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Comparative assessment of F_2, F_3 and F_4 generations derived from an intra-specific cross under RGA and field conditions in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

A total of 228 F_2, F_3 and F_4 lines in chickpea were evaluated under speed breeding, semi-controlled glasshouse and field conditions at International Crops Research Institute for the Semi-Arid Tropics, Hyderabad during summer 2024, *kharif* 2024 and *rabi* 2024-25 respectively. Observations were recorded for days to flowering, days to maturity, seeds per pod and grain yield per plant. Pair-wise two sample t-test across generations revealed highly significant differences for the all the traits showing progressively delayed flowering, maturity and higher yield per plant. Number of seeds per pod showed inconsistent trend with $F_2 > F_3$ and $F_4 > F_3$. Low inter-generation correlations and regressions indicated a strong influence of the environment on the performance of lines. Transgressive segregants were observed for all traits across generations. This study reveals the scope of selection in segregating populations advanced under diverse growing conditions.

Key Words: *Chickpea, Generation advancement, Inter-generation Association and Transgressive segregation*

Chickpea (*Cicer arietinum* L.) grows as an annual legume crop that belongs to the Fabaceae family. It ranks as the third most important legume crop globally and is also called as bengalgram or garbenzo bean. Its seeds serve as valuable source of protein and are consumed as falafel, hummus, dhal, curry, dhokla, boiled, fried and used as flour in breadmaking globally (Singh *et al.,* 2022). The cultivated chickpea is categorized into desi and kabuli type based on seed morphology. In India, it is grown in an area of 95.87 lakh hectares with production and productivity of 11.03 million tonnes and 1151.4 kg/ha respectively (FAOSTAT, 2024). In Andhra Pradesh, it occupies an area of 2.8 lakh hectares with a productivity of 1061 kg/ha and a production of 3.21 lakh tonnes (Directorate of Economics and Statistics, GOI, 2023-24).

Conventional chickpea breeding under field conditions requires six years to develop homozygous lines as it is a *rabi* season crop. Natural speed breeding reduces this to three years by advancing two generations within a single *rabi* season in the field (Gurumurthy *et al.,* 2024), while speed breeding could achieve it within one year by inducing early flowering with the help of artificial light and maintaining an optimum temperature of 26 °C by coolers (Samineni*et al.,* 2020). In contrast, semi-controlled glasshouse conditions maintain 26 °C with coolers and no artificial light to advance the F_3 population. To date, no study has compared phenological and yield related traits across these diverse conditions. Hence, this study focused on the performance of phenological and yield-related traits in the F_2, F_3 and F_4 generations grown under speed breeding, semi-controlled glasshouse and field conditions respectively.

MATERIAL AND METHODS

The present study involved segregating F_2, F_3 and F_4 populations developed through hybridisation between parents contrasting for seed protein content BG 3062 (16.5 %) and ICC 9848 (24.6 %) with a population size of 228 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad. During summer 2024, F_2 lines were advanced following a rapid generation advancement (RGA) facility at 26°C with artificial light for self-fertilisation and mature F_3 pods were harvested and threshed manually to obtain F_3 seeds (Samineni*et al.,* 2020). During *kharif* 2024, F_3 lines were advanced in a semi-controlled

glasshouse at 26 °C without artificial light and mature F_4 pods were harvested and manually threshed to obtain F_4 lines. A total of 228 F_4 lines were evaluated under field conditions during *rabi* 2024-25 in an augmented block design with six blocks each block containing 38 F_4 lines along with five checks namely BG 3062, JG 11, JAKI 9218, ICC 9848 and ICC 9862. Each F_4 line was sown in a single 2m row with 30cm spacing between rows and 10 cm between the plants within the row.

