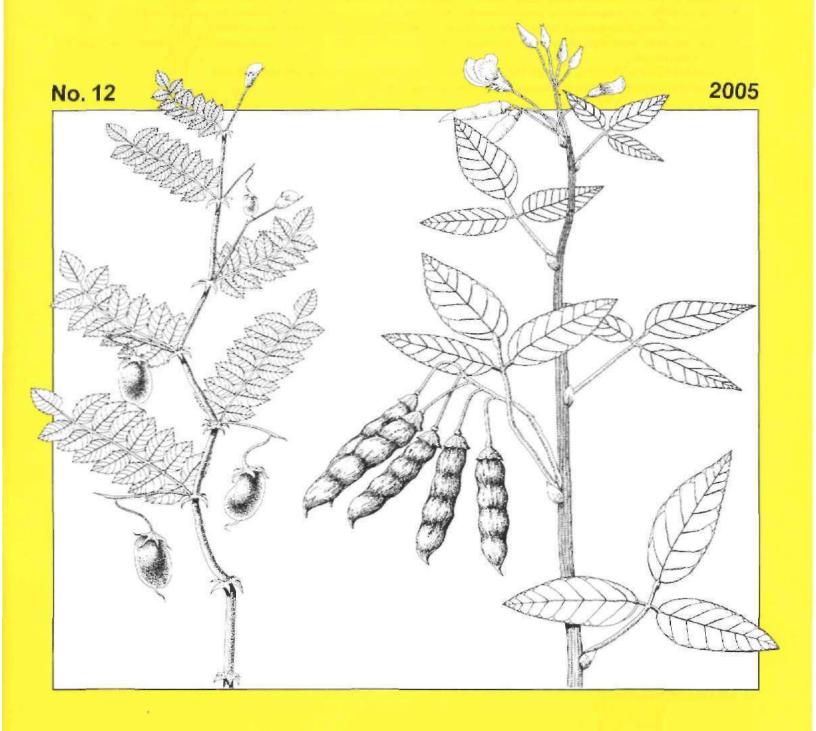
# International Chickpea and Pigeonpea Newsletter



### 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:

ICPN Editor ICRISAT Patancheru 502 324 Andhra Pradesh, India Fax +9140 30713074 Email newsletter@cgiar.org Tel +9140 30713071

#### Contents

Editorial	1
News	
CGIAR Award to ICRISAT Scientist	
Chickpea Scientists' Meet held at ICRISAT-Patancheru	
Marigold: A Diagnostic Tool for BGM Forecasting and Management in Chickpea	2
Visiting Scientists	

#### **Research Reports**

#### Chickpea

#### Genetics/Breeding/Biotechnology

Detection of Polymorphism Using CAPS and dCAPS Markers in	
Two Chickpea Genotypes PN Rajesh, Kevin McPhee and Fred J Muehlbauer	
Nutritional Constituents in Chickpea Varieties R Bhatnagar, JP Yadavendra and KV Patel	
A New Kabuli Chickpea "Vihar" for South India BM Jamadagni, LB Mhase and DV Deshmukh	8
Performance of Kabuli Chickpea Cultivar KAK 2 in Rainfed Black Soils of Prakasam District, Andhra Pradesh, India <i>T Srinivas, MC Obaiah</i> and SP <i>Moula</i>	
Identification of Chickpea Genotypes with Combined Resistance to Ascochyta Blight and Fusarium Wilt RS Waldia, BPS Malik, Hari Chand, VS Lather, IS Solanki and Ram Kumar Yadav	
Induction of Androgenesis as a Consequence of Wide Crossing in Chickpea Nalini Mallikarjuna, Deepak Jadhav, Heather Clarke, Clarice Coyne and Fred Muehlbauer	
Chefe (ICCV 92318) - A New Kabuli Chickpea Variety for Ethiopia <i>Ketema Daba, Geletu Bejiga, Yadeta Anbessa, PM Gaur, Jagdish Kumar</i> and <i>BV Rao</i>	
Leafand Pod Characters as Selection Criteria for Large-Seeded Kabuli Chickpea JS Sandhu, SK Gupta, Pritpal Singh, TS Bains and Ajinder Kaur	

## Agronomy/Physiology

Low Temperature Effects on Early Maturing Chickpea Genotype ICCV 96029 S Kumar, H Nayyar, TS Bains, G Kaur and RK Bhanwra	
Response of Chickpea ( <i>Cicer arietinum</i> ) to Irrigation, FYM and Sulphur on a Sandy Clay Loam Soil <i>RA Patel</i> and <i>RH Patel</i>	
Effect of Soaking Seeds on Polyphenols of Chickpea S Paramjyothi and B Anjali	
Increased Chickpea Yield and Economic Benefits by Improved Crop Production Technology in Rainfed Areas of Kurnool District of Andhra Pradesh, India <i>A Ramakrishna, SP Want, Ch Srinivasa Rao</i> and <i>U Srinivas Reddy</i>	
A Method for Germinating Perennial Cicer Species CJ Coyne, T Sharp-Vincent, MJ Cashman, CA Watt, W Chen, FJ Muehlbauer and Nalini Mallikarjuna	
Pathology	
Antifungal Metabolites from Arachniotus sp for the Control of Wilt Disease of Chickpea Iftikhar A Khan, S Sarwar Alam, M Sarwar, M Jahangir Iqbal and Abdul Jabbar	
Management of Root Knot Nematode and Fusarium Wilt Disease Complex by Fungal Bioagents, Neem Oilseed Cake and/or VA-Mycorrhiza on Chickpea Rajesh Kumar Pandey, BK Goswami and Satyendra Singh	
Screening of Chickpea Lines for Resistance to Ascochyta Blight SM Iqbal, A Bakhash, SR Malik and AM Haqqani	
Entomology	
First-instar Helicoverpa punctigera Larvae: Feeding Responses and Survival on Desi Chickpea and the Wild Relative Cicer bijugum SL Clement, J Ridsdill-Smith and S Cotter	
Screening of Chickpea for Resistance to Pod Borer <i>Helicoverpa armigera</i> (Hubner) at Rahuri, Maharashtra, India <i>MM Sanap</i> and <i>BM Jamadagni</i>	
Preliminary Evaluation of Chickpea Genotypes for Resistance to Pod Borer and Wilt Complex Harminder Kaur, SK Gupta, Daljeet Singh and Kuldip Singh	

#### Pigeonpea

#### Genetics/Breeding/Biotechnology

Identification of Dwarf and Extra-early Mutant of Pigeonpea [ <i>Cajanus cajan</i> (L.) Millsp.] <i>Ram Dhari</i> and <i>RS Waldia</i>	
ICP 7035 - A Sterility Mosaic Resistant Vegetable and Grain Purpose Pigeonpea Variety <i>KT Rangaswamy, V Muniyappa, P Lava Kumar, KB Saxena, M Byregowda,</i> <i>N Raghavendra, K Pandurangaiah, R Vijay Kumar, F Waliyar</i> and <i>AT Jones</i>	
Agronomy/Physiology	
Effect of Improved Crop Production Technology on Pigeonpea Yield in Resource Poor Rainfed Areas A Ramakrishna, SP Wani, Ch Srinivasa Rao and U Srinivas Reddy	
Pathology	
Occurrence of U <i>rentius hystricellus</i> (Richt.) on Pigeonpea in the Net-House SC Dhawan, RD Gautam and JN Govil	
Response of Resistant Germplasm to Different Races/Populations of Pigeonpea Cyst Nematode, <i>Heterodera cajani</i> <i>Satish Kumar Mehta</i> and <i>Harish K Bajaj</i>	
Entomology	
Biological Activity of Lectins from Grain Legumes and Garlic against the Legume Pod Borer, <i>Helicoverpa armigera</i> <i>Richa Arora, HC Sharma, E Van Dreissche</i> and <i>KK Sharma</i>	

#### Publications

SATCRIS Listing	

#### Editorial

I wish to welcome all the ICPN readers. For this issue of ICPN, 34 manuscripts were received for consideration of which 25 have been accepted and included in this issue. Five manuscripts were found unsuitable for ICPN, and four corresponding authors did not review and respond to the remarks in time. I suggest that the contributors follow the guidelines (on the inside cover) while preparing the manuscript, and respond to the reviewing queries in time so as to bring out the newsletter issues conveniently and promptly. News about the researchers and the crops, and short research articles should be the focus of the newsletters, and I request the contributors to consider the same. It would give me immense satisfaction if all the personnel engaged with the research and development of chickpea and pigeonpea take interest in sharing and distributing the information using this newsletter.

I thank the contributors and the authors of this issue, and particularly the reviewers of the manuscripts, namely, SL Dwivedi, PM Gaur, L Krishnamurthy, K Krishnappa, JVDK Kumar Rao, N Mallikarjuna, S Pande, RPS Pundir, LJ Reddy, OP Rupela, KL Sahrawat, DVSSR Sastry, KB Saxena, HC Sharma, P Singh, Sube Singh, V Vadez (ICRISAT), PK Agrawal, SC Goswami, GT Gujar, J Kumar (1ARI, New Delhi), Shiv Kumar (IIPR, Kanpur), and SB Sharma (Department of Agriculture, Australia), and the Library at ICRISAT compiling SATCRIS listing.

We are updating the mailing list of ICPN. Therefore, kindly furnish the particulars in the attached form and send it back to us before 30 November 2005 or email your response to newsletter@cgiar.org. It may be difficult to process any request after the deadline.

ICPN team wishes its readers a very happy Christmas and a healthy, productive and prosperous 2006.

