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Research Article

Genotypic variability for root nodulation and identification of high yielding chickpea (*Cicer arietinum* L.) genotypes

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Abstract
Evaluating diverse chickpea genotypes for growth, root nodule, and yield parameters can provide valuable insights to identify genotypes with desirable traits for improved crop production. The investigation on root nodule parameters can shed light on the nodulation potential and nitrogen-fixing efficiency of the genotypes and its role in enhancing grain yield as well as soil health. Hence, the present study was carried out involving 30 diverse chickpea genotypes including 3 checks and was evaluated under natural soil condition for agronomic traits associated with high root nodulation. Under natural soil condition (Sandy loam) chickpea nodule formation and nitrogen fixation ability were observed to vary significantly. The significant differences observed among the genotypes for all the parameters indicate the presence of genotypic variability. The flowering and pod formation stages demonstrated a significantly higher number of nodules per plant compared to other stages. Regarding the distribution of root nodules, it was found that the pre-flowering stage had the highest number of nodules on the primary roots. However, as the plant progressed into the later stages of flowering and podding, the nodules shifted to the secondary roots. Several traits have been found to correlate positively and significantly with seed yield. The genotypes namely BRHT-8, Sabour chana-2, BRHT-12, JG-218, ICC-67, BRHT-1, and BRHT-6 exhibited considerably high nodulation and seed yield/hectare.

Keywords:- Root nodules, Growth stages, Genotypic variability, Correlation.

INTRODUCTION

The symbiotic relationship between legumes and rhizobia, leading to nitrogen fixation allows leguminous plants to convert atmospheric nitrogen into a usable form, thus enriching the soil with nitrogen, an essential nutrient for plant growth. Legumes have long been recognized and valued for their ability to enhance soil quality through biological, chemical, and physical means. The process of biological nitrogen fixation not only benefits the legume plants themselves but also has a positive impact on the surrounding soil ecosystem. It helps increase soil fertility, as the fixed nitrogen becomes available for succeeding crops and organisms in the soil, leading to improved overall soil health. As the demand for food increases due to a growing global population, the use of biofertilizers, including rhizobial inoculants, has gained significance in crop production.

Pulses, being an important group of leguminous crops, play a significant role in providing protein-rich food for human consumption. Additionally, they contribute high-quality crop residues that can serve as valuable animal feed, further enhancing the overall agricultural productivity (Herridge *et al.,* 1995; Siddiqi and Mahmood, 2001; Kantar *et al.,* 2007, Shaikh *et al.* (2020)). Moreover, the biological nitrogen fixation ability of pulses helps reduce the dependence on synthetic nitrogen fertilizers, which can be costly and have adverse environmental impacts like soil degradation and water pollution (Roy *et al.*, 2018).

Chickpea (*Cicer arietinum* L.) is the third most important legume crop in the world after dry beans (*Phaseolus vulgaris*) and dry peas (*Pisum sativum*). India is the largest chickpea-producing nation in the world with 10.74 million hectare area yielding 13.54 million tonnes and 1261 kg/ha productivity (Directorate of Economics and Statistics, Department of Agriculture Cooperation and Farmers Welfare 2021 -22). Gujarat, Madhya Pradesh, Telangana, Andhra Pradesh, Maharashtra, Rajasthan, and Uttar Pradesh are the major chickpea-producing states of India. However, in Bihar, an area of 0.52 lakh ha under chick pea yields 0.54 lakh tonnes with a productivity of 1052 kg/ha (Directorate of Economics and Statistics, Department of Agriculture Cooperation and Farmers Welfare 2020-21). Chickpea contain about 18 to 22 per cent protein, 61-62 per cent carbohydrates, 4.5 per cent fat, 280 mg/100 g calcium, 301 mg/100 g phosphorus and trace vitamins and iron (NFSM 2016). Its leaves are rich in malic and citric acid. It helps in curing stomach ailments and acts as a blood purifier.