The number of days from sowing to first flowering in each F_2 and F_3 line, while the number of days to 50 % flowering in each F_4 line was recorded. Similarly, the number of days from sowing to complete pod maturity in each F_2 and F_3 line, while the number of days to 50 % maturity of each F_4 line was recorded. Three pods per plant were randomly selected, threshed separately and the average number of seeds per pod was calculated for F_2, F_3 and F_4 lines. The pods harvested from single plants were threshed separately, cleaned, weighed and the seed yield per plant was expressed in grams. Single-plant phenotyping data from the F_2 and F_3 populations were used to generate descriptive statistics, correlations and regressions in excel. For the F_4 population, the data was analysed using an augmented block design in RStudio to perform an analysis of variance (ANOVA), descriptive statistics and the estimation of genetic parameters. Trait-wise comparisons among generations, inter-generation correlation and regression analysis and transgressive segregant analysis was performed to assess the consistency of trait expression and scope for selection across generations.

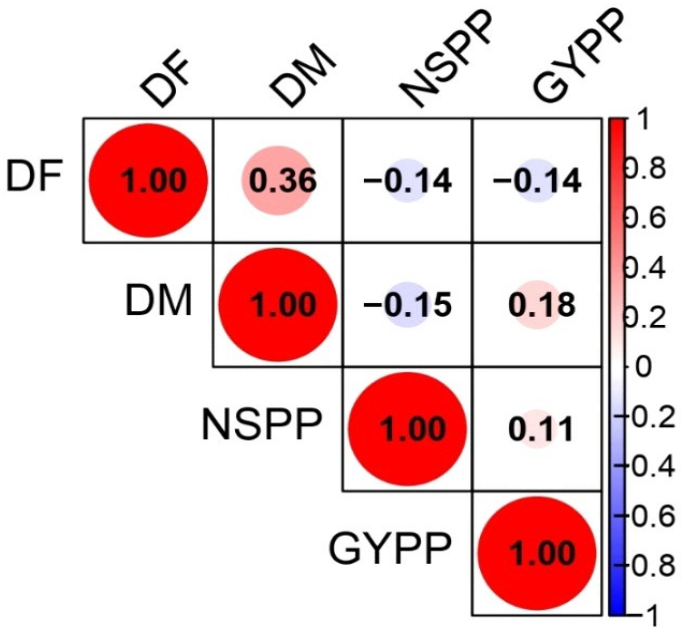
RESULTS AND DISCUSSION

A perusal of descriptive statistics for the F_2 population showed days to flowering (DF) ranging from 19 to 41 days with a mean of 24 days, mode of 22 days, a positive skewness (1.81) and a leptokurtic distribution (8.58). Days to maturity (DM) ranged from 24 to 61 days (mean = 51.96 days and mode = 49 days) with strong negative skewness (-3.08) and leptokurtic distribution (18.89). Number of seeds per pod (NSPP) varied from 1 to 2.5 (mean of 1.6 and mode of 1.7) and exhibited near symmetry (Skewness = 0.07) with platykurtic distribution (-0.56). Grain yield per plant (GYPP) among F_2s ranged from 0.4g to 2g with a mean of 1.3g and a mode of 1.4g. The negative skewness (-0.15) and platykurtic distribution (-0.22) were observed in GYPP (Table 1). Days to flowering showed a moderate positive correlation (r = 0.360) with days to maturity. Correlation coefficients are illustrated in Figure 1. Regression showed that days to flowering, days to maturity and number of seeds per pod explained only 2%, 3% and 1% grain yield variation respectively and left most of the variation unclarified (Table 2).

Descriptive analysis of F_3 population revealed that days to flowering ranged from 30-64 days (mean = 45.33 days, mode = 46) and days to maturity varied from 69-106 days with a mean maturity of 86.68 days,

Table 1. Descriptive statistics for phenological and yield related traits in F_2 population

S.No	Statistic	Days to flowering	Days to maturity	Number of seeds per pod	Grain yield per plant
1	**Minimum**	19	24	1	0.4
2	**Maximum**	41	61	2.5	2
3	**Range**	22	37	1.5	1.6
4	**Mean**	24	51.96	1.6	1.3
5	**Median**	24	53	1.7	1.3
6	**Mode**	22	49	1.7	1.4
7	**Standard error**	0.17	0.27	0.02	0.02
8	**Standard deviation**	2.54	4.1	0.34	0.29
9	**Sample variance**	6.44	16.8	0.12	0.08
10	**Skewness**	1.81	-3.08	0.07	-0.15
11	**Kurtosis**	8.58	18.89	-0.56	-0.22
12	**Count**	228	228	228	228
13	**Confidence level (95.0%)**	0.33	0.53	0.04	0.04