#### HD Upadhyaya

#### News

#### CGIARAwardtoICRISATScientist

P Lava Kumar, Special Project Scientist - Virology, of ICRISAT, received the "CGIAR Young Scientist Award 2004", for his contribution to identification of the causal agent of pigeonpea sterility mosaic disease (SMD), a widespread problem in the Indian subcontinent that drastically cuts the pigeonpea yields, causing over US\$300 million worth of grain loss. His work lead to the development of disease diagnostic tools and improved methods of controlling it. He was also the recipient of "MillenniumICRISATScienceAward 2004" as promising young scientist for contribution to the sustainable management of SMD.

#### Chickpea Scientists' Meet Held at ICRISAT-Patancheru

A one-day Chickpea Scientists' Meet was organized at ICRISAT on 6 January 2005 for the scientists of National Agricultural Research System (NARS), India. The meeting was attended by 45 scientists that included 28 Indian NARS scientists from 12 states and 17 ICRISAT scientists. The objective of the meeting was to facilitate interaction between ICRISAT and NARS scientists and provide opportunity to NARS scientists to see ICRISAT's chickpea experiments and select breeding lines and germplasm of their interests.

The meeting was inaugurated by JDH Keatinge, the Deputy Director General (Research) of ICRISAT, after a formal welcome by CLL Gowda, the Leader for Global Theme - Crop Improvement. Masood Ali, Director, Indian Institute of Pulses Research, Kanpur, made a presentation on significant achievements and future opportunities for ICRISAT-Indian NARS collaboration in chickpea research. PM Gaur, ICRISAT's Chickpea Breeder, presented highlights of the recent developments in chickpea research at ICRISAT. It was emphasized that ICRISAT-Indian NARS partnership has been very fruitful in chickpea research as 25 varieties, including some very popular varieties such as ICCV 2, ICCV 10, ICCC 37, JG 11, JG 16, JG 130, KAK 2, JGK 1, Vishal, and BG 1053, have been developed through this partnership. The ICRISAT-Indian NARS collaborative varieties had 37% share in the total indent of chickpea breeder seed in the country for 2004/05.

The participants witnessed various experiments on physiology, pathology, entomology, genetic resources, wide hybridization, genetics and breeding of chickpea, and had interactions with the scientists. They selected germplasm and breeding materials of their interests and submitted indents to ICR1SAT for the supply of seed.

*Contributed by* PM Gaur ICRISAT, Patancheru, India

#### Marigold: A Diagnostic Tool for BGM Forecasting and Management in Chickpea

Botrytis gray mould (BGM) is a disease that mainly attacks the reproductive structures of a chickpea plant. Flower abortion is a common symptom of the disease (Fig. 1) which remains undiscovered until the damage is visible on the canopy. As a result, timely application of fungicides is hampered in the integrated disease management. The predictive models (Shtienberg and Elad 1997) to estimate disease severity and timing are based on complex mathematical calculations, and they do not account for inoculum pressure. To identify an alternative indicator for a reliable diagnosis, forecasting and management of BGM, several ornamental plants commonly grown during the chickpea season as a collateral host of *Botrytis cinerea* were evaluated.

The controlled environment investigations on host pathogen interaction were carried out with marigold (*Tagetus erecta* L.). Flowering plants of marigold when spray-inoculated with *B. cinerea* ( $3 \times 10^5$  conidia mL<sup>-1</sup>) from chickpea and incubated in an environment ( $15^{\circ}$ C and 100% RH) needed for BGM development, produced symptoms on the leaves, flowers, flower buds and stems. Six days after inoculation (DAI), dark lesions were observed on a fully bloomed flower (Fig. 2A). Concurrently, all the young buds appeared completely rotted, but did not support sporulation (Fig. 2B). By 12 DAI, masses of wind blown grey sporulation on flowers and flower buds were clearly visible (Fig. 2C and 2D). Between 15 and 20 DAI, profuse grey sporulation was observed on all the aerial plant parts (Fig. 2E).

The early infection of B. cinerea causing moldy infection on marigold clearly identified its usefulness to farmers as a diagnostic tool to predict BGM epidemics and its management in chickpea. Marigold as an indicator plant to apply prophylactic fungicidal protection to chickpea crop in Nepal has been successfully validated. Infection of B. cinerea on the flowers of marigold and

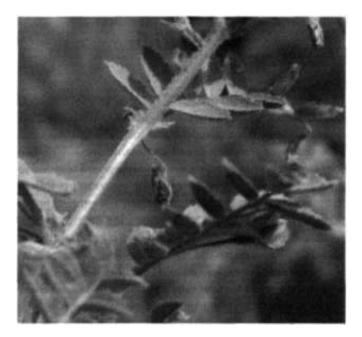
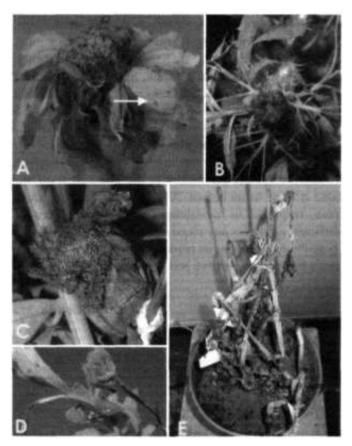


Figure 1. BGM infection on chickpea flowers.



**Figure 2.** Progressive symptoms of *Botrytis cinerea* infection on marigold: (A) Initial lesion development and sporulation on bloomed flowers and (B) rotted young buds; (C) sporulation on flowers; (D) sporulation on flower buds and lesion development on leaves; (E) sporulation on all the aerial plant parts.

Dahlia, grown at Ishurdi and Jessore in Bangladesh, indicates the possible integration of this farmer friendly, low-cost B G M forecasting system.

#### Reference

Shtienberg D and Elad Y. 1997. Incorporation of weather forecasting in integrated, biological-chemical management of *Botrytis cinerea*. *Phytopathology* 87:332-340.

Contributed by Suresh Pande, G Krishna Kishore and J Narayana Rao Crop Improvement Theme ICRISAT, Patancheru, India

#### Visiting Scientists

**SL Dwivedi** has joined ICRISAT Genetic Resources Unit as a Visiting Scientist to work on Generation Challenge Program supported chickpea project on "assessing the genetic diversity and allelic variation associated with beneficial traits in global composite chickpea core collection" in partnership with ICARDA, which is another CGIAR Center participating in this project. This composite core consists of 3000 accessions, drawn from vast collection of chickpea germplasm maintained at the ICRISAT and ICARDA gene banks chickpea core collection, elite germplam, advanced lines/ cultivars, unique germplasm with specific traits, and wild *Cicer* species. Using ABI3700 and SSR markers, the accessions will be molecularly profiled at MS Swaminathan Applied Genomics Laboratory, ICRISAT, to define the genetic structure of the global composite collection, and to form a subset of 300 accessions representing the maximum diversity for the isolation of allelic variants of candidate gene associated with beneficial traits. It is expected that molecular biologists and plant breeders will have ample opportunities to use diverse lines in functional and comparative genetics, in the mapping and cloning of gene(s) of particular interest, and in applied breeding to diversify the genetic base of the populations which leads to the development of cultivars with superior performance.

Ranjana Bhattacharjee joined the Genetic Resources Unit (GRU), ICRISAT, as a Visiting Scientist for the project "molecular characterization of pigeonpea composite collection." The project is supported by the Generation Challenge Program of the Consultative Group on International Agricultural Research (CGIAR). Dr Bhattacharjee has a PhD on establishing pearl millet core collection, which she pursued at the GRU, ICRISAT, and at the Haryana Agriculture University. Following this, she worked at the International Institute of Tropical Agriculture (IITA), Nigeria, as Postdoctoral Fellow on cocoa molecular genetics. In her new stint at ICRISAT, she will be involved in characterizing pigeonpea accessions using micro-satellite markers to determine the genetic structure of the global pigeonpea composite collection. The results of this study will further diversify the genetic base of populations, and assist in mapping and cloning gene(s). Data generated will also contribute to comparative and functional genetics. Breeders will have opportunity to use genetically diverse parents in their program to develop broad based cultivars.

# **Research Reports**

#### Chickpea

Genetics/Breeding/Biotechnology

#### Detection of Polymorphism Using CAPS and dCAPS Markers in Two Chickpea Genotypes

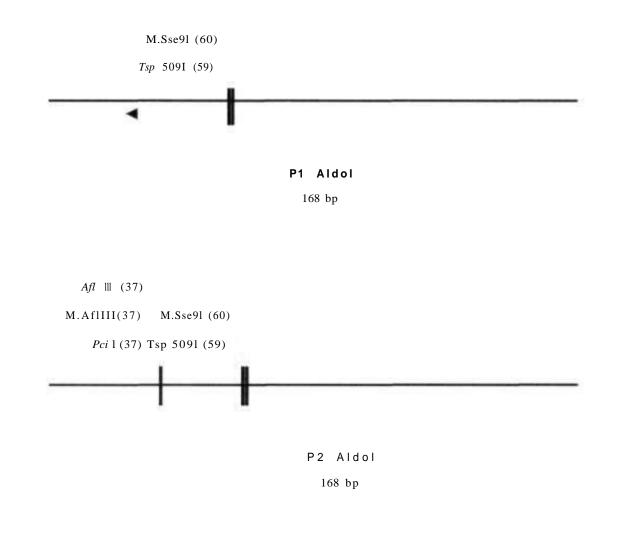
**PN Rajesh, Kevin McPhee** and **Fred J Muehlbauer\*** (USDA-ARS, Dept of Crop and Soil Sciences, Washington State University, Pullman, WA 99164-6434, USA) \*Corresponding author: muehlbau@wsu.edu

Cleaved amplified polymorphic sequence (CAPS) and derived cleaved amplified polymorphic sequence (dCAPS) are sequence-based and co-dominant markers. CAPS markers result from differential restriction digestion of gene / allele specific polymerase chain reaction (PCR) products based on the loss or gain of restriction enzyme recognition sites due to the presence of single nucleotide polymorphisms (SNPs) or insertion / deletion mutations. In dCAPS analysis, a restriction enzyme recognition site that includes the SNP is introduced into the PCR product by a primer containing one or more mismatches to template DNA (Neff et al. 1998). These markers were developed previously and have shown utility in other plant species but have not been used in chickpea genome analysis prior to this report. Using available DNA sequences from BAC ends and gene specific markers, we studied the usefulness of CAPS and dCAPS markers to identify polymorphism in a region of the chickpea genome lacking visible polymorphism.

