International Chickpea and Pigeonpea Newsletter

No. 13 2006

International Chickpea and Pigeonpea Newsletter

Publishing objectives

The International Chickpea and Pigeonpea Newsletter (ICPN) is published annually by ICRISAT. It is intended as a worldwide communication link for all those who are interested in the research and development of chickpea (*Cicer arietinum* L.), and pigeonpea (*Cajanus cajan* (L.) Millsp.), and their wild relatives. Though the contributions that appear in ICPN are peer-reviewed and edited, it is expected that the work reported will be developed further and formally published later in refereed journals. It is assumed that contributions in ICPN will not be cited unless no alternative reference is available.

ICPN welcomes short contributions (not exceeding 600 words) about matters of interest to its readers.

What to contribute?

Send us the kind of information you would like to see in ICPN.

- **Contributions should be current, scholarly, and their inclusion well-justified on the grounds of new information.**
- Results of recently concluded experiments, newly released varieties, recent additions to germplasm collections, etc.
- Genome maps and information on probe-availability and sequences, and populations synthesized for specific traits being mapped. Glossy black and white prints of maps should be included, if possible. Partial maps can also be submitted.
- Short reports of workshops, conferences, symposia, field days, meetings, tours, surveys, network activities, and recently launched or concluded projects.
- Details of recent publications, with full bibliographic information and 'mini reviews' whenever possible.
- Personal news (new appointments, awards, promotions, change of address, etc).

How to format contributions?

- Keep the items brief – remember, ICPN is a newsletter and not a primary journal. About 600 words is the upper limit (no more than two double-spaced pages). **As the newsletter is devoted to the chickpea and pigeonpea crops, authors should refrain from providing a general introduction to these crops, except if they are being grown in a new area.**
- If necessary, include one or two small tables (and no more). Supply only the essential information; round off the data-values to just one decimal place whenever appropriate; choose suitable units to keep the values small (eg, use tons instead of kg). Every table should fit within the normal typewritten area of a standard upright page (not a 'landscape' page).
- Black-and-white photographs and drawings (prepared in dense black ink on a white card or a heavy-duty tracing paper) are welcome – photocopies, color photographs, and 35-mm slides are not. Please send disk-files (with all the data) whenever you submit computer-generated illustrations.
- Keep the list of references short – not more than five references, all of which should have been seen in the original by the author. Provide all the details including author/s, year, title of the article, full title of the journal, volume, issue, and page numbers (for journal articles), and place of publication and publishers (for books and conference proceedings) for every reference.
- Express all the quantities only in SI units. Spell out in full every acronym you use.
- Give the correct Latin name of every crop, pest or pathogen at the first mention.
- Type the entire text in double spacing. Send a file, which should match the printout, on a double-sided/high density IBM-compatible disk using **Microsoft Applications**.
- Contact the Editor for detailed guidelines on how to format text and diskettes.
- **Include the full address with telephone, fax and email numbers of all authors.**

The Editors will carefully consider all submitted contributions and will include in the Newsletter those that are of acceptable scientific standard and conform to requirements. The language of the Newsletter is English, but where possible, articles submitted in other languages will be translated. Authors should closely follow the style of the reports in this issue. Contributions that deviate markedly from this style will be returned for revision, and could miss the publication date. Communications will be edited to preserve a uniform style throughout the Newsletter. This may shorten some contributions, but particular care will be taken to ensure that the editing will not change the meaning and scientific content of the article. Wherever substantial editing is required, a draft copy of the edited version will be sent to the contributor for approval before printing.

Contributions should be sent before 31 March to:

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Editorial

World chickpea area has increased by 5.3% and yield by 8.0% in the last two decades, from 1986–2005. This expansion has occurred mostly outside of South Asia and has resulted in an increase in the Simpson Index of diversity. However, South Asia's share of world area has fallen from 77.4% to 73.0% but production increased from 75.0% to 80.3% over the same period. South Asia is projected to have a substantial deficit in chickpea in 2010 to the extent of 1.6 million tons and Africa will also have a deficit. On the other hand West Asia and North Africa (WANA), Latin America and the Caribbean (LAC)), and Australia are expected to have trade surpluses. India remains the dominant producer of pigeonpea. However, India's share of world pigeonpea production has reduced from 87.7% in 1986–1995 to 78.7% in 1996–2005. At the same time, there is newfound interest in China for multiple uses of pigeonpea, and as fodder in USA and in other countries. We solicit both formal and informal articles on these two crops from the different countries where they have shown promise, especially for alternative uses, that provide livelihood opportunities for the rural poor.

Water remains the primary constraint throughout the SAT, with competition in its use for domestic and industrial purposes, apart from its agricultural uses. Hence the need of the hour is to breed drought-tolerant genotypes, through judicious use of drought-tolerant germplasm, landraces and their wild ancestors on one hand, and deployment of both conventional and molecular breeding methods on the other. We look forward to an increase in submissions along these lines in the newsletter and also submissions from Africa, and other countries of Asia. We have sent 1500 copies of ICPN 12 to members and libraries (as per the existing mailing list in 2005) with a request to express their willingness to receive future issues of ICPN. But, unfortunately we have received responses from only 300 members. It has therefore been decided to **send ICPN 13 only to the respondents and libraries** to minimize expenditure on printing and mailing costs. From this issue onwards we plan to send a copy of the newsletter to the corresponding authors who have submitted article(s) for ICPN. He/she can circulate the copy amongst the coauthors and let us know at **newsletter@cgiar.org** whether anyone wishes to receive future issues including this one so that we can update our mailing list accordingly.

I thank the contributors and the authors of this issue, and particularly the reviewers of the manuscripts, namely, SL Dwivedi, PM Gaur, JVDK Kumar Rao, S Pande, RPS Pundir, KPC Rao, LJ Reddy, HC Sharma, MM Sharma, RP Thakur, V Vadez, RK Varshney from ICRISAT; and R Ahmad, PS Basu, Jyoti Kaul, ND Majumder, Shiv Kumar, Vishwa Dhar from the Indian Institute of Pulses Research (IIPR), Kanpur, India; and the Library at ICRISAT for compiling the publications listing.

The ICPN team wishes its readers a very productive and prosperous 2007.

HD Upadhyaya

Research Reports

Chickpea

Genetics/Breeding/Biotechnology

Construction of a Lambda Phage Library of the Chickpea Blight Pathogen *Ascochyta rabiei* Genome

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Ascochyta blight of chickpea, caused by *Ascochyta rabiei* (Pass.) Lab., can result in 100% yield loss and occurs anywhere the crop is grown. Two pathotypes of *A. rabiei* were found in the US (Chen et al. 2004) and pathotype-dependant resistance has been investigated (Cho et al. 2004). However, its pathogenic mechanisms are unknown. The isolation and cloning of the genes responsible for pathogenesis can be facilitated by constructing a genomic DNA library of the *A. rabiei* that can be screened. We have used the bacteriophage lambda to construct and amplify a genomic library of the pathotype II strain AR628.

High molecular weight DNA was isolated from strain AR628 using a standard method, and was partially digested with the restriction enzyme ApoI and size fractionated on a 0.8% low melting point agarose gel. Fragments corresponding to 7–10 kb were isolated and treated with agarase enzyme. The fractionated AR628 DNA was mixed with phage arms and ligated in the presence of T4 Ligase for 3 h at 25°C. Ligated arms were packaged using Gigapack III packaging extracts at 25°C for 2 h followed by chloroform extraction. Packaging extracts were titered and amplified by infecting *Escherichia coli* strain XL-1 Blue cells.

The recombinant clear plaques and non-recombinant blue plaques were screened in the presence of 5-bromo-4-chloro-3-indolyl-beta-D-galactoside (X-gal) and isopropylthio-beta-D-galactosidase (IPTG). Ten randomly selected recombinant plaques were used for plasmid rescue using ExAssist® helper phage and the *E. coli* host strain SOLR under ampicillin selection. Recovered plasmid DNA was digested with ApoI enzyme and separated in 1% agarose (Fig. 1). Based on the average size of the insert DNA in the recombinant phage, the number of recombinants recovered, and the low percentage of non-recombinants recovered, we calculated this *Ascochyta* library to have approximately three times genome coverage.

This is the first *A. rabiei* phage library constructed from *A. rabiei* and should sufficiently represent the genome for the recovery of genes involved in pathogenesis. To begin to identify the genetic components of pathogenesis we previously generated random mutations from strain AR628 using T-DNA insertional mutagenesis via *Agrobacterium*-mediated transformation (White and Chen 2006). A dozen transformants screened for pathogenicity using a minidome assay (Chen et al. 2005) showed significantly reduced pathogenicity compared to the wild type strain.

Using Southern and Inverse PCR techniques we have determined that each transformant contains a single T-DNA insertion in a unique position in the genome; however these techniques do not allow for the recovery of the complete gene disrupted by the T-DNA. With the phage library we can now recover larger DNA sequences corresponding to the insertion sites for further analysis. Large fragments recovered from the library will be used in complementation studies as well as further mutational analysis. In addition, other candidate genes that have been shown to be involved in pathogenicity in related fungi can be recovered from the phage library. For example, partial regions of the polyketide synthase I gene of *Glarea lozoyensis* (Zhang et al. 2003) and the *cps* gene of *Cochliobolus heterostrophus* (Lu et al. 2003) can be amplified from *A. rabiei*. These short regions can now be used as probes to isolate complete copies of the gene from the phage library. Taken together, construction of this library represents a important step towards determining the genetic factors required pathogenesis in *A. rabiei*.

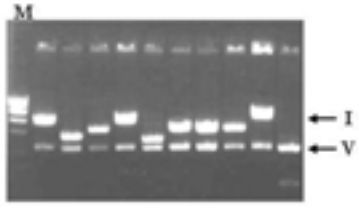

Figure 1. Insert size determination of ten randomly selected clones (M = Lambda DNA digested with HinDIII; I = Insert; and V = Vector).

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Identification of Large-Seeded High-Yielding Diverse Kabuli Accessions in Newly Assembled Chickpea Germplasm

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Chickpea (*Cicer arietinum* L.) is an important grain legume grown for easily digestible quality protein and its nitrogen fixing capability that improves soil fertility. It is cultivated on 10.38 million ha in 45 countries across the globe producing 8.57 million tons with productivity of 0.83 t ha^{-1} (FAO 2004), which is rather low. India, Pakistan, Myanmar, Turkey, and Iran in Asia; Mexico in North Central America; and Ethiopia in Africa are the largest chickpea producing countries. Of late chickpea is being cultivated on considerable area in Canada, Australia, and USA. Two types of chickpeas – kabuli and desi – are recognized. The kabuli types have owl-shaped, large beige colored seeds with thin seed coat and white colored flowers; while the desi types have angular-shaped seeds with thick seed coat, generally colored flowers and seeds. Kabuli types account for about 15% of the world chickpea production. However, about two-thirds of chickpea-growing countries cultivate only the kabuli types (Singh 1987). Kabuli types fetch higher prices in markets. In India the price of kabuli chickpeas is up to 100% more than that of the desi chickpeas. In Canada, where chickpea is grown as a cash crop mainly for export to other countries, kabuli chickpeas with seed weight of 50 g 100 seed^{-1} fetch 60% higher price than the small seeded (25 g 100 seed^{-1}) desi chickpeas (Liu et al. 2003). A similar premium on kabuli types prevails in Australia. Over 67000 accessions of chickpea germplasm have been conserved globally. ICRISAT holds in trust 17258 chickpea accessions and USDA has over 4900. However, there has been very limited use of these accessions in genetic enhancement of chickpea (Upadhyaya et al. 2001), leading to cultivars with narrow genetic base and low genetic gain. The aim of our study is to identify large-seeded high-yielding kabuli germplasm accessions in the 335 newly introduced kabuli chickpea germplasm accessions from USDA, Pullman, USA.

ICRISAT assembled 996 desi accessions (originating from 28 countries), 335 kabuli accessions (originating from 27 countries) and 11 pea shaped accessions (originating from seven countries), from USDA, Pullman, USA in August 2004. These newly assembled germplasm accessions were evaluated in an augment design with five control cultivars (Annigeri, G 130, ICCV 10, KAK 2, and L 550). Annigeri, ICCV 10, and G 130 are early, medium, and late maturing desi type cultivars, respectively. KAK 2 is an early-maturing and L 550 is a medium-duration kabuli cultivar. A control cultivar was repeated after every 19 test entries on a rotational basis. The experiment was conducted under high input (100 kg ha^{-1} diammonium phosphate as basal dose, and protection against insect pest and diseases, and two irrigations) on a Vertisol (Kasireddypally series- Isohyperthermic Type Pellustert) field at ICRISAT center, Patancheru, India (18°N, 78°E, 545 m.a.sl., and 600 km inland) during the 2004-2005 postrainy season. Each plot consisted of a 3 m row on a ridge, with 60 cm distance between rows and 10 cm between plants within a row. Data was recorded following IBPGR, ICRISAT, and ICARDA (1993) descriptors.

Table 1. Geographic origin and agronomic characters of selected kabuli chickpea accessions evaluated at ICRISAT Patancheru, India, 2004–2005 season.

EC_No	Identity	Origin	Days to 50% flowering	100-seed weight (g)	Plot yield (kg ha^{-1})	Plant yield $^{-1}$ (g)	Productivity (kg ha^{-1} day^{-1})
EC543451	W6 30	Morocco	50	44.1	1522	6.5	13.7
EC543533	W6 10543	USA	37	45.5	1700	9.0	14.9
EC543562	W6 12855	Morocco	62	40.3	1463	6.4	12.9
EC543582	W6 17590	Mexico	38	40.4	1531	11.6	13.9
EC543583	W6 17591	Mexico	42	40.0	1846	16.0	15.4
EC543584	W6 17592	Mexico	46	47.3	1690	13.0	14.5
EC543586	W6 17594	Mexico	43	41.9	1698	9.6	14.9
EC543587	W6 17595	Mexico	45	40.8	1568	13.0	13.8
EC543588	W6 17596	Mexico	57	40.3	1430	9.4	13.3
EC543593	W6 17601	Mexico	45	54.9	1645	18.0	14.5
EC543594	W6 17602	Mexico	54	40.2	1881	15.6	15.5
EC543597	W6 17605	Mexico	44	42.3	1746	7.6	14.9
EC543598	W6 17606	Mexico	51	45.7	1856	13.6	15.4
EC543599	W6 17607	Mexico	36	53.1	1906	16.6	15.8
L550		India	58	20.2	1695	16.0	14.8
KAK2		India	39	40.9	1406	9.8	13.6
Trial Mean			59.4	18.7	1557	9.57	13.92
SE ±			3.17	3.33	308.02	0.05	2.28
CV (%)			5.9	20.6	36.6	52.3	41.3

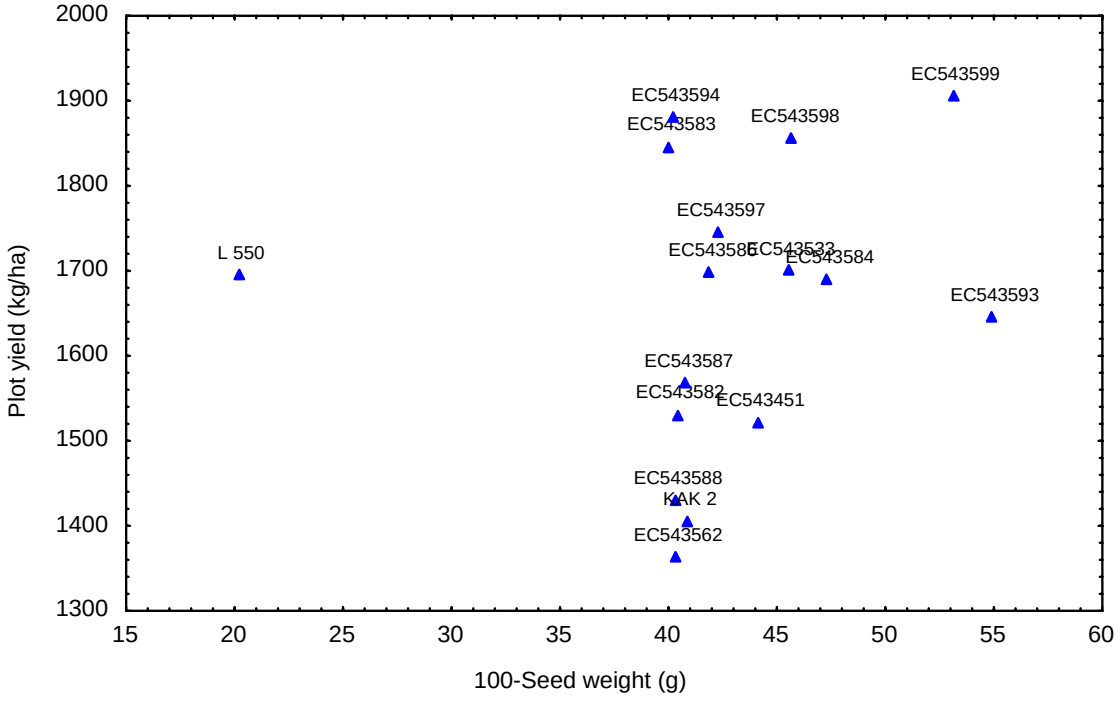

Figure 1. Scatter plots of 100-seed weight (g) and plot yield (kg ha^{-1}) in 14 selected kabuli chickpea accessions and two control cultivars.

Data were analyzed using random model of Residual Maximum Likelihood (REML) in Genstat 8.1. Variance components due to genotype (δ^2g), error (δ^2e) and their standard errors (SE), and broad sense heritability (h^2) were estimated. Best Linear Unbiased Predictors (BLUPs) were calculated for all quantitative traits. Fourteen kabuli accessions with more than 40 g 100-seed^{-1} weight and having greater or similar seed yield to the kabuli control cultivars (KAK 2, L 550) were identified. Principal component analysis (PCA) on standardized data of 18 agronomic (days to 50% flowering, flowering duration, plant height, plant width, days to maturity, number of basal primary and secondary branches, number of apical primary and secondary branches, tertiary and total number of branches, number of pods per plant, number of seeds per pod, 100-seed weight, plot and plant yields, productivity per day, and SPAD (Soil Plant Analyses Development) chlorophyll meter reading) traits was performed. Cluster analysis of selected 14 accessions and two control cultivars, using scores of first 5 Principal Components (PC) was performed following the Ward (1963) method.

REML analysis of data of all the 1342 accessions revealed significant genotypic variance for days to 50% flowering, flowering duration, plant height, plant width, apical primary, basal secondary, and tertiary branches, seed per pod, 100-seed weight, plot yield and SPAD chlorophyll meter reading. Genotypic variances were significant for all the traits except apical secondary branches and SPAD reading in the kabuli accessions (335). It indicated that even within this set of kabuli accessions, there is scope for selecting large-seeded accessions with different maturity duration and seed yields.

Fourteen selected large-seeded kabuli accessions produced an average of 8.2% more seed yield and 44.3% larger seeds than the average of the two kabuli control cultivars, and had 9.8% higher 100-seed weight and produced 18.8% higher seed yield than the best control cultivar KAK 2 (Table 1). EC 543533 (originating from USA) and EC 543599 (Mexico) were early flowering and took 36 and 37 days to flower, had large seeds (45.5 and 53.1 g 100 seed^{-1}), and produced high seed yield (1700 and 1906 kg ha^{-1}) compared to control KAK 2 (39 days; 40.9 g; and 1406 kg ha^{-1}) and L 550 (58 days; 20.2 g; 1695 kg ha^{-1}) (Table 1). Furthermore, scatter plot of plot yield and 100-seed weight revealed that ECs 543594, 543598, 543599, 543583, 543586, 543533, 543584, 543593, and 543597 had large seeds (40.0 g–54.9 g) and produced higher yields (1645 to 1906 kg ha^{-1}) (Fig. 1).