DF-Days to flowering, DM-Days to maturity, NSPP-Number of seeds per pod and GYPP-Grain yield per plant

Figure 1: Correlation co-efficients of phenological and yield-related traits in the F_2 population

a mode of 85 days both showing slight negative skewness (DF = - 0.3 and DM = - 0.04). The number of seeds per pod ranged from 1 to 2 exhibiting a mean of 1.18 with a mode of 1 and the grain yield per plant ranged from 0.62 to 5.67g (mean: 2.35g, mode: 2.84g) with both showing positive skewness of 1.15 and 0.46 respectively. All traits exhibited platykurtic distributions (Table 3). Association analysis revealed moderate positive correlation (r = 0.557) between days to flowering and days to maturity and zero correlation between seeds per pod and yield per plant. Salimath and Patil (1990) and Kumhar *et al.* (2024) observed a strong positive correlation between seeds per pod and grain yield per plant in the F_3 generation and a significant negative correlation with seed yield per plant in the F_3 lines by. The shaded correlation matrix is depicted in Figure 2. The coefficient of determination (R^2) explained 7.2%, 8.2% and <1% of the variation in the grain yield per plant by days to flowering, days to maturity and number of seeds per pod, respectively, leaving 93 to 99.99% variation unexplained (Table 4).

Analysis of variance of augmented block design for F_4 population revealed highly significant treatment effects for days to flowering (79.89**) and days to maturity (49.26**) indicating the significant variation among F_4 lines for these traits. However the treatment effects are non-significant for NSPP (0.3) and GYPP (111.09). The mean squares for days to flowering (282.15**) and grain yield per plant (5313.1**) were high for test *vs* check, indicating significant differences for these traits between test and check. Detailed ANOVA results for phenological and yield-related traits are presented in Table 5. Days to flowering (35 to 78 days, mean 60.93 days) and days to maturity (72 to 118 days, mean 98.34 days) showed negative skewness and a leptokurtic distribution. Seeds per pod (0.67 to 3, mean 1.59)

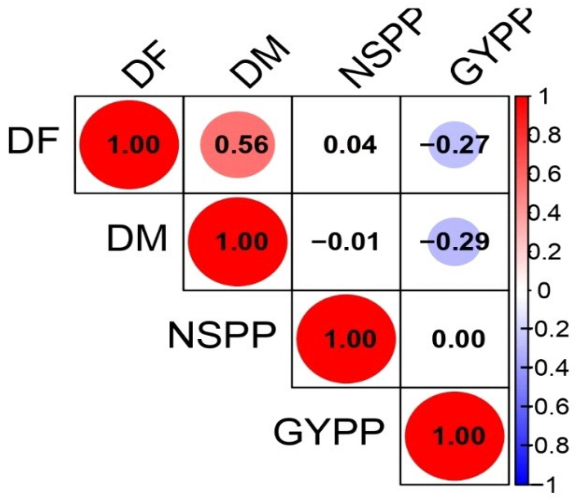
Table 2: Regression statistics of the F_2 population

S.No	Statistic	DF *vs* GYPP	DM *vs* GYPP	NSPP *vs* GYPP
1	**Multiple R**	0.14	0.176	0.11
2	**R Square**	0.02	0.031	0.012
3	**Adjusted R Square**	0.015	0.027	0.008
4	**Standard Error**	0.285	0.284	0.286

Note: DF: Days to flowering, DM: Days to maturity, NSPP: Number of seeds per pod, GYPP: Grain yield per plant

Table 3. Descriptive statistics for phenological and yield-related traits in the F_3 population

S.No	Statistic	Days to flowering	Days to maturity	Number of seeds per pod	Grain yield per plant
1	**Minimum**	30	69	1	0.62
2	**Maximum**	64	106	2	5.67
3	**Range**	34	37	1	5.05
4	**Mean**	45.33	86.68	1.18	2.35
5	**Median**	47	85	1	2.28
6	**Mode**	46	85	1	2.84
7	**Standard error**	0.47	0.56	0.02	0.06
8	**Standard deviation**	7.04	8.39	0.23	0.92
9	**Sample variance**	49.5	70.31	0.05	0.85
10	**Skewness**	-0.3	-0.04	1.15	0.46
11	**Kurtosis**	-0.37	-1.02	1.02	0.05
12	**Count**	228	228	228	228
13	**Confidence level (95.0%)**	0.92	1.09	0.03	0.12



