

Advanced backcross strategy for alien introgression for productivity enhancing traits in chickpea (*Cicer arietinum* L.)

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ABSTRACT

A chickpea (*Cicer arietinum* L.) cultivar, GPF2, was crossed with two accessions, EC556270 and ILWC21, of its wild relative *C. reticulatum* with the objective to introgress productivity enhancing traits from wild to cultivated chickpea. The F_1 s were backcrossed to cultivated parent to generate backcross derived generations and also selfed to generate F_3 progenies. In BC_1F_1 and BC_2F_1 generations, plants showing superiority for fruiting branches, pods and seed yield over the recurrent parent were recovered. A set of 77 BC_1F_2 and F_3 progenies along with recurrent parent was grown to record data on various morphological traits, yield components and seed yield were recorded. There was significant improvement in number of pods, number of primary and secondary branches and seed yield. Some BC_1F_2 progenies recorded 30-32% higher seed yield as compared to recurrent parent. Many backcross progenies were superior to the cultivated parent for more than one trait. It was observed that F_2 and F_3 progenies were inferior as compared to the backcross derived progenies due to the undesirable characters like prostrate growth habit, seed shape and dull seed colour which were inherited from the wild parent. Results showed that the wild donors contributed several positive alleles for yield and yield contributing traits. The study also suggested that one or two backcrosses are required to reduce linkage drag of undesirable traits from the wild donors.

Key words: Alien introgression, Back cross, Chickpea, Genetic variability, Productivity traits.

INTRODUCTION

Chickpea production showed only a marginal growth in last two decades due to the non availability of high yielding varieties that are resistant to abiotic and biotic stresses. This is the major problem in achieving higher productivity level even in highly productive environment in North India (Chaturvedi and Nadarajan 2010). Constraints in breeding of cultivated chickpea include narrow genetic base because of its single domestication event (Zohary 1996) and very high self pollination rate of 98-100% (Gowda 1981). The improvement through conventional breeding is very slow because adequate sources of resistance to abiotic and biotic stresses and productivity traits are not available within the cultivated germplasm. This has stimulated interest to use wild species for the improvement of chickpea (Mallikarjuna *et al.* 2007). There are convincing evidences for the use of wild progenitors as donors of productivity alleles in some crops such as rice (Xiao *et al.* 1996), tomato (Tanksley and Nelson 1996) and chickpea (Singh and Ocampo 1997, Singh *et al.* 2005). Ladizinsky and Adler (1976 a,b), Jaiswal and Singh (1989) and Verma *et al.* (1990) exploited the possibilities of introgression of desirable alien genes from wild to cultivated chickpea. Studies have shown that besides disease resistance and drought tolerance, the

wild *Cicer* species possess genes for higher number of fruiting branches and pods per plant contributing towards higher productivity (Singh *et al.* 1994). Singh and Ocampo (1997) successfully transferred some productivity enhancing genes into cultivated chickpea from the annual species *C. echinospermum* and *C. reticulatum*. As the quantitative trait loci (QTLs) for many economic traits are present in wild species, the advanced backcross lines containing these QTLs can be used in novel breeding strategies like advanced back cross quantitative trait loci (AB-QTL) for the restricted introgression without much linkage drag. Considering the narrow genetic base of cultivated chickpea, the present investigation aimed at introgression of productivity traits into elite chickpea cultivar.

MATERIALS AND METHODS

The experimental material consisted of *Cicer arietinum* cv. GPF2 which was used as recurrent parent and two wild accessions, EC556270 and ILWC21, of *C. reticulatum* which were used as donor parents. The interspecific F_1 plants derived from the two crosses and their recurrent parent formed the base material from which the backcross generations were developed in the background of elite chickpea line, GPF2. The data were recorded for days to flowering, primary and secondary branches per plant, pods

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per plant, seeds per 10 pods, 100-seed weight, seed yield per plant, growth habit, seed colour and seed shape for all the generations. F_{1s} derived from two crosses (GPF2 x *C. reticulatum* Acc. EC556270 (cross I) and GPF2 x *C. reticulatum* Acc. ILWC21(cross II)) were backcrossed to cultivated recurrent parent to generate BC_1F_1 during rabi 2014-15 in Experimental Area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The seeds derived from the crosses and the F_2 populations were advanced during off-season (2015) at PAU Off-season Research Station, Keylong. About 8-10 visually selected BC_1F_1 plants from each cross were again backcrossed with their recurrent parent (GPF2) to generate BC_2F_1 seeds. The selected plants were hand emasculated and then pollinated at appropriate stage. Later these plants were harvested individually thus comprising BC_2F_1 populations.

