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Identification of Transgressive Segregants and Variability Studies in Segregating Generations of Four Crosses in Chickpea

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ABSTRACT

Background: An impressive progress has been made in development and promotion of extra-large *kabuli* chickpea varieties in India during the past decade. As a result, India has become from importer to exporter of *kabuli* chickpea. The breeding programmes need to continue efforts on enhancing genetic gain in breeding *kabuli* chickpea varieties through increasing genetic variability and precision and efficiency of selection. This study was aimed at evaluating early segregating generations of chickpea to assess genetic variability for various important traits.

Methods: Four large-seeded *kabuli* chickpea genotypes (JGK 2, KAK 2, KRIPA and ICC 17109); were crossed with a common small-seeded *kabuli* genotype (ICC 16644). F_1, F_2 and F_3 along with parents were evaluated under normal field conditions and observations were recorded on various phenological, morphological and yield traits. Data was analysed to estimate genetic variability, heritability and genetic advance in the segregating populations.

Result: Considerably high variability was observed in F_2 and F_3 populations of all the crosses. Heritability estimates in broad sense were high coupled with high genetic advance as per cent of mean for days to first flower, days to pod initiation, number of pods per plant, number of seeds per plant and 100-seed weight which indicated the presence of additive gene action for these traits. Large number of transgressive segregants was identified for number of seeds per plant followed by number of pods per plant and yield per plant. The most promising transgressive segregants could be used in future breeding programme.

Key words: Chickpea, Heritability, Transgressive segregants, Variability.

INTRODUCTION

Chickpea (*Cicer arietinum* L.), a self-pollinated diploid (2n=2x=16) with a genome size of 740 Mb belonging to the family Leguminosae and subfamily Papilionaceae, is the most important food legume crop of South Asia and the third most important food legume crop in the world after beans (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.) in terms of annual production (FAOSTAT, 2017). Chickpea is a valuable source of dietary protein in many parts of the world for humans and, in some cases, animal feed. The subsequent crop after chickpea is benefited by improved soil fertility through symbiotic nitrogen fixation by *Rhizobium*. Globally, it is grown over an area of 14.56 million hectares with a production of 14.77 million tonnes and a productivity of 1014 kg per hectare (FAOSTAT, 2017). India is the largest chickpea producing country in the world with a share of 64.4% (9.07 million tonnes) in production and 65.5% (9.53 million hectares) in area. The other major chickpea producing countries include Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, Mexico, Canada and USA (FAOSTAT, 2017).

Within cultivated chickpea, two distinct groups are found; *desi* type (mostly pink flowers, angular shaped brown coloured small seeds) and *kabuli* type (white flowers, owl's head shaped, beige coloured large seeds). Large-seeded *kabuli* types are gaining importance in the market because price of *kabuli* chickpea is up to twice that of *desi* chickpea (Upadhyaya *et al*., 2006). Earlier, India used to import large amounts of extra-large seeded *kabuli* chickpea from Turkey, and Mexico as such varieties were not grown in India. An impressive progress has been made in development and promotion of extra-large *kabuli* chickpea varieties in India during the past decade. As a result, India has become an exporter of kabuli chickpea. The breeding strategy for improvement of chickpea, a self-pollinated crop, generally involves selection of superior genotypes. Hence, it is essential to study the existing variability in the population and utilizing that in hybridization and isolation of superior genotypes from segregating generations. Role of genetic variability in crops is important for selection of the best genotypes for improvement in yield and other important traits. Transgressive segregation refers to the phenomenon

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of appearance of offspring having a mean value for a specific trait studied out of the range of their respective parents. Unlike heterosis, transgressive segregants can be fixed. Therefore, an experiment was carried out to evaluate early segregating generations of chickpea for genetic variability, heritability, genetic advance and identification of superior transgressive segregants.