DF-Days to flowering, DM-Days to maturity, NSPP-Number of seeds per pod and GYPP-Grain yield per plant

Figure 2: Correlation co-efficients of phenological and yield-related traits in the F_3 population

and grain yield per plant ranged from 0.33g to 63.45g with a mean of 16.6g indicating slight positive skewness with near normal distribution *i.e.* mesokurtic. The descriptive statistics of the F_4 generation are detailed in Table 6.

Genetic variability analysis revealed phenotypic variance (PV) and genotypic variance (GV) for days to flowering (77.32 and 64.81 respectively) and days to maturity (PV = 49.76 and GV = 37.89) suggesting that these traits were largely governed by genetic factors. High heritability (DF = 83.82% and DM = 76.14%) and moderate genetic advance (DF = 15.21 and DM = 11.08) suggested effective improvement through simple selection. Similarly high heritability and moderate GA was observed in the F_4 generation for grain yield per plant (Patil *et al.,* 2024). In contrast, the number of seeds per pod (PV = 0.3, EV = 0.22) and grain yield per plant (PV = 81.51, EV = 70.29) were mainly influenced by environment with low heritability (26.24% and 13.77%) and low genetic advance indicating that direct selection for these traits would be ineffective. Genetic parameters are presented in Table 7 and Figure 3. The mean performance of F_4 lines exhibited wide variability. Days to flowering ranged from 35-78 days (mean = 60.93) with ICC×211045-250-1-1 flowering earliest. In the present study 107 lines flowered earlier than the mean and 20 earlier than check ICC 9862 (51). Days to maturity ranged from 72-118 days (98.34 mean) with

Table 4. Regression statistics of the F_3 population

S.No	Statistic	DF *vs* GYPP	DM *vs* GYPP	NSPP *vs* GYPP
1	**Multiple R**	0.269	0.287	0.005
2	**R Square**	0.072	0.082	2.73E-05
3	**Adjusted R Square**	0.068	0.078	-0.004
4	**Standard Error**	0.89	0.885	0.924

Note: DF: Days to flowering, DM: Days to maturity, NSPP: Number of seeds per pod, GYPP: Grain yield per plant

Table 5. ANOVA for augmented design for phenological and yield-related traits in the F_4 population

S.No	Source of variation	df	Days to flowering	Days to maturity	Number of seeds per pod	Grain yield per plant
1	**Block (ignoring Treatments)**	5	8.31	49.32**	0.09	129.15
2	**Treatment (eliminating Blocks)**	232	79.89**	49.26**	0.3	111.09
3	**Test**	227	77.32**	49.76**	0.3	81.51
4	**Check**	4	166.47**	86.03**	0.48	327.55**
5	**Test *vs.* Check**	1	282.15**	11.38	0.03	5313.1**
6	**Test and Test *vs.* Check**	228	78.37**	48.61**	0.3	107.29
7	**Treatment (ignoring Blocks)**	232	79.74**	50.22**	0.3	108.3
8	**Block (eliminating Treatments)**	5	15.57	4.54	0.06	258.43*
9	**Residuals**	20	12.51	11.87	0.22	70.29