Chickpea have the remarkable ability to fix atmospheric nitrogen through a symbiotic relationship with nitrogen-fixing bacteria called rhizobia. This process takes place within specialized structures known as root nodules, which are formed as a result of the interaction between the legume plant's roots and the rhizobia. Root nodules are knob-like protuberances that develop on the roots of leguminous plants. Inside these nodules, the atmospheric nitrogen is converted into ammonia, a form of nitrogen that the plant can utilize for its growth and development. This biological nitrogen fixation is a crucial process as it allows chickpea to access nitrogen from the air, which is otherwise unavailable to most plants. The formation of root nodules typically occurs around 40 to 50 days after the emergence of plant primordial and the nodules provide a favorable environment for the growth of rhizobia, allowing them to convert nitrogen gas into ammonia within the plant tissues (Velde *et al.,* 2006). Furthermore, the nodules help absorb ammonia produced during nitrogen fixation, preventing its loss from the soil. When the legume plant dies or is harvested, the fixed nitrogen present in the nodules is released into the soil, enriching it with available nitrogen. This residual nitrogen benefits subsequent crops, making legumes valuable as green manure or cover crops.

A well-designed study on root nodules is essential to address critical aspects of chickpea breeding program to avoid huge dependency on synthetic fertilizers. So identifying chickpea genotypes with the highest number of root nodules under natural soil conditions is crucial in selecting parents for breeding programs. Evaluating these traits will provide insights into how efficiently different genotypes fix nitrogen from the atmosphere and utilize it for their growth and development naturally. The study's also focus on exploring the association between yield parameters and root nodulation is particularly significant. Understanding the relationship between these two traits can help breeders identify genotypes that exhibit both high yield potential and efficient nitrogen fixation. Such genotypes would be highly desirable for sustainable agriculture, as they can contribute to increased crop productivity while reducing the reliance on external nitrogen fertilizers.

Overall, this study contributes to the advancement of chickpea breeding and crop improvement efforts. By characterizing the genetic diversity and root nodulation traits of various desi chickpea genotypes, breeders can make informed decision to develop improved varieties that are better adapted to local agro-climatic conditions and possess superior agronomic traits. Ultimately, this research can lead to the identification of chickpea cultivars that enhance food production, improve soil health, and promote sustainable agricultural practices.

MATERIALS AND METHODS

The pilot research work was initiated at the pulse research farm of Bihar Agricultural University Sabour. The farm area selected for the research had loamy sand soil with low soil Nitrogen content, specifically less than 30 parts per million (PPM). The geographical location of the farm falls under the Middle Gangetic plain region, which is categorized as agro-climatic zone IIIA of Bihar. The exact coordinates of the site are approximately 25°15'40" N latitude and 87°2'42" E longitude. The farm is situated at an elevation of 46 meters above the mean sea level.

During the initial screening phase, 70 chickpea genotypes with different genetic backgrounds were evaluated for root nodulation and yield during the *Rabi,* 2020. Among the 70 genotypes evaluated, some showed significant variability for root nodulation under natural soil conditions. A total of 27 selected genotypes from the previous year's evaluation, which exhibited significant variability for root nodulation traits, were sown along with three checks (PG-186, Sabour chana-2, and Pusa-256) during *rabi,* 2021. The experimental design adopted was the Randomized Complete Block Design with three replications. Each genotype was sown in rows of 4 meters length with the spacing of 30 cm x 10 cm. Standard agronomic practices and management strategies were meticulously implemented to promote optimal crop growth.

The natural variation in nodulation and related traits in chickpea genotypes without external inoculation of rhizobium was assessed at four different physiological stages namely pre-flowering (at 45 days), flowering stage (at 65 days), podding stage (at 95 days), and maturation stage (at 105 days). The procedure for assessment involved taking five random plants from each genotype in each replication at each stage. These plants were gently uprooted, and special care was taken to lift and wash the root systems to avoid damage. Observations were recorded on number of nodules per root system, number of nodules on primary root, number of nodules on secondary roots, length of primary roots, length of secondary roots, and dry weight of root nodules. For the determination of root nodule dry weight, the chickpea root systems were cleaned of soil, and the number of nodules per root system was counted. Nodules were carefully detached and placed in aluminium foil. These nodules were later oven-dried at 80°C for 36 hours and then weighed. Diseased, chlorotic, or atypical nodules were excluded from the sample to ensure accurate results. Along with root-related traits, data on harvest index (%), seed yield (kg/ha), and number of pods/plant were recorded. Data on chlorophyll content (mg/g) were also recorded to investigate the relationship between nodule number and chlorophyll pigment content. Chlorophyll content (mg/g) was estimated by following the method proposed by Arnon (1949).

Mean data of all the traits were subjected to Analysis of Variance (ANOVA) as per Panse and Sukatme (1985). Significance of means were tested based on critical difference. Phenotypic correlation coefficient was estimated from the components of variance and covariance as per Johnson *et al.* (1955). All the analyses were carried out in R- software (R version 4.2.1).