Cluster analysis performed on the scores of first five PCs (total variation 90.77) resulted in four clusters (Fig. 2). Two control cultivars formed separate clusters. KAK 2 occurred in first and L 550 in the third cluster. ECs 543598, 543594, 543584, 543597, 543586, 543583, 543599, 543593, 543582 from Mexico, and 543533 from USA formed a second cluster. ECs 543451, 543562, 543587, and 543588 formed the fourth cluster. The delineation of the first cluster from the other three was mainly on maturity related traits indicated by significantly

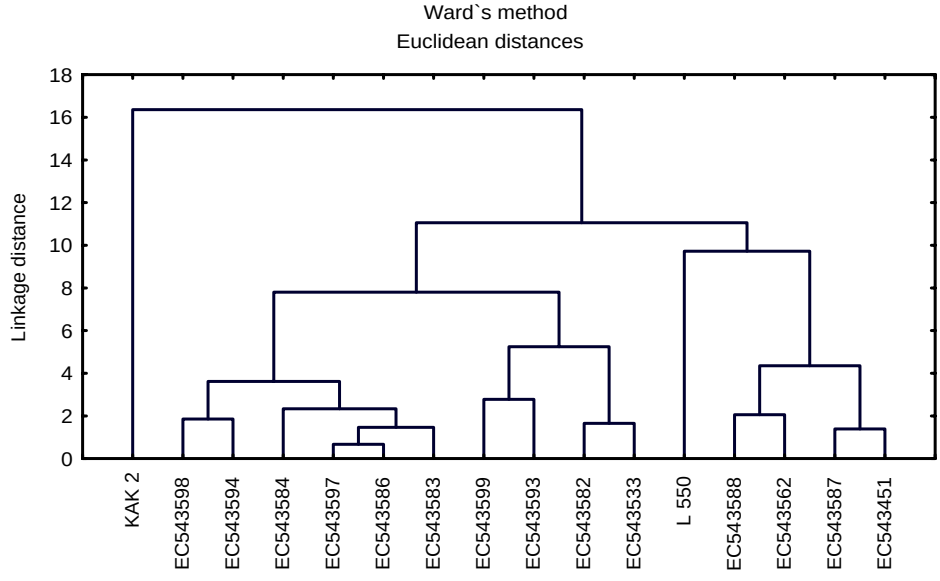

Figure 2. Dendogram based on first five principal components of 18 quantitative traits of 14 large-seeded kabuli chickpea accessions with two control cultivars.

lower mean values than the other clusters for flowering duration and maturity. Large-seeded accessions with high seed yield with early and medium duration, high per-day productivity and SPAD reading were included in cluster 2. Cluster 4 included medium to long duration accessions with low yield per plant and plot.

The identification of the large-seeded, early-maturing and agronomically superior diverse parents will prompt breeders to use them in crop improvement programs (Upadhyaya et al. 2006)**.** Early maturity is advantageous in chickpea to avoid terminal drought and make adequate use of available soil moisture during growth, as chickpea is usually grown on conserved soil moisture, where soil moisture reduces towards maturity. In the present study, a few more very early-flowering genotypes such as ECs 543533, 543582, and 543599 were identified. As mentioned earlier, large seed size has a price premium in trade. In this study we have identified ECs 543533, 543584, 543593, 543598, and 543599 as additional sources of large seed size for improvement in chickpea. While selecting the exotic germplasm lines for inclusion in the breeding programs, it is important to consider the genetic background and agronomic performance of the lines, as it will be useful in predicting its behavior in hybrid combinations with the adapted genotypes.

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Extra-Large Kabuli Chickpea with High Resistance to Fusarium Wilt

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There is an increasing international market for extra-large (>50g 100-seed^{-1}) kabuli chickpea. Such chickpeas are being sold at about three times the price of desi chickpea and about two times the price of medium-seeded (~25 g 100 seed^{-1}) kabuli chickpea in India, the largest chickpea importing country. None of the kabuli chickpea varieties released to date in India has seed size larger than 40 g 100 seed^{-1}. Thus, the Government of India has launched a 3-year project from 1 April 2006 on breeding extra-large kabuli chickpea with resistance to fusarium wilt under the Integrated Scheme of Oilseeds, Pulses, Oil Palm and Maize (ISOPOM).

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp *ciceri*, is the most important root disease of chickpea in the semi-arid tropics (SAT), where the chickpea growing season is dry and warm. Resistance to FW is required in all chickpea cultivars targeted for SAT and other FW-prone areas of the world. There are many sources with high resistance to FW available in desi type, while resistance sources in kabuli type are limited. A world collection of over 13,500 germplasm accessions from 40 countries was evaluated for race 1 of *Fusarium oxysporum* at ICRISAT-Patancheru. Of the 160 resistant accessions identified, only 10 accessions were of kabuli type (Haware et al. 1992). Desi × kabuli crosses have been widely used at ICRISAT for enhancing FW resistance of kabuli chickpea. However, most kabuli varieties that involved one or more desi parents in the pedigree have a brown tinge in seed color, e.g. Swetha (ICCV 2), KAK 2 (ICCV 92311), JGK 1 (ICCV 92337), and Vihar (ICCV 95311), while the market prefers cream to white (zero tannin) seed color in kabuli chickpea. Thus, it is important to identify additional sources of FW resistance in kabuli chickpea, particularly in the large-seeded category, so that large-seeded kabuli varieties with high resistance to FW and typical kabuli type seed (ram's head shape and white seed color) can be developed from kabuli × kabuli crosses.

We selected 50 large-seeded kabuli chickpea germplasm from ICRISAT's genebank and evaluated these for agronomic traits at ICRISAT-Patancheru during the 2004/05 postrainy season. From these, 12 accessions having seed size larger than 50 g 100 seed^{-1} were selected for further

evaluation. During the 2005/06 postrainy season, one set of these 12 genotypes was grown in wilt-sick plot for screening against FW and another set in wilt-free area for evaluation of agronomic traits.

Two accessions, ICC 14194 and ICC 17109, originating from Mexico, showed complete resistance (0% plant mortality) to FW, whereas other lines showed 11–100 % plant mortality (Table 1). The resistant control (WR 315) had 0% plant mortality, whereas the early-wilt susceptible check (JG 62) had 100%, and the late-wilt (K 850) susceptible check had 87% mortality. Both the resistant accessions had pinnate (fern) leaves, which is the common

Table 1. Morphological and agronomic characteristics of twelve extra-large kabuli chickpea germplasm evaluated during postrainy season 2005/06 at ICRISAT-Patancheru.

Accession	Origin	Leaf type	Days to flower[1]	Days to mature[1]	100-seed mass (g)[1]	Wilt reaction (%)[2]
ICC 7344	Mexico	Pinnate	38	100	50.2	95.2
ICC 8155	USA	Simple	45	112	62.2	100.0
ICC 11742	Chile	Pinnate	64	130	51.9	86.4
ICC 11883	Spain	Pinnate	56	130	58.7	90.9
ICC 13821	Ethiopia	Simple	50	118	51.0	92.0
ICC 14194	Mexico	Pinnate	38	97	52.9	0.0
ICC 14195	Mexico	Simple	50	109	60.2	52.2
ICC 14198	Mexico	Pinnate	42	94	50.2	70.8
ICC 14202	Mexico	Pinnate	46	118	58.1	75.0
ICC 15576	Mexico	Pinnate	52	120	55.6	81.0
ICC 16670	USA	Simple	45	110	50.1	11.1
ICC 17109	Mexico	Pinnate	46	115	63.2	0.0
WR 315 (Resist. check)	India	Pinnate	44	102	13.5	0.0
K 850 (Late wilting sus. check)	India	Pinnate	56	109	28.9	87.0
JG 62 (Early wilting sus. check)	India	Pinnate	42	103	15.8	100.0

1. Data from crop grown in wilt-free field.
2. Data on resistance to race 1 of *Fusarium oxysporum* f. sp *ciceri* from screening in wilt nursery.

ICCV 2 KAK 2 ICC 17109

Figure 1. The seed of fusarium wilt resistant extra-large (63 g 100-seed^{-1}) kabuli accession ICC 17109, the medium-seeded (25 g 100-seed^{-1}) kabuli variety ICCV 2, and the large-seeded (38 g 100-seed^{-1}) kabuli variety KAK 2.

leaf type in chickpea. ICC 14194 was very early (97 days), while ICC 17109 had medium maturity (115 days). A comparison of the seeds of a medium-seeded variety ICCV 2 (25 g 100 seed^{-1}), a large-seeded variety KAK 2 (38 g 100 seed^{-1}) and an extra-large-seeded kabuli line ICC 17109 (63 g 100 seed^{-1}) is shown in Figure 1.

Early maturity is important in chickpea for its adaptation to short-season environments and for escape from terminal drought, which is the number one constraint to chickpea productivity in the SAT. The development of medium- to large-seeded (25–40 g 100 seed^{-1}) early-maturing kabuli varieties, particularly ICCV 2 and KAK 2, has helped expansion of kabuli chickpea area to southern India, which has typically short-season tropical environment (Gowda and Gaur 2004). Of the 12 accessions evaluated in this study, two (ICC 14194 and ICC 14198) were very early (days to maturity <100 days) and had 50–53 g 100 seed^{-1}, suggesting that it is possible to breed early-maturing kabuli varieties with extra-large seed.

It is hoped that these new FW resistance sources will be very useful in breeding extra-large kabuli varieties with FW resistance and typical kabuli type seed. The seeds of these accessions are available for distribution at ICRISAT's genebank.

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Relationships of Pinnate (Fern) and Simple (Unifoliate) Leaf Traits with Seed Yield and Seed Size in Kabuli Chickpea

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Chickpea typically has pinnate type of compound leaves in which the leaf lamina (blade) is differentiated into a rachis and a number of leaflets. These leaflets are generally odd in number and borne directly on the rachis. Mutants have been identified that have simple (unifoliate) leaves in which the lamina is not differentiated into rachis and leaflets, though there may be deep incisions in the lamina. A single recessive gene is known to control the simple leaf trait (Pundir et al. 1990). Most chickpea cultivars released in different countries have normal pinnate leaves. The simple leaf mutants have also been exploited in chickpea breeding and some cultivars, mainly kabuli type, with simple leaves have been released, e.g. Surutato 77 and Macarena in Mexico; Dwelley, Sanford, Evans and Sierra in USA; and CDC Diva and CDC Xena in Canada (FJ Muehlbauer, personal communication; Warkentin et al. 2003).

This study was conducted to determine if the leaf type has any relationship with seed yield and major seed yield components, particularly number of pods per plant and seed weight, in kabuli chickpea. Three crosses, ICCV 2 × ICC 14195, ICCV 2 × ICC 14215 and ICC 16644 × ICC 16670, were selected from ICRISAT's chickpea breeding program. The parents of each cross differed in leaf type and seed size. ICCV 2 and ICC 16644 have pinnate leaf and medium seed size (23–25 g 100 seed^{-1}), while ICC 14195, ICC 14215 and ICC 16670 have simple leaf and large seed size (50–59 g 100 seed^{-1}). The F_2 populations from these crosses were grown at ICRISAT-Patancheru during the postrainy season 2005/06 keeping row-to-row distance of 60 cm and plant-to-plant distance of approximately 10 cm. In each cross, observations were recorded on all plants individually. There were 226 plants in ICCV 2 × ICC 14195, 247 plants in ICCV 2 × ICC 14215, and 244 plants in ICC 16644 × ICC 16670. Observations were recorded on leaf type, number of pods per plant, number of seeds per plant, 100-seed weight and seed yield per plant. In each cross, the F_2 plants were classified into two groups based on leaf type (pinnate-leaved and simple-leaved) and then mean value of each trait was calculated for each group.

The significance of difference between the mean values of two groups for each trait was tested using t-test.

The pinnate-leaved plants and the simple-leaved plants gave a good fit to the expected 3:1 ratio in two crosses (ICCV 2 × ICC 14215 and ICC 16644 × ICC 16670), but showed distorted segregation in one cross (ICCV 2 × ICC 14195) (Table 1). The pinnate-leaved plants gave significantly higher seed yield (44% in ICCV 2 × ICC 14215, 53% in ICCV 2 × ICC 14195 and 62% in ICC 16644 × ICC 16670) than the simple-leaved plants, mainly because of higher number of pods per plant (Table 1). On an average, the pinnate-leaved plants produced 23–31 pods per plant, whereas simple-leaved plants produced 14–19 pods per plant. The increased number of pods per plant in pinnate-leaved plants resulted in increased number of seeds per plant and ultimately increased yield per plant. Seed size of pinnate-leaved plants and simple-leaved plants did not differ significantly in any of the crosses.

It is interesting to note that most simple-leaved kabuli germplasm accessions (e.g. ICC 8155, ICC 8156, ICC 13821, ICC 14195, ICC 14206, ICC 14215, and ICC 16670) and cultivars (e.g. Surutato 77, Macarena, Dwelley, Sanford, Evans, Sierra, CDC Diva and CDC Xena) have large seeds (>40 g 100 seed^{-1}). This gives the impression that simple-leaf trait may be associated with large seed size. In pinnate-leaved plants, it is well-established that the large-seeded varieties have large leaflets (Dahiya et al. 1988; Sandhu et al. 2005). Thus, it also indicates that the simple-leaf trait may affect seed size. However, results of this study suggest that the simple- and pinnate-leaf types have no relationship with seed size in kabuli chickpea, and the same relationship is expected to be true for desi chickpea.

One disadvantage of using simple-leaf trait reported earlier is the higher susceptibility of simple-leaved cultivars to the foliar disease ascochyta blight, caused by *Ascochyta rabiei* (Gan et al. 2003). The results of this

Table 1. Differences in mean values of yield and major yield components between pinnate-leaved and simple-leaved plants in F_2 of kabuli × kabuli chickpea crosses.

			Mean±SE			
Cross	Category of plants	No of plants	No of pods/plant	No of seeds/plant	Seed yield/plant (g)	100-seed weight (g)
ICCV 2 × ICC 14195	Pinnate-leaved	185	30.7±1.1	32.3±1.2	11.8±0.4	37.5±0.5
	Simple-leaved	41	19.1±1.6	20.3±1.7	7.7±0.6	39.5±1.2
	χ^2 for a 3:1 ratio (probability)	5.67 (0.02–0.01)	–	–	–	–
	t-value (probability)	–	5.96 (<0.001)	5.77 (<0.001)	5.65 (<0.001)	1.63 (0.12) NS
ICCV 2 × ICC 14215	Pinnate-leaved	196	29.3±1.3	30.5±1.4	10.8±0.5	36.5±0.5
	Simple-leaved	51	18.7±1.5	19.9±1.6	7.5±0.7	37.9±1.1
	χ^2 for a 3:1 ratio (probability)	2.49 NS (0.90-0.10)	–	–	–	–
	t-value (probability)	–	5.75 (<0.001)	5.44 (<0.001)	3.77 (<0.001)	1.38 (0.22) NS
ICC 16644 × ICC 16670	Pinnate-leaved	192	23.3±1.6	26.7±2.0	7.3±0.5	27.8±0.7
	Simple-leaved	52	14.4±2.5	15.3±2.5	4.5±0.7	29.3±1.6
	χ^2 for a 3:1 ratio (probability)	1.77 NS (0.90–0.10)	–	–	–	–
	t-value (probability)	–	3.19 (0.002)	3.70 (<0.001)	3.33 (<0.001)	0.87 (0.39) NS

study reveal another negative effect of simple-leaf trait, the reduction in seed yield per plant. Thus, it is recommended that selections should be practiced for pinnate-leaved plants in crosses involving simple-leaved and pinnate-leaved types.

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JG 412 – A Large-Seeded, Short-Duration, High-Yielding Chickpea Variety for Western Madhya Pradesh

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Chickpea is a major cool season pulse crop in Madhya Pradesh, India. The production of the crop is low mainly because of unavailability of suitable genotypes for specific agroclimatic regions. In Madhya Pradesh, long duration varieties of chickpea are subjected to terminal drought stress leading to substantial yield losses. Therefore, the development of early-maturing varieties assumes great importance, particularly in areas where chickpea is sown after the harvest of rainy season crops on the conserved soil moisture with minimum tillage. Recently, a large-seeded, short-duration cultivar, JG-412 has been developed with an aim to stabilize yield under semi-arid zone of Madhya Pradesh.

The cultivar JG 412 was developed through a three way cross i.e., (Phule G-5 × *Narsingpur* Bold) × ICCC-37, by pedigree selection. The parent Phule G-5 is wilt tolerant, whereas *Narsingpur* Bold is large-seeded with seed weight of 26 g 100 seed^{-1} and ICCC-37 is early maturing (95–100 days). The cultivar was recommended for commercial cultivation in western Madhya Pradesh, especially for *Malwa* plateau, *Jhabua* hills and parts of *Nimar* valley zones.

The cultivar JG 412 has high average yield (1880 kg ha^{-1}), and is large seeded (26 g 100 seed^{-1}) with good parching quality, good storage ability and early in maturity (100 days) as compared to JG 218 the commonly cultivated variety with average yield of 1690 kg ha^{-1}, medium-seeded (18.5 g 100 seed^{-1}), average parching quality, average storage ability and late in maturity (120 days). Being early type, JG 412 is highly suitable for "Soybean-Potato-Gram" cropping sequence and also suitable for rainfed, irrigated and late-sown conditions (25 November to 10 December), as is evident from experimental results.

The yield performance of JG 412 in various trials conducted in Madhya Pradesh from 1992–93 to 2003–04 and in Central, NWP and NEP Zones from 1994–95 to

Table 1. Performance of JG 412 in state multilocation and coordinated trials in Madhya Pradesh and Central Zone, North Western Plain Zone, North Eastern Plain Zone; 1992 to 2003–04.

Location	Grain yield (kg ha^{-1})		
	JG 412	Ujjain-21/JG 218 (st. ch.)	BG 256 (nc)
State trials			
1992–93	2102 (3)[1]	1779	–
1993–94	2144 (5)	1795	–
1994–95	2453 (6)	1892	–
1995–96	1406 (8)	1353	–
2002–03	1930 (4)	1808	–
2003–04	1814 (2)	1515	–
Coordinated trials			
1994–95	1924 (16)	–	1663
1995–96	1344 (8)	–	1271
Mean	1880 (52)	1690 (33)	1467
% Increase of JG 412 over			
Ujjain-21/JG 218	11.22		
BG 256	28.15		

1. Figures in parentheses indicate test locations.
st. ch. = State check; nc = National check.

Table 2. Reaction of JG 412 to root rot, dry root rot, collar rot and foot rot diseases of chickpea (1994–95).

Entry	Root rot %	Dry root rot %	Collar rot %	Foot rot %
JG 412	6.9 (2)	12.4 (2)	62.5	8.2
BG 256 (nc.)	18.4 (2)	14.3 (2)	100.0	100.0
ICC-4951 (W.S.Ch)	100.0 (2)	41.0 (2)	100.0	100.0

Source: Rabi pulse pathology report, 1994-95, Table-20, page-72
Figures in parentheses indicate test locations.
W.S.Ch = Wilt-susceptible check.

1995–96 is summarized in Table 1. In 52 trials conducted at different locations, JG 412 gave an average seed yield of 1880 kg ha^{-1} as compared to 1467 kg ha^{-1} of control cultivar BG 256, reflecting an increase of 28%. Similarly, in five agronomy trials, JG 412 gave a mean seed yield of 2087 kg ha^{-1} compared to 1752 kg ha^{-1} of Ujjain 21.

The new cultivar JG 412 is promisingly stable, with tolerance to root rot (6.9%), dry root rot (12.4%) and foot rot (8.2%) diseases under sick condition compared to control BG 256 and susceptible control ICC 495 as is evident from testing over seasons (Table 2).