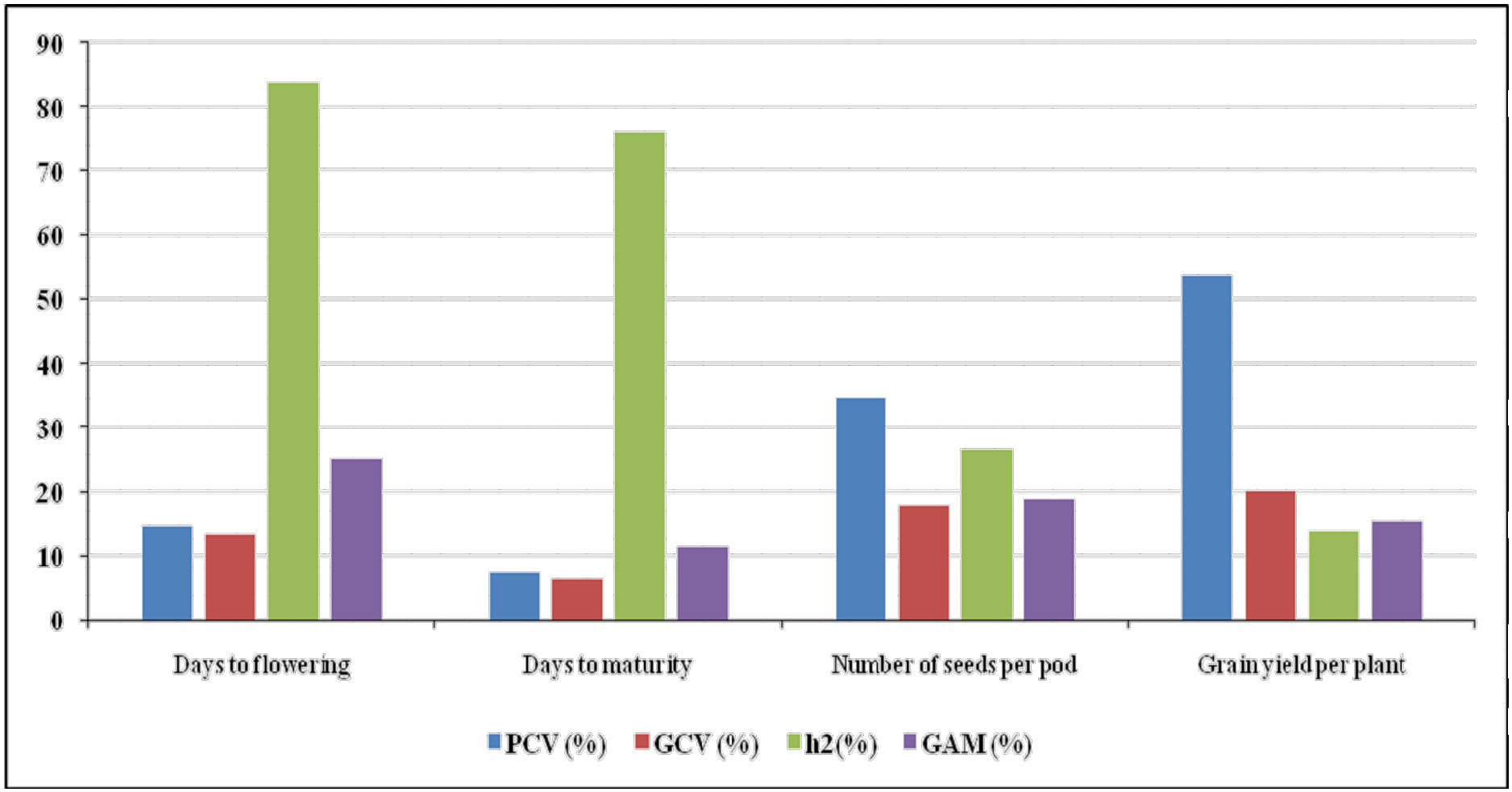
Note: df: degree of freedom, *, ** is significant at $p < 0.05$ and $p < 0.0$ respectively

Table 6: Descriptive statistics of the F_4 generation for phenological and yield-related traits

S.No	Trait	Count	Min	Max	Mean	Skewness	Kurtosis	Standard error	Standard deviation
1	**Days to flowering**	233	32.5	78.5	60.93	-0.56**	3.7*	0.59	8.96
2	**Days to maturity**	233	72.9	119	98.34	-0.39*	4.79**	0.46	7.03
3	**Number of seeds per pod**	233	0.56	3.15	1.59	0.42**	2.82	0.04	0.55
4	**Grain yield per plant**	233	0	56.5	16.81	0.27	2.91	0.68	10.41

Table 7: Genetic variability parameters for phenological and yield related traits in F_4 generation

