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Exploitation of wild *Cicer* species for yield improvement in chickpea

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Abstract Chickpea (*Cicer arietinum* L.) ranks third in the world, and first in the Mediterranean basin, for production among pulses. Despite its importance as a crop and considerable research effort, traditional breeding methods have so far been unable to produce cultivars with a large impact on chickpea production. Interspecific hybridization is known to improve yield in many crops. Therefore, an attempt was made to increase the seed yield in chickpea through the introgression of genes from wild relatives at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, from 1987 to 1995. Four crosses, ILC 482 (*C. arietinum*) × ILWC 179 (*C. echinospermum*) and ILC 482 × ILWC 124 (*C. reticulatum*) and their reciprocals, were made. Pedigree selection was used to advance the material. Heterosis was recorded visually in F_1s, and single plant measurements for seed yield were recorded in F_2 populations. Promising and uniform progenies were bulked in the F_5 generation. Out of 96 F_6 lines, 22 were selected on the basis of seed yield and other agronomic characters, and evaluated in a replicated trial for seed yield and 14 agronomical, morphological and quality characters. A high level of heterosis was observed in F_1s. Several F_2 plants produced two to three times more seed yield than the best plant from the cultigen. Nine F_7 lines out-yielded the cultigen parent by up to 39%. Over 2 years, 12 lines had a higher yield than the cultigen parent. These lines were not only high yielding but also free of any known undesirable traits from the wild species, such as spreading growth habit, pod dehiscence, and non-uniform maturity. Quality traits, such as seed shape, type, colour, weight, and testa texture, protein content, cooking time and an organoleptic test of a Middle East dish, *Homos Bi-Tehineh*, were also similar to the cultigen parent. Both *C. echinospermum* and *C. reticulatum* contributed towards the increased yield.

Key words Chickpea · *Cicer arietinum* · Wild *Cicer* species · Interspecific cross · Yield · Introgression of genes

Introduction

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in the Mediterranean basin and South Asia, though it ranks third in the world (FAO 1995). However, its world average yield of 700 kg ha^{-1} is much below its potential (Singh et al. 1994). Traditional breeding methods have not produced cultivars with a large impact on chickpea production. As a result, chickpea yield continues to be low.

Wild species have contributed substantially to crop improvement for many characters including seed yield (Stalker 1980; Harlan 1984; Prescott-Allen 1988). However, exploitation of wild species is mainly confined to major cereal and cash crops. In chickpea, attempts to exploit wild *Cicer* species are recent and much of the research has been conducted at the International Center for Agricultural Research in the Dry Areas (ICARDA). Singh et al. (1994) report genes in *Cicer* species for resistance to ascochyta blight [*Ascochyta rabiei* (Pass.) Lab.], fusarium wilt [*Fusarium oxysporum* Schlecht. emend Snyd. & Hans. f.sp. *ciceri* (Padwick) Snyd. & Hans.], leaf miner (*Liriomyza cicerina* Rondani), seed beetle (*Callosobruchus chinensis* L.), cyst nematode (*Heterodera ciceri* Vovlas, Greco et Di Vito), and cold. However, Robertson et al. (1995) report the

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Joint contribution from ICARDA and ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), Patancheru P.O., A.P. 502 324, India

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lack of useful genes of morphological, physiological and agronomical importance in eight wild annual *Cicer* species.

Ocampo et al. (1992) classified nine annual species into two groups using karyotype symmetry indices. The first group included *C. arietinum* L. (cultivated), *C. reticulatum* Ladiz. and *C. echinospermum* P. H. Davis, while the second group comprised the remaining species. Labdi et al. (1996) studied the phylogenetic relationships among the annual *Cicer* species as revealed by isozyme polymorphism and confirmed the grouping of three species in the first group. The remaining six species were grouped in three clusters.

Ladizinsky and Adler (1976 a, b) successfully crossed *C. reticulatum* with chickpea and also considered this species as the wild progenitor. Singh and Ocampo (1993) succeeded in crossing chickpea with *C. echinospermum*. Jaiswal and Singh (1989) studied the F_2, F_3 and F_4 generations of a cross between *C. arietinum* and *C. reticulatum* for yield and yield-related traits. Their results indicate the possibility of improving yield through the introgression of genes from *C. reticulatum* into the cultigen. Unfortunately, they did not pursue their study further. Singh and Ocampo (1993) report 28–153% hybrid vigour in the F_1s of crosses between chickpea and *C. echinospermum* and *C. reticulatum*, compared with the 75% reported in intraspecific crosses (Singh et al. 1984). Singh and Ocampo (1993) also found numerous transgressive segregants for high yield in F_2 populations. The performance of these lines suggested that genetic re-shuffling, originating from interspecific hybridization, could produce favourable combinations of genes expressing high yield. Therefore, the objective of this study was to determine the gain in seed yield in lines developed from interspecific hybridization.