The new cultivar JG 412 can easily be distinguished from the existing cultivars in growth characteristics including semi-erect plant type, dark green foliage, pink flowers and yellow-brown, slightly wrinkled large seed (26 g 100 seed^{-1}).

Anther Development and Microsporogenesis in *Cicer arietinum* L. Plants Treated with Ethrel

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Ethrel or Ethephon (2-chloroethyl phosphonic acid) is an ethylene-generating synthetic compound that acts as a plant growth regulator and affects plant growth, flowering and ripening of fruits. The gametocidal property of ethrel was shown by Rowell and Miller (1971) and Keys and Sorrells (1990) in wheat; Colhoun and Steer (1983) in barley; Chauhan and Chauhan (2003) in broad beans; and Gupta and Chauhan (2005) in cotton. However, the origin of abortive process in the anthers of ethrel-treated crops at light and electron microscopic level has received little attention (Bennett and Hughes 1972; Colhoun and Steer 1983).

This paper deals with the development of anther and microsporogenesis in *Cicer arietinum* plants treated with ethrel.

The plants of *Cicer arietinum* L. cultivar Rachna were treated with 0.1, 0.2 and 0.3% ethrel at different developmental stages (Table 1). A group of 90 plants was sprayed a week before floral bud initiation (T_1). Leaving 30 plants from T_1 treatment, the other 60 plants were sprayed again three days after the first treatments (T_2). A group of 30 plants that had received T_1 and T_2 treatments were sprayed a third time (T_3) at the time of anthesis. Pollen fertility was tested at regular intervals throughout the flowering period with 1% Tetrazolium chloride and Fluoro Chromatic Reaction tests.

The anthers of plants sprayed thrice with 0.3% ethrel (exhibiting 100% pollen sterility) were fixed in 3% glutaraldehyde in 0.1M phosphate buffer. Post fixation was done in 0.1% osmic acid in the same buffer. These were dehydrated in a graded ethanol series and embedded in Epon medium. Semi-ultra thin sections were cut at 0.5–20 μm and stained with a solution of 0.5 w/v toludine blue in 1% w/v sodium borate. For transmission electron microscope (TEM) studies, ultra-thin sections were cut at 0.75-1.5μm and stained with uranyl acetate and lead citrate and observed under Phillips (CM-10) transmission electron microscope at EM Facility, All India Institute of Medical Sciences, New Delhi.

In control plants, anther wall formation was of dicotyledonous type. At sporogenous tissue stage, the anther wall consisted of an epidermis, a single layer of endothecium, a middle layer and a single layer of secretory tapetum. The epidermis consisted of a single layer of cells which elongated tangentially with age. The cells in middle layers were found to degenerate at vacuolated pollen stages. The endothecial cells elongated radially after tapetal degeneration and characteristic fibrous thickenings developed on their radial walls at late vacuolated pollen stage (Fig. 1a). The tapetal cells were uni-nucleate and their degeneration commenced at the microspore tetrad stage. The tapetal cytoplasm consisted of a large number of vacuoles and lipid-containing plastids, some with starch grains. At the vacuolated microspore stage, the tapetal cells disorganized with the release of a large number of Ubisch bodies. These bodies were discernible outside the tapetal plasmalemma. At the pollen grain stage, the tapetal cells lysed completely and left a large number of Ubisch bodies near the pollen grains (Fig. 1c). The pollen grains were spherical, tricolporate and engorged with reserves. The exine of pollen consisted of tectum, baculum, and foot layer (Fig. 1e). The intine was thin and present well below the foot layer. The pollen cytoplasm contained a large round nucleus with various well organized cell organelles.

The development of anthers in ethrel-treated plants showing 100% pollen sterility was found to be similar to their control plants until meiosis in pollen mother cells. The endothecial cells failed to enlarge radially and formation of fibrous thickening was fully inhibited. The degeneration of tapetal cells was seen to be delayed till anthesis. The intact tapetal cells were radially elongated, highly vacuolated and stained more intensely than the outer anther wall layers. The tapetal protoplasm consisted of degenerated nucleus with deformed cell organelles (Fig. 1b). The plastids in tapetal cells

Table 1. Description of ethrel treatments.

Chemical	Conc. (%)	Treatments
Ethrel	0.1	T_1: Plants sprayed a week before floral bud initiation
	0.2	T_2: Plants sprayed again three days after the first treatment
	0.3	T_3: Plants sprayed a third time at the time of anthesis

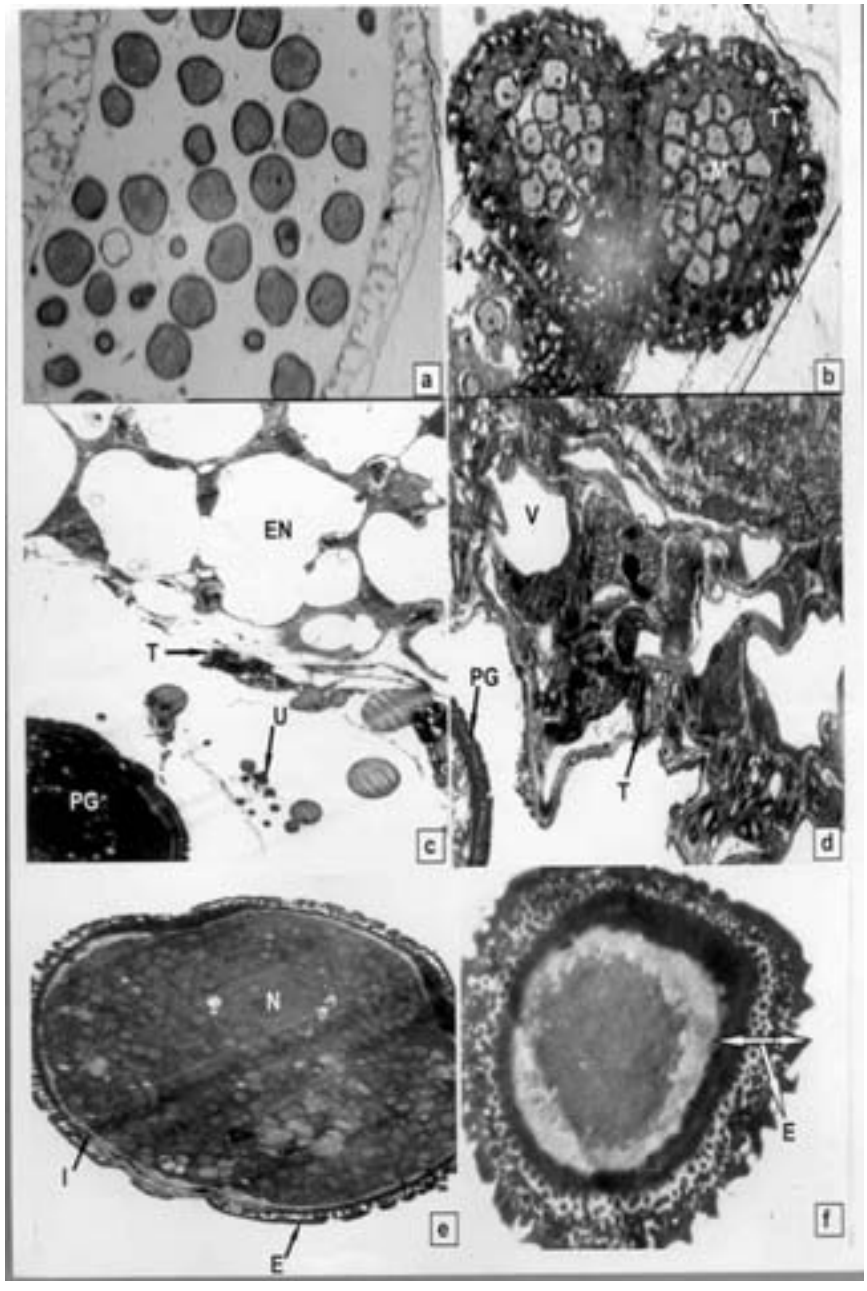

Figure 1. LM and TEM microphotographs showing anther development

a. LM of fertile anther at pollen grain stage
b. LM of two microsporangia of 0.3% ethrel treated plant at microspore stage, showing intact tapetum (T) and nonviable microspores (M)
c. Part of fertile anther under TEM showing degenerated tapetum (T) with large number of Ubisch bodies (U) on outer membrane and part of mature pollen grain (PG).
d. Part of sterile anther under TEM showing intact tapetum cells with degenerated protoplast (PG : pollen grain, T: tapetum, V : vacuole).
e. TEM of fertile pollen with well developed exine (E), intine (I) and nucleus (N).
f. TEM of sterile pollen with abnormally thick exine (E) and degenerated protoplast.

possessed developed starch grains. A large number of small Ubisch bodies occurred at outer zone of tapetal plasmallema (Fig. 1d). At mature pollen grain stage, the tapetal cells degenerated and pollen grains of various shapes and sizes were discernible. The exine was very irregular and significantly thick but failed to differentiate into tactum, baculum and foot layer. The intine was conspicuous by its absence. The pollen protoplasm failed to differentiate into cytoplasm and nucleoplasm. The cell organelles were also completely degenerated (Fig. 1f). Thus, we can conclude that pollen abortion in ethrel-treated plants of *Cicer arietinum* is associated with abnormal behavior of tapetum and disorganized cell organelles.

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Abnormal Tapetal Mitochondria Associated with Pollen Abortion in the Anthers of *Cicer arietinum* L. Plants Treated with a Detergent – Surf Excel

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Male gametocides or chemical hybridizing agents are used for inducing male sterility in plants (Cross and Schulz 1997). Surf Excel, a synthetic detergent, has been successfully used for inducing pollen sterility in *Brassica juncea* (Chauhan and Singh 2002, Singh and Chauhan 2003), *Vicia faba* (Chauhan and Chauhan 2003), *Lycopersicon esculentum*, *Capsicum annuum* and *Abelmoschus esculentus* (Chauhan and Agnihotri 2005) and *Cicer arietinum* (Chauhan and Gupta 2005).

This paper describes a study of the changes at light and electron microscopic levels in sterile anthers of *C. arietinum* L. plants treated with Surf Excel.

Cicer arietinum L. cultivar Rachna plants were sprayed with 0.5% aqueous Surf Excel solution a week before floral bud initiation, a 0.1% solution three days after the first treatment, and a 1.5% solution at the time of anthesis. Individual plant received 15 ml of each concentration. Untreated plants of cultivar Rachna were sprayed with distilled water to serve as control. Pollen fertility was tested throughout flowering period with 1% tetrazolium chloride.

Anthers of treated and untreated plants were fixed in 3% glutaraldehyde in 0.1M phosphate buffer pH 6.8 for 24 h at 4°C and post fixation in 0.1% osmic acid in the same buffer. These were then dehydrated, cleared and embedded in Epon medium using common customary procedures. Sections were cut at 0.5–2.0 μm and stained with toludine blue in 1% sodium borate. For transmission electron microscope (TEM) studies, ultra-thin sections cut at 0.75 to 1.5 μm were stained with uranyl acetate and lead citrate and observed under Phillips (CM-10) TEM at the All India Institute of Medical Sciences, New Delhi.

Anther development in male fertile plants (controls). At sporogenous tissue stage, the anther wall consisted of an epidermis, a single middle layer, a single layer of endothecium and a secretory tapetum. Cytoplasm of isodimetric tapetal cells was found to be intensely stained with a prominent nucleus. The vesicular cytoplasm possessed thin walled mitochondria and pleomorphic

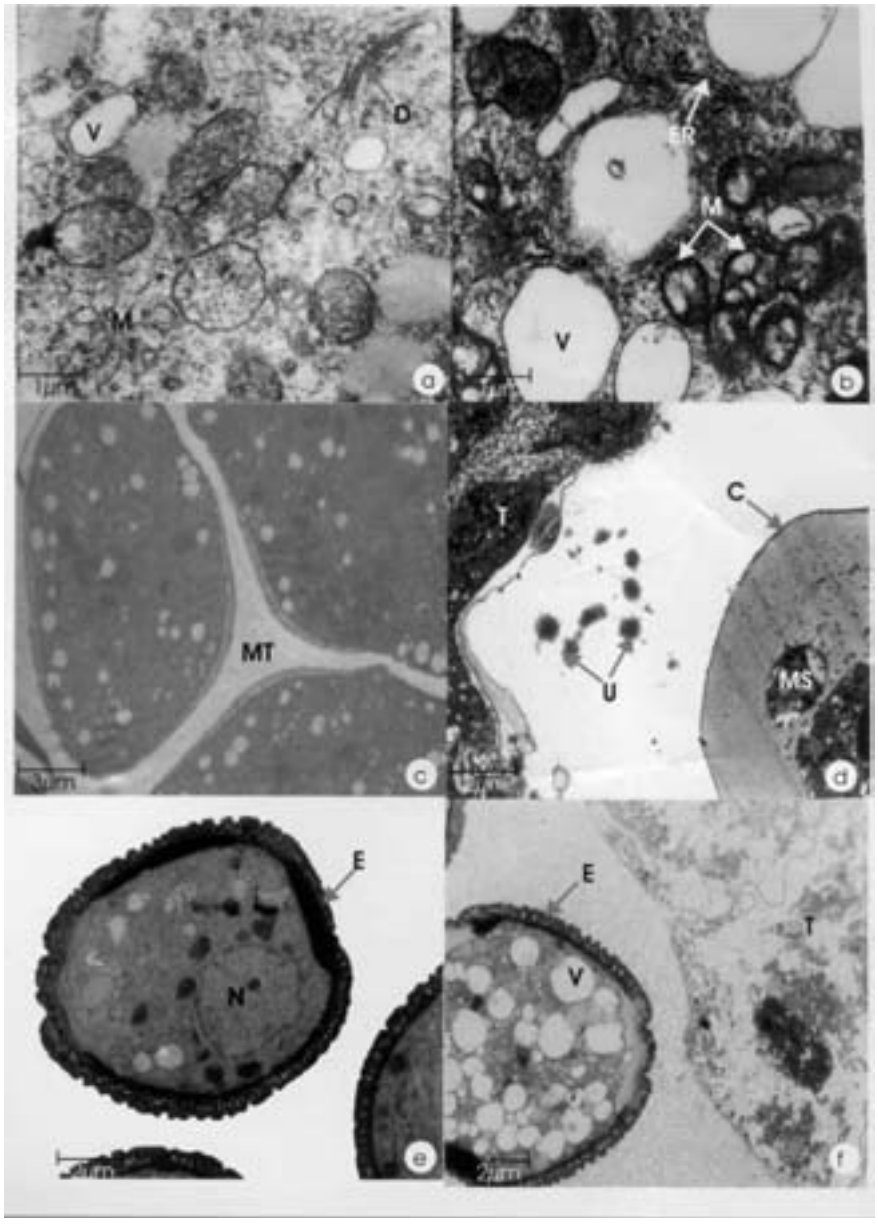

Figure 1. TEM microphotograph showing anther development in malefertile (MF) and surf excel treated (SET) plants of *Cicer arietinum* L.

a. At sporogenous tissue stage in MF plants, the cytoplasm of tapetal cells contained thin walled mitochondria (M), small vacuoles (V) and dictyosomes (D) with some other cells organelles.
b. At sporogenous tissue stage in SET plants, the cytoplasm of tapetal cells contained thick walled deformed mitochondria (M), large vacuole (V) and endoplasmic reticulum (ER).
c. MF plants at microspore tetrad (MT) stage showing the dense cytoplasm with various small vacuoles.
d. SET plants at microspore tetrad stage showing intact tapetum (T) releasing large number of ubisch bodies (U). Note the presences of degenerated microspores enclosed with in thick callose wall (C).
e. Mature pollen grain showing well developed exine (E) and an organized nucleus (N).
f. Intact tapetum (T)with degenerated protoplast at pollen grain stage. Note the highly vacuolated pollen grains (PG) with the presence of normal exine (E).

plastids. Inflated strands of rough endoplasmic reticulum (ER) were found ramified throughout the cell cytoplasm. The degeneration of tapetal cells was found to start at microspore tetrad stage. Tapetal mitochondria at young microspore stage remained essentially unchanged with sharp, open cristae and dark matrix (Fig.1a). At late vacuolated pollen grain stage, the tapetal cells were more or less completely absorbed except for some degenerated narrow bands remaining at places. Condensed sporopollenin and Ubisch were present bodies outside the tapetal plasmallema.

The pollen mother cells underwent normal meiotic division to produce microspore tetrads encased in callose wall. The microspore cytoplasm consisted of plastids, mitochondria, rough ER and vesicles (Fig. 1c). A thin inner intine and a thick outer exine wall developed in each microspore to grow into pollen grains. Mature pollen grains were more or less spherical, tricolpate, and engorged with reserves (Fig. 1e).

Anther development in treated plants. In treated plants exhibiting 100% pollen sterility, the anther development was found associated with abnormally intact tapetal cells. At sporogenous tissue stage, highly vacuolated tapetal cells were discernible with intense staining. It was interesting to note that the number of mitochondria in tapetal cells increased but they were in degenerated form; with their outer as well as inner walls significantly thick with degenerated matrix (Fig.1b). Degenerated form of tapetal protoplast increased further at microspore tetrad stage but it continued to secrete large quantity of sporopollenin and released a large number of Ubisch bodies in the anther locule (Fig. 1d). Tapetal cells remained intact even up to the formation of mature pollen grains but all their organelles degenerated (Fig. 1f). Similar tapetal behaviour is well known in large number of chemically treated sterile male plants (Cross and Schulz 1997). Association of abnormal behaviour of tapetal mitochondria and alteration in their genome is now well known in large number of cytoplasmic male sterile plants (Chauhan and Kinoshita 1995). However, such studies in chemically induced sterile male plants are lacking and should be undertaken in the light of the fact that pollen abortion in presently studied chemically induced male sterile *C. arietinum* is associated with abnormal behaviour of tapetal mitochondria.

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Agronomy/Physiology

Variation of SPAD Chlorophyll Meter Readings (SCMR) in the Mini-Core Germplasm Collection of Chickpea

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Drought is one of the major causes of yield losses in chickpea (*Cicer arietinum*). A large portion of such losses can be avoided through crop improvement. Simple analytical models are often used to dissect out and to understand the effects of model parameters on the final yield. Passioura (1977) proposed one such model where yield is considered a function of transpiration, transpiration efficiency (TE) defined as crop biomass production per unit water transpired, and harvest index. Among these three components, genetic enhancement of TE has been taken up as a major research effort in crop improvement programs throughout the world (Bindu Madhava et al. 2003). Although TE is considered a highly useful trait, it was also categorized as a difficult one to screen. Therefore, it becomes necessary to identify surrogate traits that are closely associated with TE for rapid screening of a large number of genotypes. A direct close relationship of TE with SPAD Chlorophyll Meter Readings (SCMR) was reported in groundnut (Nageswara Rao et al. 2001; Bindu Madhava et al. 2003) and SCMR is a direct linear relationship through extracted leaf chlorophyll (Yadava 1986) and also related leaf nitrogen concentration (Kantety et al. 1996; Bullock and Anderson 1998). The advantages such as easy and rapid measurement, nondestructive method and light weight made SPAD meters the best choice for use in the trait-based groundnut breeding program to improve the drought tolerance of groundnut at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Serraj et al. 2004).The same strategy can be applied to chickpea, provided baseline information is available on genetic diversity of SCMR in chickpea. The chickpea mini-core collection has been chosen to collect such information as the number is manageable for initial exploratory efforts and it represents the diversity of the whole germplasm collection (Upadhyaya and Ortiz 2001), Thus, the main objective of this study was to document the extent of variation available for the SCMR readings in the mini-core germplasm of chickpea, and also to identify accessions with contrasting SCMR.