S.No	Trait	Days to flowering	Days to maturity	Number of seeds per pod	Grain yield per plant
1	**Mean**	60.93	98.34	1.59	16.81
2	**Phenotypic variance**	77.32	49.76	0.3	81.51
3	**Genotypic variance**	64.81	37.89	0.08	11.23
4	**Environmental variance**	12.51	11.87	0.22	70.29
5	**Genotypic co-efficient variance (%)**	13.21	6.26	17.61	19.93
6	**Phenotypic co-efficient of variance (%)**	14.43	7.17	34.31	53.7
7	**Environmental co-efficient of variance (%)**	5.8	3.5	29.44	49.86
8	**Broad sense heritability (%)**	83.82	76.14	26.34	13.77
9	**Genetic advance**	15.21	11.08	0.3	2.57
10	**Genetic advance as percent of mean**	24.96	11.27	18.64	15.26



Note: PCV (%): Phenotypic co-efficient of variance, GCV (%): Genotypic co-efficient of variance, h2(%): Broad sense heritability, GAM(%): Genetic advance as percent of mean

Figure 3: Pattern of genetic variability parameters for phenological and yield related traits in F_4 population

Table 8. Trait-wise mean differences among F_2, F_3 and F_4 generations grown under diverse conditions using a two-sample t-test

S.No	Trait	Compariso	Mean1	Mean2	Variance1	Variance2	t Stat	df	P value	Significanc
1	Days to flowering	F_2 *vs* F_3	24.00	45.33	6.44	49.50	-43.0791	285	8.18E-127	**
		F_3*vs* F_4	45.33	61.00	49.50	77.32	-21.0006	433	4.90E-68	**
2	Days to maturity	F_2 *vs* F_3	51.96	86.68	16.80	70.31	-56.162	330	5.92E-171	**
		F_3*vs* F_4	86.68	98.36	70.31	49.76	-16.0947	441	3.49E-46	**
3	Number of seeds per pod	F_2 *vs* F_3	1.60	1.18	0.12	0.05	15.4443	396	1.87E-42	**
		F_3*vs* F_4	1.18	1.59	0.05	0.30	-10.424	305	5.80E-22	**
4	Grain yield per plant	F_2 *vs* F_3	1.30	2.35	0.08	0.85	-16.4563	271	1.16E-42	**
		F_3*vs* F_4	2.35	16.29	0.85	81.51	-23.1954	232	2.28E-62	**

Note: df: degree of freedom, *, **significant at $p < 0.05$, $p < 0.01$ respectively

Table 9: Inter-generation correlation and regression analysis for phenological and yield-related traits across the F_2, F_3 and F_4 generations

BG 3062 × ICC 9848					
S.No	Traits	F_2-F_3		F_3-F_4	
		Correlation Coefficient	Regression Coefficient	Correlation Coefficient	Regression Coefficient
1	Days to flowering	0.219	0.607	0.158	0.197
2	Days to maturity	0.111	0.228	0.053	0.045
3	Number of seeds per pod	0.029	0.019	0.005	0.013
4	Grain yield per plant	-0.092	-0.296	-0.09	-0.885

Table 10. Transgressive segregants for phenological and yield-related traits across the F_2, F_3 and F_4 generations

Transgressive segregants in the F_2 Population					Transgressive segregants in the F_3					Transgressive segregants in the F_4 Population				
Parent	**DF**	**DM**	**NSPP**	**GYPP**	**Parent**	**DF**	**DM**	**NSPP**	**GYPP**	**Genotype**	**DF**	**DM**	**NSPP**	**GYPP**
BG 3062	26	54	1	1.43	**BG 3062**	45	98	1.67	3.67	**BG 3062**	69	107	1.33	44.53
ICC 9848	21	51	2	1.6	**ICC 9848**	40	74	1	2.3	**ICC 9848**	65	97	2	15.22
S.No	**Trait**	**Range**	**Lower than LP**	**Higher than HP**	**S.No**	**Trait**	**Range**	**Lower than LP**	**Higher than HP**	**S.No**	**Trait**	**Range**	**Lower than LP**	**Higher than HP**
1	DF	21 - 26	10	31	1	DF	40 - 45	52	143	1	DF	65 - 69	154	38
2	DM	51 - 54	81	53	2	DM	74 - 98	17	11	2	DM	97 - 107	76	23
3	NSPP	1 to 2	0	6	3	NSPP	1 - 1.67	0	3	3	NSPP	1.33 - 2	67	24
4	GYPP	1.43 - 1.6	160	25	4	GYPP	2.3 - 3.67	116	20	4	GYPP	15.22 - 44.53	110	1