The diversity analysis was calculated by Mahalanobis D^2 statistic as explained by Rao (1952). In D^2 analysis, the comparative importance of a trait is known by the percentage variation produced by the trait and therefore avoiding the bias in choice. Hence, by using the D^2 values the genotypes bearing significant difference in root nodules were grouped into various clusters.

RESULTS AND DISCUSSION

For all the traits under study, the mean sum of squares due to genotypes was found to be significant, for all the traits indicating that there was a significant level of genotypic variability among the genotypes (**Table 1**). Pravallika and Lavanya, 2022, Roy *et al.* (2018), Priyadarsini *et al.* (2017), Khanam *et al.* (1994) and Gupta and Namdeo (1996a) also reported a similar observation for number of nodules plant^{-1}, nodule dry weight, and seed yield plant^{-1} among the chickpea germplasm and Rahim *et al.* (2017) in soybean.

Nodules "the factories of nitrogen" are an important symbiotical and economic trait in the pulse family. The nodule number and its dry weight are the indexes of the degree of infection leading to the development of nodules. So, root nodule assessment was carried out at four different physiological stages such as pre-flowering (at 45 days), flowering stage (at 65 days), podding stage (at 95 days), and maturation stage (at 105 days). Out of the total stages studied flowering and podding stages showed a significantly higher number of nodules/plant. Earlier research work also showed that the formation of root nodules typically occurs around 40 to 50 days after the emergence of plant primordial and the nodules provide a favorable environment for the growth of rhizobia, allowing them to convert nitrogen gas into ammonia within the plant tissues (Velde *et al.,* 2006). All root nodule were found effective/ fertile (pink color) till podding stages later on color changed to blackish brown and was waned subsequently senescence of root nodules taken place at maturity. The number of nodules ranged from 2.25 (Pre-flowering) to 26.5 (podding). It has also been observed that at the pre-flowering stage maximum number of root nodules were found on primary roots, however, it was shifted to the secondary root in the later stages (flowering, podding) (Plates 4, 6, 8 are the highest nodulating genotypes and Plates 2, 3, 5, 7 are the lowest nodulating genotypes at 45 days, 65 days, 85 days, and 105 days respectively). By evaluating the number of nodules per root system and the dry weight of the nodules, its efficiency for nitrogen-fixing process and the extent to which the legume plant benefits from the symbiotic relationship could be assessed. These traits provide valuable information for plant breeders and researchers in selecting superior genotypes with strong nodulation capabilities. Genotypes with higher nodule numbers and larger nodule dry weights are indicative of stronger symbiotic interactions, resulting in increased nitrogen fixation and consequently, better nitrogen availability for the plants (Roy *et al*., 2018; Lamptey *et al*., 2014 and Pedersen, 2009).

The mean of 10 best genotypes for all the traits and the overall mean, range, CV, and CD, of all 30 genotypes for all the traits has been presented in **Table 2 (a) and 2 (b).** Nodule-related observations that were taken at different stages, were averaged and correlation was assessed with number of pods per plant, harvest index, chlorophyll content (mg/g), and seed yield (kg/ha). The length of root ranged from 9.17 cm to 13.73 cm with a mean of 11.23 cm at 45 days, from 11.1 cm to 18.38 cm with a mean of 14.46 cm at 65 days and from 14.43 cm to 20.83 cm with a mean of 16.52 cm at 85 days whereas the length of the secondary root varied from 8.6 cm to 13.57 cm with a mean of 10.61 cm at 45 days, from 10.95 to 19.88 cm with a mean of 14.85 cm at 65 days and from 14.73 to 21.33 cm with a mean of 17.44 cm at 85 days. Number of nodules per plant varied from 2.25 to 6 with a mean of 3.96 at 45 days, from 10.5 to 18.25 with a mean of 13.42 at 65 days, from 15 to 26.5 with a mean of 21.46 at 85 days and from 5.5 to 14.25with a mean of 8.9 at 105 days. Nodules dry weight varied between 3.31-9.31 mg/plant with a mean of 5.67 mg/plant at 45 days, from 17.28 mg/plant to 29.6 mg/plant with a mean of 22.07 mg/plant at 65 days, from 35.98 mg/plant to 60 mg/plant with a mean of 48.6 mg/plant at 85 days and from 9.53 to 26.6 mg/plant with a mean of 16.81 mg/plant at 105 days. Number of pods per plant ranged from BRHT-3 (22.53) to BRHT-8 (62.53) with an average of 38.55. The range of the harvest index varied between 34.24 % (BRHT-9) to 55.46 % (BRHT-6) with a general mean of 45.38 %. ICC-67 (52.77 %), GP-24 (52.85%), BRHT-2 (53.47 %), BRHT-6 (55.46%), and BRHT-12 (55.03%) were found significantly superior to the best check Sabour chana-2

