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Development and characterization of inter-specific crosses involving cultivated and wild species of chickpea (*Cicer arietinum* L.)

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Abstract
The crossability of cultivated chickpea (*Cicer arietinum* L.) with two wild annual species *C. reticulatum* (ILWC 46) and *C. echinospermum* (ILWC 239, ICC 17159) was estimated under field condition at two environmental locations *viz*., Palampur and Sangla. *C. reticulatum* showed higher crossability to chickpea as evident from higher seed set (14.36%) than *C. echinospermum* (seed set = 8.49%). Crosses involving *C. reticulatum* also had higher pollen viability (93-97%) than crosses involving *C. echinospermum* (33-67%). Weather parameters with day temperature regimes of 15.9-29.4°C; relative humidity of 38-41% and photoperiod of 8.9 hours were most appropriate for successful hybridization. Positive heterosis for seed yield per plant was observed in HC-2 × ILWC239 (148%) and HC-1 × ILWC46 (71.70%). Inter-specific crosses were also characterized for different traits and sufficient genetic variability existed for all the traits under study. Seed yield per plant for inter-specific hybrids was 12.70 g as compared to 12.64 g for chickpea and 6.79 g for wild species. Correlation studies revealed that higher seed yield per plant can be obtained by selecting derivatives with high biological yield per plant, pods per plants, seeds per plant and harvest index.

Keywords: Chickpea, *C. reticulatum*, *C. echinospermum*, Wide hybridization

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop in the world after dry bean (*Phaseolus vulgaris* L.) and dry pea (*Pisum sativum* L.). Chickpea is a self-pollinated (Gowda 1981) [6], diploid (2n=2x=16) crop with a relatively small genome (740 Mb). Of the nine annual species of genus *Cicer*, *C. arietinum* (chickpea) is the only cultivated species. Chickpea germplasm, however, lacks several desirable agronomic traits such as resistance to biotic and abiotic stresses that are vital for development of high yielding varieties of chickpea (Millan *et al*., 2006) [14]. These abiotic and biotic stresses are the major bottlenecks in increasing chickpea productivity as sources of resistance to those are not available in the cultigen. The genetic base of chickpea is narrow and inters-specific breeding programmes have further narrowed genetic base of the present day cultivars (Chaturvedi and Nadarajan 2010) [3]. Genetic advance for yield is also low in chickpea owing to limited genetic variation present in the germplasm, consequently leading to classification of chickpea as a recalcitrant crop (Van Rheenen *et al*., 1993) [31]. A potential mean of increasing the genetic variation and introducing resistant genes in cultivated species is inclusion of wild species in the breeding programme (Van der Maesen and Pundir 1984 [30]; Van Rheenen *et al*., 1993 [31]). However, interspecific or wide hybridization involving wild *Cicer* species remained under-exploited.

Wild species of *Cicer* possess agronomically important characters such as resistance/tolerance to biotic and abiotic stresses. The *C. reticulatum* and *C. echinospermum* are of special significance because they grow vigorously and possess resistance to two fungal diseases i.e. *Ascochyta* blight and *Fusarium* wilt and tolerance to cold stress and are cross compatible with cultivated chickpea (Ladizinsky and Adler 1976a [12]; Verma *et al*., 1990 [32]; Singh and Ocampo 1993 [24]; Singh and Ocampo 1997 [25]; Van der Maesen *et al*., 2007 [29]; Mallikarjuna *et al*., 2011 [13]). Thus, these can be exploited for gene transfer to cultivated chickpea *via* hybridization. This will also provide an opportunity to broaden the genetic base of cultivated chickpea by incorporating several traits from these species. Keeping in view the above considerations, the present study deals with development and characterization of inter-specific crosses involving chickpea, *C. reticulatum* and *C. echinospermum* with the aim to broaden genetic base of chickpea by incorporating useful traits.

