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EVALUATION OF *CICER* SPECIES FOR RESISTANCE TO *ASCOCHYTA* BLIGHT

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Abstract

A collection of 64 wild *Cicer* accessions from seven different species (*C. bijugum* K.H. Rech., *C. cuneatum* A. Rich., *C. echinospermum* Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jaub. & Spach, *C. reticulatum* Ladiz., and *C. yamashitae* Kitamura) were screened for resistance to *Ascochyta* blight *(Ascochyta rabiei* (Pass.) Lab) by creating artificial epiphytotic conditions in the field. Resistance was identified in accessions from six wild *Cicer* species. Variation for resistance within accessions of *C. bijugum, C. echinospermum, C. judaicum, C. pinnatifidum, C. reticulatum* and *C. yamashitae* was recorded. All the accessions of *C. cuneatum* were highly susceptible to *Ascochyta* blight. Resistant accessions of *C. echinospermum* and *C. reticulatum* belong to primary gene pool of *Cicer* species and can be crossed easily with *Cicer arietinum* and fertile hybrids can be obtained.

Introduction

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in Pakistan. It is a major food legume in many countries of the world. Chickpea seed is an important source of plant based dietary protein and around 95% of its total annual production ($8.8x10^6$ tons) occurs in developing countries (Anon., 2002). The average yield of 550-650 kg ha^{-1} is rather low in Pakistan. Unfortunately the chickpea crop is susceptible to a range of biotic and abiotic stresses, which can be devastating to crop yield by about one third every year (Haware, 1993). There are 47 diseases and 54 insect pests reported from chickpea, of which 6 diseases and 2 insect pests resulted in a severe losses of chickpea yield (van Rheenen 1991; Singh *et al*., 1994). However, major biotic factor limiting chickpea yield worldwide are the fungal diseases, *Ascochyta* blight (*Ascochyta rabiei* (Pass.) Lab) and Fusarium wilt (*Fusarium oxysporum* Schleeht. Emend. Synd. & Hans. f. sp. *Cicer* [Padwick] Synd.& Hans). Botrytis grey mould (*Botrytis cinerea* Pers. Ex Fr.) and Phytophythora root rot (*Phytophythora megsperma* Drechts) are constraints to chick pea production in India, Pakistan and some parts of Australia (Singh *et al*., 1994, 1998; Siddique *et al*., 2000; Knights & Siddique, 2002). To minimize the yield losses, introduction of resistant species is necessary and screening is the best tool in this regard. Screening program of cultivated chickpea germplasm has not been able to identify stable and high level resistance to a number of diseases (Singh & Reddy 1993; Singh *et al*., 1994). Chemical control of these diseases might be effective, however, use of resistant cultivars would be the most effective one. Limited germplasm of chickpea resistant to *Ascochyta* blight is found in existing chickpea species so it is, necessary to search out new sources of resistance to this disease (Reddy & Singh, 1984).

Table 1. Annual wild *Cicer* species germplasm and their country of origin.