Fig. 1. Root nodules at the pre-flowering stage (45 days)

Fig. 2. Root nodules at the flowering stage (65 days)

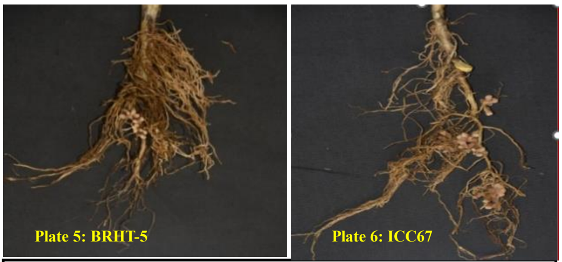

Fig. 3. Root nodules at the podding stage (85 days)

Fig. 4. Root nodules at the maturation stage (105 days)

Table 1. Analysis of Variance of root nodule and yield related traits

	Traits		Mean sum of squares (MSS)		
			Replication	Treatments	Error
1	Number of root nodules/ plant	Pre-flowering	0.31	4.32***	0.02
		Flowering	3.21	13.13**	0.21
		Podding	6.63	45.82***	0.87
		Maturation	0.40	19.74**	0.10
2	Number of nodules on primary roots	Pre-flowering	0.02	0.1**	0.00
		Flowering	0.10	0.35***	0.02
		Podding	0.48	0.5***	0.04
		Maturation	0.03	3.75**	0.01
3	Number of nodules on secondary roots	Pre-flowering	0.08	4.58**	0.01
		Flowering	1.29	14.15***	0.10
		Podding	6.51	48.35***	0.53
		Maturation	0.40	21.47**	0.05
4	Dry Weight of total root nodules	Pre-flowering	0.46	9.14**	0.05
		Flowering	5.66	34.34***	0.60
		Podding	27.65	266.67***	4.28
		Maturation	4.71	75.45**	0.32
5	Length of primary root	Pre-flowering	1.89	3.47**	0.16
		Flowering	4.21	8.99**	0.22
		Podding	1.77	7.21**	0.32
6	Length of secondary root	Pre-flowering	0.63	5.85**	0.13
		Flowering	2.72	12.02**	0.28
		Podding	2.03	5.86**	0.34
7	Chlorophyll content (mg/g FW)		0.04	0.08**	0.00
8	Number of pods/plant		123.84	182.14***	96.56
9	Harvest index (%)		142.65	88.85***	4.34
10	Seed Yield (kg/ha)		292369.10	629302.02**	12229.11

Footnote: P=0.05…*; P=0.01….** ; P=<0.01….***

Table 2 (a): Mean performance and range of 10 best genotypes for various root related traits

