across both environments. However, genotype NKC-5-S-24 exhibited minimum days to 50% flowering (124.83 days) and maximum nodules plant^{-1} across both environments. Highest plant height (66.83 cm) across both systems was showed by NKC-5-S-15 and NKC-5-S-21. Genotype NKC-5-S-14 took minimum days (168.5 days) to mature. Yield plant^{-1} showed highly significant and positive correlation with pods plant^{-1}(r_i = 0.729) (r_n = 0.824), seeds plant^{-1} (r_i = 0.862) (r_n = 0.865) and harvest index (r_i = 0.885) (r_n = 0.828) under inoculated (r_i) and non-inoculated (r_n) environments. Therefore, pods plant^{-1}, seed plant^{-1} and harvest index can be exploited as indirect selection criteria for selecting high yielding chickpea genotypes. NDC-5-S-11 performed better for yield and yield associated traits across the two environments, moreover its performance under inoculated environment was more prominent, therefore, this genotype can be used in future breeding programs and is recommended for crop rotation program to get maximum yield.

Keywords: Biozote Thal-08; Chickpea; Correlation;Rhizobium inoculation

Introduction

Chickpea (*Cicer arietinum* L.) is an important winter pulse crop that belongs to genus *Cicer*, tribe Cicereae and family Fabaceae. The name *Cicer* is of Latin source, derivative of the Greek word 'kikus' meaning force or strength [1]. The word *arietinum* is also found to have Latin origin, that equivalent of Greek word *'krios'*, used for chickpea[2]. It originated in southeastern Turkey [3]. According to archeological evidences, earliest domestication of chickpea is reported in the Middle East and indo-pak sub-continent. Facts also explain that it has been cultivated in Ethiopia and Mediterranean area, since the distant past [4].

Chickpea is one of the world's most important leguminous crops that provide food for human as well as for livestock. Chickpea seeds are consumed fresh as green vegetables, fried, roasted, and boiled, as snack food. Seeds are ground and the flour can be used as soup, dhal, and to make bread. Dhal is the split chickpea without its seed coat, dried and cooked into a thick soup [5]. Sprouted seeds are also utilized as a vegetable or added to salads. Young plants and green pods are eaten like spinach. Animal feed is a further use of chickpea in many developing countries. Gram husks, and green or dried stems and leaves are used as fodder.

Pakistan with an annual production of 842 thousand tones is ranked second in the production of chickpea after India (5970 thousand tones) in the world [6].From the total cropped area of Pakistan 7% area is under the cultivation of pulses out of which 73% is under chickpea cultivation. In Pakistan, chickpea production is 496 thousand tons which is produced from a canopy of 1055 thousand hectares with an average yield of 471 Kg ha^{-1}[6]. In Khyber Pakhtunkhwa it is cultivated on an area of 37.6 thousand hectares with a production of 21.0 thousand tons and an average yield of 532 kg ha^{-1}[7].In KP about 75% chickpea is grown on rainfed land and its cultivation is concentrated in the southern districts of the province including D. I. Khan, Tank, Lakki Marwat, Bannu and Karak.

Genetic variability is a basic source for improvement of crops as it provides raw material to professional breeders to recombine the genes of various traits in same plant for advancement of desirable variety. Plant genetic resources are the basis of worldwide food security. They hold diversity of genetic material contained in crop wild relatives, other wild species and synthetic cultivars. Sensible utilization of plant genetic resources can be used as a key to meet the mounting demand of ever-increasing population [8].

Amino acids are needed by plants to form proteins and nitrogen is one of the most essential elements for the production of amino acids. Moreover nitrogen acts as a vital element for plant metabolism. Plants cannot consume nitrogen in atmospheric form rather they can utilize it only in ionic form of either nitrate (NO_3) or ammonium (NH^+). Chickpea is considered to sustain cropping system productivity due to its capability to fix atmospheric nitrogen. This crop bears nodules on its roots where *rhizobium* resides with a specific function of renovating the atmospheric nitrogen into plant accessible form called biological nitrogen fixation (BNF). Through BNF, considerable amount of cost nitrogen is deposited in the soil which can be exploited by the same crop and the subsequent one [9]. The effectiveness of such crop in fixing maximum nitrogen depends upon the cultivar, number of nodules and the competent strain of the *rhizobia* existing in their root nodules.