Species	Access. no.	Plant no.	Origin	Species	Access. no.	Plant no.	Origin
Cicer arietinum	Control	K-850		*C. judaicum*	593714	LR-126	Syria
C. bijugum	458550	ILWC 34	Turkey		593715	LR-135	Syria
	458551	ILWC 7	Turkey		593717	ILWC 41	Syria
	458552	ILWC 8	Turkey		599054	ILWC 43	Syria
	599037	ILWC6 8	Turkey		599055	ILWC 44	Syria
	599039	ILWC 32	Israel		599056	ILWC 45	Syria
	599046	050689-0203	Turkey		599057	ILWC 46	Syria
	599047	050689-0301	Turkey		599077	ILWC255	Jordan
	599048	060689-0101	Turkey		599078	ILWC 256	Jordan
	599049	090689-0301	Turkey		599095	ILWC 279	Syria
	599051	100689-0401	Turkey		599104	ILWC 272	Lebanon
	599065	LR-051	Syria		599105	ILWC 273	Lebanon
	599066	W6 10149	Iraq		599106	ILWC 274	Lebanon
	599075	ILWC 42	Syria	*C. pinnatifidum*	458555	ICCW 37	Turkey
	10150	W6 10150	Turkey		458556	ICCW 38	Turkey
C. cuneatum	458554	ICCW 47	Ethiopia		458557	ILCW 9+ 33	Turkey
	ICP 17162		Ethiopia		510654	ILWC 226	Turkey
C. echinospermum	489776	ICCW 44	Turkey		510663	ILWC 19	Turkey
C. judaicum	458558	ILWC 38	Lebanon		518860	110785-401	Turkey
	458559	ICCW 35	Israel		518861	110785-0601	Turkey
	458560	ICCW 30	Israel		518862	120785-0101	Turkey
	504291	ILWC 31	Jordan		518863	130785-0301	Turkey
	510658	ILWC 4	Lebanon		593716	5119	Syria
	510659	ICCW 34	Israel		599043	040689-0603	Turkey
	510661	ILWC 29 b	Israel		599045	040689-0703	Turkey
	510662	ILWC 30	Israel		599059	ILWC 49	Syria
	568217	W6 1016	Morroco		599060	ILWC 51	Unknown
	572536	ILWC 33-5/8	Syria		599070	LR-193	Syria
	593710	ILWC 47	Syria		599071	LR-198	Syria
	593711	ILWC 48	Syria	*C. reticulatum*	489778	ILWC 17+21	Turkey
	593712	BMV 23-9	Syria	*C. yamashitae*	504550	ILWC 215	Afghanistan
	593713	BMV 26-1	Syria		510657	ILWC 214	Afghanistan
					510664	ILWC 3	Afghanistan

Wild relatives of crops often possess genes that confer resistance to biotic stresses (Malhotra *et a*l., 2000). Singh *et al*., (1994) reported genes in *Cicer* species for resistance to *Ascochyta* blight, fusarium wilt, leaf miner (*Liriomyza cicerina* Rondani), seed beetle (*Callosobruchus chinensis* L.), cyst nematode (*Heterodera ciceri* Volvas, Grco et Di Vito), and cold. Sources of resistance to *Ascochyta* blight have been identified in a limited number of annual wild *Cicer* species, as reported for *C. pinnatifidum* Jaub. & Sp. and *C. judaicum* Boiss. (Singh *et al*., 1981), *C. bijugum* K.H. Rech. (Haware *et a*l. 1992), *C. echinospermum* P. H. Davis and *C. reticulatum* Ladiz. (Stamigna *et al*., 1998) and *C. judaicum* and *C. pinnatifidum* (Singh & Reddy, 1993). The aim of this study was to evaluate seven annual wild *Cicer* species against local isolates of *A. rabiei* in order to identify potential sources of resistance for chickpea breeding programs.

Materials and Methods

Chickpea germplasm: The material consisted of 64 accessions of seven annual wild *Cicer* species which were obtained from ICRISAT Hyderabad India, ICARDA Syria and WRPIS Pullman USA. Different species screened for resistance to *Ascochyta* blight were *C. bijugum* K.H. Rech., *C. cuneatum* A. Rich., *C. echinospermum* Davis, *C. judaicum* Boiss., *C. pinnatifidum* Jaub. & Spach, *C. reticulatum* Ladiz., and *C. yamashitae* Kitamura. The list of accessions of all the species along with their country of origin is presented in Table 1.

Isolation: Infected pods, stems or leaflets were collected and sterilized in 5% sodium hypochlorite for 1 minute and dried on sterile filter paper. The material was plated on 2% water agar and incubated at 20^{o}C with a 12 h light/dark cycle for 5-7 days for fungal growth. Fungal colonies growing from the plant material were sub cultured onto chickpea seed-extract agar (CSEA) consisting of an extract from 60 g chickpea seed and boiling the seed in deionized water for 30 minutes. Sucrose (20g) and Agar (20g) were added to the extract and volume was made upto 1 liter with distilled water. Incubation on this medium for 1-2 weeks resulted in the development of colonies of the fungus with pycnidia.