The entire mini-core germplasm collection of *C. arietinum* (211 accessions) along with five genotypes (Annigeri, ICC 4958, Chafa, ICCV 2, and ICC 898) as references were evaluated by measuring the SCMR in a precision Vertisol field (fine montmorillonitic isohyperthermic typic pallustert) in ICRISAT during the 2005/06 postrainy season. The seeds were sown on 15 November 2005. Before sowing, 18 kg N ha^{-1} and 20 kg P ha^{-1} as di-ammonium phosphate were applied. The experiment was conducted in a Split Plot design with two different irrigation treatments (rainfed and optimally irrigated) in three replications. In optimally irrigated treatment, furrow irrigation was applied at 27, 50 and 66 days after sowing (DAS) besides the post-sowing irrigation. The SCMR measurement was taken at 62 and 90 DAS by using SPAD-502 meter (Minolta Konica Co. Ltd., Japan).

SCMR at different leaf positions from the topmost expanded to 6th that compose the plant canopy surface was measured among randomly selected 9 accessions in the irrigation treatments prior to the first measurement at 62 DAS. A significant difference was obtained for SCMR among the leaf positions (Fig. 1). The top and second leaf had significantly lower SCMR than the other leaves; on the other hand there was no significant difference in SCMR among the leaves below the third leaf. This suggests that the third leaf can be considered as representative of the plant canopy for SCMR measurement. Therefore, the third leaf was used for further SCMR measurements.

At 62 DAS, differences in SCMR readings among the entries were significant at <0.001 level in both rainfed and optimally irrigated conditions (Fig. 2a and b). The overall mean of rainfed condition (57.6) was significantly higher than the overall mean in irrigated condition (47.4).

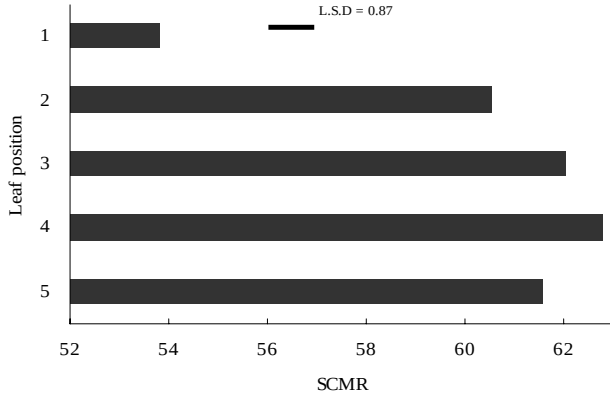

Figure 1. SCMR of different leaf positions in of chickpea accessions (Note: The values are means of 5 replications.)

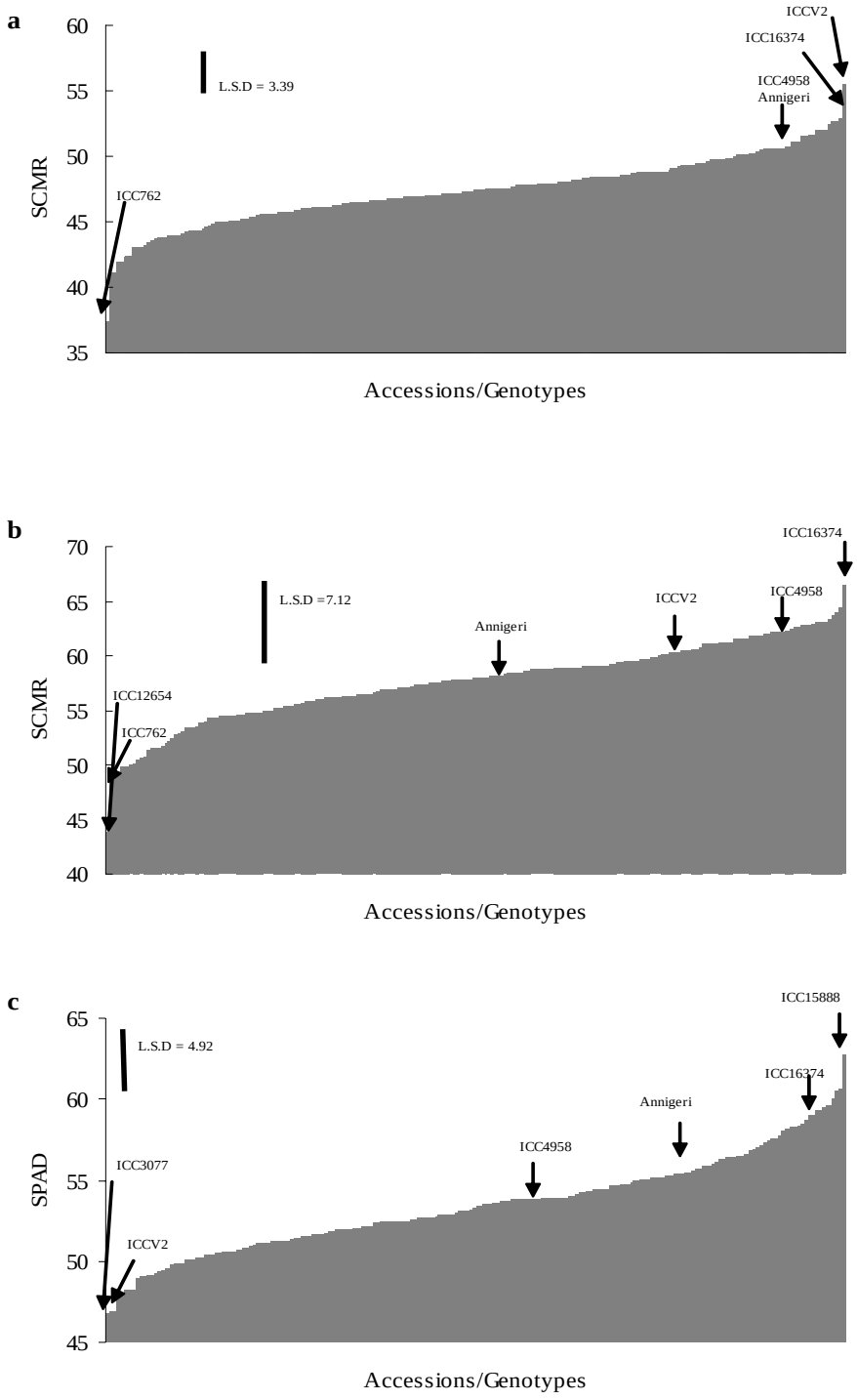

Figure 2. SCMR of the mini-core chickpea germplasm accessions (n=211), 5 cultivated genotypes: (a) in rainfed condition at 62 DAS; (b) irrigated condition at 62 DAS; (c) irrigated condition at 90 DAS (Note: The values are means of three replications.)

This irrigation environment influence might be due to relatively less restricted leaf expansion and with relatively less chlorophyll formation in irrigated condition. Also the differences on crop growth rate and the nitrogen fixation ability between the irrigated and rainfed treatments possibly influence the chlorophyll concentration. It is also likely that the irrigation treatments influence the specific leaf area. There was no genotype by irrigation ($G \times I$) interaction observed. Also, there was a significant correlation in SCMR between in the rainfed and irrigated conditions ($r = 0.534$, $p<0.01$). In rainfed condition, ICCV 2 showed the highest SCMR reading (55.5), and both ICC 4958 and Annigeri showed 51.6, with a rank of 11th. Regardless of the irrigation schemes, ICC 16374 had a superior SCMR with 66.4 (1st rank) in irrigated conditions and its rank was 4th in rainfed environment. ICC 4958 also had a better SCMR irrespective of the irrigation schemes (11th rank in rainfed, 3rd in irrigated).

At 90 DAS, the SCMR measurement was taken in optimally irrigated treatment only as most of the entries in rainfed condition had senesced and matured. There was a significant difference on SCMR among the entries (Fig. 2c). ICC 15888 had the highest SCMR value of 62.7. The accession ICC 16374 also showed a higher SCMR value (59.0); ranking 10th. On the other hand, ICCV 2 which had the highest SCMR in rainfed condition at 62 DAS was 2nd lowest with 46.8. Being extra-early in maturity, ICCV 2 matured on 97 days after sowing under irrigated condition. And as a consequence, the process of senescence and remobilization had already started in this and other early genotypes, leading to poor SCMR values. Although there was a significant linear correlation between at 62 and 90 DAS observations within the optimally irrigated treatment ($r = 0.276$, $p<0.01$), there also existed a significant $G \times I$ interaction ($p<0.001$) reflecting the effects of duration on SCMR observation. This would suggest that meaningful observations can be obtained at early stages of crop growth.

The germplasm accession ICC 16374 showed superior and more consistent SCMR readings than the others. The new genotypes identified, though the results need to be confirmed, could be utilized as valuable breeding sources to improve the drought resistance of chickpea. Also, ICC 4958, a well known drought resistant genotype with a deep and prolific root system (Junichi Kashiwagi et al. 2005) had better, SCMR possibly due to its strong root systems.

This screening of the mini-core germplasm is being repeated during 2006/07 to confirm the results obtained. Any queries related to this study may be directed to Dr J Kashiwagi, Associate Scientist, Crop Physiology, ICRISAT.

Acknowledgment. The authors thank the staff of Gene Bank, ICRISAT for supplying the seeds of mini-core chickpea germplasm in this study and the staff of Crop Physiology Lab and Gene Bank for their technical help.

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Relationships between Transpiration Efficiency and Carbon Isotope Discrimination in Chickpea (*C. arietinum* L)

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Since major cultivation areas of chickpea (*Cicer arietinum* L.) are in the arid and semi-arid zones, terminal drought is one of the major constraints limiting its productivity. Simple analytical crop models can help in identifying key strategies to improve the chickpea productivity under drought. For example, Passioura (1977) had proposed that the yield is a function of transpiration, transpiration efficiency (TE) defined as the biomass production per unit of water transpired, and harvest index. As improvement of TE means maximization of crop production per unit of water use, it is one of the important components for improving the drought resistance (Turner et al. 2001). Although TE had been recognized as a highly relevant trait, so far very little research effort had been made towards field screening for it, especially due to the difficulties in measuring TE in any screening method. The method developed by Farquhar et al. (1982) for estimating TE through measuring the discrimination against ^{13}C by leaves during photosynthesis, and establishment of a close relationship between the carbon isotope discrimination ($\delta^{13}C$) and TE in many legume crops such as bean, cowpea, groundnut, and soybean has provided an useful method of screening. This gave scope for using $\delta^{13}C$ as an indirect screening tool for TE. In chickpea, however, there is no information available on the relationship between $\delta^{13}C$ and TE. The major objectives of this study were to check if there are any variations available for $\delta^{13}C$, to investigate the relationship between $\delta^{13}C$ and TE, and to ascertain the possibility of using $\delta^{13}C$ as a surrogate for TE measurements.

Ten chickpea (*C. arietinum* L.) genotypes (Annigeri, ICC 10448, ICC 13219, ICC 14199, ICC 1882, ICC 283, ICC 4958, ICC 5337, ICC 5680 and ICC 8261) with contrasting growth duration, type (desi or kabuli), growth habits, and root systems were used. The pot experiments were conducted in a randomized block design (RBD) with two irrigation schemes plus pre-irrigation treatment harvest set in 5 replications in a greenhouse facility at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in 2004. At 30 days after sowing (DAS), pre-irrigation treatment (five) plants were harvested inclusive of roots from each genotype. At the same time, the soil in pots of both irrigation treatments was saturated with water to bring it to field capacity. All pots were then covered with polyethylene bags, leaving the plants outside to avoid evaporation, and short straw pipes were inserted for further irrigations. The daily transpiration was estimated as the difference in pot weight between two subsequent days. In the well-watered pots (control), the water lost in a day was added back, whereas in the water stress-imposed pots the water, which is equivalent to 70–90% of daily transpiration, was given to avoid the rapid build up of soil water stress. To monitor the daily available soil moisture, the daily transpiration rates (TR) in the stress condition were normalized against the transpiration rates measured in control plants on each day. The experiment was terminated when the TR of water-stressed plants fell below 0.1 (less than 10% of transpiration of control), which is considered as the point where plants are no longer able to take up water from the soil, and where all the physiological processes contributing to growth are fully inhibited. At this time, the 4th and the 5th most fully expanded leaves from the top leaf on the main stem were collected in all plants for $\delta^{13}C$ estimations. At the same time the entire plant parts, including the roots, were harvested to estimate final plant biomass. The total transpiration was calculated as a sum of the daily transpiration from the initial day when plants were bagged to the day when plants were

Table 1. Analysis of variance and its significance for water schemes, genotypes, and their interaction for carbon isotope discrimination ($\delta^{13}C$), and transpiration efficiency (TE) in ten chickpea genotypes grown under well-watered (control) and drought stress conditions in a pot experiment.

	Mean sum of squares and significance level[1]	
Source of variation	$\delta^{13}C$	TE
Irrigation scheme	146.83***	20.78***
Genotype	1.25***	0.49***
Genotype × Irrigation scheme	1.56***	0.05 NS
Residual	0.16	0.03

1. Significant at *** = <0.001 level and NS = Not significant.

harvested. The TE, therefore, could be calculated as the plant biomass gained between the first and final plant sampling divided by total transpiration during that period.

Analysis of $\delta^{13}C$ was carried out at International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Japan with the use of an isotope ratio mass spectrometer (IRMS), ThermoFinnigan Delta XP^{plus}, Hamburg, Germany, connected with an element analyzer (EA), Carlo Erba EA Flash 1112, Milan, Italy. Total carbon in leaf samples was incinerated in a furnace of EA and separated as pure CO_2 gas. A small quantity of the gas was introduced to IRMS to measure the ratio of $^{13}CO_2/^{12}CO_2$ as the different mass weight of 45/44 to obtain $\delta^{13}C$ (‰).

There were significant differences in $\delta^{13}C$ among the ten genotypes, and the $\delta^{13}C$ in stress condition was significantly higher than that in the well-watered control (Table 1). Genotype ICC 5337 showed the highest $\delta^{13}C$ (−26.0‰) in the stress condition. ICC 4958, a well known drought resistant variety, had a superior $\delta^{13}C$ value than the other genotypes. Also ICC 4958 ranked second (−27.2‰) under stress condition and the first (−28.4‰) in the well-watered control condition. The genotype by irrigation (G × I) interaction was significant for $\delta^{13}C$.

Among the ten genotypes, significant difference in TE was observed in both irrigated and stress conditions (Table 1). Genotype ICC 5337 showed the highest TE irrespective of irrigations of 3.9 g kg^{-1} under stress and 2.8 g kg^{-1} under well-watered control. The TE under stress was significantly higher than TE under control. There was a significant correlation in TE between the stress and control conditions ($r = 0.881$, $p<0.01$), and there was no G × I interaction observed. This is indicative of the genotypic difference in TE and their rankings would remain across different soil water environments.

A significant positive correlation between $\delta^{13}C$ and TE was observed ($r = 0.857$, $p<0.01$) under the stress condition (Fig. 1). This relationship agrees with the theoretical relationship between $\delta^{13}C$ and TE as observed in several other legumes. However, no significant correlation was observed between $\delta^{13}C$ and TE when the plants were grown under well-watered conditions. A similar result has been obtained in sunflower (Virgona et al. 1990). This would indicate that the ^{13}C discrimination ability manifests into TE under water-limited conditions whereas under well-watered conditions the stomatal closure-led CO_2 limitation no longer becomes a constraint to C sequestration in plants. Our results in chickpea may

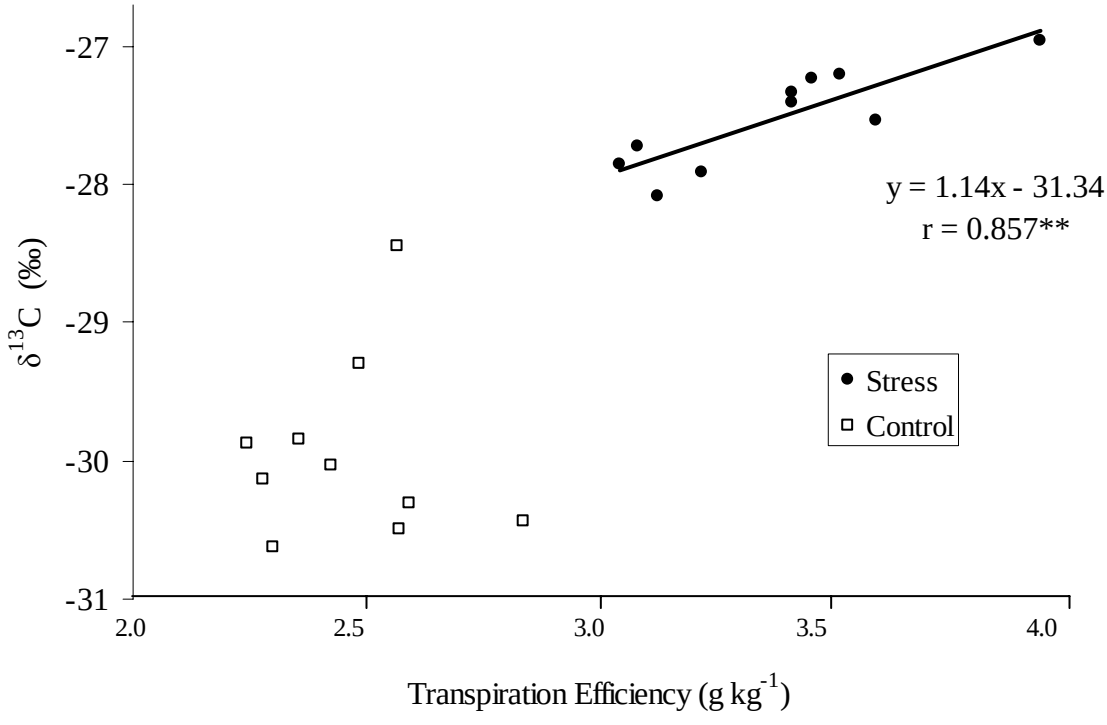

Figure 1. Relationship between transpiration efficiency (TE) and carbon isotope discrimination ($\delta^{13}C$) in ten chickpea genotypes grown under the well-watered (control) and drought stress conditions in a pot experiment.

indicate that the differences in TE are brought about by changes in stomatal conductance rather than by changes in mesophyll efficiency.

This is the first report to show the existence of a clear relationship between $\delta^{13}C$ and TE in chickpea. This result shows that TE of chickpea grown under drought conditions could be estimated through $\delta^{13}C$ measurement. Further evaluation of these chickpea genotypes for TE in field grown conditions is being carried out during 2006/07 to confirm the results obtained. Any queries related to this study may be directed to Dr J Kashiwagi, Associate Scientist, Crop Physiology, ICRISAT.

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Selection for Tolerance to Postemergence Herbicides in Chickpea Cultigen

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Chickpea yield can be doubled when the sowing time is shifted from spring to winter in the Mediterranean region, but weeds are one of the most significant unsolved problems (Toker et al. 2006). When the crop is sown in autumn or winter, it competes very poorly with weeds due to its slow initial growth. Yield loss due to weeds depends on differences in intensity of infestation and species of weeds, and has been reported up to 98% (Solh and Pala 1990). Although herbicides were economically used as a weed control method (Bhan and Kukula 1987; Bhan and Mishra 1997; Yaduraju and Mishra 2004), preplanting and preemergence herbicides barely affect weeds germinated during the late seedling stage in winter-sown chickpea. Farmers need a postemergence herbicide to be able to control weeds without affecting the crop. Therefore, this study was aimed at screening for tolerance to postemergence herbicides in the chickpea cultigen in winter-sown chickpea.