MATERIALS AND METHODS

The experimental materials were comprised of five *kabuli* chickpea genotypes (ICC 16644, JGK 2, KAK 2, KRIPA and ICC 17109); F_{1s} developed by crossing the common genotype (P_1); ICC 16644 with the remaining four genotypes (P_2) *viz*., JGK 2, KAK 2, KRIPA and ICC 17109 and respective F_2 and F_3 populations. In the study, the crosses ICC 16644 × JGK 2, ICC 16644 × KAK 2, ICC 16644 × KRIPA and ICC 16644 × ICC 17109 were designated as C_1, C_2, C_3 and C_4, respectively. As the maternal effects were not observed in any of the cross combinations earlier for the traits under study, reciprocal crosses were not studied. The details of parents are given in Table 1. The F_1, F_2 and F_3 along with the respective parents of each cross were sown in the field in November 2013 at ICRISAT. The experiment was laid out in a compact family block design with three replications. Each replication was divided into four compact blocks which consisted of single cross each. Each block was divided into five plots containing five basic generations of each cross. The crosses were randomly assigned to each block and the five generations of each cross were randomly allotted to individual plot within the block. The plots of various generations contained different number of rows *i.e*., 2 rows of parents, 1 row of F_1 and 6 rows each of F_2 and F_3. Seeds were treated before sowing with a mixture of 2 g of Thiram and 1 g of Carbendazim per kilogram of seeds to avoid infestation by soil-borne pathogens. The seeds were sown at a wider spacing of 60 cm × 20 cm with single seed per hill in the rows of 4 m. Care was taken to sow the seeds at uniform depth (5 cm). All the recommended agronomical practices and necessary plant protection measures including basal application of 100 kg/ha Diammonium phosphate (18 kg N and 46 kg P_2O_5) fertilizer were followed to raise a healthy crop (Gaur *et al*., 2010). One intercultural operation was done to control the weeds and three sprays of Indoxacarb (@ 20 mL/ha in 300 L water) were done to manage pod borer (*Helicoverpa armigera*). One light irrigation was given at 30 days after sowing to overcome moisture stress. Observations were recorded on individual plants (20 plants in parents and F_1, 210 plants each in F_2 and F_3 per cross) for days to first flower, days to pod initiation, days to maturity, plant height (cm), number of pods per plant, number of seeds per plant, number of seeds per pod, grain yield per plant (g), biological yield per plant (g), harvest index and 100-seed weight (g). The day first flower fully opened was recorded as days to first flower. The weight of 100 randomly selected seeds from each plant was recorded as 100-seed weight.

The data were subjected to analysis of variance (ANOVA) for compact family block design as described by Panse and Sukhatme (1985). Here, crosses and generations within each cross were taken as families and progenies, respectively. The analysis was carried out in two stages. Firstly, the data of main plots, the variance between crosses and the corresponding error were calculated by treating the experiment as one in simple randomized blocks. Secondly, the analysis for progenies under each family was done separately for each trait using the data of sub plots to get the variance between different generations and the corresponding error. Genotypic and phenotypic coefficients of variation were estimated according to Burton and Devane (1953). The broad sense heritability (Allard, 1960) and genetic advance as percentage of mean (GAM) were also calculated (Johnson *et al*.,1955).

RESULTS AND DISCUSSION

The ANOVA was performed for 11 traits for comparing crosses and generations of each cross (Panse and Sukhatme, 1985). The mean squares from ANOVA presented in Table 2 showed significant differences among the crosses for all the traits except harvest index, indicating considerable amount of variability in the crosses for ten traits. Likewise, the mean sum of squares among the progenies (generations) for all the traits in all the crosses revealed that the variations among the five generations of each cross were significant except for harvest index. The results are in agreement with the observations of Kumar *et al*. (2013) for days to maturity, plant height, number of pods per plant, grain yield per plant (g), biological yield per plant (g) and 100-seed weight (g).

The estimates of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for all the traits are presented in Table 3. The estimates of PCV were higher than their corresponding GCV estimates following the classification of low (0-10%), moderate (10 - 20%) and high (>20%) given by Deshmukh *et al*. (1986). PCV ranged from 4.26% (number of seeds per pod in C_1) to 25.09% (biological yield per plant in C_4), while GCV varied

Table 1: Description of parental lines used in the study.

Trait	JGK 2	KAK 2	KRIPA	ICC 17109	ICC 16644
Biological status	Cultivar	Cultivar	Cultivar	Landrace	Landrace
Maturity	Medium	Medium	Medium	Late	Super early
Seed size	Medium	Medium	Large	Large	Small
Growth habit	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-spreading
Seed type	Kabuli	Kabuli	Kabuli	Kabuli	Kabuli

Table 2: Analysis of variance for different traits in five generations and four crosses of chickpea.