Genotypes	Length of Primary root (cm)			Length of secondary root (cm.)			Number of root nodules/ plant @				Number of nodules on primary roots/plant			
	45 days	65 days	85 days	45 days	65 days	85 days	45 days	65 days	85 days	105 day	45 days	65 days	85 days	105 day
BRHT-8	10.57	13.10	15.30	9.55	11.05	17.93	5.50	18.25	25.50	14.25	1.25	4.25	4.50	2.25
BRHT-12	12.82	18.38	20.83	10.18	13.32	16.80	5.50	17.00	19.00	8.50	1.00	4.00	4.50	1.25
JG 218	11.55	13.60	17.23	9.57	13.43	18.77	5.75	16.25	23.50	14.25	1.00	3.75	4.50	1.50
BRHT-1	11.25	13.67	15.43	8.92	19.88	19.90	6.00	16.50	17.00	12.25	1.00	3.75	4.50	1.75
BRHT-6	12.82	13.77	17.15	12.02	15.93	21.33	5.00	15.00	20.75	12.50	1.50	4.00	4.50	2.00
ICC-67	10.55	15.37	17.57	12.42	13.53	16.72	6.50	15.50	26.50	13.25	1.25	3.75	4.75	4.25
BRHT-4	13.37	13.70	14.78	9.40	12.10	14.73	5.00	13.50	22.50	9.75	1.00	4.50	4.50	3.25
BRHT-2	11.02	11.90	16.58	12.98	12.92	15.60	4.00	13.50	21.00	7.50	1.00	3.25	5.00	0.50
JG 14	11.37	16.80	18.17	13.57	15.43	18.75	4.25	14.00	20.25	10.25	1.25	3.50	4.75	4.75
BAU Heat-37	11.15	16.88	17.60	9.78	17.50	18.83	4.50	14.50	18.50	10.50	1.00	3.25	5.25	1.75
PG-186 (C1)	10.15	12.88	14.52	12.22	14.40	15.83	4.00	13.25	22.00	9.00	1.00	4.25	5.25	1.50
SABOUR CHANA-02 (C2)	12.75	13.18	15.23	11.68	14.20	16.82	6.00	17.75	26.00	13.25	1.00	3.50	4.75	4.00
Pusa 256 (C3)	12.78	14.90	16.10	9.28	16.93	18.80	4.50	13.00	24.50	8.00	1.25	3.50	4.25	2.00
Over all Mean	**11.23**	**14.46**	**16.52**	**10.61**	**14.85**	**17.44**	**3.96**	**13.42**	**21.46**	**8.90**	**1.16**	**3.87**	**4.80**	**2.22**
RANGE Max	**13**	**18.38**	**20.83**	**13.57**	**19.88**	**21.33**	**6.50**	**18.25**	**26.50**	**1.75**	**4.50**	**5.75**	**4.75**	**12.25**
Min	**9.17**	**11.10**	**14.43**	**8.60**	**10.95**	**14.73**	**2.25**	**10.50**	**15.00**	**1.00**	**3.25**	**4.25**	**0.50**	**4.50**
CV (%)	**3.51**	**5.91**	**6.71**	**3.35**	**5.76**	**3.35**	**5.23**	**3.41**	**4.87**	**3.53**	**2.97**	**3.47**	**3.59**	**4.32**
CD at P=0.05	**0.64**	**0.77**	**0.93**	**0.58**	**0.87**	**0.96**	**0.23**	**0.75**	**1.52**	**0.51**	**0.06**	**0.22**	**0.34**	**0.13**

Note: C1 (Check 1); C2 (Check 2) and C3 (Check 3)

(47.98%). Chlorophyll content ranged from 0.97 to 0.47 with a mean of 0.72. The mean grain yield ranged from 1219.44 kg/ha (BRHT-3) to 2850 kg/ha (BRHT-8) with a general mean of 1923.14Kg/ha. BRHT-6, BRHT-1, ICC-67, JG-218, BRHT-12, and BRHT-8 were found significantly superior to the best check Sabour chana 2 (2380 kg/ha). Among the 30 chickpea genotypes, the desi type *viz*., BRHT-8, BRHT-12, BRHT-6, Sabour Chana-2, JG-218, ICC-67 and BRHT-1 showed significantly high yield and more nodules per plant. The ten best performing genotypes with their mean were shown in **Table 2(a) and 2(b)**. In this investigation, it has been noticed that the pre-flowering stage exhibited the least number of nodules and dry weight of nodules. The maximum number of nodules and dry weight of nodules were observed at the podding stage. This shift in nodulation pattern suggests a dynamic allocation of resources within the plant as it progresses through different growth stages. The results of Roy *et al.* (2018); Pedersen (2009); and Lamptey *et al.* (2014) are in synchrony with this outcome. The increased number of nodules at the pod initiation stage (Pedersen, 2009), helped to maintain a high number of pods, which in turn improved the yield at maturity. The deterioration of nodular tissues at physiological maturity stage (105 days) was attributed to the modest values of nodule-related features that were present during the pod maturing stage.

The phenotypic correlation coefficients (**Table 3)** provide valuable insights into the relationships between different traits under study in the chickpea genotypes. Grain yield showed a significant and positive association with dry weight of root nodules/plant (0.720**), number of nodules/ plant (0.613**), number of nodules on secondary roots/ plant (0.655**), number of pods per plant (0.586**), and harvest index (0.345 **). Length of primary roots showed significant positive correlation with length of secondary roots (0.225*) while length of secondary roots did not show any significant correlation with any of the traits under study. Number of nodules per plant showed a highly significant positive correlation with number of nodules on secondary roots/plant (0.858**), dry weight of root nodules/ plant (0.652**), number of pods/plant (0.610**), harvest index (0.373**), and seed yield (0.613**). It also showed a positively significant correlation with chlorophyll