Spore suspension: Chickpea seed were softened by boiling for 15-30 minutes in distilled water, drained and autoclaved for 30 minutes at 121^{o}C and 15 psi in a conical flask. The sterilized seed were inoculated by *Ascochyta rabiei* from CSEA slants and incubated at 20-22^{o}C for 10 days. Spore suspension was prepared from fungal cultures by adding sterile distilled water and gently mixing it with a glass rod. The suspension was filtered through four layers of muslin cloth. The concentration of the spore suspension was determined with a haemocytometer and adjusted to 4×10^{4} spores /ml with sterile distilled water. Tween 20 (one drop/100mL) was added to the spore suspension as a surfactant agent for sticking the spore to leaves of chickpea plants.

Cultivation and inoculation: All the accessions were sown in the last week of October. The screening was carried out in *Ascochyta* Blight Screening Nursery at NIAB, Faisalabad during 2002-03 where sprinkle system for creating artificial humidity (about 70-80%) by producing mist was developed. To initiate the germination, the seeds of wild *Cicer* species were scarified by incising the seed coat carefully avoiding the area of the hilum and embryo, to allow water to penetrate into the seed. Seed of each accession (20 plants) was sown (about 2cm deep) in a single 2 meter row plot with inter and intra-row

spacing of 30 and 15cm, respectively The experiment was conducted in a randomized block design with three replications. Variety K850 (highly susceptible to *Ascochyta* blight and resistant to *Fusarium* wilt) was used as check after every two lines of wild species to monitor possible variation in the level of infection. The plots were fertilized with 125 Kg ha^{-1} DAP at the sowing time. Hand weeding was done three times during cropping season. Wild *Cicer* accessions germinated more slowly than cultivated chickpea, with *C. bijugum* being the slowest growing accession. During the first week of February, when the plants were about eight to ten-leaf stage, they were inoculated by spraying approximately 5 ml of the spore suspension per plant with a hand plastic/steel sprayer until run-off.

Disease rating: The disease reactions of individual plants were scored 14 days after inoculation on 1-9-scoring scale, modified from Reddy & Singh (1984), where:

1= No lesions of disease is visible on the plant;
2= Highly resistant, infection on 1–10% of leaves;
3= Resistant, infection on 11–20% of leaves;
4= Moderately resistant, infection on 21–30% of leaves and stem (s);
5= Tolerant, infection on 31–40% of leaves and stems and/or stem girdling;
6= Moderately susceptible, infection on 41–50% of leaves and stems and/or stem girdling and breakage;
7= Susceptible, infection on 51–75% of leaves and stems including stem girdling and breakage;
8= Highly susceptible, infection on 76–98% of leaves and stems, including stem girdling and breakage; and
9= Lesions profuse on all parts of plant and stem girdling cause drying of young shoots and branches, resulting in the death of the plant.

Resistance for an individual plant was defined as a disease score less than, or equal to, four. Accessions possessing mean disease scores of less than five were described as resistant. Mean disease scores were subjected to analysis of variance (ANOVA) in order to detect differences between different accessions. Difference between mean disease scores of cv. K-850 the susceptible control and mean disease scores of individual accessions were calculated using *t*-test.

Results and Discussions

Forty-six (72 %) out of a total of 64 *Cicer* accessions were categorized as resistant and eighteen (28 %) as susceptible to *ascochyta* blight (Table 2). This figure shows that a lot of resistant genes to biotic stress are available in wild species of crop (Malhotra *et al*., 2000), which can be utilized in the breeding program for increasing stress tolerance in chickpea crops.

The analysis of variance table (ANOVA) revealed that the variation among accessions of seven wild *Cicer* species were highly significant. The mean disease scores and their standard errors (SE) for all *Cicer* accessions tested in the trial are given in the Table 3. Accessions possess significantly lower (at $p \geq 0.05$ and $p \geq 0.01$) mean disease scores than that of cv. K850 (Susceptible check). Control plants were given same treatment like other wild *Cicer* species but they did not survive.

Table 2. Reaction of accessions of *Cicer* species to *Ascochyta blight*.