Materials and Method

Wide hybridization at field conditions was carried out during *rabi* season at two different location *viz*., the Experimental Farm, Department of Crop Improvement, CSK HPKV, Palampur, Kangra and during offseason at Experimental Farm, Mountain Agriculture Research and Extension Center, Sangla, district Kinnaur HP. Experimental material was comprised of five cultivated chickpea genotypes (HC-1, HC-2, HPG-17, BGD-112 and GPF2) used as female; *C. reticulatum* Ladiz accession ILWC 46 and *C. echinospermum* Davis accessions ILWC 239 and ICC 17159 used as male. Staggered sowing was done at intervals of 15 days to synchronize flowering for hybridization. Fifteen all possible inter-specific crosses were made. The flowers were i. emasculated in morning (08:30-10:00h) followed by immediate pollination, and ii. emasculated in evening (15:00-16:30h) followed by pollination in the next morning (09:00-10:00h). Stage 14 flowers (Kiran *et al*., 2019) [11] where corolla was clearly visible and was slightly above the calayx; gynoecium was longer than (about 1 mm) stamens and anther arrangement was 5 + 4 + 1 were selected for emasculation. Only flowers with purple peduncles were selected in *desi* cultivars because flowers without anthocyanin pigmentation often drop naturally (Pandya, 1977) [16]. Emasculation and pollination was carried out as per the procedure described by (Auckland and van der Maesen 1980 [2], Tullu and Rheenen, 1989 [28], Arora and Jeena, 2000 [1]). Sharp-pointed stainless steel forceps was used to remove the front sepal. Thereafter, an incision was made on the upper end of the keel to open the flower. All the ten anthers were gently removed and care was taken to avoid damage to stigma. Pollens were harvested from stage 15 flowers (Kiran *et al*. 2019) [11] and pollen was transferred to stigma of emasculated flower by gently pressing the keel (full of pollen) against the stigma of the emasculated flowers. A solution of growth hormones containing 120mg/L GA_3, 30mg/L NAA and 15mg/L Kinetin was applied to a cotton pad with the help of dropper at the base of the pedicel of the pollinated buds about half an hour after pollination for two consecutive days after pollination (Singh *et al.*, 2005) [26] to prevent premature flower abscission.

Number of chickpea flowers pollinated and number of crossed pods formed, was used to calculate per cent pod set as following formula:

$$\text{Per cent pod set} = \frac{\text{Total number of crossed pod set}}{\text{Number of chickpea flowers pollinated}} \times 100$$

F_1 hybrids along with their parents were grown in pots following Complete Randomized Design (CRD) with unequal replications, as number of F_1 seeds varied for different crosses. Seeds from crosses were mechanically scarified prior to sowing to speed up the germination. There hybridity was confirmed through morphological markers like growth habit, leaf size and leaflet shape as compared with their parents. Five randomly selected *F_1* hybrids along with parents were characterized for plant type (erect, semi-erect, semi-spreading and spreading, prostrate) on 1-5 scale, number of nodes per plant at first flowering, days to first flowering, days to first pod setting, days to 75 percent maturity, reproductive phase duration, plant height (cm), primary branches per plant, number of nodes per plant at near maturity, biological yield per plant (g), root length (cm), pods per plant, seeds per plant, seed yield per plant (g), 100-seed weight (g) and crude protein content (%). For pollen viability analysis, 4-6 mm flower buds of F_1 crosses and parents having intact anthers were fixed between 8.30 to 10.00 h in Farmer's fixative (3 ethyl alcohol: 1 glacial acetic acid, *v/v*) for 1 to 3 days and then stored in 70 per cent alcohol at 4°C. These buds were used for pollen viability study within 15 days.

The agronomical data obtained from each cross combinations were analysed using the Statistical Analysis System *(*SAS software). The analysis of variance was done as per CRD (Cochran and Cox 2006) [4] based on the following linear model of Fisher (1954) [5]:

$$Y_{ij} = \mu + g_i + e_{ij}$$

Where

Y_{ij}=Phenotypic effect of the i^{th}genotype of j^{th} treatment,
μ = general mean,
g_i = effect of i^{th}genotype and
e_{ij}= random error associated with i^{th}genotype of j^{th} treatment

Results and Discussion

Crossability and weather parameter on interspecific crosses

The *C. reticulatum* (ILWC 46) as well as *C. echinospermum* (ILWC 239) genotypes were cross compatible with chickpea whereas *C. echinospermum* genotype ICC 17159 was cross incompatible with chickpea. Hybrid pod set was significantly higher in chickpea x *C. reticulatum* (14.36% & 10.28%); than with chickpea x *C. echinospermum* (8.49% & 7.71%) at Palampur and Sangla, respectively. Inter-specific hybrids between BGD-112 × ILWC-46 had highest pod set (22%) followed by GPF2 x ILWC 46 (21.21%), HC-2 x ILWC 46 (14.93%), HC-2 x ILWC 239 (10.75%) at Palampur (Table 1). The higher pod set in flowers emasculated in the evening followed by pollination in the morning might be attributed to higher stigma receptivity. The stages 14 (stage for emasculation) and 15 (stage for pollination) differ by about 12 h (Kiran *et al.*, 2019) [11] and hence a gap of about 14-16 h between emasculation to pollination showed better hybrid seed formation that that when emasculation was followed by immediate pollination.