Sources of variation and df	Days to first flower	Days to pod initiation	Days to maturity	Plant height	No. of pods per plant	No. of seeds per plant	No. of seeds per pod	Grain yield per plant	Biological yield per plant	100-seed weight	Harvest index
Analysis of variance between crosses											
Cross (3)	24.14**	22.45**	50.35**	130.38**	2527.76**	2994.02**	0.02**	36.20	658.65**	2057.05**	31.15
Analysis of variance between generations within crosses											
ICC 16644 × JGK 2 (C_1)											
Gen (4)	170.06**	172.60**	49.19**	22.79	1236.31**	931.84**	0.06**	66.12*	237.87**	42.73**	12.94
ICC 16644 × KAK 2 (C_2)											
Gen (4)	211.53**	208.14**	92.03**	14.77*	564.43**	357.28*	0.03**	52.23*	146.04*	85.28**	45.62
ICC 16644 × KRIPA (C_3)											
Gen (4)	203.27**	206.71**	93.99**	73.88**	510.64*	662.01*	0.02*	21.44	88.07	142.33**	80.73
ICC 16644 × ICC 17109 (C_4)											
Gen (4)	206.22**	203.87**	108.94**	51.35**	546.29**	622.50**	0.06**	43.68*	283.65**	50.00*	94.94

Gen = Generation; df = Degrees of freedom; *Significant at 5% level of significance; ** Significant at 1% level of significance.

from 3.79% (plant height in C_1) to 23.98% (biological yield per plant in C_4). The number of pods per plant had high estimates of GCV and PCV in the crosses C_1 and C_4. For the rest of the traits, the estimates of GCV and PCV were moderate in all the crosses except C_4 where estimates were high for biological yield per plant and low for 100-seed weight. These findings are in conformity with the results of Yadav *et al*. (1999); Arora and Jeena (2000); Shivkumar *et al*. (2013); and Monpara and Dhameliya (2013). On the contrary, Ali *et al*. (2010) reported high estimates of coefficients of variation for grain yield per plant and low for days to first flower. The GCV expresses the true genetic potential of the genotypes. In the present study, the PCV values were higher than the corresponding GCV values suggesting the existence of substantial environmental variation.

Heritability estimates were classified as low (5-10%), medium (10-30%) and high (30-60%) (Dabholkar, 1992). The heritability (in broad sense) estimated for 11 quantitative traits ranged from 17.60% (plant height in C_1) to 98.77% (days to first flower in C_3). Days to first flower, days to pod initiation, days to maturity, number of pods per plant, number of seeds per plant, number of seeds per pod and 100-seed weight had high estimates of heritability in all the crosses. This implies that the expected gain from selection would be high if these traits are used as selection criteria in chickpea breeding.

Heritability is usually adopted as a reliable indicator for making effective improvement in the character for which selection is practiced. According to Johnson *et al*. (1955) high heritability should be accompanied with high genetic advance to arrive at desired level of improvement in a particular trait, but it may not be necessary that a character exhibiting high heritability will have high genetic advance. Several researchers (Malik *et al*., 1983; Ghafoor *et al*., 1990 and Ghafoor *et al*., 2000) have emphasized the utility of the estimates of heritability and genetic advance for the prediction of response of quantitative traits to selection in chickpea. The genetic advance as per cent of mean (GAM) (Table 3) for the traits ranged between 3.28% (plant height in C_1) to 40.01% (days to first flower in C_3). Johnson *et al*. (1955) categorized GAM as high (≥20%), moderate (10-20%) and low (0-10%). In the present study, high heritability along with high GAM was exhibited by days to first flower, days to pod initiation, number of pods per plant, number of seeds per plant and 100-seed weight for all the crosses indicating that selection based on mean values would be effective for improving these traits. Anbessa *et al*. (2006) and Bicer and Sakar (2008) reported high broad-sense heritability and GAM for days to first flower in chickpea. For 100-seed weight high broad sense heritability was reported by Dubey and Srivastava (2007), Sharma and Saini (2010), Hossain *et al*. (2010), Srinivasan *et al*. (2011), Karami and Talebi (2013), Sharma *et al*. (2013) and Shivkumar *et al*. (2013) in chickpea. For grain yield per plant high heritability coupled with high GAM was exhibited in C_1. For grain yield

Shivkumar *et al*. (2013) reported high heritability along with high GAM, while Ali *et al*. (2010) reported heritability along with low GAM. Biological yield per plant had high heritability associated with high GAM in C_1 and C_4. These results are in accordance with the findings of Dubey and Srivastava (2007), Bicer and Sakar (2008), Sharma and Saini (2010) and Karami and Talebi (2013).

ICC 16644 was early for phenological traits (days to first flower and days to maturity) and had lower values for plant height, biological yield per plant, grain yield per plant and 100-seed weight than the other parents. The range was high for yield and its component traits (number of pods per plant, number of seeds per plant, 100-seed weight) in segregating generations. In general, F_3 exhibited wider range than that of F_2 for number of pods per plant, number of seeds per plant and grain yield per plant in all the crosses.100-seed weight exhibited wide range in F_2 (C_2 and C_3) and in F_3 (C_1 and C_4). The range of variation in quantitative traits depends on its genetic complexity. The observed wide range of variation for different traits might be due to parental diversity. Similar results were recorded in F_2 and F_3 of cowpea (Salimath *et al*., 2007).