Primers were designed from the ends of 4m 10, 1509 BAC clones and the partial sequences of Aldolase (visit

http:// frodo. wi. mit. edu/cgibin/primer3/primer3 www.cgi). These primers were used to amplify genomic DNA of FLIP 84-92C and PI 599072 which are parental lines that resulted in monomorphic bands of expected size. To develop CAPS and dCAPS markers, the amplified products were run on 1% agarose gels, and the fragments eluted from the agarose gels using DNA gel extraction kit (Millipore, USA) were cloned into the pGEM-T easy plasmid vector (Promega, USA). The cloned DNA fragments were sequenced on an ABI Prism 377 DNA sequencer (Applied Biosystems, USA) using the dideoxy sequencing method with T7 universal primer. In CAPS analysis, the sequences of both parental DNAs were compared using Vector NTI Advance 9.0 software (www.informaxinc.com) for SNP detection and restriction mapping. The SNPs that conferred differential restriction enzyme sites between the parents were used for further analysis. Amplified product size of aldolase primers is 168bp and the polymorphism was detected by Afl 111 restriction enzyme digestion (Fig. 1).

CAPS analysis did not detect polymorphism in the product amplified with the primer (MF) designed from forward end of 4m10 BAC clone. In this case we used dCAPS technique by designing primers with a single nucleotide mismatch adjacent to SNP position creating restriction site in the amplified PCR product of one parent but not the other. The primers for dCAPS analysis were designed using a web-based software package and the program is available on http://helix.wustl.edu/dcaps/ dcaps.html. The size of the product amplified by MF is 553bp and Taq 1 restriction site was created by replacing an adenosine with a thymidine at the third position 5' to the SNP. The amplified products were digested using Taq 1 enzyme and separated on 2% agarose gel to detect polymorphism between the parental lines and the segregating population (Fig. 2). Metaphor agarose gel or 6% acrylamide gel is recommended for improved resolution of the digested bands.



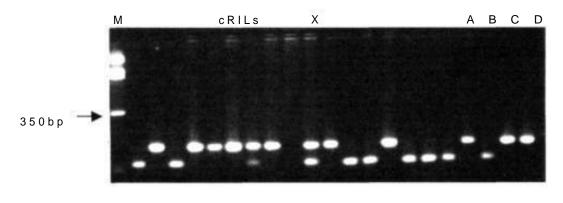




Figure 1. CAPS analysis using Aldolase specific primers.

AACTTGAAGATATTTAATATGGCAC **T**C -dCAPSMF primer

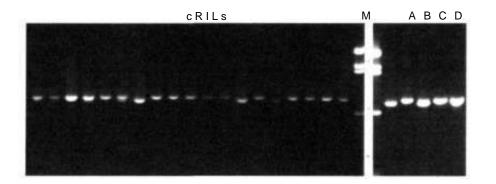
AACTTGAAGATATTTAATATGGCACAC**A**AACA -FLIP84-92C AACTTGAAGATATTTAATATGGCACAC**G**AACA -PI599072

Taq 1 TCGA

Taq1 digestion

A A C T T G A A G A T A T T T A A T A T G G C A C **T** C **A** A A C A

A A C T T G A A G A T A T T T A A T A T G G C A C **T** / C **G** A A C A



A - Taq1 digested FLIP 84-92CD - Undigested PI599072B - Taq1 digested PI599072M - Lambda Bst-N1 markerC - Undigested FLIP84-92CcRILs - chickpea recombinant inbred lines

Figure 2. dCAPS analysis using BAC end primers.

SNP detection between parental allelic sequences was verified by comparing replicate sequences. Although the frequency of SNPs present in the chickpea genome is not known, SNPs arc reported to be abundant in plant genome (Griffin and Smith 2000). Development of CAPS and dCAPS markers is simple and does not require expensive instruments. It involves common laboratory methods such as polymerase chain reaction, restriction digestion and agarose electrophoresis. Application of these markers in chickpea mapping where absence of polymorphism is a constraint is expected to improve generation of high density maps necessary for map-based cloning and integration of physical and genetic maps.

#### References

**Neff M, Neff J, Chory J** and **Pepper A. 1998.** dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. The Plant Journal 14(3):387 392.

**Griffin TJ** and **Smith LM. 2000.** Single-nucleotide polymorphism analysis by MALDI-TOF mass spectrometry. Trends in Biotechnology 18:77-84.

#### Nutritional Constituents in Chickpea Varieties

R Bhataagar\*, JP Yadavendra and KV Patel (Department of Biochemistry, BA College of Agriculture, Anand Agricultural University, Anand 388 110, Gujarat, India) Corresponding author: bhatnagarramesh@yahoo.com

Chickpea (Cicer arietinum) is an important source of dietary protein of the predominant vegetarian population of the Indian sub-continent. It is also adopted as protein supplement food by the people of European countries (Viveros et al. 2001). The green chickpea is used for vegetable purpose whereas, dry seed is consumed in the form of whole seed, *dhal*, and in the form of fried items from the chickpea flour. However, many people use the chickpea in flour form. Limited information on proximate composition of chickpea is available (Gopalan et al. 1971; Sotelo et al. 1987). Therefore, here an attempt was made to evaluate different varieties of chickpea for nutritional constituents and tannin contents in the flour of dry seeds of ten varieties including the dominant varieties of the region viz Dohad Yellow and GG 1.

Seeds often varieties were collected from a replicated breeding trial conducted at the Plant Breeding Farm, Gujarat Agricultural University, Anand, during winter season of 1999-2000. The dry seeds were ground in mechanical grinder from each replicate and 60-mesh powder was used for chemical analysis. The analysis was done for oil, ash and protein, as per AOAC standard whereas crude fiber and methionine were estimated according to the procedures described by Maynard (1978) and Mc Carthy and Paille (1959), respectively. Total carbohydrate percentage was determined by subtracting the sum of the percentage of crude protein, crude fiber, fat, ash and moisture from 100%. The energy value of seeds was calculated (Osborne and Voogt 1970). Total phosphorus and iron was determined colorimetrically. The procedure of AOAC (1970, 1980) was used to determine the calcium.

Tannin and anti-nutritional factor was determined as per the procedure described by Sadasivam and Manikam (1992). All these above mentioned analytical observations of three replicated samples for individual components were used for the analysis of variance by Randomized Block Design (RBD) (Steel and Torrie 1980). The results obtained for various parameters are presented in Table 1. The flour moisture content was found highly variable indicating variable moisture holding capacity of flour. This was also reported in cereals and pulses (Patel and Parameswaran 1992).

Table 1. Bie	l'able 1. Biechemical composition (%) of chickpea flor	position ("X	i) of chickpes	ı Ösar samples	sples.							
•	Moisture	10	Protein	Ash	Crude fiber	Total Carbohydraie	Energy content	Methionine	Mű	Mineral contem	E	Tannin
Variety	(%)	8	8	8	8	(%)	kcal/100 g	(% pro.)	Ca(%)	P(%)	Fe(%)	2
Pust 267	7,63	6.58	18.53	3.50	18.1	61.72	380.40	0.83	0.18	07.1	0.50	0.81
Pusa 5003	8.41	5,42	19.03	3,45	2.26	61.72	370.55	0.63	0.23	1.13	0.51	0.82
Pusa GS	5.96	6.02	17.29	3.48	5.79	61.38	371.30	0.61	0.13	1.15	0.54	1.02
GCP 105	6.74	6.12	18.53	3.25	81 é	57.15	358.02	0.65	0.23	8	0.25	1,19
GCP 106	6.B4	5.07	19.33	2.87	7,07	58.79	357.46	0.49	0.18	<b>8</b> :	0.59	1.08
GCP 9516	6,00	6.39	18.53	2.93	6.81	59.41	369.21	0.47	0.13	80	0.35	66.0
GCP 9707	4.42	5.58	17.29	3.14	5.84	63.79	374.07	0.60	0.20	1.03	0.38	10.
GCP 9605	5.68	5.9	17.36	3.50	5.97	61.2}	368.61	0.68	0.20	<b>8</b> 0	0.41	1.38
199	6.34	5.97	17.58	3.62	4.79	61.66	372.18	0.65	0.23	1.02	0.46	1.12
D. Yellow	6.24	5.31	19.92	3.32	6.28	10.92	363.21	0.48	0.18	1.17	0.52	.12
SEm (±)	0.26	0.07	0.15	0.0	0.43	8.63	1.76	0.05	0.02	0.07	0.01	0.12
CD (0.05)	0.77	0,20	0.44	0.14	1.37	NS	S.56	0.15	NS	NS	20-0	ŝ
ر ج ک	7,02	2,04	1.42	9671	2.3	21.5	0.68	34.1	17.1	8.7	42	16.0

The analysis of variance for chemical constituents of the chickpea varieties revealed significant differences for oil, protein, ash, crude fiber, iron and energy content among the varieties. Maximum protein content was observed in variety Dohad Yellow (19.9%). However, its methionine content (0.48%) was the lowest. Across the varieties, there was no significant difference in total carbohydrates, calcium, phosphorus and anti-nutritional factor such as tannin. High fat and carbohydrate makes variety Pusa 267 a good source of energy (Table 1) besides its high protein content. There was a wide range of crude fiber content, a non-nutritional constituent required for maintenance of good health, (1.81 to 8.18%) that was lower than that of the Mexican chickpea (9.1%) reported earlier (Sotelo et al. 1987). It was also reported that cooking diminished only the ash content and the Mexican variety Poranero was only one that had a high amount of crude fiber, as far as the varieties evaluated globally.