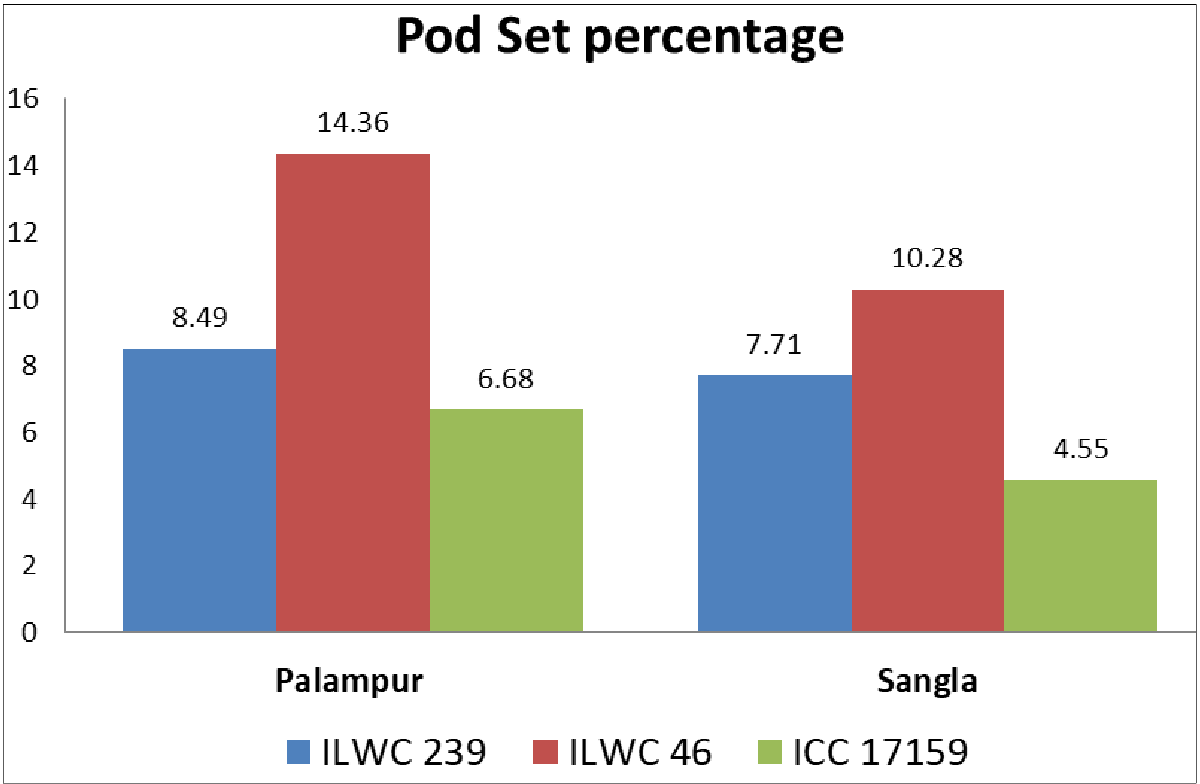
Table 1: Number of buds emasculated, pod set and percentage of pod set during *rabi* 2011-12 at Palampur and offseason 2013 at Sangla

S. No.	Cross combinations	Number of buds emasculated		Number of pod set		Percentage of pod set (%)	
		Palampur	Sangla	Palampur	Sangla	Palampur	Sangla
1.	HC-2 x ILWC 46	288	48	43	5	14.93	10.42
2.	HC-1 x ILWC 46	233	48	22	3	9.44	6.25
3.	HPG-17 x ILWC 46	265	51	19	2	7.17	3.92
4.	BGD-112 x ILWC 46	200	60	44	8	22.00	13.33
5.	GPF2 x ILWC 46	198	75	42	11	21.21	14.67
	Average performance	1184	282	170	29	14.36	10.28
6.	HC-2 x ILWC 239	279	50	30	8	10.75	16.00
7.	HC-1 x ILWC 239	237	47	19	7	8.02	14.89