A total of 229 genotypes including Turkish chickpea core collection of 101 accessions along with five popular cultivars (Akcin, Er, Gokce, Kusmen and Uzunlu) grown in Turkey and 123 lines from the International Center for Agricultural Research in Dry Areas (ICARDA), and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) were evaluated for herbicide tolerance at Antalya location (approximately 30° 44' E, 36° 52' N, 51 m asl), Turkey. Genotypes were sown in one m single row and 45 cm row spacing with two replications in the first week of December in 2004 and third week of February in 2006. Quizalofop-p-tefuryl, fluazifob-p-butyl and aclonifen were applied postemergence at seedling stage at a rate of 2, 0.75 and 1.5 liters a.i. ha^{-1}, respectively. The herbicides were applied at two weeks intervals. Aclonifen and quizalofop-p-tefuryl provided limited weed control among herbicides. Aclonifen and fluazifob-p-butyl negatively affected the chickpea genotypes while the latter provided effective control of some weeds. After application of herbicides, genotypes were evaluated after one week using herbicide tolerance score on a 1–9 scale, where 1 = very highly herbicide tolerant (free from herbicide effects), 2 = highly herbicide tolerant (up to 10% leaves showing chlorosis damage), 3 = herbicide tolerant (11–20% leaves showing chlorosis damage), 4 = moderately herbicide tolerant (21–30% leaves and up to 20% of branches withering and drying, no plant death after one week), 5 = intermediate (31–60% leaves and 21–40% branches withering, up to 10% plant death), 6 = moderately herbicide susceptible (61–80% leaves and 41–60% branches withering and drying, 11–25% plant death), 7 = herbicide susceptible (81–99% leaves and 61–80% branches withering and drying, 26–50% plant death after one week), 8 = highly herbicide susceptible (100% leaflets and 81–99% branches withering and drying, 51–99% plant death), and 9 = very highly herbicide susceptible (100% plant death in harvest).

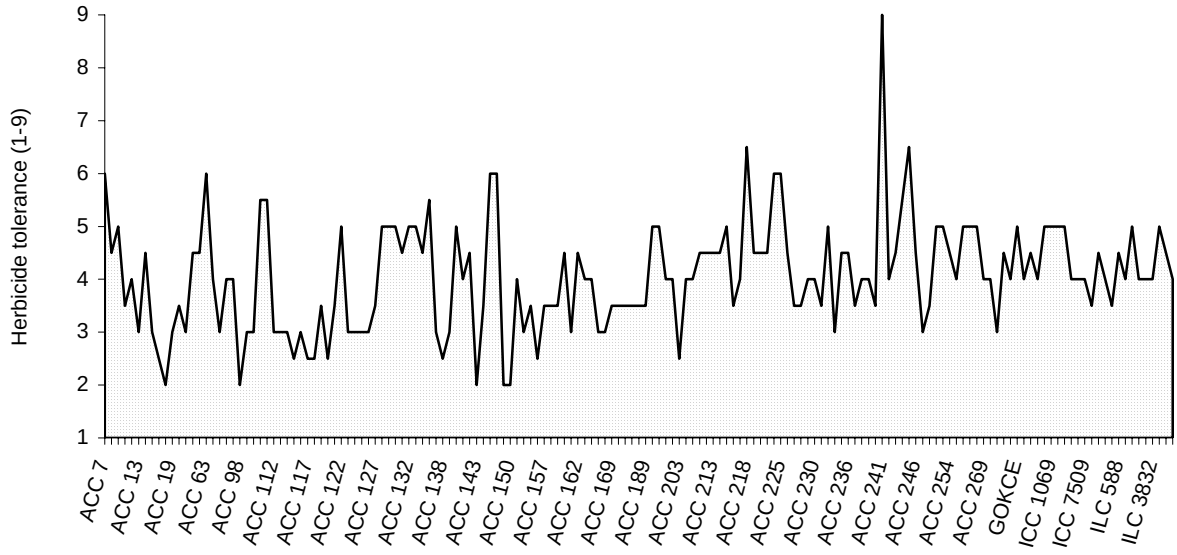

Figure 1. Herbicide (Aclonifen and fluazifob-p-butyl at a rate of 1.5 and 0.75 liter a.i. ha^{-1}, respectively) tolerance on a 1–9 scale in chickpea cultigen. Areas are means with average standard errors of 0.55.

Anthemis chia, Emex spinosa, Sinapsis arvensis, Melilotus officinalis, Lamium amplexicaule, Fumaria parviflora, Avena fatua, Cynodon dactylon, Anagallis arvevsis var. *arvensis* and *Anagallis arvevsis* var. *caerulea* were detected as sensitive weeds. Thirty-six genotypes were scored 2 and 3 on the scale, while one accession (ACC 241) died. ACC 18, ACC 98, ACC 143, ACC 149 and ACC 150 had scores of 2, while ICCV 2, scored 4 on 1–9 scale. Commercial cultivars grown in Turkey and some germplasm lines registered cold (ILC 8262, ILC 8617 and CA 2969) and drought tolerant (ICC 4958) scored 4 to 5 on the 1–9 scale (Fig. 1).

Herbicide tolerant chickpea genotypes could be further evaluated for winter sowing in breeding programs as gene sources. This study has addressed one of the most important unsolved problems in winter-sown chickpea to combat weeds by using postemergence herbicide tolerant genotypes. Selected herbicide tolerant genotypes could later be recommended for commercial production.

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Selection for Tolerance to Postemergence Herbicides in Annual Wild *Cicer* Species

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There are eight annual wild species in the genus *Cicer* and they have generally been grouped into three categories on the basis of genetic similarities to the cultigen. The primary gene pool of *C. arietinum* consists of *C. reticulatum* Ladiz. and *C. echinospermum* P.H. Davis, because they can be easily crossed with the cultigen. The second closest gene pool encompasses *C. bijugum* K.H. Rech., *C. judaicum* Boiss and *C. pinnatifidum* Jaub. & Sp.; while *C. chorassanicum* (Bge) M. Pop., *C. cuneatum* Hochst. ex Rich and *C. yamashitae* Kitamura are in the third gene pool due to being the most distinct from the cultigen (Croser et al., 2003). Although the annual wild species are not important for direct production, there is interesting variability in their agronomic traits (Robertson et al. 1997). Moreover, wild *Cicer* species in the first and the second gene pools are superior to the cultigen on the basis of resistance to some stresses (Singh et al. 1998). Furthermore, they have a higher level of cold tolerance than the cultigen (Toker 2005). However, there is a gap with respect to evaluation for herbicide tolerance in the first and the second gene pools of annual wild chickpeas. This study therefore aimed to screen for tolerance to post-emergence herbicides in the first and the second gene pools of annual wild chickpeas.

A total of 36 accessions in the first and the second gene pools of annual wild chickpeas was evaluated for herbicide tolerance at Antalya location (approximately 30° 44' E, 36° 52' N, 51 m asl), Turkey. Accessions were sown in one m single row and 45 cm row spacing with two replications in the first week of December in 2004 and third week of February in 2006. Quizalofop-p-tefuryl, fluazifob-p-butyl and aclonifen were applied postemergence at seedling stage at a rate of 2, 0.75 and 1.5 liters a.i. ha^{-1}, respectively. The herbicides were applied at two week intervals. Herbicides negatively affected some accessions. After application of herbicides, genotypes were evaluated after one week using herbicide tolerance score on a 1–9 scale (Ceylan and Toker 2006).

AWC 641, an accession of *C. reticulatum* Ladiz., was scored 2, highly herbicide tolerant. Nine accessions of *C. reticulatum* Ladiz. were herbicide tolerant, while all lines of *C. bijugum* K.H. Rech., *C. judaicum* Boiss. and *C. pinnatifidum* Jaup. & Spach, were intermediate. Three accessions of *C. echinospermum* P.H. Davis, showed moderately tolerant reaction (Fig. 1). AWC 641 had the highest level of herbicide tolerance among some germplasm lines registered cold (ILC 8262 and ILC 8617) and drought tolerant (ICC 4958) (Ceylan and Toker 2006). Herbicide tolerance scores of accessions ranged from 2 to 6. Also, some agronomic, pnenologic and morphologic characters are given in Table 1. Some accessions had interesting variability in their agronomic traits (Table 1).

Table 1. The mean, standard error, minimum and maximum values of yield components in annual wild *Cicer* species.

Characters*	Mean	Standard Error	Minimum	Maximum
Herbicide tolerance (1–9)	4.04	±0.12	2.0	6.00
Days to flowering (days)	134.91	±0.44	128.00	140.00
Days to maturity (days)	170.70	±0.80	164.00	190.00
Seeds per pod	1.11	±0.05	1.00	4.00
Pods per node	1.04	±0.02	1.00	2.00
Pods per plant	32.18	±1.89	7.50	70.00
100 seed weight (g)	14.75	±0.85	1.20	32.60
Branches per plant	7.31	±0.25	3.00	12.00
Plant height (cm)	14.79	±0.89	5.60	37.00
Canopy width (cm)	41.78	±1.72	17.30	69.50
Biological yield (g $plant^{-1}$)	34.30	±3.31	5.00	140.00
Seed yield (g $plant^{-1}$)	21.80	±2.88	0.30	123.60

*IBPGR/ICRISAT/ICARDA. 1993. Descriptors for chickpea (C*icer arietinum* L.). ICRISAT, Patancheru, India.

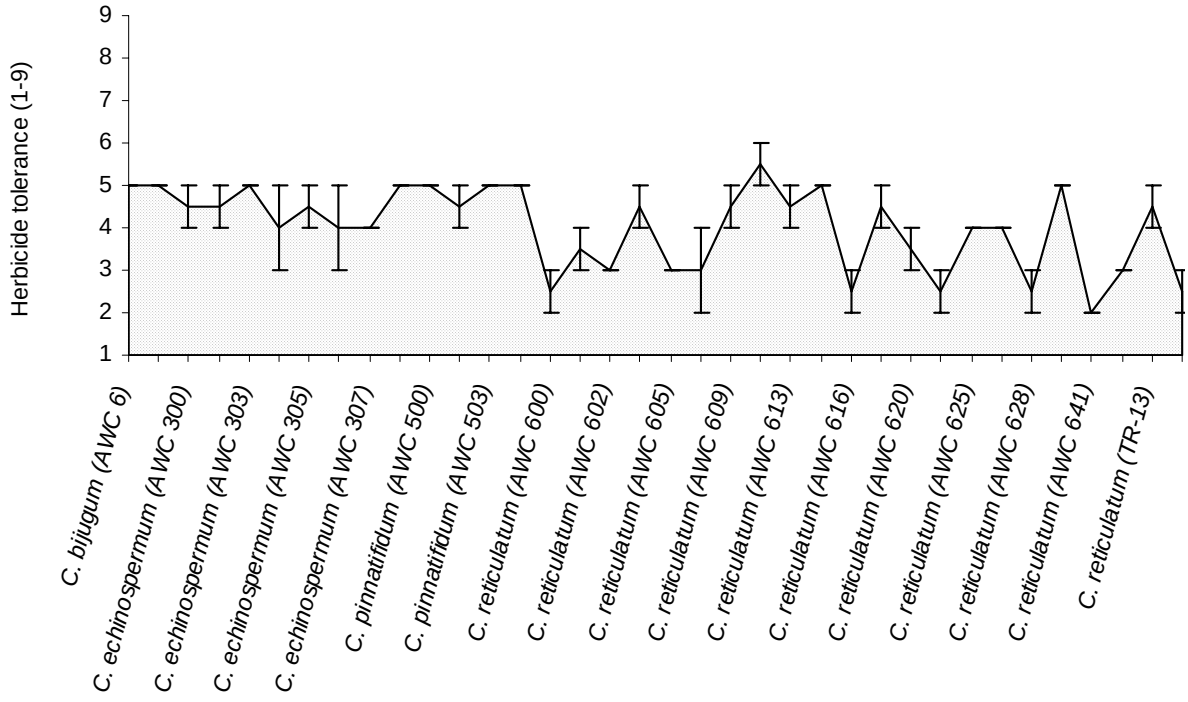

Figure 1. Herbicide (Aclonifen and fluazifob-p-butly at a rate of 1.5 and 0.75 L a.i. per hectare, respectively) tolerance on a 1–9 scale in annual wild *Cicer* species. Areas are means ± standard errors.

Some accessions, especially in *C. reticulatum* Ladiz., had a high level of herbicide tolerance as the best cultigens. Tolerant genotypes will be used in breeding programmes as gene sources for winter sowing.

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Pathology

Evaluation of Wild *Cicer* Species for Resistance to Ascochyta Blight and Botrytis Gray Mold in Controlled Environment at ICRISAT, Patancheru, India

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Chickpea (*Cicer arietinum* L.) is the third most important food legume crop grown over 45 countries across five continents. It maintains soil fertility through biological nitrogen fixation and contributes to the sustainability of cropping systems in cereal-legumes rotation.

Ascochyta blight (AB, caused by *Ascochyta rabiei*) and Botrytis gray mould (BGM, caused by *Botrytis cinerea*) are destructive fungal foliar diseases of chickpea (Davidson et al. 2004; Pande et al. 2004 and Pande et al. 2005) that can cause up to 100% yield losses. Cool and wet weather favour these diseases and their epidemic development. Management of AB and BGM rely on fungicides, but these are not effective when the disease pressure is high. Deployment of resistant genotypes could be an effective way to minimize yield losses due to AB and BGM. Since adequate levels of disease resistance are not available in the cultivated chickpea germplasm, wild *Cicer* spp. have been identified as good sources of resistance to these diseases and there is a potential to transfer resistance genes from these species into cultivated *C. arietinum* species (Singh et al. 1992 and Haware et al. 1992). Therefore, in our quest to identify durable levels of resistance to AB and BGM, we initiated a large-scale screening of wild *Cicer* accessions under optimal disease development conditions at ICRISAT.

Ascochyta blight. Following the controlled environment screening technique (CEST), 148 wild accessions belonging to seven *Cicer* spp. *viz.*, *C. bijugum*, *C. cuneatum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum* and *C. yamashitae* were evaluated for AB resistance. Eight seedlings each of the test entry and a susceptible genotype were raised in rows in plastic trays filled with sand-vermiculite mixture (4:1) in a greenhouse. Nine test entries and a susceptible check Pb7 were sown in each tray. These trays with 12-day-old seedlings were transferred to controlled environment facility (CEF) maintained at 20±1°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with the conidial suspension (5×10^4 conidia ml^{-1}) till runoff. The *A. rabiei* conidia were produced on the autoclaved seeds of chickpea and harvested into sterile distilled water to prepare the conidial suspension for inoculation. After inoculation, the seedlings were allowed to dry partially for 30 min; thereafter 100% relative humidity (RH) was maintained till the end of the experiment. Disease severity was recorded on a 1–9 rating scale 10 days after inoculation (Pande et al. 2005). The experiment was repeated once. Based on the mean disease score of two repetitions (16 seedlings), individual chickpea lines were categorized as asymptomatic (disease score 1.0), resistant (disease score 1.1–3.0), moderately resistant (disease score 3.1–5.0), susceptible (disease score 5.1–7.0) and highly susceptible (disease score 7.1–9.0).

Out of 148 accessions evaluated, five accessions of *C. judaicum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038) were resistant. Of the remaining lines, 55 accessions were moderately resistant, 61 were susceptible and 27 were found to be highly susceptible to AB infection (Table 1).

Botrytis gray mold. One hundred and forty-eight wild *Cicer* accessions belonging to seven *Cicer* spp. *viz.*, *C. bijugum*, *C. cuneatum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum* and *C. yamashitae* were raised similar to AB resistance screening procedures in the greenhouse and tested for BGM resistance in CEF. There were eight seedlings of each of the nine test genotypes and a BGM susceptible line (JG 62 as indicator) in each tray. Trays with 12-day-old seedlings were transferred to CEF adjusted at 15±2°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with the conidial suspension (3×10^5 conidia ml^{-1}) till runoff. After inoculation, the seedlings were allowed to dry for 30 min; thereafter 100% RH was maintained till the end of experiment. The *B. cinerea* inoculum was multiplied on autoclaved petals of marigold (*Tagetus erecta)* flowers for 8 days at 25°C and 12 h photoperiod. Conidia from the profusely sporulating culture were harvested into sterile distilled water and used for inoculations. The experiment was repeated once. Disease severity was recorded on a 1–9 rating scale as

done for AB at 20 DAI, and based on the mean disease score of two repetitions (16 seedlings) individual chickpea lines were categorized as asymptomatic, resistant, moderately resistant and susceptible or highly susceptible.

Of the 148 wild accessions evaluated, 29 accessions were found to be resistant. Out of 29 resistant accessions 23 were from *C. judaicum* (ICC 17194, ICC 17205, ICC 17149, ICC 17148, ICC 17204, IG 69977, IG 70033, IG 72931, IG 72932, IG 17150, IG 69959, IG 69969, IG 70032, IG 70038, ICC 17151, ICC 17190, ICC 17192, ICC 17195, IG 69943, IG 69997, IG 69998, IG 70034 and IG 70037); three from *C. bijugum* (IG 69981, IG 70023 and IG 70006) and three from *C. reticulatum* (IG 72959, IG 72933 and IG 72941). The remaining 107 were categorized as moderately resistant (50), susceptible (51) and highly susceptible (6) to BGM (Table 2). Twelve lines did not germinate.

Ascochyta blight and Botrytis gray mold. Five AB resistant accessions belonging to *C. judaicum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038) were separately evaluated for AB and BGM twice in the CEF to identify combined resistance to both the diseases. Procedures for raising the seedlings, inoculum preparation, inoculations, and disease scoring were similar to AB and BGM evaluations explained earlier. Two accessions (IG 70037 and IG 70038) were found to be resistant (≤3.0, on 1–9 scale) to both the diseases and the remaining three (ICC 17211, IG 69986 and IG 70030) were moderately resistant (Table 3). These wild *Cicer*

Table 1. Evaluation of wild *Cicer* accessions for resistance to Ascochyta blight in controlled environment.

Cicer species	No. of lines tested	Reaction to Ascochyta blight infection[a]				
		A	R	MR	S	HS
C. bijugum	30	–	–	7	20	3
C. cuneatum	3	–	–	1	2	–
C. echinospermum	4	–	–	–	3	1
C. judaicum	47	–	5	34	8	–
C. pinnatifidum	27	–	–	13	13	1
C. reticulatum	31	–	–	–	15	16
C. yamashitae	6	–	–	–	–	6
Total	148	–	5	55	61	27

a. Based on the disease score the wild accessions were categorized for their reaction to Ascochyta blight infection as follows: 1 = asymptomatic (A); 1.1–3.0 = resistant (R); 3.1–5.0 = moderately resistant (MR); 5.1–7.0 = susceptible (S); 7.1–9.0 = highly susceptible (HS).

Table 2. Evaluation of wild *Cicer* accessions for resistance to Botrytis gray mold in controlled environment.

Cicer species	No. of lines tested[b]	Reaction to Botrytis gray mold infection[a]				
		A	R	MR	S	HS
C. bijugum	28	–	3	18	7	–
C. cuneatum	3	–	–	3	–	–
C. echinospermum	2	–	–	1	–	1
C. judaicum	45	–	23	18	4	–
C. pinnatifidum	26	–	–	4	20	2
C. reticulatum	27	–	3	6	18	–
C. yamashitae	5	–	–	–	2	3
Total	136	–	29	50	51	6

a. Based on the disease score the wild accessions were categorized for their reaction to Botrytis gray mold infection as follows: 1 = asymptomatic (A); 1.1–3.0 = resistant (R); 3.1–5.0 = moderately resistant (MR); 5.1–7.0 = susceptible (S); 7.1–9.0 = highly susceptible (HS).

b. 12 lines did not germinate.

Table 3. Identification of combined resistance to Ascochyta blight and Botrytis gray mold diseases in controlled environment.