A significant variation in the ash content of different chickpea varieties was also prevalent. Iron content differed significantly among the varieties studied. Maximum iron content was observed in the variety GCP 106 (0.59%). Pusa 267 had the maximum methionine percent (0.83%). Wide range of tannin content, the antinutritional factors, had been found in most of the legumes. Tannins, which reduce the digestibility of proteins, were found to be the highest in GCP 9605 (1.38%).

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#### A New Kabuli Chickpea "Vihar" for South India

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Kabuli chickpea is mostly suitable for the region where the span of winter season is long. Its cultivation has attracted several progressive farmers in the south zone as it fetches premium price when compared to desi. Hence developing a cultivar of kabuli chickpea suitable for the region with mild and short winter, large seed size and resistance to fusarium wilt are the basic requirements. On this background, the variety Phule G-95311 released by 'All India Co-ordinated Research Project on Chickpea' has fulfilled a long awaited demand. Twenty advanced breeding lines of kabuli chickpea were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, in 1996-97 at Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri. ICCV 95311 derived from a multiple cross of [ICCC-32 x ICCL-80004) x [(ICCC-49 x FLIP-82-8C) x ICCV-3] was found promising for yield and seed size. It was, therefore, tested in Station Trial and Regional Varietal Trial during 1996-98. On the basis of its performance, it was promoted to State Multilocation Trial in 1998-99. Further, it was included in All India Co-ordinated trials where it performed extremely well in Initial Varietal Trial, Advance Varietal Trial-1 and Advance Varietal Trial-2, especially for southern zone. Considering its high yield performance in comparison with the standard check ICCV 2, KAK2 and BG 1003, it was released for general cultivation in the south zone in

Character	
Yield (t ha <sup>-1</sup> )	1.81
Height (cm)	35-43
Number of branches/plant	6-9
Flower color	Whitish
Duration of flowering (days)	38-43
Maturity period (days)	105-115
Test weight in g (100 seeds)	34-36
Color of seed	Creamy white

Table 2. Percent increase in yield of 'Vihar' over standard control cultivars.

Variety			Yield (kg ha <sup>-1</sup> )	Increase over (%)
Vihar	(1	7) <sup>1</sup>	1811	-
ICCV 2	(10)		1490	21.54
KAK	2	(5)	1357	33.46
BG 1003	(11)		943	92.05

1. Figures in parentheses indicate number of trials.

 Table 3. Reaction of 'Vihar' to fusarium wilt and Helicoverpa armigera (1997-2003).

Genotypes		Wilt (%)	Pod borer (%)
Vihar		7.04	12.19
ICCV 2		35.72	18.97
L 550		77.90	17.10
KAK	2	13.98	-
BG 1003		37.98	-
JG 62		100.00	-

2002 under the name 'Vihar'. The important features of this variety are given in Table 1.

Typically, in 17 trials conducted at different locations, Vihar has given average grain yield of 1811 kg ha<sup>-1</sup> as against 1490 kg ha<sup>-1</sup> of ICCV 2, 1357 kg ha<sup>-1</sup> of K A K 2, and 943 kg ha<sup>-1</sup> of BG 1003 (Table 2). Thus, the increase in yields over the three controls were 21.54, 33.46 and 92.05 percent, respectively.

The grain yield in Agronomy trial revealed that 'Vihar' was significantly superior under irrigated (3241 kg ha<sup>-1</sup>) and rainfed (1070 kg ha<sup>-1</sup>) conditions as compared to other genotypes.

The variety has shown high resistance to fusarium wilt (7.04%) as against 35.72% in check ICCV 2, 77.90% in L 550 and 13.98% in KAK 2 over 5-year period in

fusarium wilt sick plot at Rahuri (Table3). In addition, this variety showed least pod borer damage (12.19%) than ICCV 2 (18.97%) and L 550 (17.10%), showing high degree of tolerance to *Helicoverpa*.

The Central Variety Release Committee, New Delhi, identified this variety for cultivation in south zone in December 2002. This variety is expected to fulfill the demand of Indian market for extra bold seed size.

#### Performance of Kabuli Chickpea Cultivar KAK 2 in Rainfed Black Soils of Prakasam District, Andhra Pradesh, India

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Prakasam district, the southern part of Andhra Pradesh state has about 100,000 ha under rainfed black soils. In the last few decades, the major crops in this farming situation were tobacco and cotton. During 2000-2001, due to declaration of crop holiday for tobacco, there has been a shift from tobacco crop to chickpea (Cicer arietinum L.), and now chickpea occupies 67 percent of the rainfed black soils. Most farmers in the district grow a desi chickpea variety Annigeri. To increase the net income of the farmer, the District Agricultural Advisory and Transfer of Technology Centre, Ongole, AP, India, initiated testing of two kabuli chickpea varieties KAK 2 and ICCV 2 along with the popular cultivar Annigeri in the farmers' fields (during 2001-2002 and 2002-2003). Each variety was sown in an area of about 500 m<sup>2</sup> replicated five times in Randomized Block Design (RBD).

The sowings were done during the first fortnight of November and the crop was harvested during the first fortnight of February. The seed was sown with seed drill and in each plot the plant population was approximately 33 plants m<sup>-2</sup> A fertilizer dose of 20 kg N and 50 kg  $P_2O_5$  per hectare was applied as basal dose in the form of urea and single super phosphate. All the operations during the crop growth period such as seed treatment with fungicides, weeding at 30 days after sowing, and plant protection measures were followed for the management of pod borers, viz, *Helicoverpa armigera, Spodoptera exegua.* 

Year	Variety	Plant height (cm)	Number of pods plant <sup>-1</sup>	Yield (t ha <sup>-1</sup> )	100-seed weight (g)
2001-2002	KAK 2	57.0	95.0	2.75	39.2
	ICCV 2	41.5	32.5	1.65	25.0
	Annigeri	41.0	68.0	2.40	24.3
SEd±				0.058	0.602
CD at 5%				0.16	1.67
CV(%)				14.3	12.4
2002-2003	KAK 2	55.8	82.0	2.82	38.2
	ICCV 2	40.0	28.0	1.58	24.5
	Annigeri	39.2	63.4	2.34	23.3
SEd±	•			0.061	0.555
CD at 5%				0.17	1.54
CV(%)				16.8	14.5

Table 1. Performance of chickpea varieties under rainfed condition in black soils of Prakasam district of Andhra Pradesh, 2001-2002 and 2002-2003.

Table 2. Cost benefit particulars of chickpea varieties grown under rainfed conditions in black soils of Prakasam district of Andhra Pradesh, 2001-2002 and 2002-2003 seasons.

	2001-2002			2002-2003			
Parameters	KAK 2	ICCV 2	Annigeri	KAK 2	ICCV 2	Annigeri	
Yield (t ha <sup>1</sup> )	2.75	1.65	2.40	2.82	1.58	2.34	
Market rate in Rs per kg	20.00	18.00	14.50	22.00	20.00	15.00	
Gross returns (Rs)	55000	29700	34800	62040	31600	35100	
Total cost of cultivation (Rs)	15737	13827	13337	15425	13515	13025	
Net returns (Rs)	39263	15873	21463	46615	18085	22075	
Cost benefit ratio	1:2.49	1:1.15	1:1.61	1:3.02	1:1.34	1:1.69	

The data on plant height and number of pods per plant were recorded on ten plants selected at random in each plot. Seed yield and 100-seed weight were recorded for each plot. The cost benefit ratios for all the cultivars were calculated for both the years by using the following formula:

Cost benefit ratio (C:B)

#### Net returns Total cost of cultivation

The cultivar KAK2 gave the highest yield in both the years (2.75 t ha<sup>-1</sup> during 2001-2002 and 2.82 t ha<sup>-1</sup> during 2002-2003) while ICCV 2 gave the lowest yields. The 100-seed weight was also significantly higher in KAK2 as compared to that of Annigeri and ICCV 2 which had

similar seed size. The number of pod plant<sup>-1</sup> and yields of ICCV 2 were significantly less compared to Annigeri and KAK 2 during both the years (Table1).

The cost benefit ratios were calculated based on the market price during the test years (Table2). Due to higher cost of seed, the cost of cultivation was high for KAK 2 and ICCV 2 compared to *desi* chickpea varieties. The highest net returns were obtained with KAK 2 (Rs 39,263 during 2001-02 and Rs 46,615 during 2002-03) followed by Annigeri. The cost benefit ratio for KAK 2 was 1:2.49 during 2001-02 and 1:3.02 during 2002-03. The ICCV 2 gave least net returns due to poor yields. Though the yield differences were not much between Annigeri and KAK 2, the high relative net returns were

obtained with KAK 2 (Rs 17,800 during 2001-2002 and Rs 24,540 during 2002-2003) due to high market price for KAK 2 produce. From the results it is clear that, kabuli variety KAK 2 can be cultivated by the farmers in rainfed black soils of Prakasam district so as to obtain highest net returns.

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Identification of Chickpea Genotypes with Combined Resistance to Ascochyta Blight and Fusarium Wilt

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Chickpea is mainly cultivated as rainfed crop and is grown on residual moisture. In such environments,

fusarium wilt is the common root disease, which cause heavy losses. Among foliar diseases, ascochyta blight is the most important disease and its occurrence can completely destroy the crop. Ascochyta blight is more prevalent in humid and sub-tropical climates. These diseases are the major limiting factors for higher production and stable performance. There is a need to develop high yielding genotypes with combined resistance to these diseases. Genetic variability exists among chickpea genotypes for resistance to one or more different diseases (Gaur et al. 1992; Haware et al. 1994). However, a few genotypes exhibit multiple resistance to two or all the major diseases (Singh and Hari Chand 1996). Such genotypes can ensure higher productivity and stable performance across the various growing conditions and environments.