Cross hybridization often produces progenies with wider phenotypic variation than their parents, called as transgressive segregants. A good number of transgressive segregants were identified for number of pods per plant, number of seeds per plant, grain yield per plant and 100-seed weight in all the crosses (Table 4). The highest number of transgressive segregants was identified for number of seeds per plant (114) followed by number of pods per plant (112) in C_1. These results are in agreement with the findings of Shivkumar *et al*. (2013). The lowest number of transgressive segregants was identified for 100-seed weight (8) in C_2. Among the combinations of two yield traits, the highest number of transgressive segregants was recorded for number of pods per plant and number of seeds per plant in F_2, whereas the lowest number of transgressive segregants was identified for 100-seed weight and number of pods per plant in F_3. Among the combinations of three, the highest number of transgressive segregants was identified for number of pods per plant, number of seeds per plant and grain yield per plant in F_3, whereas the lowest number was recorded for number of pods per plant, number of seeds per plant and 100-seed weight in F_3. In C_1, the highest number of transgressive segregants (38 in F_2 and 20 in F_3) having extreme phenotypes for number of pods per plant, number of seeds per plant, grain yield per plant and 100-seed weight and one transgressive segregant in F_2 of each cross in C_2, C_3 and C_4 were identified. Gene recombination with positive effects is responsible for production of more transgressive segregants in F_3. The frequency of transgressive effects depends on cross combinations i.e. on genotypes of parents. Transgressive segregants mostly result from the appearance, in individual genotypes with combination of alleles from both parents that have effects in same direction *i.e*. complementary gene

Table 3: Estimates of genetic parameters for 11 different traits in four crosses of chickpea.

Genetic parameter	ICC 16644 × JGK 2 (C_1)				ICC 16644 × KAK 2 (C_2)				ICC 16644 × KRIPA (C_3)				ICC 16644 × ICC 17109 (C_4)			
	PCV (%)	GCV (%)	h^2 (%)	GAM	PCV (%)	GCV (%)	h^2 (%)	GAM	PCV (%)	GCV (%)	h^2 (%)	GAM	PCV (%)	GCV (%)	h^2 (%)	GAM
Days to first flower	19.24	18.63	93.77	37.16	19.69	19.52	98.22	39.18	19.67	19.54	98.77	40.01	19.73	19.54	98.13	39.88
Days to pod initiation	17.3	16.7	93.18	33.2	17.6	17.43	98.08	35.55	17.79	17.61	97.93	35.89	17.57	17.42	98.28	35.58
Days to maturity	4.94	4.53	84.24	8.57	6.31	6.22	97.19	12.63	6.32	6.2	96.13	12.51	6.7	6.59	96.77	13.35
Plant height	9.04	3.79	17.6	3.28	5.62	4.12	53.84	6.23	9.99	9.26	85.13	17.6	9.2	7.72	70.36	13.34
No. of pods per plant	22.28	20.47	84.39	38.73	17.36	15.04	75.04	26.84	20.32	16.22	63.71	26.68	21.29	20.43	83.29	36.53
No. of seeds per plant	18.77	16.53	84.39	38.73	13.36	10.73	75.04	26.84	20.84	17.27	63.71	26.68	21.04	20.19	83.29	36.53
No. of seeds per pod	4.26	3.91	84.25	7.4	7.99	7.61	90.72	14.93	6.79	5.7	70.35	9.84	4.73	4.37	85.45	8.33
Grain yield per plant	16.92	13.79	66.44	23.15	16.78	12.04	51.51	17.8	13.26	10.47	23.84	16.51	16.51	12.91	51.99	17.69
Biological yield per plant	18.85	15.57	68.19	26.48	14.95	11.54	59.56	18.34	13.88	10.09	41.98	12	25.09	23.98	91.31	47.19
100-seed weight	12.44	11.89	91.32	23.4	17.25	16.23	88.45	31.34	19.6	18.17	85.95	34.71	8.8	6.33	61.71	20.37
Harvest index	4.37	2.99	46.85	4.22	8.77	6.91	45.4	8.2	12.75	8.19	23.55	6.18	13.47	8.27	18.8	5.6

GCV = Genotypic coefficient of variation; PCV = Phenotypic coefficient of variation; GAM = Genetic advance as per cent of mean.