When it comes on the farmer's field the average yield of chickpea is very low than its potential yield. The reasons reported for the dramatic decline of the chickpea production with respect to its requirements are unavailability of good quality seed, absence of effective *rhizobial* strain and severe damage by blight and pod borer attack. Moreover the soils of Pakistan are generally deficient in nitrogen; which is most important element for the plant metabolism and protein synthesis. Its deficiency in soil usually results in low crop yield. Inoculation of chickpea seeds by artificial means in such soils which are deficient in native effective *rhizobia* is a very useful practice for improving root nodulation and yield of the crop [10]. Moreover Inoculations with *Rhizobium* strains produce a greater number of nodules and thus fix greater amount of nitrogen which not only increase the production of inoculated crop but also abscond a good amount of nitrogen in the soil, which benefits the subsequent crops [11].

The information of interrelationship among different economic and morphological characters facilitate professional breeder to set the criteria of selection for the breeding of improved cultivars [12]. Correlation studies deliver a better understanding of the association of various characters with grain yield [13, 14]reported that several of the traits in chickpea were strongly associated among themselves as well as with seed yield. Correlation analysis of yield contributing trait and their relationship with the yield is fundamental to launch selection criteria.

The production can possibly be increased up to much extent by exploiting better colonization of the roots and rhizospheres through the application of effective nitrogen fixing bacteria to the seed or to the soil. Inoculation treatment of competitive rhizobial strain to good responsive cultivars can minimize uses of nitrogenous fertilizer, which is very costly in our country. Using high yielding varieties/advanced lines of chickpea in combination with effective rhizobial strains along with management practices can enhance the yield. Hence the objectives of this study were to study the effect of *rhizobium* inoculation on different morphological and yield contributing traits in chickpea genotypes, to evaluate and compare the association of various characters and their contribution to determine seed yield under inoculated and uninoculated production systems and to identify the genotype by rhizobium interaction in terms of yield contributing traits and nodules plant^{-1}.

Materials and methods

Fourteen chickpea genotypes were evaluated as independent experiments under non-inoculated and inoculated production systems The experiment was plotted in randomized complete block (RCB) design comprising three replications under both production systems (non-inoculated and inoculated). Each genotype was planted in a plot of three rows, each of 4 meter length, with a row to row and plant to plant distance of 30 and 10 cm, respectively. Plots were separated with a path distance of 60 cm. Rhizobial inoculant Biozote “Thal-08” was collected from Land Resources Research Institute, National Agriculture Research Centre, (NARC) Islamabad. To prepare the inoculation slurry, first 5% sugar solution was prepared and then 40 g of inoculant was added in 300 ml of sugar solution. The mixture was stirred well. Sugar solution improves the level of adhesion of inoculum to the seed. After pouring the inoculation slurry on seed, it was mixed in a clean glass jar so that all seeds would have uniformly been coated with it. The whole inoculation operation was carried out in the shady place as sunlight damages the bacteria. Data were recorded on number of nodules plant^{-1}, plant height, days to maturity, number of pods plant^{-1}, number of seeds plant^{-1}, yield plant^{-1} and harvest index.Collected data were analyzed Repeated Measures Design in statistical software STATISTIX in order to reckon Genotype × Environment Interaction. Further comparison among the mean values for each parameter under both production schemes was made by using least significant difference (LSD) test at 5% probability lavel.Correlation among different characters and their involvement to define seed yield was determined for both inoculated and non-inoculated systems by following procedure of Pearson correlation by using the following formula[15].

$$r = \frac{N\Sigma xy - (\Sigma x)(\Sigma y)}{\sqrt{[N\Sigma x^2 - (\Sigma x)^2]\left[N\Sigma y^{2}{}^{2}(\Sigma y)\right]}}$$