Evaluation of BC_1F_2 / BC_2F_1 / F_3 progenies introgression lines/plants for yield and yield components was done during Rabi 2015-16 in Experimental Area, Department of Plant Breeding and Genetics, PAU, Ludhiana. All the progenies were planted in plant to progeny rows in Randomized Complete Block Design with two replications in paired rows of 1.5 m length. The cultivated recurrent parent and donor parents were also planted along with the progenies. All the recommended cultural practices were followed to raise a healthy crop. The data were recorded on 5 randomly taken plants from each progeny, recurrent parent and the donor parents. Analysis of variance was performed as per standard procedures (Fisher 1935).

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among the lines for all the seven traits studied (Table 1). It was observed that the recurrent parent was superior for most of the traits as compared to the wild accessions which were inferior for most of the traits, except for number of pods, number of primary and secondary branches per plant and number of seeds per 10 pods. Many progenies obtained from these crosses were superior for different characters like primary and secondary branches per plant, pods per plant, number of seeds per 10 pods, 100-seed weight and seed yield per plant (Table 2). But at the same time, there were also some undesirable characters those were inherited from the wild donor parents. The backcross progenies and selfed progenies of both the crosses were

compared. The differences between two crosses were due to wild parents as the cultivated parent was same for both the crosses.

The mean number of days to flowering was lesser in recurrent parent (98) than the donor parents (*C. reticulatum* EC556270 and *C. reticulatum* ILWC21) (110-114), hence most of the progenies in BC_1F_1 , F_2 and F_3 generations were having late flowering than the cultivated parents. However, the earliness was improved by backcrossing, as the BC_2F_1 progenies were early flowering as compared to other generations. The BC_2F_1 progenies namely, progeny # 3 (91 days), 4 (94 days), 6 (90 days), 9 (92 days), 10 (91 days), 16 (93 days), 31 (92 days), 33 (93 days) and 56 (94 days) took lesser number of days for flowering than recurrent parent, GPF2 (98 days) (Table 2).

Even though the donor parents were superior over the recurrent parent for number of primary branches, there was no considerable increase in primary branches in F_2 and F_3 progenies, however an increase up to 58% was observed in BC_1F_2 generation. Maximum number of primary branches was observed in progeny # 4 (4) of cross I in BC_2F_1 generation. Overall, the mean of number of primary branches was higher in cross II (GPF2 X *C. reticulatum* ILWC21) than cross I (GPF2 X *C. reticulatum* EC556270). There was a considerable variation in number of secondary branches among the progenies. As donor parents (21.0-23.0) were far superior to the recurrent parent (11.2) for secondary branches, there was a considerable increase in the number secondary branches of the progenies over the cultivated parent. The BC_2F_1 generation of cross II had the maximum value of mean number of secondary branches. There was only a slight increase (9-14%) in BC_1F_1 generation, while an increase of 38-73% was observed in F_2 and in F_3 generations, there was an increase of 4-32%. Verma *et al.* (1995) also recovered some interspecific F_2 recombinants with large number of secondary branches. The BC_1F_2 and BC_2F_1 generations were found superior to selfed (F_2 , F_3) generations. Some progenies having 3 to 4 times higher secondary branches than the recurrent parent were also recovered. The progeny #6 in BC_2F_1 generation of cross II had the highest number of secondary branches (42) among the progenies. Cross II was found to be superior to cross I in different generations for number of secondary branches.

