Identification of stable determinate growth habit genotypes in the RIL population of chickpea (*Cicer arietinum* L.)

Comment [PB1]: It may be excluded from title

ABSTRACT

Chickpea is a cool-season annual legume crop with lion share in India, accounting for 65% of annual world production. Despite its domestic production, it is unable to meet the current requirement. To encounter this, the identification of superior genotypes with high yield stability is essential. The present study was conducted in two locations to identify determinate type chickpea genotypes with high stable yield. Combined analysis of variance revealed significant genotype × environment interaction. Results of stability analysis identified the recombinant inbred lines viz., 183 (L1- 4866.66 kg/ha, L2- 1825 kg/ha), 12 (L1- 4416.66 kg/ha, L2-2325 kg/ha), 173 (Determinate type) (L1- 3466.66 kg/ha, L2- 2050 kg/ha), 165 (Determinate type) (L1- 3433.33 kg/ha, L2- 2030 kg/ha), 92 (Determinate type) (L1- 2708.33 kg/ha, L2- 1733.33 kg/ha) and 77 (L1- 4096.66 kg/ha, L2- 2051.66 kg/ha), exhibited superior performance at both the locations compared to the checks. Identified stable, superior genotypes in this investigation, could be further utilized for enhancing production and productivity eventually achieving the needed demand and food security in chickpea.

Keywords: ~~*Chickpea*~~*, recombinant inbred lines, Seed yield, and Stability analysis*

Comment [PB2]: Chickpea is also mentioned in the title, use keywords that help to access your manuscript.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's second most significant cool-season annual legume, grown in more than 50 countries across the world, after dry bean. It belongs to the family *Leguminosae*, sub-family *Papilionoideae,* tribe *Cicereae* Alef., and genus *Cicer* L. The cultivated species have a diploid chromosome number of 2n = 2x = 16 and an estimated genome size of 738 Mb, with ~28,269 genes (Varshney *et al.,* 2013). It is an important food legume crop of the semi-arid tropics (SAT), particularly in the Indian subcontinent with 65% of the annual world's production. Globally, it is cultivated in an area of 15 million ha with 15.87 million tons of production (FAOSTAT, 2021). Since 1961, chickpea production per unit area has risen at a steady but gradual rate of about 6 kg/ha per annum. Over 2.3 million tons of chickpea enter world markets annually to supplement the needs of countries unable to meet demand through domestic production (Merga and Haji, 2019). Thus, it is necessary for ~~the~~ plant breeders to identify adaptable, high yielding genotypes for horizontal expansion over new niches.

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Stability analysis corresponds to identifying stable, high yielding genotypes with greater agroecological adaptability. Seed yield is a complex character that largely relies on its component characters and their interactions with the environment. G × E interactions make it more challenging to

judge the genetic potential of a genotype because the presence of it, may reduce the correlation between phenotype and genotype. Thus, the study of G × E interaction through the cantered scatter plots method acts as a reference and aids in the selection of genotypes appropriate for specific environmental niches.

Comment [PB5]: In introduction mentioned about determinate and indeterminate growth habits of chickpeas and its affect on yield and production

Therefore, the objective of this study was to identify determinate chickpea genotypes with higher yield and stable performance, which was assessed over two locations ICRISAT, Patancheru and ARS, Tandur.

2. MATERIALS AND METHODS

The experimental material comprised of chickpea lines developed at ICRISAT, Patancheru assessed over two locations *viz,* ICRISAT, Patancheru and ARS, Tandur. A total of 252 genotypes (248 $F_{7:8}$ RILs derived from the cross, BGD 9971 × NBeG 47 long with 4 checks (among them, 2 are parental lines)) were used as experimental material for evaluation at ICRISAT, Patancheru and Agricultural Research Station, Tandur, using alpha-lattice design (Supplementary Table 1).

Each genotype was sown with a spacing of 60 x 20 cm (inter and intra) in an area of 1.2 m^2 plot. At ICRISAT, Patancheru, planting was completed during the second fortnight of November 2022, while in ARS, Tandur, it was during the first fortnight of December 2022. A basal dose of fertilizer was applied at the rate of 20:40:20 NPK kg per hectare. Seeds were treated with 2g Thiram + 1g carbendazim kg^{-1} seed before sowing for reducing seed and fungal diseases. All the other recommended package of practices were followed during the crop growth to raise a good crop.