Results and discussion

Combined analysis for divulgence revealed significant differences between environments for nodules plant^{-1}, plant height and days to maturity. Genotypes were found significant for plant height, pods plant^{-1} and seeds plant^{-1}. Similarly all studied traits significantly differed for genotype × environment interaction (Table 1). The most promising genotype across both environments was NDC-5-S-11 for pod plant^{-1}(52.5), seeds plant^{-1} (56.5 cm), yield plant^{-1} (15.27 g) and harvest index (37.18%). Genotype NKC-5-S-14 took minimum days to maturity (168.5 days), whereas NKC-5-S-24 showed maximum number of nodules plant^{-1} (92.83), Maximum plant height (66.83 cm) was recorded for NKC-5-S-15.

Highest number of nodules plant^{-1} (134) were recorded for inoculated environment while lowest (27) for that of non-inoculated (Table 2).These results are the confirmation of prior scientific findings of **[16, 17]**. Shortest plant (40.27 cm) was observed in inoculated while tallest plants (70 cm) in control (Table 2). **[18, 19]** also stated similar kind of results in chickpea. For days to maturity non-inoculated environment showed greatest (177 days) while inoculated environment showed least magnitude (162 days) (Table 3). Findings of **[20]** were found similar to that of our results. Minimum pods plant^{-1}(14.67) were observed for non-inoculated while maximum (59.33) for treated environment (Table 2). **[18, 19]** also supported the increasing trend of measure of fruit plant^{-1} due to rhizobium inoculation in chickpea. Maximum seeds plant^{-1} (63.67) were produced in inoculated environment while control displayed minimum (15.67) value for the same trait (Table 3).These findings are in accordance with the result of **[19]**.Inoculated environment showed maximum yield plant^{-1} (15.87 g), while control displayed minimum value for yield plant^{-1} (5.73 g) (Table 3). This is probably due to greater nitrogen fixation by compatible rhizobium colonies in nodules and eventually the yield would have been enhanced. Such positive effect of rhizobia on yield plant^{-1} has also been reported by **[9, 20, 21]**. Harvest index reflected excellent performance under inoculated trail as maximum value (39.59%) was attain by it, while minimum H.I. (15.67%) was recorded under non-inoculated conditions (Table 4). These results are the conformation of previous scientific findings of **[18]**.

The coefficient of correlation revealed that yield plant^{-1} had a strong positive significant correlation with pods plant^{-1} (r_i = 0.729) (r_n = 0.824), seeds plant^{-1} (r_i = 0.862) (r_n = 0.865) and harvest index (r_i = 0.885) (r_n = 0.828) under both inoculated (r_i) and non-inoculated (r_n) environments (Table 5). **[22]** Previously reported positive association yield with seeds plant^{-1} and total dry weight in chickpea. More over **[23]** reported a direct relation of pods plant^{-1} with yield plant^{-1} in chickpea.

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Table 1. Mean squares for number of nodules plant^{-1}, plant height number of pods plant^{-1}, days to maturity, number of seeds plant^{-1}, yield plant^{-1} and harvest index of 14 chickpea genotypes across two cropping systems (inoculated and non-inoculated) at University of Agriculture, Peshawar during 2011-12

Sources	d.f	Number of nodules plant^{-1}	Plant height	Pods plant^{-1}	Days to maturity	Seeds plant^{-1}	Yield plant^{-1}	Harvest index
Systems (S)	1	57200.8**	362.337**	30.964	1085.76**	10.012	0.9219	64.638
Rep w/n sys.	4	16.2	33.558	19.488	5.51	8.976	0.8869	9.472
Genotypes (G)	13	978.0	215.433**	467.378**	29.05	383.016	24.7678	155.874*
G × S	13	538.8**	47.083**	124.426**	27.17**	174.679**	11.2670**	58.781**
Pooled error	52	13.8	13.981	16.962	5.06	13.592	0.8493	15.053
CV (%)		5.47	7.43	14.75	1.32	10.55	9.42	14.57

d.f = Degree of freedom, * = Significant at 5% probability, ** = Significant at 1% probability