Fifteen high yielding chickpea genotypes and two control cultivars, viz, JG 62 for wilt and a mixture of L 550 and Pb 7 for ascochyta blight (Table 1), were screened for wilt plant mortality (%) in wilt sick plots and for ascochyta blight in separate fields. Each genotype was sown in a 2.5 m row with inter-row spacing of 30 cm and plant-to-plant spacing of 10 cm, mixture of L 550 and Pb 7 was used as the susceptible control after every two test genotypes throughout the field for ascochyta blight

			Average yield	Dise	ease reaction
S. No.	Genotype	Pedigree	(kg ha <sup>-1</sup> )	FW (%)	AB(1-9 Scale
1	H92-67 (1996-97 to 1999-2000)*	(GG 588 x H81-73) x (BG 257 x H81-73)	2264	0-4.6	8-9
2	H00-256(2000-01 to 2003-04)*	HC -1 x C. reticulatum	2116	0 -4.4	3-5
3	H97-93(2000-01 to 2003-04)*	HC -1 x E 100 Ym	2033	0-9.0	3-5
4	H00-216 (2001-02 to 2003-04)*	DCP92-3 x PDG 84 -16	2142	0-6.7	7-9
5	HO 1-07(2001-02 to 2003-04)*	DCP92-3 x PDG 84 -16	2139	0-6.7	7-9
6	H01-08 (2001-02 to 2003-04)*	DCP92-3 x PDG 84 -16	2168	1.66-3.7	8-9
7	H01-09 (2001-02 to 2003-04)*	(HC-1 x NARC 9006) x NARC 9006	2070	1.69-4.2	8-9
8	H01-10 (2001-02 to 2003-04)*	(HC-1 x NARC 9006) x NARC 9006	2129	3.92-9.5	8-9
9	HO 1-67(2001-02 to 2003-04)*	(H 91 - 40 x H 91-38) x H 91 - 38	2180	0-9.1	3-5
10	H01-74 (2001-02 to 2003-04)*	HC -3 x GIGAS	2146	0 9.6	6-8
11	H01-79 (2001-02 to 2003-04)*	HC-3 x NARC 9006	2164	0-8.1	3-5
12	H01-80 (2001-02 to 2003-04)*	H 92-68 x NARC 9006	2014	0-27.6	3-5
13	H00-02 (2000-01 to 2002-03)*	HC -1 x H 91 - 37	2112	3.3-26.1	3-5
14	H00-126 (2000-01 to 2002-03)*	(H 94-67 x H 92-67)x NARC 9006	2234	6.8-17.9	3-4
15	H00-249 (2000-01 to 2002-03)*	HC -1 x C. reticulatum	1995	4.3- 11.36	3-5
16	JG 62(1996-97 to 2003-04)*	Check (not available)	Nil	100	-
17	Mixture of L 550 & Pb 7 (1996-97 to 2003-04)*	Check (not available)	Nil	-	8-9

Table 1. Reaction of chickpea genotypes to Fusarium wilt (FW) and Ascochyta blight (AB).

\* Year of testing

screening and JG 62 after every two test genotypes throughout the fields for wilt screening. Ascochyta rabiei was multiplied on chickpea flour agar medium at  $20 \pm 1^{\circ}$ C for 20 days for inoculation. At the flowering stage, when the average environmental temperature was 18-20°C, the crop was inoculated with a spore suspension containing approximately 30000 spore mL<sup>-1</sup> water. High humidity was maintained with perfo-irrigation up to three weeks after the inoculation. Disease score was recorded 30 days after inoculation on 1-9 scale (1 = no infection and 9 =completely killed). Whereas for wilt, the material was planted in wilt sick plot maintained at the Pulses Research Area, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar. The disease reactions for genotypes at Sr. No. 1 to 3 were tested for 4 years and for the rest for 3 years (Table1). The genotype, H92-67, H97-93, H00-216, H00-256, H01-07, H01-08, H01-09, H01-10, H01-67, H01-74 and H01-79, were resistant to wilt (< 10% mortality), whereas, H00-02, H00-126, H00-249 and H01-80 were resistant to ascochyta blight (3 to 5 score). H97-93, H00-256, H01-67 and H01-79 were resistant to wilt and ascochyta blight. It is suggested that the genotypes which provide resistance to more than one disease should be used as donor parents to transfer resistance to adapted promising genotypes for higher productivity and stable performance.

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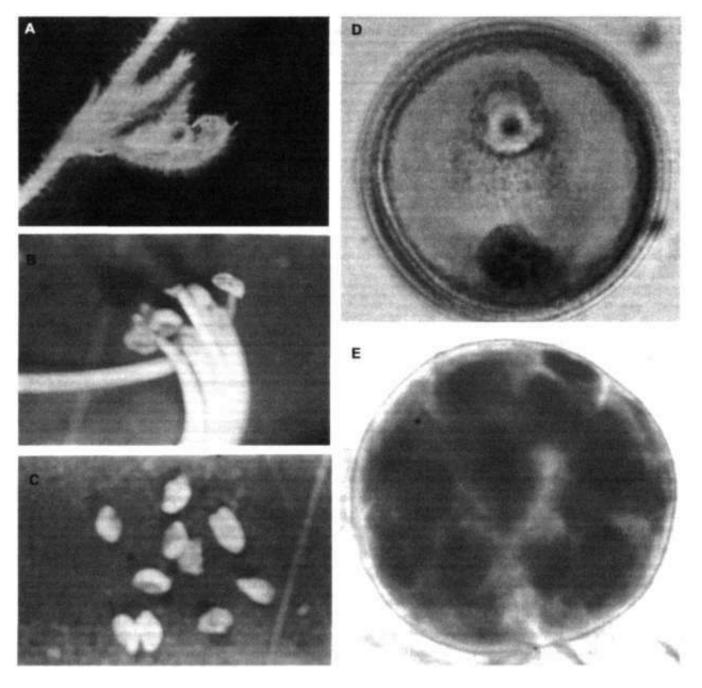
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#### Induction of Androgenesis as a Consequence of Wide Crossing in Chickpea

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The value of haploids in genetics and plant breeding has been known for a long time. Natural haploid embryos and plants have been described in about 100 species of angiosperms, and documented in detail by Kimber and Riley (1963). However, haploids occur rarely in nature. Doubled haploids are equivalent to inbred lines, with normal fertility, retaining the advantage of homozygosity, which by conventional program of producing pure lines would require 6-7 generations of selfing to achieve a satisfactory level of homozygosity.

Three principal methods of haploid production include 1. parthenogenesis, 2. wide crosses chromosome elimination, and 3. haploid plants from anther/ovule culture. In the first method of haploid production, haploids arise from both an unfertilized egg and from a male gamete. Gynogenetic haploids arise as a result of stimulation of the unfertilized egg, and in a few cases the offsprings resembled the male parent and hence were thought to have originated from the pollen (Clausen and Laments 1929; Kostoff 1929; Rhoades 1948). The doubled haploid method used in barley, is an example of preferential chromosome elimination in the cross between barley and Hordeum bulbosum, where the chromosomes of H. bulbosum were gradually eliminated. In that method, a cross is made between cultivated barley (Hordeum vulgare) and H. bulbosum. During embryo development, the chromosomes of H. bulbosum are gradually eliminated resulting in haploid plants (Subrahmanyam and Kasha 1973). The chromosome elimination phenomenon is quite prevalent among wide crosses between wheat and H. bulbosum as well (Barclay 1975). A more recent procedure to produce haploid plants is by anther culture/microspore culture (Maheshwari 1996; Guha and Maheshwari 1966; Melchers 1972). The culture of anthers or microspores gives rise to haploid plants whose chromosomes can be doubled by suitable treatment to produce homozygous diploid plants. Later Rangan (1994) and Keller and Korzun (1996) reported parthenogenesis of the egg in culture.



- A Fragile buds from the cross C. arietinum x C. pinnatifidum.
- B & C anther bundle and anthers from the cross C. areitinum x C. pinnatifidum.
- D A normal pollen grain undergoing the microsporogenesis.
- E A multicellular pollen grain from the hybrid.

Plant No.	Total microspores	No. Normal microspores	No. Androgenic microspores (%)	Maximum no. of cells in <b>a</b> microspore
1	57	43	14 (25)	3-4
2	122	109	13(11)	3-4
3	73	73	0	
4	46	18	28(61)	2-4
5	28	23	5(18)	4-6
6	27	12	15(56)	2-4
7	83	51	32 (39)	2-4
8	86	86	0	
9	151	143	8(5)	4-6
10	31	12	19(61)	2-4
11	35	35	0	
12	74	74	0	
13	43	36	7(16)	2-4
14	16	0	16(100)	8-10
15	65	62	3 (5)	
16	11	0	11 (100)	8-10

Chickpea procedures for developing haploid plants have not been reported, and induction of and rogenesis by anther culture is of a very low frequency (Mallikarjuna, personal observation). Androgenesis was observed in a wide cross of Cicer arietinum x C. pinnatifidum. Hybrids between C. arietinum x C. pinnatifidum were obtained after rescuing the hybrid embryos in vitro. The hybrids were initially devoid of any chlorophyll pigment and were albinos. Upon continuous culture in a zeatin-rich medium and in the presence of light, the hybrids turned semi-green (Mallikarjuna 1999). Hybrid shoots were grafted to chickpea root stocks to obtain hybrid plants. None of the hybrid plants flowered. When the nutrient solution with zeatin (1 mg/L) was added, flower buds were observed on the hybrid plants. Flower buds were fragile, albino to semi-green, but with normal morphology (Fig. 1A). Anthers (Figs. 1B and 1C) were squashed in acetocarmine and divisions were observed in some of the microspores (Fig. 1E). The number of divisions varied from 4-6. Adding nutrient solution with zeatin (1 mg/L) to in vivo grown chickpea plants did not induce divisions in the microspores.