Table 2 (b): Mean performance and range of 10 best genotypes for various root nodule related and yield traits

Genotypes		Number of nodules on secondary roots/plant				Dry Weight of nodules/ Plant (mg/plant)				Chlorophyll content	Number of pods/ plot	Harvest Index (%)	Seed Yield
		45 days	65 days	85 days	105 days	45 days	65 days	85 days	105 days	(mg/g)			(Kg/ha)
BRHT-8		4.75	12.50	19.00	12.75	5.46	18.62	53.90	13.30	0.90	53.44	45.89	2850.00
BRHT-12		4.25	14.00	21.00	12.00	7.72	27.89	58.30	24.56	0.80	62.53	49.77	2612.50
JG-218		5.25	11.75	21.75	9.00	8.57	26.00	56.15	19.72	0.49	55.10	52.77	2581.94
BRHT-1		3.50	11.00	16.25	10.50	8.02	27.70	50.03	24.64	0.67	45.67	55.46	2545.83
BRHT-6		4.00	9.00	18.00	6.50	7.27	24.02	59.55	20.74	0.61	43.20	41.84	2523.61
ICC-67		4.50	13.00	14.50	7.25	8.08	26.05	51.86	24.50	0.64	44.07	55.03	2504.17
BRHT-4		5.00	12.75	12.50	10.50	7.19	23.17	50.63	21.74	0.55	53.77	46.90	2375.00
BRHT-2		3.00	10.50	15.50	5.50	5.75	22.34	49.38	12.86	0.73	45.23	47.34	2338.89
JG-14		3.50	11.25	13.25	8.75	6.33	23.12	42.35	20.39	0.90	39.07	46.82	2305.56
BAU Heat-37		3.00	10.25	16.00	7.00	6.53	24.57	47.56	19.98	0.58	44.97	53.47	2237.50
PG-186 (C1)		3.00	9.00	16.75	9.00	5.23	21.65	60.00	16.85	0.62	39.83	37.72	2219.44
SABOUR CHANA-02 (C2)		5.20	14.25	21.25	13.50	8.90	28.57	54.29	26.70	0.85	53.33	47.97	2380.18
Pusa 256 (C3)		3.25	9.50	20.25	8.00	6.42	21.84	51.67	15.63	0.95	39.83	47.00	1859.72
Over all Mean		**2.80**	**9.55**	**16.64**	**6.93**	**5.67**	**22.07**	**48.6**	**16.81**	**0.72**	**38.55**	**45.38**	**1987.63**
RANGE	**Max**	**5.25**	**14.25**	**21.75**	**9.31**	**29.61**	**60**	**26.7**	**26.70**	**0.97**	**62.53**	**55.46**	**2850.00**
	Min	**1.00**	**6.25**	**10.00**	**3.31**	**17.28**	**35.98**	**9.53**	**9.53**	**0.47**	**22.53**	**34.24**	**1219.44**
CV (%)		**3.35**	**5.32**	**3.38**	**6.81**	**4.02**	**3.5**	**3.31**	**3.34**	**5.16**	**6.27**	**4.59**	**7.53**
CD at P=0.05		**0.15**	**0.52**	**1.19**	**0.35**	**0.37**	**1.26**	**3.38**	**0.92**	**0.06**	**3.25**	**3.41**	**180.74**

Note: C1 (Check 1); C2 (Check 2) and C3 (Check 3)

Table 3. Phenotypic correlation between root nodule related traits in 30 Desi chickpea genotypes

Traits	LPR	LSR	RNPP	RNPRPP	RNSRPP	DWPP (mg/plant)	CC (mg/g)	NPPP	HI (%)	SY (Kg/ha)
LPR	1	0.225*	0.109	-0.053	0.120	0.150	-0.206	-0.007	0.186	0.064
LSR		1	-0.120	-0.076	-0.110	-0.121	-0.195	-0.020	0.150	-0.115
RNPP			1	0.099	0.858**	0.652**	0.210*	0.610**	0.373**	0.613**
RNPRPP				1	-0.106	0.226*	-0.326*	0.128	0.149	0.139
RNSRPP					1	0.619**	0.252*	0.544**	0.309*	0.655**
DWPP (mg/plant)						1	0.097	0.653**	0.310 *	0.720**
CC (mg/g)							1	-0.108	-0.315*	0.128
NPPP								1	0.388**	0.586**
HI (%)									1	0.345 **