Table 2. Means for Number of nodules plant^{-1}, plant height and number of pods plant^{-1}of 14 chickpea genotypes across two cropping systems (inoculated and non-inoculated) at University of Agriculture, Peshawar during 2011-12

Genotypes	Number of nodules plant^{-1}			Plant height			Pods plant^{-1}		
	Inoculated	Non-inoculated	Avg.	Inoculated	Non-inoculated	Avg.	Inoculated	Non-inoculated	Avg.
NDC-15-4	96.33	27.00	61.67	44.63	56.47	50.55	18.00	21.33	19.67
SL-05-53	110.33	47.00	78.67	45.73	49.47	47.60	40.67	33.00	36.83
NDC-4-20-2	100.33	50.67	75.50	47.80	55.00	51.40	16.67	23.00	19.83
SL-08-14	104.33	51.33	77.83	40.27	45.33	42.80	36.00	22.00	29.00
NDC-5-S-11	106.33	37.00	71.67	53.27	53.33	53.30	59.33	45.67	52.50
SL-03-15	115.67	42.67	79.17	48.73	46.47	47.60	30.67	26.67	28.67
NDC-15-1	83	31.33	57.17	55.73	51.13	53.43	20.00	18.67	19.33
NKC-5-S-13	66	50.67	58.33	44.33	55.00	49.67	15.33	27.00	21.17
NKC-5-S-14	59.67	32.67	46.17	42.30	49.27	45.78	25.67	28.33	27.00
NKC-5-S-15	86.33	42.67	64.50	62.67	71.00	66.83	20.33	29.33	24.83
NKC-5-S-17	83.67	29.67	56.67	52.73	47.03	49.88	31.33	14.67	23.00
NKC-5-S-20	100.33	46.00	73.17	43.68	45.47	44.57	28.33	31.33	29.83
NKC-5-S-21	69	42.33	55.67	42.93	48.13	45.53	21.67	29.67	25.67
NKC-5-S-24	134	53.67	93.83	50.47	60.33	55.40	35.33	31.67	33.50
Mean	93.95	41.76	67.86	48.23	52.39	50.31	28.52	27.31	27.92
LSD for separate env	8.17	3.29		6.53	6.00		7.44	6.32	
LSD for G averaged over env			----			8.558			13.91
LSD for env			10.94			3.234			----
LSD for G × E			6.0813			6.1262			6.4779

Table 3. Means for days to maturity, number of seeds plant^{-1} and yield plant^{-1} of 14 chickpea genotypes across two cropping systems (inoculated and non-inoculated) at University of Agriculture, Peshawar during 2011-12

`	Days to maturity			Seeds plant^{-1}			Yield plant^{-1}		
	Inoculated	**Non-inoculated**	**Avg.**	**Inoculated**	**Non-inoculated**	**Avg.**	**Inoculated**	**Non-inoculated**	**Avg.**
NDC-15-4	169.33	175.00	172.17	36.67	38.33	37.50	10.07	9.17	9.62
SL-05-53	162.67	177.00	169.84	47.67	39.33	43.50	11.97	11.3	11.63
NDC-4-20-2	173.33	176.67	175.00	33.00	36.67	34.83	7.17	8.33	7.75
SL-08-14	162.00	176.67	169.34	42.67	30.00	36.33	12.27	8.2	10.23
NDC-5-S-11	168.33	173.67	171.00	63.67	49.33	56.50	15.87	14.67	15.27
SL-03-15	163.33	172.67	168.00	36.67	32.00	34.33	7.63	8.93	8.28
NDC-15-1	164.00	173.33	168.67	40.33	35.00	37.67	10.17	8.6	9.38
NKC-5-S-13	171.67	176.33	174.00	26.00	40.67	33.33	10.6	13.23	11.92
NKC-5-S-14	162.33	174.67	168.50	22.67	40.33	31.50	6.3	11.5	8.9
NKC-5-S-15	170.33	172.33	171.33	22.00	29.33	25.67	7.47	9.37	8.42
NKC-5-S-17	171.00	176.00	173.50	34.00	15.67	24.83	10.9	5.73	8.32
NKC-5-S-20	170.67	172.33	171.50	30.33	34.67	32.50	7.87	9.73	8.8
NKC-5-S-21	169.67	175.00	172.34	22.67	31.00	26.83	6.93	9.33	8.13
NKC-5-S-24	165.33	173.00	169.17	35.67	32.00	33.83	10.37	10.4	10.38
Mean	167.43	174.62	171.02	35.29	34.60	34.94	9.68	9.89	9.79
LSD for separate env	4.67	2.60		8.11	3.28				
LSD for G averaged over env			----			----			
LSD for env			2.457			----			
LSD for G × E			3.6867			6.0403			0.7537