A total of 16 hybrid plants were obtained. The number ofmicrospores/pollen grains in an anther varied from 11-151 compared to more than 500 pollen grains in cultivated chickpea. The number of pollen grains, which had undergone microsporogenesis and induction of androgenesis, varied from plant to plant. Percent androgenic pollen grains varied from 0-100%. Plant no. 8, 11 and 12 (Table 1) did not have any androgenic pollen grains, whereas in plant no. 14 and 16, all the pollen grains were androgenic, or in other words had multicellular microspores. The number of cells in multicellular microspores in plant no. 14 and 16 varied from 8-10 (Fig. 1E) unlike 4-6 cells in multicellular microspores in other hybrid plants which had androgenic microspores.

This is the first report in literature wherein multicellular microspores have been consistently produced as a result of wide crossing. Wide crosses are not only important in gene transfer from wild species but also in the production of haploid plants by *in vitro* culture of anthers with multicellular microspores.

Next logical step would be to explore the feasibility of androgenesis from wide crosses, for rapid development of homozygous lines.

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#### Chefe (ICCV 92318) - A New Kabuli Chickpea Variety for Ethiopia

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Ethiopia is the largest chickpea growing country in Africa, with a share of about 37% in area and 48% in production. During 2003/2004, Ethiopia produced 195,800 t of chickpea from an area of 176,554 ha (FAOSTAT 2004). There has been an increase of about

10% in the area and 42% in the production of chickpea during the past decade (1994/95 to 2003/04). Most of the chickpea production is used for domestic consumption. However, there has been a substantial export of chickpea by Ethiopia during the past five years, with the highest of 48,549 t (valued at US14.7 million) during 2002 (FAOSTAT 2004).

The Debre Zeit Agricultural Research Center (DZARC) is the premier institute for chickpea research in Ethiopia. It has collaborated with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, and the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, in chickpea improvement and released eight chickpea varieties in Ethiopia. Of these, three (DZ 10-4, DZ 10-11, and Dubie) were developed from its own breeding materials, four (Mariye, Worku, Akaki and Shasho) from the breeding materials supplied by ICARDA. Three of these varieties (DZ 10-4, Shasho and Arerti) are kabuli type and the remaining are desi type.

The Ethiopian chickpea production is predominated by *desi* chickpea (about 95%). However, in recent years, there has been an increase in the interest of farmers in growing large-seeded *kabuli* varieties due to their higher price in the market. The market price for one ton *kabuli* chickpea currently varies from 3000 to 4000 Birr (US\$344 to 459) depending on the seed size, while the *desi* chickpea is sold at about 2000 Birr (US\$230). The first *kabuli* chickpea variety released in Ethiopia (year 1974) was *DZ 10-4* with a very small seed size (10-11 g 100 seed<sup>-1</sup>) and is now almost out of cultivation. The



Figure 1. Seed of kabuli chickpea variety Chefe.

					Location					
		Debre		Chefe					Arsi	
Variety	Minjar	Zeit	Akaki	Donsa	Enewari	Adet	Sirinka	Ambo	Negale	Mean
1999/2000										
ICCV 92318	1231	3274	4778	3129	1879	2515	3117	-	-	2784
Arerti	1728	3844	4608	3091	1669	3867	1989	-	-	2971
DZ 10-4	501	2057	3892	2614	1790	3338	1519	-	-	2244
2000/2001										
ICCV 92318	2739	3513	3861	3542	2794	1543	-	2010	-	2858
Arerti	3804	3730	4054	4321	3320	1426	-	2915	-	3367
DZ 10-4	3173	3997	2913	3524	2580	1455	-	1469	-	2730
2001/2002										
ICCV 92318	1247	1493	2749	-	-	-	-	-	2499	1997
Arerti	1397	1791	2953	-	-	-	-	-	2875	2254
DZ 10-4	1066	1069	1329	-	-	-	-	-	1754	1305
Over all mean										
ICCV 92318	1594	2760	3798	3336	2337	2029	3117	2010	2499	2546
Arerti	2310	3122	3872	3706	2495	2647	1989	2915	2875	2864
DZ 10-4	1580	2374		3069	2185	2397	1519	1469	1754	2093

Table 1. Mean seed yield (kg ha<sup>-1</sup>) of chickpea variety (ICCV 92318) as compared to standard check (Arerti) and local check (DZ-10-4) across locations and over years.

other two kabuli varieties, Arerti and Shasho, with medium (26 g 100-seed<sup>-1</sup>) and large seeds (30 g 100-seed<sup>1</sup>), respectively were released in 1999.

ICCV 92318, a breeding line developed from a 3-way cross (ICCV 2 x Surutato) x ICC 7344 at ICRISAT, Patancheru, was received by DZARC from ICRISAT along with many other advanced breeding lines. After preliminary yield evaluation at the station, it was selected for multilocation evaluation along with the controls DZ 10-4 (local check) and Arerti (standard check). The trials were conducted at seven locations each during 1999/ 2000 and 2000/2001 and at four locations during 2001/ 2002. The overall average yield of ICCV 92318 was 2546 kg ha<sup>-1</sup> against 2864 kg ha<sup>-1</sup> for the standard check Arerti and 2093 kg ha<sup>-1</sup> for the local check DZ 10-4 (Table 1). Though ICCV 92318 was not superior to Arerti in yield, it was selected for release primarily because of its attractive and larger (35 g 100-seed<sup>-1</sup>) seeds (Fig. 1) as compared to Arerti (26 g 100-seed<sup>-1</sup>) and high resistance to fusarium wilt. It was released as "Chefe" in 2004 by the National Variety Release Committee. Chefe is one of the research stations of DZARC where chickpea productivity is always very high.

A high preference by farmers was observed for the new variety *Chefe* during on-farm trials because of its large pods. We presume that the increased price in the international market for the large-seeded *kabuli* varieties will help in faster adoption of the variety. Also there is a large market for chickpea immature fresh seeds, for human consumption in Ethiopia and large-seeded varieties are preferred for this purpose. Thus, the new variety also has potential for this local market. Ethiopian Seed Enterprise and private commercial farmers are multiplying this variety for further distribution as seed and also for export.

Acknowledgment. We are thankful to Canadian International Development Agency (CIDA) for funding the project "Improving income of farmers in eastern Africa through increased chickpea yield" during 2000 to 2003 under the CGIAR-Canada Linkage Fund (CCLF).

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#### Leaf and Pod Characters as Selection Criteria for Large-Seeded *Kabuli* Chickpea

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Large seed size is a premium trait of kabuli chickpea because of consumer's preference. Hence, large-seeded kabulis are sold at higher price in the market. Large variation (8 g to 75 g 100 - seed<sup>-1</sup>) has been recorded for this character in global germplasm (Singh and Saxena 1999). Most of the released cultivars of kabuli chickpea have a seed weight of 20-25 g 100-seed<sup>-1</sup>, except five recently released cultivars namely KAK 2, BG 1053, BG 1003, JKG 1 and Phule G 95311. Efforts are being made to develop the cultivars with more than  $30 \text{ g} 100 \text{ seed}^{-1}$ . Selection for most of the characters in the segregating generations is made visually based on the morphological traits. The advantage of this practice is that the selection of individual plant is based on a number of desirable traits. On the other hand, selection for seed size is carried out after the crop harvest. Thus, some morphological traits need to be identified which may be used as markers for large seed size while selection is practised for other traits in segregating generations. This will help identify the plants superior in number of traits simultaneously in the field itself. Keeping this in view, an attempt has been made in this study to correlate the leafand pod characters with seed size in kabuli genotypes.

The material consisted of 12 kabuli genotypes, grown in 6 row plots with row length of 4 m and rows spaced at 30 cm, in a randomized block design with three replications during the crop season 2003-04. Observations were recorded on five plants on each genotype in all replications, for plant height (cm), primary branches plant<sup>-1</sup>, leaf length (cm), leaf breadth (cm), leaflets leaf<sup>-1</sup>, leaflet length (cm), pods plant<sup>-1</sup>, pod length (cm), pod circumference (cm), days to flower, days to maturity, seed yield plant<sup>-1</sup> (g) and 100-seed weight (g). Leaf length and breadth were measured at the center of a branch. The central leaflets of these leaves were used for measuring the length of the leaflets. Pod characteristics such as pod length and pod circumference were recorded using vernier caliper. Correlations were estimated on replicated data following the methodology of Dewey and Lu (1959).