Table 1: Analysis of variance of different characters in chickpea

Source of variation	Degree of freedom	Days to flowering	Number of primary branches	Number of secondary branches	Number of pods per plant	Number of seed per 10 pods	100- seed weight (g)	Seed yield per plant (g)
Replication	1	112.07**	0.03	8.98	255.39	0.05	25.71**	31.25**
Genotype	76	57.56**	0.23**	50.18**	656.28**	11.36**	10.34**	44.73**
Error	76	20.64	0.05	3.05	73.93	4.04	2.47	4.16

** Significant at 1% level of significance

Table 2: Superior progenies of different generations of cross I (C-I) and cross II (C-II)

Cross/Generation	Progeny #	DF	NPB	NSB	NP	NSTP	100-SW (g)	SYPP (g)	Growth habit	Seed shape	Seed colour
C I-BC ₁ F ₁	6	104	2.00	17.00	115.00	14.00	12.20	22.50	Semi erect	Owl head	Segregating
C I-BC ₁ F ₁	11	98	2.00	36.00	174.00	14.00	12.70	31.00	Semi erect	Owl head	Light brown
C I-BC ₁ F ₁	15	98	2.00	23.00	121.00	17.00	13.40	27.50	Semi erect	Segregating	Segregating
C I-F ₂	1	98	2.00	33.00	94.00	14.00	13.50	17.20	Semi erect	Segregating	Light brown
C I-BC ₂ F ₁	3	91	3.00	21.00	68.00	21.00	15.86	20.77	Erect	Owl head	Light brown
C I-BC ₃ F ₁	4	94	4.00	38.00	170.00	14.00	15.37	30.05	Erect	Owl head	Light brown
C I-BC ₂ F ₁	6	90	3.00	27.00	184.00	19.00	14.69	38.31	Erect	Owl head	Brown
C I-BC ₂ F ₁	9	92	2.00	26.00	87.00	24.00	10.08	21.70	Erect	Owl head	Light brown
C I-BC ₂ F ₁	10	91	2.00	11.00	56.00	16.00	18.08	18.08	Erect	Owl head	Light brown
C I-BC ₂ F ₁	16	93	2.00	19.00	85.00	18.00	16.14	19.53	Erect	Owl head	Light brown
C I-BC ₃ F ₁	31	92	2.00	37.00	95.00	20.00	13.55	21.20	Erect	Owl head	Light brown
C I-BC ₂ F ₁	33	93	3.00	35.00	173.00	23.00	11.06	29.46	Semi erect	Owl head	Light brown
C I-BC ₂ F ₁	56	94	3.00	21.00	110.00	16.00	15.80	22.77	Erect	Owl head	Light brown
C I-BC ₁ F ₂	3	99	2.63	18.30	40.36	17.00	16.16	11.36	Semi erect	Owl head	Light brown
C I-BC ₁ F ₂	4	97	2.48	27.13	80.00	17.50	13.03	12.70	Semi erect	Owl head	Segregating
C I-BC ₁ F ₂	5	97	2.60	18.99	72.30	20.00	13.32	18.39	Semi erect	Segregating	Segregating
C I-BC ₁ F ₂	7	99	2.70	19.39	58.95	17.00	14.12	13.19	Erect	Segregating	Segregating
C I-BC ₁ F ₂	8	97	2.63	23.82	74.48	12.50	13.74	18.87	Semi erect	Owl head	Segregating
C I-BC ₁ F ₂	9	98	2.90	27.72	104.05	17.50	13.36	24.08	Semi erect	Owl head	Segregating
C I-BC ₁ F ₂	12	97	2.80	20.40	81.70	14.00	16.70	22.38	Semi erect	Owl head	Segregating
C I-BC ₁ F ₂	16	98	2.77	20.30	80.40	11.00	15.59	19.00	Semi erect	Segregating	Segregating
C I-F ₃	20	96	2.60	22.80	55.90	16.00	16.62	12.02	Semi erect	Segregating	Light brown
C I-F ₃	40	100	2.88	20.04	26.34	16.00	14.60	6.50	Prostrate	Segregating	Segregating
C I-F ₃	41	98	2.50	16.80	74.20	14.00	15.74	15.81	Prostrate	Owl head	Dark brown
C I-F ₃	44	104	3.35	16.50	25.90	13.00	15.98	6.10	Semi erect	Owl head	Segregating
C I-F ₃	46	98	2.84	28.92	58.55	15.50	16.36	13.62	Segregating	Owl head	Light brown
C II-BC ₁ F ₁	2	99	2.00	34.00	121.00	17.00	7.70	15.70	Prostrate	Segregating	Dark brown
C II-BC ₂ F ₁	6	92	2.00	42.00	55.00	21.00	11.96	25.8	Semi erect	Owl head	Dark brown
C II-F ₂	2	98	2.00	38.00	152.00	16.00	16.50	32.80	Prostrate	Owl head	Segregating
C II-F ₂	24	111	3.00	37.00	100.00	17.00	11.80	20.70	Prostrate	Owl head	Segregating
C II-BC ₁ F ₂	51	109	2.63	20.00	27.92	15.50	12.02	5.71	Segregating	Owl head	Light brown
C II-BC ₁ F ₂	52	98	2.70	19.43	65.74	18.00	14.10	14.27	Segregating	Owl head	Light brown
C II-BC ₁ F ₂	53	105	2.80	19.70	103.10	15.00	15.03	19.88	Segregating	Owl head	Dark brown
Recurrent Parent	GPF2	98	2.11	11.23	55.20	18.25	16.11	15.65	Semi erect	Owl head	Light brown
Donor Parent ¹	EC556270	110	5.00	21.00	125.00	21.00	2.80	3.80	Prostrate	Angular	Dark brown
Donor Parent ²	ILWC21	114	6.00	23.00	116.00	26.00	2.35	3.40	Prostrate	Angular	Dark brown