	Disease reaction (on 1–9 rating scale)					
	Ascochyta blight			Botrytis gray mold		
Accession No[1]	Test 1	Test 2	Mean	Test 1	Test 2	Mean
ICC 17211	2.7	2.0	2.3	4.0	3.0	3.5
IG 69986	2.5	3.5	3.0	4.5	2.5	3.5
IG 70030	3.5	2.5	3.0	4.5	2.5	3.5
IG 70037	2.0	4.0	3.0	4.0	2.0	3.0
IG 70038	2.7	3.0	2.8	3.5	2.0	2.8

1. All accessions belong to *Cicer judaicum.*

accessions, found resistant to AB, BGM and or to both the diseases, can be used in the chickpea foliar disease resistance breeding programs as resistant donor parents.

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Comparison of Greenhouse and Field Screening Techniques for Botrytis Gray Mold Resistance

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Botrytis gray mold (BGM), caused by *Botrytis cinerea* Pers. ex. Fr., is the most destructive foliar disease of chickpea in eastern India, Bangladesh, Nepal, and western Australia. Cool wet weather favors the development of BGM and can cause upto 100% yield loss. Host plant resistance (HPR) is the most economical and eco-friendly means of management of BGM. For exploitation of HPR, reliable field and controlled environment screening techniques are essential. In general, field screening techniques (FST) are used for large-scale screening of germplasm and breeding material, and controlled environment screening techniques (CESTs) are used to confirm field resistance, screening against different pathotypes/races and to carry out inheritance and race identification studies.

Several CESTs, such as whole plant screening technique (WPST), cut-twig screening technique in water (CTST-W) and cut-twig screening technique in sand (CTST-S) were standardized in a controlled environment facility (CEF) at ICRISAT, Patancheru. Components of CESTs such as optimum temperature, relative humidity, and photoperiod for BGM were identified. This study attempts to compare CESTs with FSTs.

In WPST, seedlings of the test material were grown in rows in plastic trays filled with a mixture of sterilized sand and vermiculite (4:1) in a greenhouse (Fig. 1A). One row of a susceptible cultivar JG 62 was planted as indicator

in each tray along with nine test entries. Trays with 10-day-old seedlings were transferred to CEF adjusted at 15±1°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with conidial suspension (3×10^5 spores ml^{-1}) of *B. cinerea*. After inoculation the plants were allowed to partially dry for 30 min and thereafter 100% RH was maintained till the end of experiment (Pande et al. 2002). The experiment was conducted in two replications with eight plants in each replication and repeated once.

In CTST-W, tender shoots of chickpea plants were cut from the actively growing chickpea plant (30–60 days after sowing) with a sharp edged blade in the evening. The lower portion of the detached twig was wrapped with a cotton plug and transferred to a test tube (15 × 100 mm) containing fresh water (Sharma et al. 1995), (Fig. 1B). The tubes were kept in CEF, allowed to acclimatize for 12–24 h and inoculated following standardized procedures (Pande et al. 2002).

In CTST-S, the detached twigs were planted into sterilized moist coarse sand-vermiculite medium in trays (Fig. 1C). Trays were kept in the CEF, allowed to acclimatize

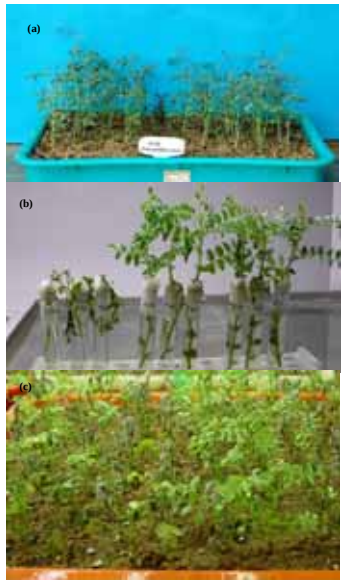

Figure 1. Controlled environment screening techniques at ICRISAT, Patancheru 502 324, Andhra Pradesh, India
(a) Whole plant (WPST) (b) Cut twig-water (CTST-W) (c) Cut twig-sand (CTST-S).

Table 1. Comparison of controlled environment and field screening techniques for Botrytis gray mold resistance.

	Disease score[1] (1–9 rating scale)[2]					
	Controlled environment			Field		
Entry	WPST[3]	CTST-W[4]	CTST-S[5]	Pantnagar	Ishurdi	Overall mean
ICC 8509	5.0	4.5	5.0	7.0	4.5	5.2
ICC 12339	4.5	4.0	5.5	5.0	4.5	4.7
ICC 89302	4.0	4.0	5.0	7.0	4.0	4.8
ICC 89303	6.0	6.0	6.5	7.0	6.0	6.3
ICC 89310	7.0	7.0	7.5	8.0	6.0	7.1
ICC 86215	6.0	5.5	4.0	6.0	5.5	5.4
ICC 86242	6.5	5.0	5.5	6.5	4.7	5.6
ICCX860030-BP-BP	6.0	6.0	7.0	7.0	6.0	6.4
ICCX860023-BP-BP-BP-3P-BH-IH-BH	6.0	6.0	7.0	8.0	6.6	6.7
ICCX880355-BH-BP-5H-BH	7.2	7.5	8.0	9.0	6.5	7.6
Susceptible check	9.0	9.0	9.0	9.0	9.0	9.0
Overall mean	6.1	5.9	6.3	7.2	5.8	
CD at 5%						
Techniques = 0.69						
Entry = 0.86						
Technique × Entry = 1.9						

1. Average of three replications.
2. Disease reaction was based on the disease score: 1 = asymptomatic; 1.1–3 = resistant; 3.1–5 = moderately resistant (MR); 5.1–7 = Susceptible; 7.1–9 = Highly susceptible (HS).
3. WPST = whole plant screening technique.
4. CTST-W = cut-twig screening technique in water.
5. CTST-S = cut-twig screening technique in sand.

Figure 2. Field screening technique, Ishurdi, Bangladesh.

for 12–24 h and inoculated following standardized procedures as explained above. The experiment was conducted in two replications with eight twigs in each replication and repeated once.

To compare the CESTs and FST for BGM resistance, 10 chickpea lines selected from the International Botrytis Gray Mould Nursery (IBGMN) were evaluated under CEF at ICRISAT and in the field at hot spot locations in Pantnagar (India) and Ishurdi (Bangladesh). In FST test lines were sown in 2–3 m long rows spaced at 30 × 10 cm. Indicator-cum-infector rows of a susceptible cultivar H208/JG 62 were sown after every two-test row. At the onset of flowering, the trial was irrigated and plants were inoculated with a spore suspension (5×10^4 spores ml^{-1}) of 10 day old culture of *B. cinerea.* From the following day, sprinkler irrigation or perfo-irrigation was run every day for about 15 min after every 1 or 2 h from 9.00 to 17.00 h depending upon the environmental conditions (Fig. 2). Inoculation with spore suspension of *B. cinerea* was repeated twice at 10-day intervals after the first inoculation (Pande et al. 2002). The trial was replicated twice at both the locations. Data on disease severity was recorded on a 1–9 rating scale after 20 days of inoculation (DAI) in WPST, 8 DAI in CTST-W and CTST-S and at the time of harvest in FST. Based on the mean disease score, individual chickpea line was categorized as asymptomatic (disease score 1.0), resistant (disease score 1.1–3), moderately resistant (disease score 3.1–5), susceptible (disease score 5.1–7) and highly susceptible (disease score 7.1–9).

Results obtained with CESTs i.e. WPST, CTST-W, CTST-S, and FST are comparable for BGM (Table 1). Analysis of variance revealed that there was no significant difference between the techniques except in the field screening at Pantnagar where disease pressure was marginally higher on a few test entries than the CESTs. However, the severity of BGM in susceptible check and in majority of test entries was uniform in all the techniques. Therefore, we can conclude that the CEST and FST are equally reliable, repeatable and economical. However, CTST-W and CTST-S are found to be rapid and economical and useful in screening segregating germplasm and breeding lines without destroying the plants and thus can be used to screen for other target traits and seed production.

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Resistance Screening to *Ascochyta* Blight Disease of Chickpea in Pakistan

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Chickpea is an important grain legume sown under rainfed conditions in Pakistan with average yields of 615 kg ha^{-1} (GOP 2003). Several biotic and abiotic factors are responsible for low yield. Among the biotic factors, blight caused by *Ascochyta rabiei* (Pass.) Lab. is a major limiting factor (Haqqani et al. 2000). The disease can be effectively controlled by the foliar application and seed dressing fungicides (Reddy and Singh 1984, Rauf et al. 1996), the use of disease-free seeds (Kaiser 1984) and destruction of plant diseased debris (Chaube and Pandey 1986); however, these approaches are not economically viable. Host plant resistance provides the cheapest and most sustainable control of chickpea blight – therefore, the present study was undertaken to identify sources of resistance for the development of blight resistant varieties of chickpea. A total of 355 chickpea germplasm lines obtained from National and International Institutes (Table 1) were planted in earthen pots (7.5 × 15 cm) filled with sterilized soil and sand (2:1) mixture. Five seeds from each accession were surface sterilized by treating with Clorox solution (0.1% available chlorine) for 2 min before sowing. A susceptible variety C 727 was sown as control. The pots were kept in a greenhouse at 20±2°C in natural light for 14 days before inoculation. Plants were sprinkled with water prior to inoculation.

The inoculum was prepared from a 15 day-old culture of *A. rabiei* multiplied on chickpea grains according to the procedure developed by Ilyas and Khan (1986). Two week old seedlings were spray-inoculated with spore suspension (5×10^5 spores ml^{-1}). The inoculated seedlings were incubated in humid chamber for 72 h at relative humidity >90%. Disease observations were scored on a 1–9 disease rating scale (Singh et al. 1981) when susceptible check was completely killed by AB infection. The genotypes were grouped into three categories on the basis of disease severity: resistant (1–3 rating), moderately resistant/tolerant (4–5 rating) and susceptible (6–9 rating).

Ten genotypes were resistant with disease rating of 3 and 32 genotypes were moderately resistant with disease rating of 4–5 (Table 1) whereas all the others were susceptible with disease rating of 6–9. Out of 10 resistant genotypes, two (FLIP03-42C, ICC 12004) were developed/ provided by ICARDA, four (ICC 3932, ICC 4033, ICC 6373, ICC 6945) from ICRISAT, two (NCS 0507, NCS 0524) from NARC and two (AZRI-7130, AZRI-17115) from AZRI (Table 1). This indicated that national and regional agricultural research institutes on chickpea are concentrating on development of blight-resistant varieties of chickpea.

Table 1. Number of chickpea accessions obtained from various sources and number of blight-resistant/ tolerant lines identified under greenhouse conditions at NARC, Islamabad.

Source	Number of genotypes				Names of resistant lines
	Total	Resistant	Tolerant	Susceptible	
International Centre for Agricultural Research in Dry Areas (ICARDA), Syria	89	2	11	76	Flip 03-42C, ICC 12004
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India	47	4	5	38	ICC 3932, ICC 4033, ICC6373 & ICC6945
National Agricultural Research Centre (NARC), Islamabad	53	2	8	43	NCS 0507, NCS 0524
Arid-Zone Research Institute (AZRI), Bhakkar	90	2	3	85	AZRI 7130, AZRI 17115
Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad	76	0	5	71	–

Rating scale: Resistant (1–3), Moderately resistant/tolerant (4–5), Susceptible (6–9).

In the present investigation obvious genetic differences were obtained among genotypes at seedling stage, suggesting that germplasm lines should be initially screened at seedling stage under greenhouse conditions to save time and labor. The resistant genotypes selected at seedling growth stage should be re-tested for adult plant resistance at flowering and/or pod formation stage under field as well as greenhouse conditions. A large number of genotypes were found to be susceptible, which indicated the effectiveness of artificial inoculation and resistance screening conditions for the development of disease.

Among the lines of chickpea e.g. ILC-72 and ILC-3279 resistant to *Ascochyta* blight that have been identified at International Centre for Agricultural Research in Dry Areas (ICARDA), Syria (Reddy and Singh 1984, Singh et al. 1984) though these showed high level of resistance in several countries, were not found resistant in Pakistan (Iqbal 2002). Therefore, resistant genotypes originating at ICARDA and elsewhere need to be re-tested with *A. rabiei* pathotypes of Pakistan before their use in breeding programs, as it is well established that the fungus *A. rabiei* is highly variable and the pathotypes present in Pakistan and India are more aggressive than those prevalent in the Mediterranean region (Singh et al. 1984).

The information on the resistance to *A. rabiei* generated in the present study indicated that there is sufficient genetic variation in chickpea for this trait that can be exploited for disease control by building disease resistance pyramids.

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Pigeonpea

Genetics/Breeding/Biotechnology

Open Flower Segregants Selected from *Cajanus platycarpus* Crosses

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Pigeonpea (*Cajanus cajan* L. Millsp.) has a typical papilionaceous flower. The flower is irregular (zygomorphic) and is made up of five petals, a standard or vexillum, two wing petals, and two petals fused together to form a keel-like structure (Fig. 1a) that encloses the anthers and stigma. Although the structure is most suited for self pollination, in pigeonpea a certain amount of cross pollination does occur with insect visitations (Saxena et. al. 1990).

The natural outcrossing was in the past considered a negative trait due to its role in the contamination of cultivar purity. However of late a lot of importance is being given to this trait for its potential role in hybrid pigeonpea research and the development of cytoplasmic male sterile systems (CMS) (Tikka et al. 1997; Saxena and Kumar 2003; Mallikarjuna and Saxena 2005). In all the CMS systems, cross pollination is essential for seed set.

Cajanus platycarpus is a wild species placed in the tertiary gene pool of pigeonpea. ICRISAT has made progress in successfully crossing *C. platycarpus* with cultivated pigeonpea (Mallikarjuna 2003). In the segregating population from the cross *Cajanus platycarpus* × *C. cajan* ICPL 85010, significant variation in flower morphology was observed in F_1BC_3 progeny. Some of the flowers were found to be abnormally completely open (Fig. 1b). Such chasmogamous flowers (Lord 1981) encourage cross pollination as the pollinating agents have free access to pollen grains in the anthers and the stigma. The percentage of abnormal flowers on each plant ranged from 5 to 86%. In these open flowers, the stamens were separate (Fig. 1b & d) instead of forming a di-adelphous bundle as usually seen in pigeonpea (Fig. 1c). The filaments of each anther were separate from each other, giving a rubiaceous flower structure. The anthers in these open flowers did not dehisce even at anthesis (Fig. 1e). Hence the pollen grains remained enclosed in the anther sacs, not available for pollination/fertilization, and for all practical purposes was similar to a male sterile trait. Anther morphology in the F_1BC_3 plants was abnormal too and anthers were not placed close to the stigma as seen in cv ICPL 85010. Nondehiscent anthers and their placement away from the stigma are traits favoring cross pollination. Pollen fertility in the anthers was assessed based on acetocarmine pollen stainability studies. Pollen grains were stained in 2% acetocarmine, a DNA specific stain, and pollen grains which picked up a bright stain were counted as fertile grains. In pigeonpea, pollen stainability is a good indication of pollen fertility (Mallikarjuna, unpublished). In this study, pollen fertility ranged from 26 to 77% but in spite of high pollen fertility, none of the plants set seeds due to self pollination. Tripping the flowers did not release the pollen grains from the anthers, which meant that the anther walls were tough, unlike anthers in cultivated pigeonpea.

Forced self pollination did not set seeds in these hybrids, but seeds were obtained when pollinated with cultivated pigeonpea ICPL 85010. This showed that that there is no female sterility in these plants, but some sort of self incompatibility mechanism seemed to be operational. Open flowers coupled with self incompatibility are desirable traits for hybrid pigeonpea breeding.

In the interspecific cross *Cajanus cajan* T-21 × *C scarabaeoides*, some of the BC_1F_2 plants showed free stamens that were all sterile, although the anther appeared normal. Histological observation revealed early degeneration of pollen mother cells (Reddy and Faris 1981). In the present study, anthers were fertile but without the dehiscence of the anther wall, hence pollen was not released from the anthers.

Further experimentation is necessary to determine if the open flower mutants of pigeonpea can be effectively utilized for the development of exclusively cross pollinating pigeonpea, and thus for use in the hybrid breeding program, where self pollination is an undesirable feature.

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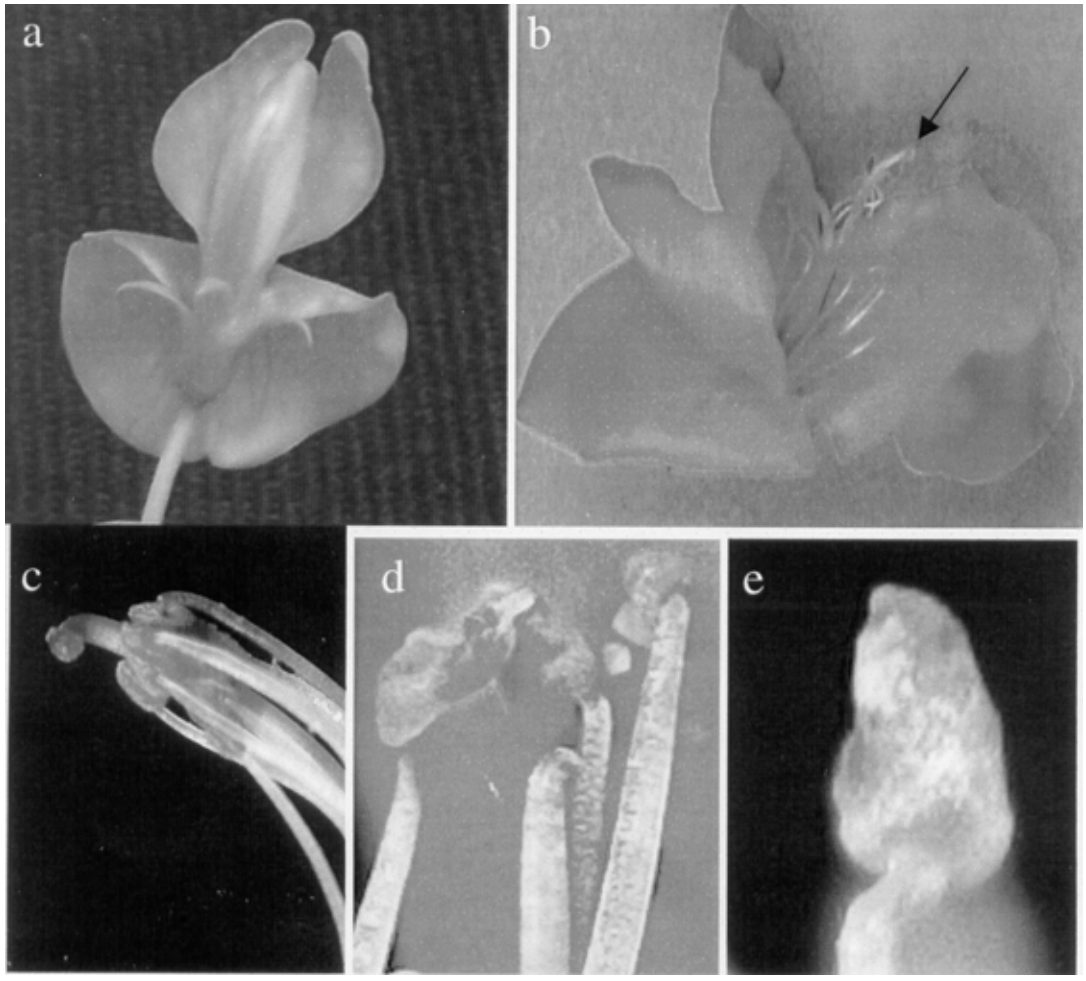

Figure 1. Open flower segregants from the cross *Cajanus platycarpus* × *C. cajan.*
a. Normal pigeonpea flower of pigeonpea cv ICPL 85010
b. Open flower (chasmogamous) from the cross *C. platycarpus* × *C. cajan*. Arrow points at the stigma.
c. Normal anthers of pigeonpea cv ICPL 85010.
d. Anthers from the cross *C. platycarpus* × *C. cajan* with abnormal morphology.
e. A close up of a nondehiscent anther from the cross *Cajanus platycarpus* × *C. cajan*.