Note: LPR- Length of primary root; LSR- Length of secondary root; RNPP-number of root nodules per plant; RNPRPP; root nodules on primary root per plant; RNSRPP- Number of nodules on secondary roots/plant; DWPP-Dry weight of nodules/plant; CC-chlorophyll content; NPPP- number of pods /plant; HI-harvest index; SY- Seed yield content (0.210*). Number of nodules on primary roots per plant showed a significant positive correlation with dry weight of root nodules per plant (0.226*) and a negative significant correlation with chlorophyll content (-0.326*). Number of nodules on secondary roots showed a highly significant positive correlation with dry weight of root nodules per plant (0.619**), number of pods per plant (0.544**), and seed yield (0.655**), while with harvest index (0.309*) and chlorophyll content (0.252*) its relation was significant and positive. Dry weight of root nodules per plant displayed highly significant positive correlations with the number of pods per plant (0.653**) and seed yield (0.720**). It also showed a significant positive correlation with the harvest index (0.310*). Chlorophyll content exhibited a significant negative correlation with the harvest index (-0.315*). Number of pods per plant demonstrated highly significant positive correlations with seed yield (0.586**) and the harvest index (0.388**). Overall, these correlation coefficients indicated which traits tend to vary together in the chickpea genotypes studied. For example, genotypes with more nodules tend to have higher seed yield, more pods, and a higher harvest index. Similarly, dry weight of root nodules is positively associated with the number of pods and seed yield. On the other hand, chlorophyll content seems to have an inverse relationship with the harvest index. Therefore, it is suggested that these traits should be used as selection criteria for yield improvement in chickpea. Similar observation was reported earlier by other workers like Raval and Dobaria (2003) where seed yield was positively and significantly associated with number of pods per plant, and harvest index. Samyuktha *et al.* (2017) and Vekariya *et al.* (2008) also observed that seed yield per plant was significantly and positively associated with number of pods per plant. Singh and Phule(1999) also observed a positive and significant correlation between number of nodules/plant and grain yield. Priyadarsini *et al.* (2017) also reported that nodule dry weight per plant, number of pods per plant, and harvest index were positively correlated with seed yield per plant at genotypic and phenotypic level. Roy *et al.* (2018) revealed that traits like number of nodules/plant, and nodule dry weight were positively and significantly correlated with seed yield at genotypic level in all three stages. Similar findings were also observed with Elias (2009) and Mohamed and Hassan, (2015). But contrary to the above findings, Gul *et al.* (2014), Bhuiyan *et al.* (2008) in chickpea and Vieira *et al.* (2001) in soybean found no significant association of seed yield with nodule number whereas they observed positive and significant correlations with nodule weight. Pravallika and Lavanya (2022) observed negative but non-significant association for main root length, and nodule dry weight. Understanding these correlations is crucial for chickpea breeding programs, as it helps breeders to identify and prioritize traits that can be targeted for improvement simultaneously. By selecting genotypes with desirable trait combinations, breeders can develop chickpea varieties that have enhanced productivity and performance under specific agro-climatic conditions

Nodule numbers at all the different stages were observed and averaged. Relationship between average root nodule dry-weight with yield is presented in **Fig. 5.** Yield of genotypes *viz*. BRHT-8, BRHT-6, JG-218, ICC67, BRHT-1 and SABOUR CHANA-02 showed positive correlation with nodules weight per plant. Increasing nodule weight corresponded with increasing yield was observed in this study but some genotypes like BRHT-10, BRHT-13 and BRHT-11 showed high average nodules weight whose yield was low.

The quantitative estimation of genetic divergence was performed by applying the Mahalanobis D^2 statistic on traits. The distribution of all 30 genotypes on the basis of