Materials and methods

Four crosses, ILC 482 (*C. arietinum*) × ILWC 179 (*C. echinospermum*) and ILC 482 × ILWC 124 (*C. reticulatum*) and their reciprocals, were made in the field at Tel Hadya (36° 35′E, 36° 51′ N and 284 masl), Syria, the main research station of ICARDA, during 1987/88. For the sake of convenience, the cross between the cultigen and *C. echinospermum* is designated as the echinospermum cross and that between the cultigen and *C. reticulatum* as the reticulatum cross. The F_1s were grown in the field during 1988/89 at Tel Hadya. Each F_1 row was flanked by its two parents to ascertain their hybrid authenticity. All the F_1 plants were bulk-harvested after recording hybrid vigour visually.

About 2000 seeds from each of four F_2 populations were grown in the field during 1989/90 along with their parents. Seeds were sown in 4-m rows, spaced 0.45 m apart and the parents were grown in the beginning and at the end of each row. Plants from reticulatum crosses were fully fertile, but plants from echinospermum crosses varied from complete sterility to complete fertility. The selection of plants from the latter cross was made among those plants which were near to 100% fertile. The plants selected from each cross were those phenotypically close to the characteristics of chickpea, such as growth habit (erect to semi-spreading), tall height, absence of pod dehiscence, uniform maturity, heavy beige-coloured seeds, owl or round shaped seeds, and high seed yield. Each of 138 plants was individually harvested and advanced to the next generation by pedigree selection.

Sowing of all generations from F_1 to F_8 was done during winter in early December with harvesting during summer in June. Seeds of wild parents were always sown after scarification, whereas crossed seeds were scarified up to the F_3 generation only. Weeds were controlled using herbicides and ascochyta blight using a fungicide spray.

Sixty six F_3 progenies from the echinospermum cross and 72 F_3 progenies from the reticulatum cross were grown during 1990/91. Each progeny was grown in a single 4-m row plot, spaced at 0.45 m apart. After every ten test entries, one row of each of the three parents was grown. Selection was first carried out among progenies, followed by the selection of 3–5 plants within each selected progeny. The selection criteria were as described for the F_2 generation. Exceptions were that the selected plants had seeds like the kabuli (ram-head-shaped, large size, beige colour) or desi type (small, angular coloured seeds). Each plant was harvested individually. Following this procedure, the material was advanced to F_5. In the F_5, 48 uniform and promising progenies from the reticulatum cross and 87 progenies from the echinospermum cross were selected and individually harvested in 1992/93.

In a preliminary yield trial, 96 F_6 progenies (61 from the echinospermum cross and 35 from the reticulatum cross) and three parents were evaluated in an incomplete block design with two replications during 1993/94. The plot size was two rows, 4-m long, spaced 0.45 m apart. The plant population in the plot was maintained at 33 plants per m^2. On the basis of high seed yield, tall plants and heavy seed, 22 lines were selected. In an advanced yield trial, 22 F_7 lines and three parents were evaluated in a randomised complete block design with three replications during 1994/95. The plot size was the same as in the preliminary yield trial.

Observations were recorded on 15 characters: days to 50% flowering, days to maturity, uniformity in maturity, pod dehiscence, biological yield, seed yield, harvest index, 100-seed weight, plant height, seed colour, seed shape, seed type, growth habit, germinability, and cooking time. An organoleptic test for *Homos Bi-tehineh*, a Middle East delicacy, was also conducted.

The statistical computer programs MSTAT-C Version 2.10 and Genstat 5 (release 3) were used for the analysis of variance.

Results

The success in crossing using the cultigen as a female parent with both echinospermum and reticulatum parents was over 75%. However, when echinospermum and reticulatum were used as female parents, the success rate was low at 21 and 5%, respectively.

The F_1s showed hybrid vigour (Fig. 1). This suggested that the genes associated with traits which affect yield in the cultivated and the two wild relatives may be different and combining them in a single genotype could improve yield in chickpea. This finding encouraged us to measure the seed yield of plants from F_2 populations. Numerous transgressive positive segregants for agronomic traits were present in the F_2 generation (Table 1). This result further suggested that high-yielding lines could be developed from interspecific hybridization in chickpea. Although the means of four segregating populations were not different from the parents, the difference in range in F_2s was greater than in the parents, permitting the selection of high-yielding plants.