8.	HPG-17 x ILWC 239	173	58	7	0	4.05	0
9.	BGD-112 x ILWC 239	183	93	18	7	9.84	7.53
10.	GPF2 x ILWC 239	188	102	16	5	8.51	4.90
	Average performance	1060	350	90	27	8.49	7.71
11.	HC-2 x ICC 17159	110	34	8	1	7.27	2.94
12.	HC-1 x ICC 17159	112	34	9	2	8.04	5.88
13.	HPG-17 x ICC 17159	88	26	1	0	1.14	0
14.	BGD-112 x ICC 17159	44	30	5	3	6.82	10.00
15.	GPF2 x ICC 17159	50	30	4	1	8.00	3.33
	Average performance	404	154	27	7	6.68	4.55
	Overall performance	2648	786	287	63	10.84	8.02

This indicated that both maternal and paternal genotype effected the success of pod formation. These findings are in agreement with the earlier studies by Naik (1993) [15], Singh and Ocampo (1997) [25]. Van der Maesen *et al.*, (2007) [29], and Mallikarjuna *et al.*, (2011) [13] reported high crossability of *C. reticulatum* than *C. echinospermum* with cultivated chickpea.

Hybrid pod formation and indicator of success in wide hybridization, differed was significantly between Palampur and Sangla region (Graph 1). Per cent hybrid pod set was highest when the average maximum/minimum day temperature was 29.4/15.9°C, rainfall 0mm, morning/evening, relative humidity 41/38% and photoperiod was 8.9 hours. This indicated that these factors affected pod formation in chickpea. Similar findings were also reported by Sharma and Lal (1994) [21] in lentils, while Pratap and Chaudhary (2007) [17] in Wheat ×Triticale crosses.

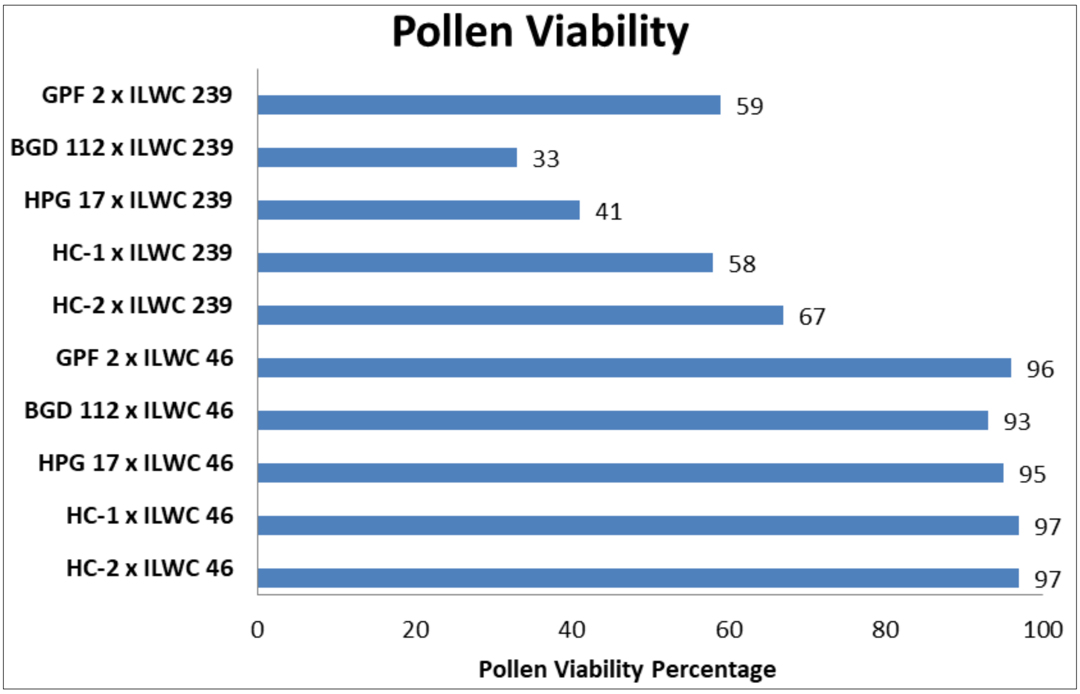


Graph 1: Percent hybrid pod setting at two different crossing locations – Palampur and Sangla