The range and mean values showed that wide variation (Table1) was available for characters under study. The leaflet

Table 1. Range and mean for haf, pod and seed characte	te and men	un for keaf, po	d and seed		ttice in <i>ka</i> b	ristics la kabuli chickpea	-						
Character	Plant	Primary	Leaf	Leaf	Leaflets	Leaflet	Pods	P.	Pod	Days to	Days to	Seed	[00-seed
:	height (cm)	branches Plant	kength (cm)	breacht. (cm)	leaf" (no.)	kength (cm)	plant' (no.)	(cm)	circum <del>feren</del> ce (cm)	flower (no.)	(filo.)	yiekl plant' (g)	weight (g)
Range	50.4	2.30-	4.37-	2.07-	12.00-	-00.1	47.63-	2.43-	3.57-	-00.06	152.00-	7.70-	16.50
•	67.33	3.33	6.00	3.43	13.67	1.63	106.87	3.47	4,43	102.00	158.00	22.57	36.10
Mean	58.6	2.8	5.2	2.8	12.6	<b>£</b> .1	68.6	3.1	4.1	97.2	154.7	13.8	26.2

I able 2. Phenotypic cerrelations among leaf, ped and	relations amo	ag ital, po		characteris	veed characteristics in <i>kubuli chick</i> per	ali chickpe	,					
	Primery								Days	Days	Sced	
	branches	l caí	Loaf	Leaflets	Leaflet	Pods	Pod	Pod	9	5	yield	100-seed
Character	plant'	length	breadth	)eaf	length	plant <sup>-1</sup>	length	circumference	flower	maturity	plant <sup>1</sup>	weight
Plant height	<u>т</u>	0.18	0.38	-0.25	0.28	81'0	0.39	0.43	0.08	60.0	0.21	0.26
Primery branches plant'	00 <sup>.</sup> 1	-0.30	-0.30	0.17	-0.42	0.27	-0.22	97. <b>9</b>	0.07	-0.27	<b>10</b> .0	<b>-</b> 6,37
Leaf length		8.	0.68*	0.02	0.72.	-0.16	0.54	0.63*	-0.44	0.13	0.20	*69'0
Leaf breach			1,00	-0.02	0.88.	10.01	0.38	0.57*	6E.Q-	0.13	0.16	0.48
Leafict leaf				1.00	-0.01	-0.24	0.00	90.0 <del>0</del>	-0.02	-0.02	-0.21	0.05
Leaflet length					8.1 1	<del>1</del>	0.54	0.68*	-0.35	0.38	0.06	<b>0</b> .60*
Pods plant <sup>2</sup>						90 1	0.09	-0.21	0.20	89	0.52	-0.25
Pod length							8.	++62'0	L0,01	0.20	0,18	0.54
Pod circumference								1.00	-0.20	0.21	<u>0</u> .0	0.61
Days to flower									8.1	0.09	-0.22	-0.26
Days to maturity										1.00	<b>60.0</b> 1	0.33
Seed yield plane"											1.8	-0.0t
• •• significant at 05 and 01 broad menacticals		يا م ا							 			

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respectively ĕ 5 ŝ X 

number was least variable while leaflet length showed the maximum variability. Pod characters, pod length and pod circumference also had high variability. The phenotypic correlations among leaf, pod and seed components revealed that leaf length, leaflet length and pod circumference had a positive and significant correlation with 100-seed weight (Table 2). All these three visually observable morphological characters were also positively correlated with each other, indicating that these were the most important components of 100-seed weight. Among these three traits, leaf length had strong positive significant correlation (r = 0.69) with 100-seed weight. In a similar study of 106 desi and kabuli genotypes, Dahiya et al. (1988) found leaflength, leaflet length and leaflet width to be significantly and positively correlated to 100-seed weight and concluded that the leaflet width was a predictor for seed characteristics.

It was concluded that easily observable morphological traits such as leaf length, leaflet length, pod length and pod circumference, could be used as a selection criterion for the large-seeded kabuli chickpea.

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#### Agronomy/Physiology

#### Low Temperature Effects on Early Maturing Chickpea Genotype ICCV 96029

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Low temperatures (< 10℃) are detrimental for reproductive growth of chickpea (Cicer arietinum L.) and induce abortion of flowers, pods, impaired seed growth and reduced yield (Srinivasan et al. 1999; Nayyar et al. 2005). Since the reproductive phase of early maturing genotypes coincides with the cold spells of northern region of India, they are expected to face varying degree of chilling stress depending upon the intensity and duration of low temperature. Low temperatures may induce anthocyanins that may allow the plant to develop resistance against stress possibly by improving sugar translocation in stressed tissues and acting as antioxidants (Chalker-Scott 1999). In the present study, we assessed the performance of an early maturing chickpea genotype ICCV 96029 under two contrasting temperature environments - low temperature conditions of the field and warm conditions of the glasshouse - in order to assess the chilling damage, if any. It was hypothesized that cold might limit the yield potential of plants as compared to those growing under warm conditions.

A super early maturing chickpea genotype ICCV 96029 (seeds procured from PAU, Ludhiana) was grown in pots (30 cm height, 25 cm diameter, 14.72 L volume) during the first week of November (2003) under low temperature conditions in the field (FD) as well as under warm conditions of the glasshouse (GH). Temperature profile during growth season under both the environmental conditions is shown in Table1. Light intensity (as photosynthetic photon flux density; PPFD) at mid-day ranged between 800 - 1100 µmol m<sup>-2</sup> s<sup>-1</sup> and 1300-2200 µmol m<sup>-2</sup> s<sup>-1</sup> in glasshouse and field conditions, respectively. Observations on the fate of flowers (retention or abortion) at different temperatures of field as well as glasshouse were recorded by tagging the flowers everyday in 25 plants and following them precisely for abortion or pod set during this period. These observations were correlated with daily temperature profiles of December and January. Observations on growth and yield were recorded on plants (50 in each case) growing in the contrasting environments. Data were subjected to t-test using SIGMASTAT software (USA).

The FD plants as compared to the GH plants showed marked reduction in plant height as well as delayed vegetative and reproductive growth in terms of days to flowering, days to podding, days to pod maturity and days to crop maturity (Table2), whereas the number of primary branches (basal) increased markedly in the former. The duration of flowering did not differ significantly between the two conditions whereas duration of podding was reduced significantly in the FD plants. The total number of flowers produced plant<sup>-1</sup> in FD plants during the season was almost threefold of GH plants, but the floral retention was significantly more in the latter. Though pod set was significantly lower in the FD plants than in the GH plants, the former showed appreciably more pod retention. All the traits contributing to yield, ie, pods plant<sup>-1</sup>, average pod weight, seeds plant<sup>1</sup>, seed weight plant<sup>-1</sup>, average seed weight, 1 seeded pods, 2-seeded pods, were markedly high in FD plants than in GH ones. There was no difference between the numbers of infertile pods in the two environments.

One of the peculiarities observed in the FD plants was the appearance of anthocyanins in the pedicels of flowers, which was in contrast to the GH plants. The FD grown plants had an average of 30 single flowers plant<sup>-1</sup> with purple pedicel (43% of total flowers) while GH plants had no such flowers but had 14.4 single flowers plant<sup>-1</sup> with green pedicel (60% of total flowers) (Table

Table 1. Average			•	. ,		,	. ,			- h
	IN	lov.	D	ec.	Ja	n.	Feb		Mar	cn
Environment	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min
Warm	30	15	25	15	25	15	30	15	38	25
Cold	25	10	20	8	17	7	23	10	32	17

Table 1 Average maximum and minimum temperature ( $\mathfrak{T}$ ) in warm (glasshouse) and cold (field) conditions

#### Table 2. Growth and yield traits per plant (Mean ± SEM) in ICCV 96029 under warm and cold conditions.

Parameters	Warm	Cold
Plant height (cm; at 60 *DAS)	51.9 ± 1.8	33.4 ± 1.5
No. of basal primary branches (at 100 DAS)	1.4±0.17	$6.0 \pm 0.69$
Days to flowering (DAS)	37.8 ± 1.5	59.7 ± 1.6
Flowering duration (days)	63.6 ± 1.5	65.3 ± 1.8 NS
Days to podding (DAS)	48.2 ± 1.8	93.6 ± 3.2
Podding duration	62.4 ± 1.9	49.2 ± 2.3
Days to pod maturity (DAS)	92.0 ± 3.2	120.0 ±2.7
Days to crop maturity (DAS)	113 ± 2.6	138 ± 2.8
Total flowers produced during the season	23.8 ± 4.9	69.2 ± 4.4
Total flowers abscised during the season	9.4 ± 1.3	$46.0 \pm 2.0$
Floral retention (%)	60.5 ± 2.5	33.6 ±2.2
Pod set (%)	$44.4 \pm 0.8$	35.4± 1.3
Pod retention (%)	$44.0 \pm 2.4$	58.9 ± 2.2
No. of Pods	$4.4 \pm 0.7$	14.4 ± 1.3
Average Pod wt. (g)	1.0±0.1	$3.2 \pm 0.3$
No. of seeds	$4.0 \pm 0.7$	15.6 ± 1.2
One-seeded pods	$2.8 \pm 0.6$	$10.0 \pm 1.4$
Two-seeded pods	1.5 ±0.17	$2.8 \pm 0.34$
Infertile pods	$2.0 \pm 0.3$	1.4 ± 0.3 NS
Seed yield plant"' (g)	0.6 ± 0.11	$2.7 \pm 0.4$
Average seed wt. (g)	0.18 ±0.02	0.18 ± 0.03

\*DAS-days after sowing

Differences between warm and cold treatments are significant at P<0.05 (t-test); NS-non-significant.

# Table 3. Observations per plant (Mean $\pm$ SEM) on single and double flowers as well as pod set under warm and cold conditions.

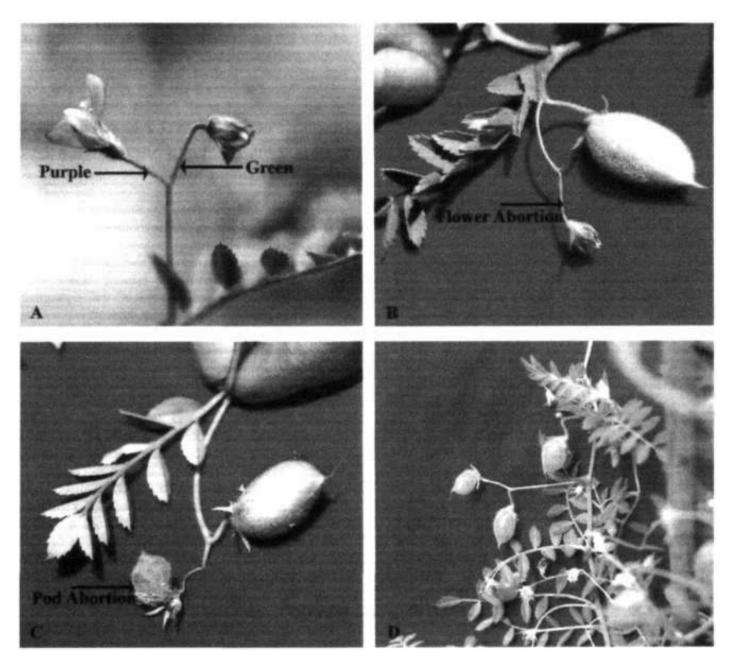
Parameters	Warm	Cold
Total flowers during the season	23.8 ± 1.0	69.2 ± 1.0
Single flower with green pedicel	$14.4 \pm 0.75$	0
Single flower with purple pedicel	0	$30.0 \pm 0.80$
Double flowers*	$4.7 \pm 0.63$	19.6 ± 0.75
Double flowers with both green pedicels	$4.7 \pm 0.63$	0
Double flowers with purple and green pedicel	0	19.6 ±0.75
Pod set in Single flower with green pedicel	8.6 ±0.75 (60 %)	0
Pod set in single flower with purple pedicel	0	24.5 ± 0.98 (82%)
Pod set in double flowers with both green pedicels	4.0 ±0.57 (85 %)	0
Pod set in double flowers with purple and green pedicel	0	7.8 ± 0.75 (40%)

\*Refers to a pair of flowers on a peduncle

Differences between warm and cold treatments are significant at P<0.05 (t-test).