Species	Total	Resistant	Susceptible
Cicer bijugum	14	14	-
C. cuneatum	2	-	2
C. echinospermum	1	1	-
C. judaicum	27	12	15
C. pinnatifidum	16	15	1
C. reticulatum	1	1	-
C. yamashitae	3	3	-
Total	**64**	**46 (72%)**	**18 (28%)**

Only *Cicer bijugum* accessions maintained uniform resistance to the disease. Some accessions of *Cicer bijugum, C. pinnatifidum* and *C. yamashitae* showed high level of resistance whereas it was only moderate in the accessions of other four wild *Cicer* species. Some variations for resistance between plants within accessions of *Cicer judaicum* and *C. pinnatifidum* were also recorded which include both types of accessions i.e. resistant and susceptible. All *C.cuneatum* accessions evaluated were as susceptible as that of check variety (cv. K-850).

Most of the accessions included in this study had not been tested previously. Only six accessions of *C. bijugum* (ILWC 32, ILWC 42, ILWC68, PI 458550, PI458551 and PI 458552) and one accessions of *C. pinnatifidum* (PI 518862) were studied by Collard *el al*., (2001) and classified as resistant these were also found resistant in our study (Table 3 & 4). Only few reports are available in the literature for the screening of *C.cuneatum.* Four accessions were classified as highly susceptible by Singh *et al*., (1998) and Stamigna *et al*., (1998) and were also classified as highly susceptible in our study. Similarly, in the case of *C. judaicum*, only three accessions were screened by Singh *et al*., (1998) and classified one accession (ILWC 46) as highly susceptible and other two accessions (ILWC255 and ILWC256) as highly resistant and our findings confirmed the above finding. In contrast to present study i.e. the three accessions of *C. yamashitae* (ILWC3, ILWC214 and ILWC215) and one accession of *C. echinospermum* (PI 489776) classified as resistant in this study were classified as highly susceptible by Singh *et al*., (1998) and by Collard *et al*., (2001) respectively. They also screened three accessions of *C. yamashitae* but did not mention the accession number in their report. The reason may be due to the disease reactions that may be caused by the differences in the races of *Ascochyta rabie*i of the region.

The worldwide germplasm collection of cultivated chickpea has very low frequency of resistance against *Ascochyta rabie*i (Reddy & Singh, 1984) and chickpea are lacking in genetic diversity that may include traits needed for effective improvement of the crop (Robertson *et al*., 1997, Collard *et al*., 2003). However, this may be overcome by looking to the wild relatives to widen the genetic bases of breeding program through interspecific hybridization (Singh & Ocampo, 1997). The accessions of *C. echinospermum* and *C. reticulatum* belong to primary gene pool of *Cicer* species and are easily crossable and can generate fertile hybrids with *Cicer arietinum* and also readily accessible source of resistance (Singh & Ocampo, 1993). The accessions evaluated in this study demonstrated that there is resistance within wild *Cicer* species to the *Ascochyta rabie*i pathotype prevalent in this region, and this may be used to develop resistant cultivars. *C. bijugum, C. judaicum* and *C. pinnatifidum* belong to secondary gene pool of wild chickpea and limited success has been reported in crossing the species in group I with group II (Ahmad *et al*., 1988, Singh *et al*., 1994, Anon., 1998). However, tissue culture methods such as