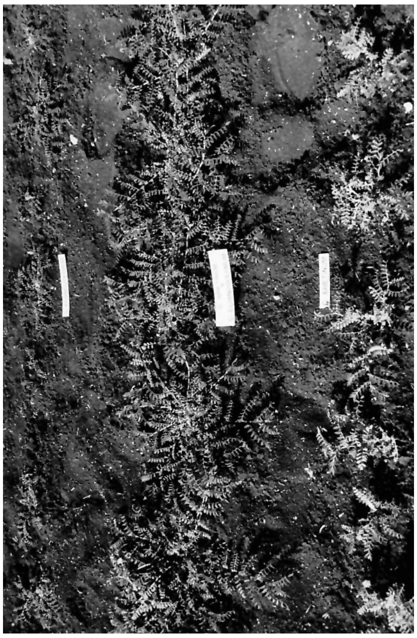

Fig. 1 Performance of F_1 hybrid (middle) derived by crossing *C. arietinum* (ILC 482) (right) and *C. echinospermum* (ILWC 179) (left) at ICARDA, Aleppo, Syria, 1988–89

Table 1 Mean and range for seed yield in F_2 populations from interspecific crosses in *Cicer* at ICARDA, Syria, 1989/90

Cross/parent	No. plants	Yield (g plant^{-1})		
		Min	Max	Mean
Cross				
AE[a]	302	0.2	54.4	10.0 ± 0.54
EA	175	0.3	29.7	8.7 ± 0.50
AR	476	0.3	27.2	5.8 ± 0.22
RA	561	0.4	64.6	8.6 ± 0.30
Parent				
ILC 482	10	3.8	15.4	8.3 ± 1.50
ILWC 124	10	1.6	23.2	8.3 ± 3.04
ILWC 179	10	1.8	17.0	6.3 ± 1.82

[a] AE = *C. arietinum* × *C. echinospermum*
EA = *C. echinospermum* × *C. arietinum*
AR = *C. arietinum* × *C. reticulatum*
RA = *C. reticulatum* × *C. arietinum*

The seed yield and other morpho-agronomic characters for the 22 F_7 lines are shown in Table 2. All the 22 derived lines from these interspecific crosses were phenotypically uniform. Nine lines produced higher seed yield than the cultigen. However, only one line (No. 55), which produced a 39% higher yield than the cultigen (ILC 482), was significantly different. Sixteen lines had a higher biological yield than the cultigen. Fifteen lines had heavier seed than the cultigen by a margin of 15–39%. Most of the new lines were similar to ILC 482 in maturity, plant height and harvest index. The wild relatives had significantly lower seed yield and harvest index, and they also had short plants and low seed weight.

All derived lines were similar to the cultigen parent in growth habit (erect to semi-spreading), uniformity in maturity, and non-pod dehiscence, whereas the two wild species had a spreading growth habit, non-uniform maturity and dehiscent pods (data not shown but available on request). Accessions of wild *Cicer* species have seed dormancy of varying length, though the wild progenitor, *C. reticulatum*, has only partial seed dormancy. When the test was conducted 3 months after harvest, the derived lines had the same germination percentage as the cultigen parent. On the other hand, the echinospermum parent had 0% germination and the reticulatum parent had only 13% germination.

The seed type, colour and shape of the derived lines were like the cultigen parent. The 100-seed weights of the new lines were similar to, or better than, ILC 482, whereas the seed size of the two wild relatives were approximately half that of the cultigen and derived lines. The cooking time of the new lines was about the same as that of the parents. The organoleptic test of a Middle East dish, *Homos Bi-Tehineh*, a popular appetizer, suggested that all new lines had good to excellent scores (data not shown but available on request).

Results based on trials of 22 test lines and three parents for seed yield, 100-seed weight and plant height in the two-seasons 1993/94 and 1994/95 are shown in Fig. 2. The performance of lines was different in the two years because of climatic differences. However, 12 lines produced a higher seed yield than ILC 482. There were several test lines which surpassed ILC 482 in 100-seed weight and plant height. The wild relatives were poor in all three attributes.