Table 4: Number of transgressive segregants for yield components in F_2 and F_3 populations of four crosses in chickpea.

Better parent mean, range and number of transgressive segregants for yield traits						
Traits	Cross (Better parent in bold)	Better parent mean	Range F_2	Range F_3	No. of F_2	No. of F_3
No. of pods per plant (1)	ICC16644 × JGK 2 (C_1)	86.70	14-208	10-275	112	105
	ICC16644 × KAK 2 (C_2)	80.80	14-211	15-250	80	108
	ICC16644 × KRIPA (C_3)	77.23	12-200	13-198	77	79
	ICC16644 × ICC17109 (C_4)	72.43	16-201	14-248	61	76
No. of seeds per plant (2)	ICC16644 × JGK 2 (C_1)	91.00	26-213	11-275	114	106
	ICC16644 × KAK 2 (C_2)	95.60	14-253	15-269	87	109
	ICC16644 × KRIPA (C_3)	95.23	12-200	13-252	54	62
	ICC16644 × ICC17109 (C_4)	82.66	16-239	18-290	52	71
Hundred seed weight (3)	ICC16644 × JGK 2 (C_1)	36.84	14.33-51.66	11.66-63.66	94	49
	ICC16644 × KAK 2 (C_2)	42.50	15.78-57.67	14.26-54.20	15	14
	ICC16644 × KRIPA (C_3)	51.78	21.54-54.78	18.34-55.94	16	10
	ICC16644 × ICC17109 (C_4)	60.57	21.98-62.88	21.33-62.87	8	06
Grain yield per plant (4)	ICC16644 × JGK 2 (C_1)	32.88	9.09-67.88	8.81-78.37	107	81
	ICC16644 × KAK2 (C_2)	35.55	8.95-71.88	8.30-77.10	46	62
	ICC16644 × KRIPA (C_3)	28.73	7.30-74.12	8.29-76.20	98	92
	ICC16644 × ICC17109 (C_4)	29.93	7.27-85.91	6.08-75.53	70	82

Number of transgressive segregants for combination of yield traits					
Trait combinations	Generation / Cross	ICC 16644 × JGK 2 (C_1)	ICC 16644 × KAK 2 (C_2)	ICC 16644 × KRIPA (C_3)	ICC 16644 × ICC 17109 (C_4)
1 and 2	F_2	112	77	52	50
	F_3	102	101	60	63
1 and 3	F_2	103	1	2	1
	F_3	22	2	0	0
1 and 4	F_2	92	44	74	56
	F_3	76	61	75	65
2 and 3	F_2	41	1	1	1
	F_3	24	2	0	0
2 and 4	F_2	92	44	53	49
	F_3	78	61	60	59
3 and 4	F_2	56	3	7	3
	F_3	27	1	6	1
1, 2 and 3	F_2	38	1	1	1
	F_3	20	1	0	0
1, 2 and 4	F_2	87	43	51	48
	F_3	74	61	58	58
1, 3 and 4	F_2	43	1	2	1
	F_3	22	0	0	0
2, 3 and 4	F_2	41	1	1	1
	F_3	24	0	0	0
1, 2, 3 and 4	F_2	38	1	1	1
	F_3	20	0	0	0

action. Transgressive segregants occur most frequently when differences between parents exist for the genes controlling the traits under consideration and the additive variance is high. The results revealed that the parents taken in the experiment differed from each other for many genes which created large amount of genetic variability in F_2 and F_3 for yield and yield component traits. The findings suggest that the materials and the parents used in the study could be used in future breeding programme. Unlike heterosis, extreme phenotypes caused by transgressive segregants are highly heritable. The identified superior transgressive segregation could be maintained and forwarded to advance generations till they reach homozygosity.

CONCLUSION

From the results, it is concluded that direct selection could be done for most of the yield attributing traits since they exhibited high genetic variability and high range of variation. A high PCV and GCV for the traits studied indicated that environmental influences on the expression of these traits were minor. High heritability coupled with high genetic advance obtained for days to first flower, days to pod initiation, number of pods per plant, number of seeds per plant and 100-seed weight for all the crosses gave an indication that desirable improvement in these traits can easily be achieved through implementation of effective selection scheme for above traits. The range of variation in traits depends on its genetic complexity. High range of variation might be due to parental diversity. The findings suggest that the materials and the parents used in the study could be used in future breeding programme. The identified superior transgressive segregants could be forwarded to advance generations till they reach homozygosity.

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Conflict of interest

All authors affirm that they have no conflict of interest.

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