Replication-wise data on various agronomic and economic traits from two locations was used for statistical analysis, to obtain site-wise and combined ANOVA, which deciphered the significance/ non-significance of genotype, environment, replication, blocks, and their interaction.

2.1 Statistical analysis

Site-wise and combined analysis of variance (ANOVA) was performed across 2 locations to assess the main and interaction effects of genotypes and environments using R software 4.1.3 with appropriate packages.

2.2 Mean performance and stability of the genotypes:

Genotypes were evaluated based on both mean performance and stability across environments. Centered scatter plot (VSN International, 2021) has been generated to compare the performance of genotypes across two environments using R software with appropriate packages.

3. RESULTS AND DISCUSSION

The individual effects and interaction effects obtained in the stability model for the genotypes evaluated in the four trials are depicted below.

3.1 Analysis of Variance (ANOVA)

Site-wise and combined analysis of variance (ANOVA) was performed across 2 locations (ICRISAT, Patancheru and ARS, Tandur) to assess the main and interaction effects of genotypes and environments, considering genotypes, environments, replication, and block as random effects, for

agronomic and economic traits to determine the contribution of genotypes and other factors to the total variation.

3.1.1 Site wise Analysis

The experimental material includes 248 RILs and four checks viz., BGD 9971, NBeG 47, ICCV 92944, and ICCV 10102, evaluated at two diverse locations namely ICRISAT, Patancheru and ARS, Tandur. The trial wise analysis of variance for agronomic traits and economic traits was described below (Table-1 (a,b) and Table 2).

The mean squares (variance) due to genotypes were highly significant (P<0.01) for DF, DM and GH (at ICRISAT, Patancheru), PRBN, SRBN, NPPP and NSPP (at ARS, Tandur) and for PH, 100 SW, SYPP and HI in both the locations indicating that there was sufficient genetic variability in the material included for the study. The non-significant results were obtained for replications (location) for all the analysed characteristics in both ~~the~~ environments except for DF indicating the homogeneity of the studied environments (Table-1 (a,b) and Table 2).

3.1.2 Combined analysis

Pooled ANOVA revealed significant main and interaction effects of genotype and environment for all the traits except for NSPP and SYPP (Table-1 (a,b) and Table 2). Results obtained depicted significant G × E interaction indicating the differential behaviour of genotypes in different environments and illustrated its ranking differences across the locations i.e., cross over or non-cross over interaction. A similar and comparable results were also reported in distinct experimental trials of chickpea by Bakhsh *et al.,* 2011; Zali *et al.*, 2012; Karpe *et al.*, 2013; Kanouni *et al.,* 2015; Erdemci in 2018; Kizilgeci in 2018; Verma *et al.,* 2019; Hajivand *et al.,* 2020.

3.2 Identification of stable genotypes at both the locations across trials

Centered scatter plot is used to compare the performance of the genotypes in two environments (or to compare the environments for two genotypes). In this study, we have drawn centered scattered plots (figure 1) to compare the performance of the genotypes in two environments viz. ICRISAT, Patancheru (X-axis) and Tandur (Y-axis).

Breeding for growth habit is an important strategy in the current scenario to increase the productivity of chickpea and the stability of their production due to changing climate. In this aspect, a total of

Table 1(a): Site-wise and combined analysis of variance for agronomic traits

Traits	DF			DM			GH		
Effect	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**
Location			4005.6**			1237.41**			0.44709
Rep(Location)	115.583	307.65	211.6	455.65	1250.6	852.75	0.00001	0.323	0.16138
Block(Rep Location)	9.037**	70.455**	28.4	7.385	35.785	21.47	0.00001	0.2166	0.11734
Genotype	146.421**	55.819	98.9**	44.614**	37.851	43.77**	0.74085**	0.2489	0.58735**
Location*Genotype			101.5**			39**			0.40725**
Residuals			26408.1			23.84			0.12437
L1	5.374			11.708			0.00001		
L2		53.187			35.499			0.24831	
Mean	41.87698	45.13228	43.50463	94.1275	95.937	95.0338			
Standard deviation	7.256288	7.389161	7.49948	4.92062	6.2577	5.700719			

Note: DF-Days to 50% flowering; DM-Days to maturity; GH-Growth Habit; L1-ICRISAT, Patancheru; L2- ARS, Tandur.