Heterosis and pollen viability in interspecific crosses

Highest positive significant heterosis was observed in HC-2 × ILWC239 (148%) and HC-1 × ILWC46 (71.70%) for seed yield per plant over better parent. The values of heterosis observed in present study were comparable to those of 28-153 percent reported earlier (Singh and Ocampo (1993 and 1997) [24, 25] for cross between *C. echinospermum* and cultivated chickpea. In crosses with *C. reticulatum*, 75 per cent hybrid vigour was recorded (Singh *et al*., 1984) [23].

Pollen viability studies revealed that F_1 hybrids of *C. arietinum* × *C. reticulatum* had higher pollen viability of 93-97%, as compared to *C. arietinum* × *C. echinospermum* (33-67%) indicating that *C. echinospermum* is more distantly related to *C. arietinum* as compared to wild progenitor *C. reticulatum* (Graph 2). Earlier 53.8 percent pollen viability for interspecific crosses with *C. echinospermum* (Pundir *et al*., 1992) [18] and 93-96 per cent pollen viability with *C. reticulatum* crosses were reported (Naik (1993) [15].



Graph 2: Pollen viability per cent among the different inter specific crosses

Characterization of interspecific crosses

Analysis of variance for parents and interspecific hybrids revealed the presence of significant differences for all the traits under study indicating the restoration of sufficient genetic variability in the experimental material upon wide hybridization. Significant differences were also reported by Singh and Singh (2009) [22] for quantitative traits in interspecific hybrids of *C. arietinum* and *C. reticulatum.* Higher estimate for range and mean values was observed for different qualitative and quantitative traits among the interspecific crosses (Table 2). Mean seed yield per plant for cultivated chickpea was 12.64 g while that of wild species was 6.79 g while it was 12.70 g per plant F_1's. Among the interspecific crosses, HC-1 x ILWC46 (19.90 g) and HPG-17 x ILWC46 (18.37g) were superior for seed yield per plant as compared to parents. High significant differences for number of primary branches, number of pods per plants, 100-seed weight, biological yield per plant, harvest index and seed yield per plant were also observed (Qureshi *et al*., 2004) [19]. Singh *et al*. (2005) [26] reported 25.2 per cent increase in yield over the female parent in inter-specific cross of *C. arietinum* and *C. reticulatum*. Similar reports have been documented by Singh and Ocampo (1993, 1997) [24, 25] and Jaiswal and Singh (1989) [8].

Table 2: Overall range and mean performance of parents and their interspecific crosses for different traits in chickpea

Characters	P_1		P_2		F_1		C.V.
	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	
Days to first flowering	84-111	96.73 ± 5.02	75-115	95.17 ± 18.84	86-144	108.92 ± 5.00	1.32
Number of nodes at first flowering	20-26	22.87 ± 0.72	19-25	21.67 ± 1.34	18-26	21.91 ± 0.62	5.92
Days to first pod set	102-128	113.93 ± 4.56	97-135	114.33 ± 16	106-156	127.94 ± 6.06	1.37
Days to 75% maturity	177-194	184.80 ± 2.50	165-188	177.17 ± 9.16	160-202	179.20 ± 3.43	1.26
Reproductive phase	74-108	88.00 ± 5.20	70-95	82.00 ± 9.67	52-88	70.28 ± 3.08	3.12
Primary branches per plant	2-4	3.07 ± 0.24	3-6	4.33 ± 1.00	2-5	3.97 ± 0.19	14.76
Number of nodes at maturity	29-37	32.67 ± 0.95	28-34	30.84 ± 1.16	25-34	30.13 ± 0.47	5.29
Plant height (cm)	50.00-80.70	62.96 ± 3.82	50.50-71.60	61.02 ± 7.19	50.00-88.00	65.44 ± 2.49	6.67
Biological yield (g)	39.40-51.40	45.32 ± 2.09	34.80-44.30	39.25 ± 2.72	34.00-63.40	48.92 ± 2.33	4.86
Root length (cm)	8.90-17.80	14.124 ± 1.43	10.50-20.50	15.55 ± 3.35	13.70-24.50	20.79 ± 1.31	7.82
Pods per plant	39-59	50.80 ± 2.66	14-78	43.34 ± 27.34	15-103	59.78 ± 8.68	10.35
Seeds per plant	47-81	67.07 ± 4.70	16-101	55.34 ± 37.34	15-137	76.03 ± 11.68	10.09
Seed yield per plant (g)	7.50-16.30	12.64 ± 1.37	2.80-12.00	6.79 ± 3.81	2.70-21.60	12.70 ± 1.70	9.61
100-seed weight (g)	10.10-29.10	18.08 ± 2.80	11.40-17.10	14.27 ± 2.06	13.10-23.00	17.51 ± 0.88	4.98
Harvest index (%)	19.01-32.47	27.63 ± 2.10	7.90-27.08	16.68 ± 8.53	7.79-35.28	24.98 ± 2.64	5.54
Crude protein (%)	18.71-22.81	20.61 ± 0.41	18.52-22.64	20.60 ± 1.16	17.12-20.16	19.86 ± 0.30	4.21