Table 3. Mean (± SE) disease score for *Cicer* accessions of annual wild species

Species	Access. No.	S.E	Class.[A]	Species	Access. No.	S.E	Class.[A]
Cicer arietinum	Control	8.8±0.1	S		593714	3.3±0.2**	R
C. bijugum	458550	2.7±0.3**	R		593715	5.7±0.9**	S
	458551	3.5±0.5**	R		593717	5.8±0.6**	S
	458552	3.8±0.8*	R		599054	5.5±0.3**	S
	599037	3.7±0.4**	R		599055	5.5±0.8**	S
	599039	3.2±0.2**	R		599056	5.0±0.6**	R
	599046	2.0±0.6**	R		599057	6.0±1.2**	S
	599047	1.2±0.2**	R		599077	3.4±0.3**	R
	599048	1.5±0.3**	R		599078	2.9±0.5**	R
	599049	1.9±0.1**	R		599095	5.0±0.2**	R
	599051	1.3±0.3**	R		599104	3.4±0.3**	R
	599065	2.0±0.9**	R		599105	5.0±0.4**	R
	599066	2.8±0.6*	R		599106	2.9±0.5**	R
	599075	2.3±0.7*	R	*C. pinnatifidum*	458555	1.7±0.6**	R
	10150	2.5±0.5**	R		458556	1.4±0.2**	R
C. cuneatum	458554	7.4±1.3[NS]	S		458557	4.7±0.9**	R
	ICP 17162	8.3±0.7[NS]	S		510654	1.5±0.3**	R
C. echinospermum	489776	5.0±0.6**	R		510663	1.2±0.4**	R
C. judaicum	458558	7.0±1.1*	S		518860	3.7±0.9**	R
	458559	7.2±0.4*	S		518861	1.6±0.3**	R
	458560	5.5±0.3**	S		518862	4.0±0.6**	R
	504291	5.0±0.6**	R		518863	3.7±0.2**	R
	510658	5.5±0.8**	S		593716	4.1±0.5**	R
	510659	7.0±1.2*	S		599043	3.1±0.1**	R
	510661	5.8±0.4**	S		599045	3.2±0.6**	R
	510662	6.1±0.5**	S		599059	1.4±0.3**	R
	568217	7.4±0.4*	S		599060	5.0±0.6**	R
	572536	7.0±1.0*	S		599070	5.5±0.4**	S
	593710	3.0±0.6**	R		599071	3.3±0.3**	R
	593711	5.4±0.3**	S	*C. reticulatum*	489778	5.0±0.2**	R
	593712	5.0±0.1**	R	*C. yamashitae*	504550	1.9±0.6**	R
	593713	3.0±0.0**	R		510657	3.1±0.2**	R
					510664	3.7±0.9**	R

Classification[A]: R, resistant S, susceptible

*Mean disease score is significantly different at P= 0.05 from cv. K-850 control

**Mean disease score is highly significantly different at P= 0.01 from cv. K-850 control

Table 4. Comparison with previous studies of resistant classification of *Cicer* accessions.

Name	Species	Classification [A]		References
		Previous study	Present study	
ILWC 32	*C. bijugum*	R	R	Collard et. *al*., (2001)
ILWC 42		R	R	Collard et. *al*., (2001)
ILWC68		R	R	Collard et. *al*., (2001)
PI 458550		R	R	Collard et. *al*., (2001)
PI 458551		R	R	Collard et. *al*., (2001)
PI 458552		R	R	Collard et. *al*., (2001)
PI 458554	*C. cuneatum*	S	S	Stamigna *et al*., (1998)
ICP 17162		S	S	Stamigna *et al*., (1998)
PI 489776	*C. echinospermum*	S	R[B]	Collard et. *al*., (2001)
ILWC 46	*C. judaicum*	S	R[B]	Singh *et. al*., (1998)
ILWC 255		R	R	Singh *et. al*., (1998)
ILWC 256		R	R	Singh *et. al*., (1998)
PI518862	*C. pinnatifidum*	R	R	Collard et. *al*., (2001)
ILWC 3	*C. yamashitae*	S	R[B]	Singh *et. al*., (1998)
ILWC214		S	R[B]	Singh *et. al*., (1998)
ILWC215		S	R[B]	Singh *et. al*., (1998)

[A] R, Resistant or S, susceptible.
[B] Denotes classification, which differ from previous studies.

embryo rescue techniques (Dey *et al*., 1993) and gene transformation technology (Kahl *et al*., 1994) may provide the means to negate crossability barriers to produce wide and interspecific hybridization in the future (Badami *et al*., 1997).

Within group II, *C. bijugum*, *C. judaicum* and especially *C. pinnatifidum* possesses very high levels of genetic diversity and were reported as sources of resistance or tolerance to biotic and abiotic stresses (Singh *et al*., 1998). Therefore, they offer great potential sources for future chickpea breeding. However, before germplasm from group II and III can be utilized in chickpea breeding, the barriers preventing interspecific hybridization needs to be overcome.

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