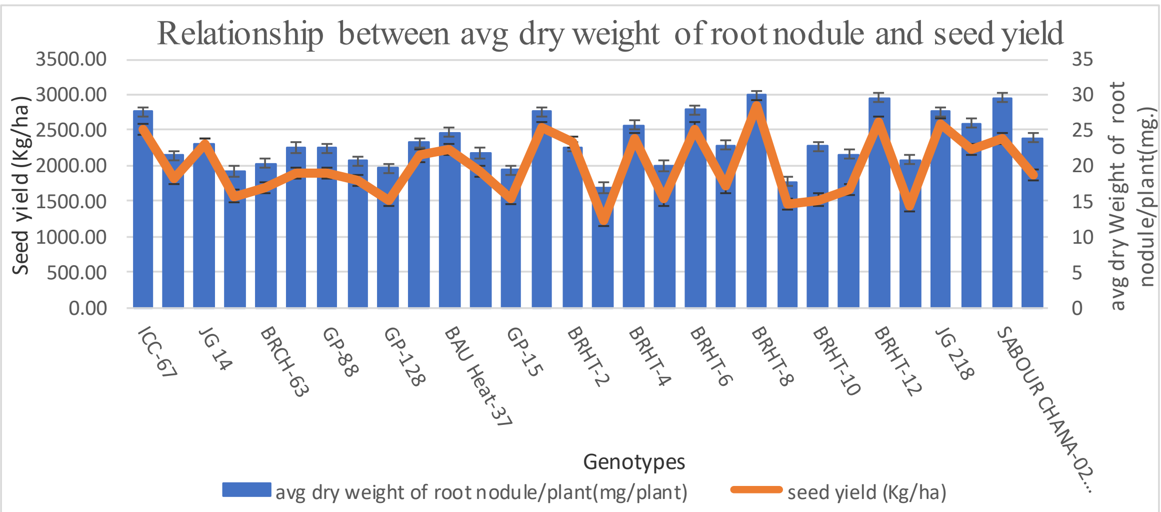

Fig. 5. Relationship between average dry weight of root nodule and seed yield

Table 4. Distribution of genotypes into various clusters

Cluster number	Number of genotypes	Name of genotypes
Cluster I	19	GP-57, GP-88, GP-15, BRHT-5, BRHT-7, BRHT-13, PUSA-256, BRHT-10, GP-86, GP-103, BRHT-11, BRHT-9, BRHT-3, BRHT-2, GP-18, HPAN, BRCH-63, **ICC-67**, BRHT-4
Cluster II	6	JG-14, BAU HEAT-37, **BRHT-6, BRHT-1, Sabour Chana-02, JG-218**
Cluster III	1	**BRHT-8**
Cluster IV	1	GP-24
Cluster V	1	GP-128
Cluster VI	1	BRHT-12

Note:- Highlighted and underlined shows the name of the genotypes having high nodulation along with high yields.

genetic divergence into distinct clusters, as per Tocher's method, is presented in **Table 4**. Out of the six clusters, cluster I had a maximum of 19 genotypes followed by cluster II (6 genotypes), while clusters III, IV, V, and VI had one genotype each. The genotypes GP 24, BRHT 12, JG 14, BAU HEAT 37, BRHT 6, BRHT 1, Sabour Chana 02, and JG 218 were identified as genetically diverse parents, which can be utilized for future crop improvement programme in chickpea. Cluster I, II and cluster III comprised if genotypes which were high yielding as well as high nodulating (**Table 4)**. Such genotypes can also be used in the breeding program for developing biparental crosses between the most diverse and closest groups to break the undesirable linkages between yield and its associated traits (Haddad *et al.* (2004)). This tendency of genotypes to occur in clusters cutting across geographical boundaries demonstrates that geographical isolation is not the only factor causing genetic diversity (Sihag *et al*., 2004). These also suggest that the genotypes within cluster may have some degree of ancestral relationship. Genotypes within the same cluster exhibit low genetic diversity, making the selection of parents from within the cluster less promising for generating superior segregants in a hybridization program. The genotypes within a group exhibited minimal variation among themselves but displayed greater divergence when compared to the genotypes of other groups. Siimilar findings have also been reported by Reena *et al*. (2023), Singh *et al.* (2017) in lentil, Sirohi and Dar (2009), Solanki *et al.* (2007), Joshi *et al.* (2006) and Kumar *et al.* (2004) in chickpea.

Thue the study indicates that nodules play quite an effective role in enhancing the seed yield which can also improve the soil fertility by fixing atmospheric nitrogen. The selection of significantly high nodulating (at flowering/ podding) as well as yielding genotype under natural soil condition would greatly reduce the doses of fertilizer and improve soil fertility which in turn reduces the cost of cultivation. Out of the total stages studied, flowering and podding stages showed significantly high number of nodules/plant. It has also been observed that at the pre-flowering stage, maximum number of root nodules was found on primary roots; however it got shifted to the secondary root in the later stages (flowering, podding). All root nodule were found effective/ fertile (pink color) till podding stages, later on the colour changed to blackish brown and waned off. Among the 30 chickpea genotypes, the desi type *viz*., BRHT-8, BRHT-12, BRHT-6, JG-218, and BRHT-1 showed significantly high yield and more nodules per plant respectively and could be exploited in future

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