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ICP 13828 – A Pigeonpea Germplasm Accession with 10-seeded Pods

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Pigeonpea [*Cajanus cajan* (L.) Millsp.] is an important source of protein for vegetarians in many countries in the semi-arid tropics. The pigeonpea germplasm assembled at ICRISAT, Patancheru is a rich source of diversity for several morpho-agronomic traits (Upadhyaya et al. 2005). In addition to many other traits, seed number per pod is also an important yield component in pigeonpea. Most cultivated pigeonpeas have 3–4 seeds per pod. However, there are several accessions with more seeds per pod (ranging from 5 to 7) in the world collection of pigeonpea germplasm maintained in the genebank at ICRISAT. A few accessions with long pods having as many as 8–9 seeds were also recorded while characterizing/evaluating the pigeonpea germplasm collection at Patancheru (Remanandan et al. 1988). These originate from diverse geographical areas and differ in other morphological traits: ICPs 8503 and 8504 (Origin: Guadeloupe, a French colony in Central America), ICP 12176 (Origin: Malawi), ICPs 13253 and 13256 (Origin: Kenya), ICPs 13555, 13828 and 13831 (Origin: Grenada) and ICPs 13961 and 13962 (Origin: Dominican Republic). Among these, ICP 8504 is an accession widely used in breeding programs for incorporating higher seed number per pod. However, for the first time we were able to locate pods with 10 well-developed seeds in the germplasm accession ICP 13828 (Fig. 1), though only three pods with 10 seeds were found from different plants grown on a 9-m row. ICP 13828 is a field collection from St. Patrick's in Grenada during an ICRISAT-initiated germplasm expedition in 1985. This accession was characterized for different morpho-agronomic traits during the 1986–87 rainy season at Patancheru. ICP 13828 is semi-spreading with indeterminate flowering habit, with 129 days to 50% flowering and 174 days to maturity. Plants grew about 130 cm tall and on average produced 50 pods. Pods were long and flat with mixed (green and purple) pod color. On an average 5.7 seeds per pod were produced. The seeds were cream colored and medium-sized (12.1 g 100 seeds^{-1}) with a seed protein content of 20.7 percent.

The number of seeds per pod is considered an important yield component (ICRISAT 1975). In regions where pigeonpea is used as a green vegetable, there is a strong consumer preference for cultivars with many seeds per pod, and the pigeonpea germplasm accession ICP 13828 could be a potential source for improving/developing cultivars for meeting such demands.

Figure 1. ICP 13828 pod with 10 seeds (left) and seeds (right).

Apart from the cultivated pigeonpea (*Cajanus cajan*, 2–9 ovules with 2–9 seeds), *Cajanus aromaticus* (8–10 seeds), *Cajanus goensis* (5–9 ovules with 5–8 seeds) and *Cajanus mollis* (8 or more ovules with 8–10 seeds) are other sources for higher number of seeds per pod (van der Maesen 1986) in the *Cajanus* genepool.

These accessions will be purified and the penetrance and expressivity of this trait studied further. Small seed samples of these accessions are available from the genebank for research use.

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Evaluation of Pollination Control Methods for Pigeonpea (*Cajanus cajan* (L.) Millsp.) Germplasm Regeneration

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Maintaining the genetic integrity of germplasm accessions during regeneration is of paramount importance in *ex situ* conservation of plant genetic resources. In pigeonpea (*Cajanus cajan* (L.) Millsp.) where outcrossing by insects ranges from 3 to 26% (Reddy et al. 2004), regeneration is costly in terms of time and resources (Remanandan et al. 1988). The problems are compounded when several hundred germplasm accessions need to be regenerated in a season. Nestor and Ramanatha Rao (1998), analyzing the information on seed germplasm regeneration, noted much conjecture and uncertainty over regeneration procedures employed by genebanks. Therefore, the development of optimal procedures for regeneration, to preclude contamination of pollination, is vital to maintain genetic integrity of pigeonpea accessions. The RS Paroda Genebank at ICRISAT conserves 13,632 accessions of pigeonpea from 74 countries, including landraces, breeding lines, cultivars and wild relatives. Bagging individual plants/branches of pigeonpea with muslin cloth bags to control outcrossing was used for the past several years while regenerating the germplasm accessions at ICRISAT and elsewhere. The disadvantages of this method include mainly the high cost of muslin bags, time and labor required for bagging and its removal, and difficulty in bagging all plants when the number of accessions to be regenerated is high, particularly when these accessions belong to the same maturity group. In addition, inadequate plant protection and high humidity and temperature within the bag result in high flower drop and low seed yield.

In view of the above limitations of the bagging method, a new method of growing accessions under net cages was developed at ICRISAT, Patancheru. In the present study, the two methods were compared for cost benefits and the performance of the crop for important agronomic traits, including seed yield.

To evaluate the two pollination control methods, six accessions of pigeonpea germplasm (ICP 28, ICP 6907, ICP 7057, ICP 8863, ICP 8865 and ICP 11289) belonging to different maturity groups and flowering patterns were sown during the rainy season 2003/04. The experiment was conducted at ICRISAT research farm, Patancheru, India, laid out in split plot design with method of pollination control as main plot and genotype as subplot with two replications. To reduce the vegetative growth and facilitate easy bagging of plants and avoid damage to the net under cage method, the crop was sown late, during the 1st week of August in both years in Alfisols (Remanandan et al. 1988). Each accession was grown on a nine-meter long ridge, spaced 75 cm apart. Plant to plant spacing was 25 cm, accommodating about 72 plants in 36 hills per accession. Crop was fertilized with 20 kg N and 40 kg P_2O_5 ha^{-1} as basal dose. The experiment was provided with life-saving irrigations and protected from pests and diseases adequately before bagging in the bagging method and throughout the crop growth period under cages.

In the bagging method, two plants of the same hill were covered with a muslin cloth bag of size 100×75 cm, after bud initiation but prior to flowering in any accession and the bag was closed tightly at the base of the plants to prevent the entry of insects. About 36 bags were used to cover 72 plants of an accession (Fig. 1). As a precautionary measure against insects, plants were sprayed with appropriate insecticide just before bagging.

The other method of pollination control used cages made of prefabricated iron frames of $3 \text{ m} \times 3$ m size and polypropylene net. Iron frames were fabricated such that

Table 1. Cost (US$) of pollination control methods in pigeonpea.

Items	Bagging	Cages
Cost of pollination control materials per year(muslin cloth bags, iron frames and net)	5625	1656
Labor (for bagging and bag removal, construction and dismantle of cages)	803	436
Plant protection	14	41
Total cost for 550 accessions	6442	2133
Cost for one accession	11.71	3.88
Cost for one accession in perpetuity	26.33	8.72
Cost for 13,632 accessions in perpetuity	359 193.86	119 018.18

they can be conveniently erected and dismantled. The iron frames can be used for 15 seasons or more and the polypropylene net can be used for 5-6 seasons. After bud initiation but prior to flowering in any accession, frames were fixed in the field and several such frames joined together to cover about 0.5 ha accommodating 550 accessions. These frames were covered with eight polypropylene net pieces measuring 25 × 25 m each stitched together. The cages were sealed all around with soil at the ground level to prevent the entry of pollinating agents and other insects as shown in Figure 2. Adequate plant protection measures were taken inside the cage.

At maturity, dry pods from all plants of an accession were harvested, bulk threshed and processed for conservation. Costs common to both methods of regeneration were not included in estimating the costs of individual pollination control methods.

To study the agronomic performance of accessions grown under two pollination control methods, observations on 10 important agronomic traits (days to 50% flowering, plant height, number of primary and secondary branches, days to 75% maturity, seeds per pod, 100-seed weight, seed yield plant^{-1}, harvest index (%) and plot seed yield (kg ha^{-1}) were recorded in accordance with the 'Descriptors for Pigeonpea' (IBPGR and ICRISAT 1993). Data were analyzed using GENSTAT 6.1. The cost of pollination control per accession in perpetuity with a regeneration frequency of 15 years was estimated using the following formula of Koo et al. (2002).

The in-perpetuity cost of an operation that is performed every nth year from zero with a cost of X is given by

$$C_0^n = X + \frac{X}{(1+r)^n} + \frac{X}{(1+r)^{2n}} + \ldots. = X \left[1 + \frac{X}{(1+r)^n} + \frac{X}{(1+r)^{2n}} + \ldots. \right] = \frac{X}{(1-a)^n}$$

Where, C= Cost of pollination control per accession in perpetuity, n = frequency of regeneration, a = 1/1+r, r = rate of interest and X= cost of one cycle of regeneration per accession.

The cost estimates revealed that pollination control using cages was 3 times less expensive than the bagging method. The estimated cost saving per accession was US$ 7.83. With a 15-year regeneration interval, the cost of pollination control per accession would be US$ 26.33 for the bagging method and US$ 8.72 for the cage method when real rate of interest is 4%. The estimated net saving in perpetuity over the entire collection of 13,632 accessions by switching to cage method would be US$ 2,40,176 (Table 1). The net savings will increase with the increase in number of accessions in the genebank. The difference in initial investment on purchase of bags (US$ 11,250) and cages (US$ 12,435) for 550 accessions is not much (US$ 1185). In addition, we need to purchase bags every alternate year.

Analysis of variance over ten agronomic characters showed significant differences ($p < 0.0001$) between the methods for plant height, number of primary branches, days to 75% maturity, 100-seed weight and highly significant differences for seed yield. All accessions except ICP 28, a short-duration and short-height accession with determinate flowering pattern, performed well under cages and yielded significantly high yields. Optimum seed yield in accessions like ICP 28 can be achieved by growing them as separate groups. This grouping will reduce the problem of shade due to tall, spreading, indeterminate and late-maturing accessions grown in adjacent rows. Grouping also facilitates adequate plant protection.

Relatively higher temperature and humidity inside the muslin cloth bag resulted in increased flower drop and reduced seed yield. It is also more likely that the microclimate within the bag may facilitate the growth of seedborne fungi, thus affecting the seed quality. Krishnasamy (1990) reported that growing eggplant crop in net cages results in the exclusion of insects that damage the crop. In addition, in the bagging method, covering all branches of two plants with a muslin cloth bag may not be possible and the seed from open pollinated branches cannot be used for conservation. It is clear from the results of the present study that we can regenerate large number of accessions at a time safely and cost-effectively under cages, even when many accessions to be regenerated belong to same maturity group. Increased seed yield under cage method minimizes the regeneration frequency of accessions, thereby reducing the maintenance costs of total collection in perpetuity.

Figure 1. Field view of pigeonpea germplasm accessions covered with muslin cloth bags to prevent outcrossing.

Figure 2. Pigeonpea germplasm accessions grown under pollination control cages to prevent outcrossing.

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Agronomy/Physiology

Effect of Carrier-Based and Liquid Inoculants on the Nodulation and Grain Yield of Pigeonpea

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The rhizosphere is characterized by greater microbial activity than the soil away from plant roots. The intensity of such activity depends on the distance to which exudations from the root system can migrate. The contribution of carrier-based *Rhizobium* inoculation in increasing crop productivity of legumes is well recognized. Liquid biofertilizer holds great promise and benefits over carrier-based inoculant in terms of saving carrier material, transport, pulverization, sterilization, convenience in handling, storage and transportation (Hegde 2002). Carrier-based inoculants cost more, whereas liquid inoculants involve lower costs and no chance of contamination (Gupta 2005). No information is available on the response of pigeonpea [*Cajanus cajan* (L.) cv. Bahar] grown in calcareous soils of Bihar to liquid inoculants. This investigation was carried out to evaluate and compare the response of pigeonpea to liquid *Rhizobium* inoculants and carrier-based inoculant.

A field experiment was conducted at Rajendra Agricultural University, Dholi Campus farm, Muzaffarpur (Bihar) during kharif 2002–03. The characteristics of the experimental soil were organic carbon 3.9 g kg^{-1}, available N 164 kg ha^{-1}, P_2O_5 17 kg ha^{-1}, and K_2O 87 kg ha^{-1}. The experiment was conducted in randomized block design with four replications. One each of carrier-based (CC-1) inoculant 10^8 rhizobia g^{-1} carrier and liquid-based inoculant (CC-1) (10^9 rhizobia ml^{-1}) broth obtained from TNAU, Coimbatore and one liquid inoculant (DHA-19) (10^9 rhizobia ml^{-1}) broth from Dholi were tested under field conditions to evaluate their relative efficiency. Seeds of pigeonpea were inoculated each with carrier-based inoculant (5 g kg^{-1}) and liquid inoculants (3 ml kg^{-1}) The viable counts of rhizobia on seeds after inoculation were recorded [carrier-based inoculant CC-1, 10^5 $seed^{-1}$ to 10^6 $seed^{-1}$, liquid-based inoculants CC-1 & DHA-19, 10^6 $seed^{-1}$ to 10^7 $seed^{-1}$]. The inoculated seeds were dried in the shade for 30 min before sowing. Nodulated plants of pigeonpea were uprooted from the soil 45 days after sowing for counting number of nodules $plant^{-1}$ and their

Table 1. Effect of inoculation of pigeonpea cv. Bahar with liquid and carrier-based *Rhizobium* on nodulation, dry matter yield and grain yield of pigeonpea.

Treatment	No. of nodules plant^{-1}	Dry wt. of nodules (mg plant^{-1})	Dry matter yield of plants (q ha^{-1})	Grain yield (q ha^{-1})	Percent yield increase over control
Uninoculated control (UIC)	56.6	31.0	55.6	10.0	–
Carrier-based inoculant of CC-1	68.0	35.0	68.3	12.0	20
Liquid inoculant of CC-1	71.0	34.4	61.6	12.0	20
Liquid inoculant (DHA-19)	75.0	36.0	63.6	11.7	17
SEm±	5.93	1.73	1.62	0.55	
CD (P = 0.05)	NS	NS	5.0	1.7	–
CV (%)	12.8	14.6	8.7	8.2	–

dry weight. At maturity, dry matter yield of plants and grain yield were recorded.

The highest number of nodules plant^{-1} was recorded in liquid inoculant treatments DHA-19 followed by that of CC-1. Carrier-based inoculant CC-1 recorded lowest number of nodules plant^{-1} as compared to the liquid inoculant. Dry weight of nodules recorded under different treatments were at par with each other; however, liquid inoculant (DHA-19) Dholi were numerically (36 mg plant^{-1}) higher than liquid inoculant CC-1. Carrier-based inoculant produced highest dry matter yield (68.3 q ha^{-1}) which was at par with liquid inoculant (DHA-19) and significantly superior over uninoculated control. This may be attributed to better compatibility and efficiency of inoculated rhizobia compared to the native rhizobia in forming effective nodules in the root system (Gupta 2005).

Grain yield is an important criterion of measuring the efficiency of a strain in the field. Carrier- and liquid-based inoculants of CC-1 recorded highest grain yield (12.0 q ha^{-1}) and were at par with liquid inoculant of DHA-19 (11.7 q ha^{-1}). Liquid inoculants of CC-1 and DHA-19 were found equally effective as carrier-based inoculants with respect to grain yield in pigeonpea. Increase in grain yield as against uninoculated control might be attributed to better nodulation, nitrogen fixation, and growth of pigeonpea due to effective *Rhizobium* inoculants. Similar results have been observed by Gupta (2005) in chickpea under field conditions.

The present study indicates that liquid inoculant of *Rhizobium* may be utilized for seed inoculation of pigeonpea to enhance biological N_2 fixation and grain yield.

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Pathology

Outbreak of Phytophthora Blight of Pigeonpea in the Deccan Plateau of India, 2005

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Andhra Pradesh, Karnataka and Maharashtra are the major pigeonpea-growing states in the Deccan Plateau (DP) of India. The area under pigeonpea in Andhra Pradesh is estimated to be around 0.42 million ha with a production of about 0.19 million tonnes, while in Karnataka it is grown on 0.49 million ha with a production of 0.26 million tonnes (Dharamraj et al. 2004). Of these three states, Maharashtra has the maximum area (1.02 million ha) with a production of about 0.77 million tonnes (http//:agricoop.nic.in/). Diseases such as wilt (*Fusarium udum* Butler) and sterility mosaic (SM Virus) are the important biotic factors limiting its production in the DP.

Phytophthora blight (PB) caused by *Phytophthora drechsleri* Tucker f. sp. *cajani* (Pal et al.) Kannaiyan et al. has been reported infrequently as a minor disease from DP. However, it is an important production constraint in Northeastern India particularly in low lying, poorly drained fields (Kannaiyan et al. 1984; Mishra and Shukla 1987 and Chauhan et al. 2002). Cloudy weather accompanied by intermittent rains followed by mean temperatures 25±1°C favours PB infection and development. In the DP, pigeonpea is sown during June-July, a period that coincides with the onset of monsoon, when wet weather prevails.

In 2005 rainy season in the months of July–August when the pigeonpea crop was 30–45 days old, exceptionally heavy rains (about 460 mm) were experienced at ICRISAT, Patancheru, Andhra Pradesh, India. These rains were also widespread in the DP, especially in the states of Maharashtra and Karnataka. In our regular monitoring of pigeonpea fields at ICRISAT farm we noticed widespread incidence of PB. Hence, a structured survey of pigeonpea fields was initiated to assess the incidence of PB at ICRISAT farm during this season. A total of 15 pigeonpea fields (7 Alfisol and 8 Vertisol fields) were surveyed and in each field, based on the availability, 2 to 35 entries were observed for PB incidence. Mean disease incidence was upto 33.9% among genotypes grown in Alfisols and upto 26.7% in the genotypes grown in Vertisol fields (Table 1).

Concurrent reports of a disease similar to PB were also received from farmers' fields in the neighboring states of Karnataka and Maharashtra. This gave the impetus to conduct a structured survey of pigeonpea-growing areas in these states of DP. The main objective of the survey was to quantify the incidence of PB in the DP. Additionally, attempts were made to collect the information on the incidence of PB in pigeonpea grown in different soil types and cropping systems. In collaboration and consultation with scientists from National Agricultural Research System (NARS), a proforma was developed to collect information on disease incidence, cropping systems, cultivars, agronomical practices and field history. The survey was conducted in August 2005. Scheduled and unscheduled stops were made after every 10–15 km. Three (1 × 1 m) quadrates were randomly selected in each field and, based on total number of plants and plants showing PB symptoms, disease incidence in the sampled field was calculated. Disease incidence of individual fields was used to calculate the PB incidence of each district and the state. Results thus obtained in surveyed states are summarized as follows:

Andhra Pradesh

Twenty nine villages in 16 talukas under four districts (Rangareddy, Mehboobnagar, Nizamabad and Medak) were surveyed. The crop was 45–60 days old at the time of survey. A range of pigeonpea cultivars, Asha (ICPL 87119), Maruti (ICP 8863), LRG 30, and Local were

Table 1. Phytophthora blight incidence (%) in Alfisol and Vertisols at ICRISAT farm, Patancheru, India, 2005.

Soils type	No. of fields surveyed	Disease incidence (%)	
		Range	Mean
Alfisols	7	16–59.4	33.9
Vertisols	8	13–46.8	26.7

Table 2. Phytophthora blight incidence (%) in Alfisol and Vertisols in major pigeonpea-growing areas of Andhra Pradesh, Karnataka and Maharashtra in Deccan Plateau, India, 2005.