Table 1(b): Site-wise and combined analysis of variance for agronomic traits

Traits	PH			PRBN			SRBN		
Effect	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**
Location			12287.7**			0.055574**			1413.01**
Rep(Location)	760.7	50.5	405.6	2.1743	0.0033	0.001672	9.145	57.99	33.57
Block(Rep Location)	12.41	169.79	47.6	3.025	0.0898	0.003936	1.7995	0.898	1.54
Genotype	188.05**	108.239**	232.2**	0.00241	0.011939**	0.006437**	1.9264	2.752**	2.4**
Location*Genotype			67.5**			0.006437**			2.33**
Residuals			42.6			0.002998			1.56
L1	16.83			0.00811			2.15387		
L2		65.5819			0.0061			0.97765	
Mean	50.57782	44.87632	47.72707	1	1.0121	1.006063	3.52205	5.455467	4.488757
Standard deviation	8.69511	9.145244	9.36481	0	0.0918	0.065166	1.44752	1.313876	1.686617

Note: PH-Plant Height; PRBN-Number of Primary Branches per Plant; SRBN-Number of Secondary Branches per plant; L1-ICRISAT, Patancheru; L2-ARS, Tandur.

Table 2: Site-wise and combined analysis of variance for economic traits

Traits	NPPP			NSPP			100 SW			SYPP			HI		
Effect	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**	**L1**	**L2**	**Pooled**
Location			28429166**			163387**			2875.2**			57856**			1.09331**
Rep(Location)	692027.5	29515	360771	896325	349.5	448337	64.65	11558.95	5811.8	550.5	10.9	281	0.13925	0.063	0.10079
Block(Rep Location)	56945.55	27491.05	46587	83218	557.95	52394	39.975	23.805	28.6	113.65	24.125	85	0.00917	0.03077	0.01303
Genotype	87650.21	26938.76*	56138	131551	321.69*	65720	40.603**	42.512**	45**	241.38*	31.9645**	143*	0.0143**	0.0234**	0.02066**
Location*Genotype			56805*			66154			38.6*			131			0.01814**
Residuals			49866			63931			30.8			114			0.01428
L1	78394.88			126559			30.965			208.4585			0.01106		
L2		21155.62			259.87			29.9427			16.5782			0.01671	
Mean	490.2593	216.0159	353.1376	753.734	96.283	425.0083	21.1026	23.86055	22.48156	31.70282	19.33118	25.517	0.43583	0.38205	0.4089362
Standard Deviation	286.3284	152.5327	267.2173	359.319	16.991	415.6783	5.87887	8.038704	7.173629	14.744889	4.744229	12.57657	0.11162	0.14059	0.1297113

Note: NPPP-Number of Pods Per Plant; NSPP-Number of Seeds Per Plant; 100 SW- 100 Seed Weight; SYPP-Seed Yield Per Plant; HI-Harvest Index, L1-ICRISAT, Patancheru; L2-ARS, Tandur.