Note: DF: Days to flowering, DM: Days to maturity, NSPP: Number of seeds per pod, GYPP: Grain yield per plant, LP: Lower parent, HP: Higher parent

ICC×211045-406-1-1 maturing earliest, 115 lines matured earlier than the mean and 57 earlier than ICC 9862 (96 days). Number of seeds per pod ranged from 0.67-3 (mean 1.59), eight lines recorded the maximum, 118 exceeded the mean and 24 outperformed checks ICC 9848 and JG 11 (2). Grain yield per plant ranged from 0.33-63.45 g with a mean of 16.6, ICC×211045-249-1-1 recorded the highest (63.45 g), 106 lines surpassed the mean and ICC×211045-249-1-1 recorded higher yield than the check BG 3062 (44.53 g).

Successive generations (F_2-F_4) showed a trend towards later flowering, later maturity and higher grain yield. Seed yield per plant increased from F_2 to F_3, but decreased from F_3 to F_4 and from F_4 to F_5 (Patil *et al.,* 2023). Seeds per pod were higher in F_2 than in F_3, but F_4 exceeded F_3. Pair wise two-sample t-test results are presented in Table 8. Inter-generation correlation and regression analysis revealed weak positive correlation for flowering time between F_2-F_3 (r = 0.219, b = 0.607) and a weaker correlation between F_3-F_4 (r = 0.158, b = 0.197). Days to maturity exhibited very weak positive correlation F_2-F_3 (r = 0.111, b = 0.228) and negligible F_3-F_4 (r = 0.053, b = 0.045). The number of seeds per pod showed negligible correlations across generations (F_2-F_3: r = 0.029, b = 0.019; F_3-F_4: r = 0.005, b = 0.013). Grain yield per plant showed weak negative correlation F_2-F_3 (r = -0.092, b = -0.296) and F_3-F_4 (r = -0.090, b = -0.885). Similar negligible non-significant positive inter-generation correlations were observed by Patil *et al.* (2023) and insignificant negative correlations among F_3 and F_4 generations were reported by Raval *et al.* (2017). These weak associations suggests influence of environmental conditions on trait expression. Detailed inter-generation correlation and regression data are presented in Table 9.

Transgressive segregation analysis across generations identified superior segregants exceeding performance of parents. In the F_2 population, 10 lines flowered earlier than ICC 9848 whereas 31 later than BG 3062. Similarly 81 lines matured earlier and 53 later than parents, six lines exceeded the parental NSPP and 25 lines exceeded the high-yielding parent. Fifty-two F_3 lines flowered earlier and 143 later, 17 matured earlier, and 11 later, 3 exceeded the parent with high NSPP and 20 lines with higher yield than the high-yielding parent. In the F_4 generation, 154 lines flowered earlier and 38 later, 76 matured earlier and 23 later, the number of lines with higher NSPP is 24 and 1 line exceeded the high-yielding parent. The transgressive segregants identified for all traits across F_2-F_4 are summarized in Table 10.

CONCLUSION

The study revealed substantial variability for phenological and yield traits along with identification of superior segregants across F_2-F_4 generations. Descriptive statistics, correlation and regression indicated wide variation for days to flowering, days to maturity, grain yield per plant whereas number of seeds per pod exhibited less variation across generations. Weak associations among traits, suggested strong environmental influence and reduced genetic variance in the traits studied. High heritability coupled with high genetic advance estimates for days to flowering and days to maturity indicated good prospects for effective selection in the F_4 population. In contrast number of seeds per pod and grain yield per plant were largely governed by environmental factors, limiting direct selection. Several superior lines were identified for earliness, maturity, seeds per pod and grain yield, demonstrating the potential of the cross for generating promising breeding material and facilitating genetic improvement in chickpea.

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