	Disease incidence (%)			
	Alfisols		Vertisols	
States	Range	Mean	Range	Mean
Andhra Pradesh[1]	5.3–22.8	14.1	10.1–21.3	14.0
Karnataka[2]	12.6–50.3	31.5	10.6–11.4	11.0
Maharashtra[3]	8.1–25.7	18.3	13.2–30.7	19.2

1. Based on four districts (Rangareddy, Mehboobnagar, Nizamabad and Medak), sixteen mandals and 29 villages. The major soil type was Vertisols.
2. Based on two districts (Gulbarga and Bidar), 10 talukas and 60 villages. The major soil type was Vertisols.
3. Based on six districts (Osmanabad, Latur, Bead, Parbhani, Hingoli and Nanded), 26 talukas and 101 villages. The major soil type was Vertisols.

found grown in surveyed villages. All the surveyed fields (Alfisol and Vertisol) were well drained without any water stagnation. Pigeonpea was grown in a range of cropping systems, from sole crop to intercropped; however, the predominant cropping system was pigeonpea intercropped with sorghum/maize. Substantial differences were not found in PB incidence with respect to soil types (Table 2). However, higher disease incidence (16.4%) was recorded in intercropping system in comparison to sole crop (10.0%). No visible difference in the mean incidence of PB was recorded among improved (14.9%) and local (13.8%) varieties grown by the farmers.

Karnataka

In all, 60 villages in 10 taluks under two districts (Gulbarga and Bidar) were surveyed. The crop was 30–60 days old at the time of survey. Maruti (ICP 8863), Gulyal Local, Benur Local, Guttali, Black Tur and Local were the common pigeonpea cultivars grown in surveyed villages. Most of the Alfisol fields were low lying with water stagnation for long periods. The predominant cropping system was sole crop or intercropped with sorghum/pearl millet. Substantial differences were not found in PB incidence with respect to cropping system and varieties grown. Mean disease incidence in the intercrop (15.6%) was at par with sole (16.0%) cropping system. Similarly, no differences were recorded among improved (12.3%) and local (11.9%) varieties grown by the farmers. However, substantial differences were recorded in PB incidence with respect to soil types (Table 2). Disease incidence was high (31.5%) in Alfisols as compared to Vertisols (11.0%).

Maharashtra

One hundred and one villages in 26 talukas under six districts (Osmanabad, Latur, Bead, Parbhani, Hingoli and Nanded) were surveyed. The crop was 45–60 days old at the time of survey. A range of pigeonpea cultivars, Maruti (ICP 8863), BSMR 736, BSMR 853, BDN 1, BDN 2, BDN 7, Gulyal Local, Black Tur, Parbhani White, Kishan, Payola, Pandri Tur and Local were found grown in surveyed villages. All the surveyed fields (Alfisol and Vertisol) were well drained without any water stagnation. The predominant cropping system in the surveyed districts was pigeonpea intercropped with soybean/cotton. Substantial differences were not found in PB incidence with respect to soil types, cropping system and varieties grown. Mean disease incidence among Alfisols (18.3%) was slightly lower than in Vertisols (19.2%) (Table 2). Similarly among cropping systems, disease incidence was slightly less in intercrop (19.8%) than sole (21.4%) crops. No difference in disease incidence was recorded in improved (21.7%) and local (21.1%) varieties grown by farmers. However, widespread incidence of PB was recorded in all the districts surveyed irrespective of soil type, cropping system and varieties grown.

High incidence of PB in Alfisol fields in the state of Karnataka may be due to topography and low lying nature of surveyed fields. Moreover, the drainage system was very poor in these fields, resulting in water stagnation due to heavy rains in August. These field conditions were optimal for the development and rapid spread of the fungus. Low incidence of PB in both Alfisol and Vertisol fields in Andhra Pradesh and Maharashtra

was attributed to the higher elevation and proper drainage system of the surveyed fields.

The survey of 190 farmers' fields in the three states revealed that PB was widespread irrespective of soil types, cropping systems and genotypes. Its incidence was higher in the low-lying fields than well drained fields. High incidence of PB in individual fields could be due to low level of field topography and poor soil surface drainage which favored the multiplication and spread of inoculum of *P. drechsleri* (Singh & Chauhan 1985).

Widespread resurgence of PB in DP in the current season is a matter of serious concern. The heavy unpredictable rains during July and August rendered the crop vulnerable to PB attack. However, it is still not clear how and where the PB pathogen *P. drechsleri* survives and causes epidemics in pigeonpea in the DP. Also our survey indicates that the pigeonpea cultivars grown by farmers do not have adequate levels of resistance to PB, at least in the three states surveyed in DP. Differential sowings and differential growth duration varieties were also in cultivation. A detailed analysis of the factors responsible for the widespread incidence of PB is, however, necessary.

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Resistance to Phytophthora Blight in the Improved Pigeonpea Lines at ICRISAT, Patancheru, India

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Phytophthora blight (PB) (*Phytophthora drechsleri* Tucker f. sp. *cajani*, Kannaiyan et al.) of pigeonpea (*Cajanus cajan* (L.) Millsp.) is a disease of endemic importance. Continuous rains and waterlogging in the seedling stage of the crop favour PB epidemics, resulting in up to 100% crop loss. Characteristic symptoms of the disease are water-soaked lesions on the leaves and slightly sunken lesions on stems and petioles. Lesions girdle the stem and the foliage dries up. The disease was first reported in 1968 at the research farm of the Indian Agricultural Research Institute (IARI) by Williams et al. (1968). Later Kannaiyan et al. (1984) reported its widespread occurrence in several parts of India.

During the 2005 rainy season, unusual and well-distributed rains (about 460 mm in 31 days) were experienced throughout the Deccan Plateau (DP). Periodical monitoring of the pigeonpea crop at the research farm of the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru during July-August indicated widespread prevalence of PB. This prompted us to conduct a structured survey of pigeonpea fields at the ICRISAT farm, with the specific objectives of quantifying the incidence of PB on improved and wild pigeonpea lines, and identifying lines with multiple resistance to PB, wilt (*Fusarium udum* Butler) and sterility mosaic (SM; pigeonpea sterility mosaic virus).

The survey was conducted between the fourth week of July and fourth week of August, 2005 when the crop was in active vegetative growth stage (30–45 days old). A total of 15 fields were surveyed, of which seven (RM 3B-1, RM 3B-2, RP 7, RP 17, RL 33, RL 17 and RCW 18B) were Alfisol and eight (BR 1A, BP 14A, BP 14B, BP 14C, BP5, BP1 and BM 15E) Vertisol. Additionally, a Vertisol field BIL 7B is a wilt and sterility mosaic screening nursery. A total of 33 lines in wilt and SM sick plot and 89 lines including wild *Cajanus* spp. were observed for PB incidence and severity. In each line three (1×1m) quadrates were randomly selected and infected plants were counted in each.

The percentage of PB incidence was calculated based on infected and total number of plants (Chauhan et al. 2002). Based on disease incidence levels the lines were categorized as resistant (≤10% incidence), moderately resistant (10.1–20.0%), moderately susceptible (20.1-40.0%), and susceptible (40.1–100%).

Varying levels of disease incidence were recorded among the improved lines. Of the 122 lines observed (33 lines in wilt and SM sick plot and 89 lines including wild *Cajanus* spp. in other fields), 33 were resistant and 61 moderately resistant, 21 moderately susceptible and 7 susceptible to PB. Of the three wild *Cajanus* species, *Cajanus sericeus* was found resistant, *C. scarabaeoides* moderately resistant and *C. cajanifolius* susceptible to PB (Table 1).

All the 33 lines observed in BIL 7B (wilt and SM sick plot) were resistant to PB and SM and only 28 of these were resistant to wilt (Table 2). Wilt susceptible check, ICP 2376 and SM susceptible check ICP 8863 also showed resistance to PB. However, these improved multiple disease resistant lines require some more testing across seasons and locations to confirm their resistance to PB, wilt and SM. There is also a need to vigorously screen wild *Cajanus* species to identify resistance sources against these diseases for strengthening the pigeonpea breeding program.

Table 1. Phytophthora blight (PB) incidence of selected pigeonpea lines at ICRISAT farm, Patancheru, India, during 2005 rainy season.

Genotypes	Number of entries	PB incidence (%)	Disease reaction[1]
AWR 74/16, Azad, Bandapaleru, *C. sericeus*, HPL 24-47, ICP 11376-5, ICP 11975, ICP 12730, ICP 12751, ICP 12755, ICPL 20093, ICPL 20096, ICPL 20099, ICPL 20100, ICPL 20101, ICPL 20104, ICPL 20105, ICPL 20109, ICPL 20114, ICPL 20115, ICPL 20122, ICPL 20124, ICPL 20125, ICPL 20126, ICPL 20127, ICPL 20128, ICPL 20135, ICPL 20136, ICPL 93179, ICPL 99044, KPBR 80-2-1, KPBR 80-2-2-1, KPL 96053	33	≤10.0	Resistant
BDN 2010, BSMR 846, *C. scarabaeoides*, DA 11, ICP 11174, ICP 12749, ICP 12759, ICP 14819, ICP 5357, ICP 6919, ICP 7870, ICP 8863, ICP 9174, ICP 9879, ICPH 2308, ICPH 2899, ICPL 20092, ICPL 20094, ICPL 20097, ICPL 20098, ICPL 20102, ICPL 20103, ICPL 20106, ICPL 20110, ICPL 20113, ICPL 20116, ICPL 20119, ICPL 20120, ICPL 20129, ICPL 20131, ICPL 20132, ICPL 20134, ICPL 20137, ICPL 20138, ICPL 87091, ICPL 87119 (Asha), ICPL 94062, ICPL 94068, ICPL 96053, ICPL 96058, ICPL 96061, IIPR lines (2032, 2033, 2035), IPA 40, JJ 65, JK cms 2A, JKPH 6101, KPBR 80-2-4, KPL 44, MAL 13, MAL 15, MAL 23, MAL 3, MA-S-DEO-74, PR 5149, PT 1037, TK 040174, V 102, V 71A, V 71B	61	10.1–20.0	Moderately resistant
ICP 12746, ICP 12942, ICP 13799, ICP 13828, ICP 6903, ICP 7035 (Kamica), ICP 8102, ICP 8610, ICP 9150, ICP 9576, ICPH 2363, ICPH 2364, ICPH 2671, ICPH 2898, ICPL 20107, ICPL 20123, ICPL 20130, ICPL 88034, ICPL 88039, MAL 20, UPAS 120	21	20.1–40.0	Moderately susceptible
C. cajanifolius, ICP 80194, ICPA 2039, ICPA 2052, ICPA 2068, ICPL 332, ICPL 85023 (Lakshmi)	7	>40.0	Susceptible

1. [Resistant (≤10.0), Moderately resistant (10.1–20.0%), Moderately susceptible (20.1–40.0%), and Susceptible (40.1–100%)].

Table 2. Reaction of pigeonpea genotypes to Phytophthora blight (PB), wilt and sterility mosaic (SM) at ICRISAT, Patancheru, India, 2005–06.

SL No.	Genotypes	Source	PB incidence (%)	Wilt incidence (%)	SM incidence (%)
1.	ICPL 20100	ICRISAT	4.0	14.7	0.0
2.	ICPL 20135	ICRISAT	4.7	2.7	0.0
3.	ICPL 20125	ICRISAT	5.2	0.0	1.3
4.	ICPL 20115	ICRISAT	5.8	0.0	1.4
5.	KPBR 80-2-2-1	IIPR, India	5.9	2.6	0.0
6.	*C. sericeus*	ICRISAT	6.0	1.3	0.0
7.	ICP 11975	ICRISAT	6.0	2.6	0.0
8.	ICPL 20124	ICRISAT	6.0	12.6	0.0
9.	ICPL 20096	ICRISAT	6.6	0.0	1.7
10.	AWR 74/16	IIPR, India	6.7	5.3	0.0
11.	ICP 12730	ICRISAT	6.7	22.6	0.0
12.	KPBR 80-2-1	IIPR, India	6.7	5.9	0.0
13.	KPL 96053	IIPR, India	6.7	0.0	0.0
14.	ICPL 20127	ICRISAT	6.8	2.1	4.6
15.	ICPL 20104	ICRISAT	7.2	9.8	0.0
16.	ICPL 20099	ICRISAT	7.4	0.0	0.0
17.	Azad	IIPR, India	7.5	55.5	0.0
18.	ICPL 20105	ICRISAT	7.6	2.1	0.0
19.	HPL 24-47	IIPR, India	7.7	20.4	9.2
20.	ICPL 20122	ICRISAT	8.1	4.9	6.7
21.	Bandapaleru	IIPR, India	8.4	1.4	0.0
22.	ICPL 20126	ICRISAT	8.4	5.9	7.0
23.	ICPL 20136	ICRISAT	8.8	3.1	9.3
24.	ICPL 20109	ICRISAT	8.9	5.4	5.7
25.	ICP 11376-5	ICRISAT	9.1	0.0	0.0
26.	ICP 12755	ICRISAT	9.1	0.0	0.0
27.	ICPL 20101	ICRISAT	9.1	3.6	5.4
28.	ICPL 20128	ICRISAT	9.2	4.7	1.7
29.	ICP 12751	ICRISAT	9.3	1.4	0.0
30.	ICPL 20114	ICRISAT	9.4	6.3	2.1
31.	ICPL 99044	ICRISAT	9.4	0.0	0.0
32.	ICPL 93179	ICRISAT	9.8	1.4	0.0
33.	ICPL 20093	ICRISAT	10.0	3.4	3.0
34.	ICP 2376[2]	ICRISAT	8.4	98.3	–
35.	ICP 8863[3]	ICRISAT	1.6	14.1	89.9

1. [Resistant (≤10.0), Moderately resistant (10.1–20.0%), Moderately susceptible (20.1–40.0%), and Susceptible (40.1–100%)].
2. Wilt susceptible check.
3. Sterility mosaic susceptible check.

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Preliminary Screening of Pigeonpea Genotypes for Multiple Disease and Insect Resistance

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Systematic efforts to utilize natural genetic variability existing in pigeonpea has been realized a long time ago as a wide range of variability exists in pigeonpea germplasm for resistance to diseases and pests and other important characteristics (Nene et al. 1990).

The major objective of the present study was to identify pigeonpea genotypes with combined resistance to three major diseases and two major insect pests: *Helicoverpa armigera* (Hub.) (pod borer) and *Melanagromyza obtusa* (Mall) (pod fly). Three major diseases known in pigeonpea and widespread in the Indian subcontinent are wilt (FW; *Fusarium udum* Butler), Phytophthora blight (PB; *Phytophthora drechsleri* Tucker f.sp. cajani) and sterility mosaic (SM; transmitted by an eriophyid mite, *Aceria cajani* Channabasavanna). The identified genotypes were further classified into long-duration maturity groups based on crop duration [210–211 days after sowing (DAS)].

Seventy-five accessions available from ICRISAT, Patancheru, were initially raised in randomized block design (RBD) with three replications and multiplied as observation nursery under screen house protection during 2001. Thirty-five accessions with ≥60% plant stand at maturity were further tested for disease and insect pest tolerance/resistance in the field for 2 years (2002–03 and 2003–04) providing recommended dose (100 kg ha^{-1}) of DAP. Standardized cultural practice was followed. The experiment was carried out in RBD with 3 replications. A minimum of 15–30 seeds/accessions were raised in a single row. A distance of 50 cm between rows was maintained and plant to plant spacing was 15 cm. No fungicide(s) or insecticide(s) were used. Established plants (10 days after emergence) were observed for appearance of any visible symptoms for the three major diseases at various stages of development to identify genotypes with combined resistance to the diseases in pigeonpea. Various stages of development coincided with vegetative stage (50 DAS), bud initiation (89 DAS) and pre-pod stage (156 DAS). Data was recorded based on appearance of visible symptoms (symptomatology) and type of damage caused. *Fusarium* wilt susceptibility was based on percentage mortality of plants following progression of disease including yellowing, drooping, drying of leaves and finally death of the whole plant. No visible symptoms of PB in the form of lesions were observed on leaves and stems of any of genotypes of cultivated species of pigeonpea. Plants with mild mosaic and stunted growth with no or little flowering were recorded as susceptible to SM (Nene et al. 1981; Reddy et al. 1993). Percent disease incidence (PDI) of FW and SM was calculated in 156 DAS old plants. Genotype susceptibility to any disease or insect pest was recorded on a 1–9 point susceptibility scale as per IBPGR and ICRISAT descriptors (1993) and expressed as five categories: 0-10% as resistant; 11–30% as moderately resistant; 31–50% as tolerant; 51–70% as moderately susceptible and 71-90% as susceptible genotypes (Nene et al. 1981).

Five genotypes were identified with multiple disease resistance to FW and SM (Table 1) and with high plant stand at maturity (61.1±38.9% to 92.9±7.2%) (Table 2).

Table 1. Pigeonpea germplasm accessions with multiple disease resistance and insect tolerance, obtained from ICRISAT, Patancheru.

ICP accessions	Known resistance/tolerance	Pedigree/identity	Origin
Accessions with combined resistance to two diseases			
ICP 10958	Wilt and *Phytophthora* blight	Banda Palera	India
ICP 11304	Sterility mosaic and *Alternaria* blight	IC-BR-Sel. 8132	ICRISAT
Insect tolerant accessions			
ICP 11965	Pod fly resistant/tolerant	1691	ICRISAT
ICP 13206	Pod borer resistant/tolerant	ICP 8127 E3-5EB	ICRISAT
ICP 13211	Pod fly resistant/tolerant	AGR 208 4EB	ICRISAT

Table 2. Plant stand, combined disease response and insect pest damage in five identified pigeonpea germplasm accessions at IARI, New Delhi, India (2001–04).

ICP accessions	Plant stand at maturity[2] (%)	Combined disease response to wilt and SM[1] (156 DAS)			Pod damage at harvest				Classification of genotypes
		PDI[3] (%)	Combined score[4]	Combined disease response[5]	Pod borer damage (%)	Pod fly damage (%)	Combined pod borer + pod fly damage	Combined score[4]	Combined response[5]	
ICP 10958	78.40±2.40	14.81±14.81	1–2	Very low to low	5.51±3.57	82.36±2.36	87.88±3.64	9	Very high	MDR[6], Insect pests susceptible genotype
ICP 11034	92.85±7.15	22.22±18.14	2	Low	0.00	96.66±0.26	96.66±0.26	9	Very high	MDR, Insect pests susceptible genotype
ICP 11965	61.11±38.89	8.33±0.70	<1	Very low	2.63±0.00	55.50±0.22	58.13±7.09	6	Inter-mediate to high	MDR, Insect pests tolerant /moderately susceptible genotype
ICP 13206	82.57±0.76	16.66±16.66	1–2	Very low to low	26.43±0.73	1.06±0.00	27.49±1.88	3	Low	MDR, Insect pests moderately resistant genotype
ICP 13211	81.81±18.18	8.33±8.33	<1	Very low	29.46±25.14	15.31±15.31	44.77±10.07	5	Inter-mediate	MDR, insect pests tolerant genotype

1. Sterility mosaic; 2. Data represents mean ± SD of 3 replications; 3. Percent disease incidence; 4. Rated on 1–9 scale, where 1 = resistant, 9 = susceptible; 5. As per IBPGR and ICRISAT descriptors (IBPGR and ICRISAT 1993); 6. Multiple disease resistant.

Testing of 35 accessions including the five mentioned above for insect pest (pod borer and pod fly) resistance/ tolerance under field conditions further identified ICP 13206 (Table 2) that has combined moderate resistance. ICP 13211 was also further identified as a pod borer and pod fly tolerant genotype (Table 2). Field screening for insect pest/s tolerance/resistance was based on pod damage characteristic of pod borer and pod fly (Reed and Lateef 1990).

Three genotypes, ICP 13206, ICP 13211 and ICP 11965 (Table 2), are being selfed, multiplied and maintained for further improvement in combined resistance to diseases and insect pests prior to their future use in the pigeonpea improvement programme.

However, there is also a need to test existence of any variability for desirable yield traits, growth components and acceptable quality to confirm them as multiple disease and insect pests resistant genotypes under artificial screening. Variations identified in germplasm for these traits have to be thoroughly quantified and exploited for their applied use.

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