Where LSD = Least significant difference at 5% probability level, G = Genotype, Env = Environment, G × E = Genotype × environment interaction

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Table 4. Means for harvest index of 14 chickpea genotypes across two cropping systems (inoculated and non-inoculated) at University of Agriculture, Peshawar during 2011-12

Genotypes	Harvest index		
	Inoculated	**Non-inoculated**	**Avg.**
NDC-15-4	30.10	21.72	25.91
SL-05-53	28.77	30.66	29.71
NDC-4-20-2	25.36	20.29	22.83
SL-08-14	34.73	27.74	31.23
NDC-5-S-11	39.59	34.77	37.18
SL-03-15	26.21	25.77	25.99
NDC-15-1	30.11	22.70	26.41
NKC-5-S-13	31.91	32.24	32.07
NKC-5-S-14	19.66	31.34	25.50
NKC-5-S-15	16.16	17.73	16.95
NKC-5-S-17	27.93	15.67	21.80
NKC-5-S-20	25.45	28.69	27.07
NKC-5-S-21	21.48	26.06	23.77
NKC-5-S-24	27.55	23.10	25.33
Mean	27.50	25.60	26.55
LSD for separate env	8.14	4.29	
LSD for G averaged over env			9.562
LSD for env			----
LSD for G × E			6.3567

Where LSD = Least significant difference at 5% probability level, G = Genotype, env = Environment, G × E = Genotype × environment interaction

Table 5. Phenotypic correlation of number of nodules plant^{-1}, plant height, days to maturity, number of pods plant^{-1} number of seedsplant^{-1} and harvest index with yield plant^{-1} among chickpea genotypes across inoculated and non-inoculated production systems

Parameters	Inoculated	Non-inoculated
Number of nodules plant^{-1}	.359	.186
Plant height	.101	.178
Days to maturity	-.155	-.080
Number of pods plant^{-1}	**.729****	**.824****
Number of seeds plant^{-1}	**.862****	**.865****
Harvest index	**.885****	**.828****

Conclusion

Genotype NDC-5-S-11 displayed highest values for number of pods plant $^{-1}$, seeds plant^{-1}, and harvest index in inoculated conditions. The same genotype (NDC-5S-11) outshined for yield (14.67 g) and yield associated traits under non-inoculated production system as well as it exhibited maximum seed yield plant^{-1}, highest pods plant^{-1} and largest harvest index across both environments. The potential of this genotype can be exploited in future breeding program. Also the same genotype can be used in crop rotation system to diminish the threat of soil degradation and to get higher yield. Correlation studies revealed that yield plant^{-1}was significantly and positively correlated with pods plant^{-1}, seeds plant^{-1} and harvest index under inoculated and non-inoculated consequences at a time. Therefore, these parameters can be used in future breeding programs as indirect selection standard to improve yield.

Authors' contributions

Conceived and designed the experiments: R Gul, Performed the experiments: MA Younis & M Adil, Analyzed the data: Muhammad Adil Younis, Contributed materials/ analysis/ tools: R Gul, MA Younis, M Adil, S Naeem, M Sajid, A Riaz & S Ali, Wrote the paper: M Adil.

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