Discussion

The three parents used in our study were carefully chosen. The cultigen parent, ILC 482, is partially resistant to ascochyta blight and cold, is high yielding and widely adapted and has been released as a cultivar in eight countries – Algeria, France, Iraq, Jordan, Lebanon, Morocco, Syria and Turkey (Singh et al. 1994). The reticulatum parent, ILWC 124, is resistant to fusarium wilt and cold, while the echinospermum parent, ILWC 179, is resistant to fusarium wilt, seed beetle and cold. The two wild representatives were agronomically the best lines available within their respective species (Robertson et al. 1995). Furthermore, they had relatively few undesirable traits. Therefore, it was expected that they would combine well and could produce positive transgressive segregants for seed yield and other agronomic traits. Our result supported our hypothesis. We developed a few lines superior to the best cultigen line under Tel Hadya conditions (Mediterranean climate with a cold and wet winter and

Table 2 Seed yield and yield-related characters of 22 F_7 lines derived from interspecific crosses at ICARDA, Syria, 1994/95

Line no.		Characters evaluated					
		DFL[a] no.	DMA no.	HGT cm	BYD kg ha^{-1}	SYD kg ha^{-1}	HI %
55	RA[d]	107	161	43	7415	3420	46
126	AE	103	161	42	6925	3160	46
41	AE	110	163	47	6762	2850	42
63	RA	109	159	42	6258	2711	43
90	AE	106	157	40	5265	2706	51
118	AE	110	159	49	6350	2687	43
86	AE	106	158	44	5664	2585	46
49	RA	111	160	43	5755	2584	44
95	AE	108	159	40	5510	2487	45
482[b]	A	106	158	42	4966	2458	50
50	RA	109	161	42	5538	2457	44
17	AR	114	160	50	6476	2450	38
5	RA	100	163	42	4966	2416	48
79	AE	107	163	53	6408	2371	37
82	AE	114	160	38	5660	2367	41
67	RA	110	163	45	6490	2355	36
80	AE	110	162	47	6041	2343	38
81	AE	110	161	50	5633	2324	41
53	RA	106	158	43	4673	2226	46
130	AE	110	160	47	5003	2077	41
51	RA	110	163	47	4871	2034	46
96	AE	110	162	43	5286	1997	38
18	AR	109	160	39	4599	1819	40
124[c]	R	106	160	27	6289	662	14
179[c]	E	120	157	13	1644	424	30
SE ±		1.1	1.2	4.4	201	331	2.7
CV (%)		1.8	1.3	18	25	23	11

[a] DFL = days to flowering; DMA = days to maturity; HGT = plant height; BYD = biological yield; SYD = seed yield; HI = harvest index
[b] ILC line
[c] ILWC line
[d] A = *C. arietinum* (ILC 482), R = *C. reticulatum* (ILWC 124), E = *C. echinospermum* (ILWC 179), AE = A × E and AR = A × R

a hot and dry summer, and average precipitation of 330 mm). Most important, these lines were not only superior in yield but were also free of any known undesirable traits from the wild species. Additionally, where sufficient seeds were available, a few of the high-yielding lines were given an organoleptic test for the Middle East dish, *Homos Bi-Tehineh*, and the quality of those lines were as good as ILC 482. All the available data indicate that a few genes associated with traits affecting yield have been transferred from the wild relatives to chickpea, resulting in an improvement in seed yield.

In selecting plants in segregating populations, we took extreme care to reject those having obviously undesirable traits from the wild relatives, and to select plants which were closer to the cultigen parent and superior in yield performance. In the cross with *C. echinospermum*, we were faced with partial sterility, but careful selection of plants against this trait eliminated it after the F_4 generation. Rejection of traits like pod dehiscence and spreading growth habit was achieved relatively easily by selecting against them.

Since our interest was in the selection of kabuli-type plants, we found a smaller percentage of plants of this type, which was about the same as Hawtin and Singh (1980) reported. They obtained 16% recovery of the kabuli type in crosses between kabuli and desi in the F_2, and 81% recovery in the F_3 from kabuli-type selection in the F_2 generation. Plants with near-kabuli-type seeds in the F_2 gave 42% kabuli-type seeds in the F_3. Hence, in selection from the F_2 to the F_4 generation, we applied pressure in favour of kabuli-type seeds. The cultigen had kabuli-type seed and the two wild relatives had desi-type seeds.