Correlation studies revealed that seed yield per plant has positive and significant correlation with biological yield per plant, pods per plant, seeds per plant and harvest index, whereas seed yield per plant was negatively correlated with days to first flowering, days to first pod set, days to 75 percent maturity and crude protein (Table 3). These finding are in conformity with earlier reports observed by Rao *et al.* (1994) [20], Jeena and Arora (2001) [9], Singh (2007) [27], Jadhav *et al.* (2014) [7] and Johnson *et al.* (2015) [10].

Table 3: Simple correlation coefficients among different agro-morphological traits in chickpea

Character	Number of nodes at first flowering	Days to first pod set	Days to 75 per cent maturity	Reproductive phase	Number of primary branches	Number of nodes at maturity	Plant height (cm)	Biological yield (g)	Root length (cm)	Number of pods per plant	Number of seeds per plant	Seed yield per plant (g)	100-seed weight (g)	Harvest index (%)	Protein content (%)
Days to first flowering	0.176	0.957*	0.574*	-0.792*	0.024	-0.078	0.761*	-0.040	0.405*	-0.570*	-0.586*	-0.308*	0.612*	-0.433*	0.381*
Number of nodes at first flowering		0.137	0.067	-0.165	-0.322*	0.643*	0.242*	-0.151	0.007	-0.268*	-0.223*	-0.042	0.375*	0.018	0.251*
Days to first pod set			0.606*	-0.715*	-0.025	-0.071	0.728*	0.007	0.344*	-0.533*	-0.556*	-0.279*	0.629*	-0.426*	0.378*
Days to 75% maturity				0.046	-0.203*	0.086	0.419*	-0.259*	-0.312*	-0.577*	-0.546*	-0.459*	0.267*	-0.532*	0.464*
Reproductive phase					-0.181	0.160	-0.616*	-0.144	-0.727*	0.265*	0.308*	0.033	-0.546*	0.132	-0.119
Number of primary branches						-0.196	0.101	-0.025	0.370*	-0.029	-0.110	-0.184	-0.095	-0.280*	-0.055
Number of nodes at maturity							0.197	-0.140	-0.191	-0.212*	-0.188	-0.036	0.227*	0.030	0.126
Plant height (cm)								0.023	0.324*	-0.477*	-0.509*	-0.164	0.698*	-0.263*	0.222*
Biological yield (g)									0.234*	0.762*	0.731*	0.899*	0.123	0.740*	-0.235*
Root length (cm)										-0.055	-0.105	0.091	0.404*	0.009	0.027
Number of pods per plant											0.984*	0.851*	-0.423*	0.812*	-0.500*
Number of seeds per plant												0.842*	-0.456*	0.812*	-0.460*
Seed yield per plant (g)													0.043	0.952*	-0.312*
100-seed weight (g)														-0.009	0.285*
Harvest index (%)															-0.334*