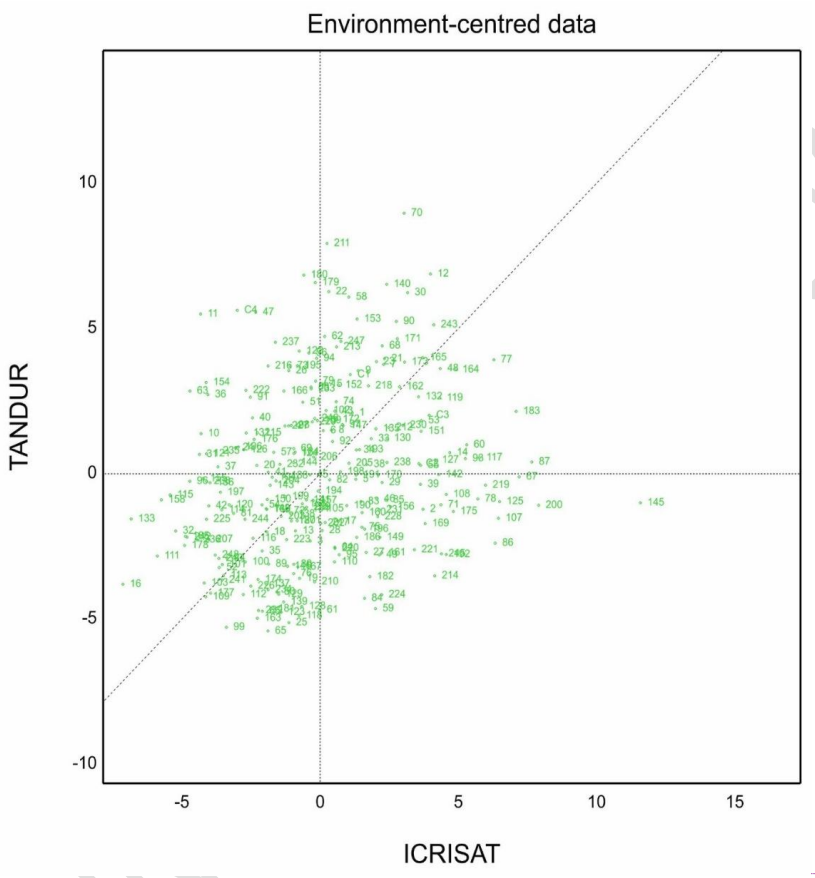

Figure 1. Centered scatter plot for seed yield at ICRISAT, Patancheru and ARS, Tandur.

252 genotypes were categorized into four groups on the basis of their performance in both the environments. The first group comprised ~~of~~ 55 genotypes performing better at Tandur, with highest seed yield per plant recorded for genotype 180 (Determinate type) (2414.96 kg/ha). In the second group comprising 72 genotypes displayed poor performance in both ~~the~~ environments. Whereas the third group with 50 genotypes, performed better at ICRISAT, with genotype 145 having highest seed yield per plant (5536.66 kg/ha). A stable performance was recorded for the remaining 75 genotypes in group-4 of the plot. The genotypes 183 (L1- 4866.66 kg/ha, L2- 1825 kg/ha), 12 (L1- 4416.66 kg/ha, L2-2325 kg/ha), 173 (Determinate type) (L1- 3466.66 kg/ha, L2- 2050 kg/ha), 165 (Determinate type) (L1- 3433.33 kg/ha, L2- 2030 kg/ha), 92 (Determinate type) (L1- 2708.33 kg/ha, L2- 1733.33 kg/ha)

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and 77 (L1- 4096.66 kg/ha, L2- 2051.66 kg/ha) were found superior than the checks C1 (L1- 2881.66 kg/ha, L2- 1976.66 kg/ha), C3 (L1- 3558.33 kg/ha, L2- 1816.66 kg/ha), and C4 (L1- 1740 kg/ha, L2- 1721.66 kg/ha.)

4. CONCLUSION

The present study is conducted to identify the stable genotypes for seed yield, which was evaluated in two different environments. Combined analysis of variance revealed significant genotype × environment interaction at both ~~the~~ locations. The recombinant inbred lines viz., 183 (L1- 4866.66 kg/ha, L2- 1825 kg/ha), 12 (L1- 4416.66 kg/ha, L2-2325 kg/ha), 173 (Determinate type) (L1- 3466.66 kg/ha, L2- 2050 kg/ha), 165 (Determinate type) (L1- 3433.33 kg/ha, L2- 2030 kg/ha), 92 (Determinate type) (L1- 2708.33 kg/ha, L2- 1733.33 kg/ha) and 77 (L1- 4096.66 kg/ha, L2- 2051.66 kg/ha) exhibited superior performance at both the locations compared to the checks, which provides an opportunity for developing determinate plant types for synchronous harvest. Thus, the current investigation identified superior stable genotypes for determinate growth habits which could be further used to boost the production and productivity of chickpea to meet demand for a secure supply of food.

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Comment [PB7]: Write references in a uniform manner as per journal guidelines