Deleterious genes introduced from *C. echinospermum* and *C. reticulatum* to the cultigen were less of a problem than expected. Therefore, backcrossing was not considered necessary. Moreover, it has been observed in many interspecific hybridization programmes that after four to five backcrosses with the cultigen lines similar to the cultigen parent are produced (personal communication with Dr. A. van Schoonhoven, CIAT, Colombia). Several backcrosses are required where a simply inherited character is to be added. Also, at least a few

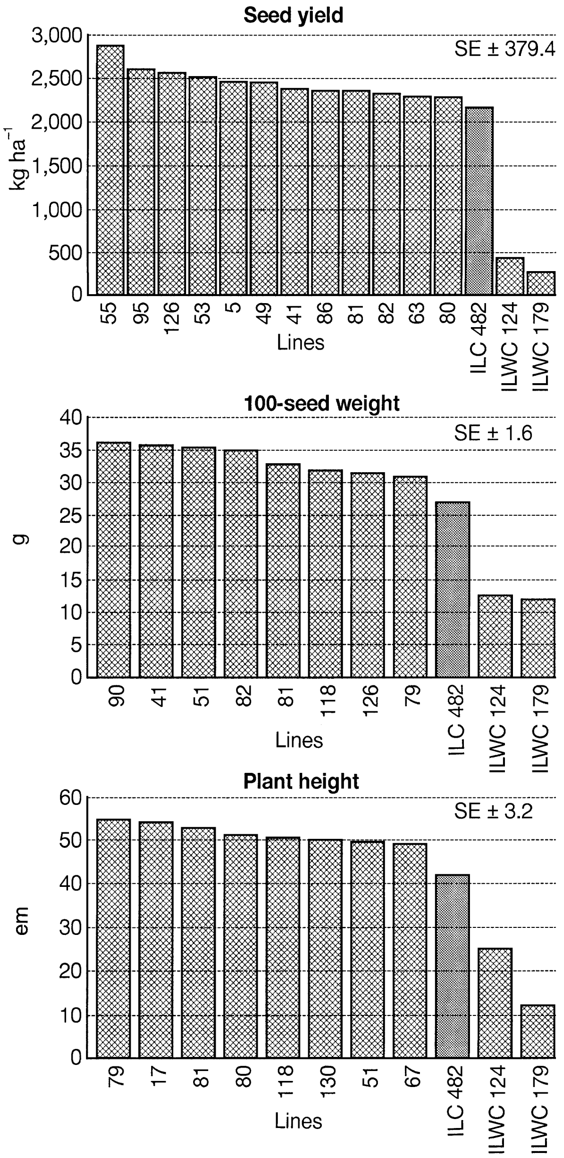

Fig. 2 Seed yield, 100-seed weight and plant height of lines exceeding the cultigen based on tests at ICARDA, Aleppo, Syria in the two-seasons 1993/94 and 1994/95

backcrosses are required where high levels of quality traits are desired. In chickpea, our goal was to increase the seed yield, which we believe has been achieved without backcrossing. Harlan (1984) compiled information on the improvement in seed yield through hybridization between cultivated species and their wild relatives. He listed 14 crops where improvement in seed yield has been increased through hybridization with wild relatives and races. These crops are pearl millet, sorghum, rice, barley, wheat, maize, egg plant, sweet potato, oats, potato, groundnut, tobacco, sugarcane and strawberries. We may now add chickpea in this list.

Comparisons between reciprocals of crosses of the cultigen with *C. echinospermum* showed that positive transgressive segregants for seed yield were recovered only from the cross using the cultigen as a female parent, suggesting strong maternal effects of this species. No such difference was noticed in crosses between the cultigen and *C. reticulatum*. Both wild species contributed towards the recovery of superior lines.

The presence of transgressive segregants for seed yield and other agronomic traits suggests genetic complementarity between *C. arietinum*, *C. echinospermum* and *C. reticulatum*. This fosters hopes of even better segregants than those already obtained. With this in mind, we have already crossed six high-yielding lines with these two wild relatives, and material from these crosses had reached the F_4 stage by 1994/95. Furthermore, the derived high-yielding lines from the present study have been crossed with lines resistant to ascochyta blight to add genes for resistance. High-yielding and blight-resistant lines will be useful to growers for stabilizing yields.

The introgression of genes from currently non-crossable species, especially *C. bijugum*, *C. judaicum* and *C. pinnatifidum*, is highly desirable because they possess genes for resistance to many stresses, and may produce higher-yielding derivatives upon crossing with the cultigen than we have achieved from the current crossing programme. The lack of cross-compatibility between *C. echinospermum* and *C. reticulatum* and the above three species precludes their introgression into the cultigen, even through bridge-crossing. But diverse crossing techniques (Stalker 1980) could aid the gene flow in crosses involving annual *Cicer* species. The rescue of ovules and their in-vitro culture could also prove a valid solution for cross success whenever genetic incompatibilities between taxa do not preclude total gene flow.

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