** Significant at $P \leq 0.05$

Fig 1: Plant morphology of Parents and Inter-specific hybrids

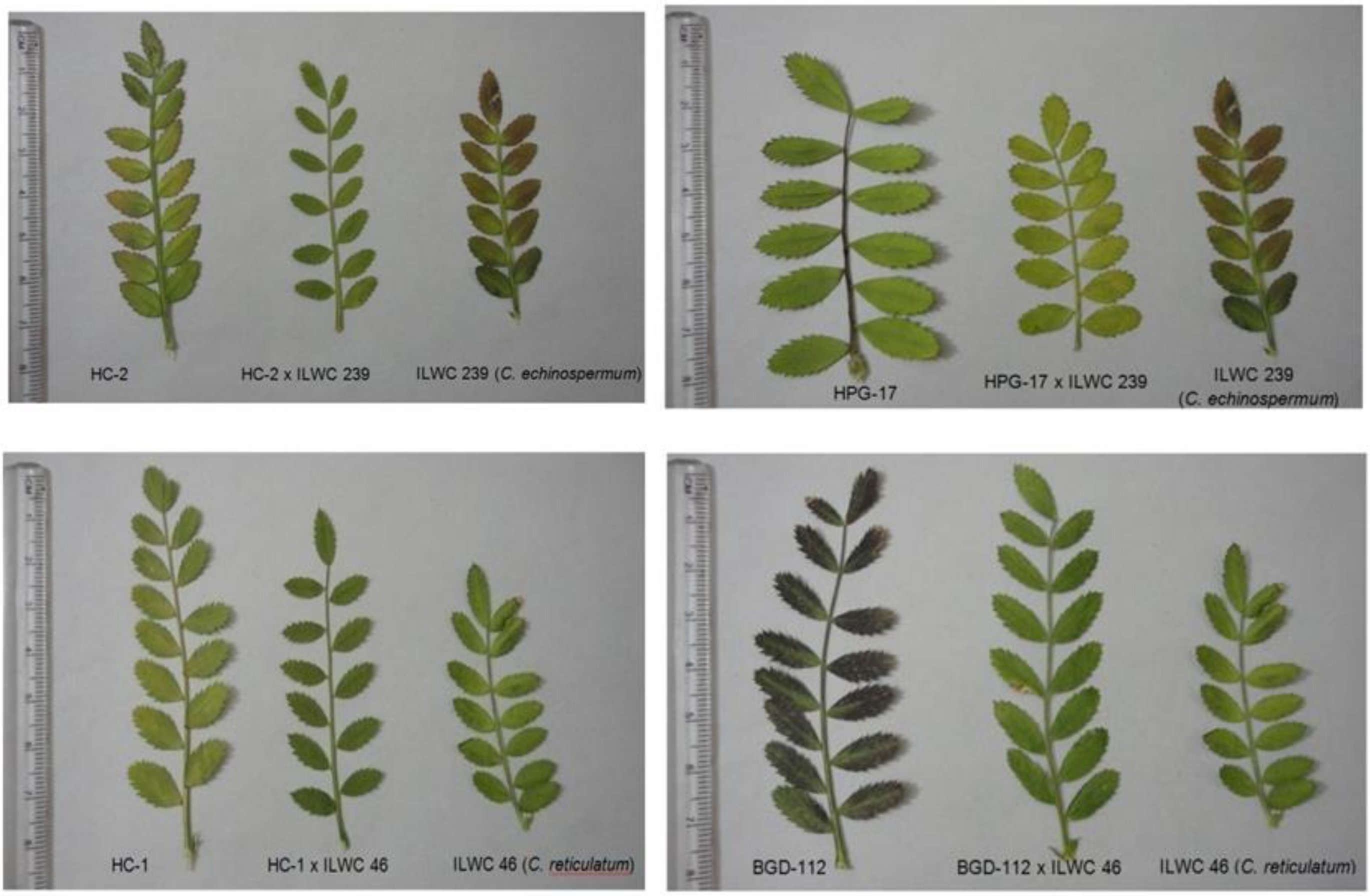


Fig 2: Leaf Morphology of Parents and Inter-specific hybrids

Conclusion

Present investigation revealed the crossability of *C. reticulatum* and *C. echinospermum* with cultivated chickpea indicating ample possibility for unhindered gene transfer via wide hybridization. Results also revealed that hybrid pod set dependednot only upon the species chosen but also on the parents of a particular species involved in the crossing programme.The inter-specific hybrids generated sufficient genetic variability thereby increasing the scope of selection for these traits apart from widening the genetic base. The study also showed that annual wild *Cicer* species i.e. *C. reticulatum* and *C. echinospermum* can be exploited successfully for chickpea improvement and broadening the genetic base of cultivated chickpea for yield and stress tolerance. Higher seed yield per plant can be obtained by selecting derivatives with high biological yield per plant, pods per plants, seeds per plant and harvest index.

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