DF= Days to flowering, NPB= number of primary branches, NSB= number of secondary branches, NP= number of pods per plant, NSTP= number of seeds per 10 pods, 100 SW= 100-seed weight, SYPP= Seed yield per plant

The donor parents contributed positive alleles for number of pods which led to the increase in seed yield in most of the progenies over their cultivated recurrent parent. From the interspecific derivatives of cultivated chickpea with *C. reticulatum*, Upadhyaya (2008) recovered the progenies with 150-250% more seed yield than their cultivated parent. Similar results were obtained in the present study also. In F_2 generation, there were progenies with number of pods 2 to 3 times greater than the cultivated parent and an increase up to 89% in seed yield was observed in backcross generations. The mean number of pods of both the crosses were compared, there was an increase in cross II with back crossing while in cross I, BC_1F_2 generation was superior. Maximum number of pods per plant (184) was observed in a BC_2F_1 progeny # 6 of cross I.

There was no increase in number of seeds per 10 pods and 100-seed weight in the progenies, except some BC_2F_1 progenies of cross I. Most of the progenies were either comparable or inferior to the recurrent parent for these traits. The reports of Singh *et al.* (2005), that most of the BC_1/BC_2 self population resembles the cultivated parent for seed weight, found to be true in this case also. There were no significant differences across selfed and back cross progenies indicating additive genes are controlling 100-seed weight as reported by Sharma *et al.* (2013).

Even though the number of pods and number of seeds per pods were higher in donor parents, the seed yield was lower as compared to the recurrent parent. This was because of the about five times higher seed weight of recurrent parent. In BC_1F_1 generation of cross I, three progenies namely, progeny # 6 (22.50 g), 11 (31.00 g) and 15 (27.50 g) were found to be superior to the recurrent parent (15.65 g) with an increase in seed yield up to 98%. Recovery of such types of positive transgressive segregants from BC_1F_1 generations was previously reported by Singh and Ocampo (1997). The possible reason for this could be unexpected epistatic effect. A wide range of variability in seed yield was observed in F_2 generation which was similar to the reports of Jaiswal *et al.* (1986). An increase in seed yield from 28% to 110 % was observed in F_2 generations, which was comparable to the seed yield increase of F_2 of a cross of *C. arietinum* with *C. reticulatum* as earlier reported by Singh and Ocampo (1997). They recovered several F_2 plants that out yielded the cultigens 2-3 times. Even though there was no increase in the overall mean yield of BC_2F_1 progenies, yet there were many progenies that out yielded the recurrent parent by 1.5 times. The maximum seed yield was recorded for progeny # 6 (38.31 g) in BC_1F_2 of cross I. In BC_1F_2 generations, superior progenies showed 27 to 54% seed yield increase over recurrent parent. An increase of 16.9-25.2% in seed yield of interspecific derivatives over cultivated parent was earlier reported by Singh *et al.* (2005) in a cross of cultivated chickpea with *C. reticulatum*. But most of the progenies in BC_1F_2 generation were inferior causing an overall reduction in the yield. Most of the superior progenies

were recovered in cross I and were better than cross II. There were no superior progenies in F_3 generations. When the mean yield was considered, the BC_1F_2 generation was better in case of cross I, while for cross II, BC_2F_1 generation was showing maximum mean yield. This suggested that an additional backcross helped to increase seed yield probably by reducing linkage drag in case of cross II.

The undesirable characters of wild donor parents like prostrate growth habit, angular seed shape and dark brown or dull seed colour were inherited to the progenies. Because of these characters, the selfed progenies were inferior and the backcrosses effectively reduced the linkage drag. Most of the progenies in F_2 and F_3 generations were showing either prostrate or segregating in growth habit. Most of the progenies in BC_1F_2 generations showed semi spreading growth habit and with another backcross, the progenies in BC_2F_1 found to have semi erect growth habit like the recurrent parent. Earlier Singh *et al.* (2005) reported that the BC_1/BC_2 derived lines resembles the cultivated chickpea in their growth habit and seed characters, while lines derived from the straight crosses were having wild characters like spreading/semi spreading growth habit with reticulated and dull coloured seeds. The reports of Tanksley and Nelson (1996) that one or two backcrosses are needed to reduce the linkage drag hold true in this case also. Also a transition from angular seed to owl head seed shape and from dark brown to light brown colour was visible across back crosses, while most of the $BC_1F_2/F_2/F_3$ plants/progenies had angular shape seeds of dark brown colour or they were segregating for seed traits. The backcross derived lines were superior to F_2 and F_3 generations and the backcrossing also improved the characters like growth habit, seed shape and seed colour.

The mean of different characters across the generations of cross I and cross II are given in the Table 3. For most of the characters, cross I was superior to cross II. As the recurrent parent was same for both the crosses, it seems that the differences in the characters were due to the donor parents. It can be concluded from the results of present study that *C. reticulatum* Acc. EC556270 was found to be the better donor with lesser linkage drag when compared with *C. reticulatum* Acc. ILWC21. This study also suggested that *C. reticulatum* can be further exploited for the introgression of desirable alleles for productivity enhancing traits. A wide range of variability for different traits suggested that the wild progenitor species could be used for broadening the genetic base of cultivated chickpea. This increases the scope of crop improvement and chances for getting better recombinants. The advanced backcross lines produced in the experiment can be used further following AB-QTL strategy which allows the restricted introgression of desirable traits with reduced genetic load of wild relatives. The superior derivatives of these interspecific crosses can be used as directly as cultivars or as genetic stocks for the improvement of elite chickpea cultivars.

Table 3: Mean of different morphological characters of cross I (C I) and cross II (C II) across generations

Cross	Generation	Days to flowering	Number of primary branches	Number of secondary branches	Number of pods	Number of seeds per 10 pods	100- seed weight (g)	Seed yield per plant (g)
C I	BC ₁ F ₁	103.48	1.97	12.31	43.90	15.90	11.15	8.27
C I	F ₂	109.97	2.15	15.54	34.60	11.86	8.48	4.05
C I	BC ₁ F ₂	98.00	2.42	18.79	56.51	16.05	15.36	13.59
C I	F ₃	101.00	2.29	14.92	36.14	14.45	14.21	7.99
C I	BC ₂ F ₁	94.82	2.48	16.26	44.61	16.58	13.26	9.53
C II	BC ₁ F ₁	115.73	2.00	12.93	28.47	13.87	12.37	5.29
C II	F ₂	114.13	2.26	19.49	27.34	11.32	9.89	3.48
C II	BC ₁ F ₂	105.00	2.63	16.84	51.96	16.10	13.58	10.64
C II	F ₃	109.00	2.08	11.71	35.76	13.35	11.51	5.28
C II	BC ₂ F ₁	98.63	2.53	24.21	71.11	17.89	12.36	13.93

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