3). The double flowers in FD plants (19.6 plant<sup>-1</sup>) had one green and another purple pedicel (57% of total flowers) unlike in GH plants (4.7 plant<sup>-1</sup>), which had both green pedicels (40% of total flowers) (Plate 1 A; Table 2). The flower with purple pedicel emerges earlier than the other with green pedicel. These flowers set pods according to the temperature of the environment. At temperature of 2-11°C, both types of flowers abort while at temperature

between 12-20°C, the flower with purple pedicel sets pods and the other with green pedicel aborts and gets abscised (Plate 1B; Table 2). The green type also shows abnormal growth and cannot complete its development in FD plants. Distortion of flower and its organs may also occur in green type as twisted anthers leading to impaired fertilization and pod abortion. Depending upon the stage of cold exposure, the flowers with green pedicel may



**Plate 1**. Cold-induced abnormalities; A - Flowers with purple and green pedicel under cold conditions; B - Flower with green pedicel aborts while the other one sets pod; C - Pod abortion in green pedicel and normal pod in purple pedicel; D - normal pod set by both the flowers under warm conditions.

either get abscised at bud or at anthesis stage. At 15-30°C conditions of the glasshouse, both the flowers set pods with higher intensity (Plate 1D; Table 2), whereas under cold conditions of the field, only flowers with purple pedicel are able to set pods and those with green pedicels abort leading to reduction in pod set (Table 2). Purple coloration in pedicels is because of accumulation of anthocyanins, which have been suggested to impart cold tolerance in several plant species (Chalker-Scott 1999) that also appears to be substantiated by the present findings. Since, this pattern was prevalent only in the FD plants, cold-induced restrictions in photosynthesis and in preferential mobilization of photosynthates towards the flowers having purple pedicel may be some of the key causative factors related to this variation (Nayyar et al. 2005). Anthocyanins are indicated to assist in sugar translocation in stressed tissues and may thus protect the purple flowers from abortion due to stress-induced starvation (Chalker-Scott 1999). Additionally, being antioxidants, they may protect the oxidative damage to stressed tissues. The exact mechanisms by which anthocyanins protect the cold-stressed tissues remain to be probed and are being investigated by us. Nevertheless, these findings indicate that anthocyanins accumulation can be employed as reliable marker in screening for coldtolerance in chickpea.

The present findings indicated that in spite of reduction in vegetative growth and delay in onset of subsequent growth phases, cold conditions did not appear to inhibit the yield potential of this genotype. The FD plants as compared to the GH plants compensated their reduced plant height by increasing the number of branches. Though cold conditions caused damage to the flowering phase, the plants produced more flowers and consequently higher number of pods as the field temperature increased in February. On the other hand, warm conditions throughout the growth season proved to be relatively much inhibitory for yield, which was in contrast to our hypothesis. Additional testing of plant response to intermediate temperature treatment may be needed to confirm the cold compensation of ICCV 96029.

It is concluded that ICCV 96029 possesses highly effective yield compensation mechanisms to face the chilling conditions. Accumulation of anthocyanins in the pedicels of flowers may be exploited as a screening trait in studies on cold tolerance. In-depth studies are underway to probe the mechanisms related to the differential effects of the temperature.

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#### Response of Chickpea (*Cicer arietinum*) to Irrigation, FYM and Sulphur on a Sandy Clay Loam Soil

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Chickpea in India is usually grown on well-conserved soil moisture. Though soil moisture depletes much after the harvest of preceding crop, which necessitates the presowing irrigation. Besides, improving physical-chemical and biological properties of soil, FYM also enhances water-holding capacity of the soil. Sulphur is essential for the synthesis of protein, vitamin and S-containing amino acids. It promotes roots and nodule development in legumes. Therefore, proper management of irrigation, FYM and sulphur, is essential for increasing the quality and productivity of chickpea.

A field experiment was conducted at the Regional Sugarcane Research Station, Anand Agricultural University, Thasra, on sandy clay loam soil during rabi season of the year 2002-2003. The soil was low in organic matter (0.37%) and nitrogen (0.032%), medium in available P (32.5 kg ha<sup>-1</sup>) and high in available K (296 kg ha<sup>-1</sup>) with pH 7.9. The experiment was conducted in split-plot design with four replications, comprised of two levels of irrigation and F Y M each in main plots and three levels of sulphur in sub plots. The crop (variety ICCC 4) was sown in rows 30 cm apart with 60 kg seed ha<sup>-1</sup>. One

pre-sowing irrigation of 100 mm depth was given before the layout was done and later on one irrigation was given at flowering as per treatment (60 mm depth). The crop was sown on 31 October 2002, and harvested on 1 March 2003 and 7 March 2003 for the treatments of only presowing irrigation and irrigation at pre-sowing plus flowering stage, respectively. N was applied in the form of urea, whereas P and S were applied in the form of diammonium phosphate and gypsum, respectively. N content in seeds was estimated by micro-kjeldhal's method (Jackson 1973). Protein content in seeds was calculated by multiplying N-content of seed (%) with the conversion factor of 6.25. Sulphur content in seed was estimated by Turbidmetric method (Chaudhary and Cornfield 1966). N, P, K and S contents in soil samples were determined through procedures described by Jackson (1973). Soil moisture at 30 cm depth on flowering (50 DAS) and pod development (90 DAS) stages were recorded in each treatment with TDR soil moisture meter.

There was a significant effect of irrigation on growth, yield attributes, yield and quality of chickpea (Table1). Application of two irrigations each at pre-sowing and at flowering stage recorded significantly higher number of branches per plant, number of pods per plant, grain and straw yields. Similar results were also observed by Sharma (1994). Quality parameters such as protein and sulphur contents in grain were also higher under two irrigations. The postharvest available soil nutrients such as N, K<sub>2</sub>O and S, were unaffected due to irrigation schedule, while postharvest available P2O5 was significantly higher under I, (Table 1). Moisture content in soil at flowering stage (50 DAS) and at pod development stage (90 DAS) differed significantly due to irrigation treatments and these were recorded considerably higher under  $I_1$  than under  $I_0$ treatment.

Number of branches per plant increased due to application of FYM. This resulted in more number of pods per plant, test weight and thereby more grain and straw yields. The protein content also improved significantly, whereas sulphur content in grain was unaffected. Further, the FYM application increased the moisture retention of soil and the postharvest available soil nitrogen and phosphorus. Available soil potassium and sulphur content did not differ much. FYM significantly improved the soil moisture content recorded at flowering and pod development stages.

Sulphur application in chickpea had important effect on almost all attributes. Number of branches and number of pods per plant as well as total grain and straw yields improved up to  $S_1$  (20 kg S ha<sup>-1</sup>), but was at par with  $S_2$ 

Table 1. Effect of different treatments on yield, growth	atments on	yieki, gi		quality par	meters of	<sup>r</sup> chickpea	t as well a	s pestha.	rveit set	ricat st	and quality parameters of chickpea as well as pestharvest natricut status and soil muldture content.	Sture content.
	Jo of	No of		, Line			A Angle	utrient status ir barvest of t (kg ha'l)	Nutrient status in soil after barvest of the crop (kg ha')	_ e	Moisture coment in soil at 30 cm depth (mm)	tture content in soil A 30 cm deptà (mm)
Treennent	branches plant <sup>1</sup>	pods	yjeld kg har	yield Xg ha	Proteín (%)	S (%)	z	°0'a	ç.	s	A1 flowering stage	At pod development stage
trrigation (l)	:											
L-At pre-sowing	5.3	68.9	1951	2612	17.6	0.25	218.7	28.6	277.8	23.2	68.2	0.95
IAt pre-cowing + at flowering	5.9	85.7	1167	3228	19.8	0.26	217,7	36.4	269.3	22.9	73.8	53.2
CD(P = 0.05)	0.6	9.5	186	173	<b>+</b> :0	0.01	NS	1.6	SN	SN	<b>1</b>	0.0
FYM (F)												
F. 0 tope FYM hz <sup>1</sup>	5.2	70.9	2002	2754	18.4	0.25	203.1	30.4	274.3	22.9	66.5	43.8
F. 10 tone FYM hat	6.0	83.7	2260	3086	0.01	0.26	233.3	34.6	272.8	23.2	75.9	48.5
ČD (P= 0.05)	0.6	9.5	<b>1</b> 86	173	1.0	SZ	27.9	9.1	SN	SZ	1.3	0.9
Stalphur (S)												
S 0 kg sulphur ha'	Q. 41	71.I	2027	2747	16.6	0.21	223.9	32.7	266.3	18.3	72.9	45.9
S -20 kg suppur ha	5.8	78.3	2151	2947	19.4	0.26	212.4	32.9	273.1	24.3	72.9	45.9
S. 40 kg subture hat	6.1	82.6	2215	3066	20.1	0.30	218.4	31.9	281.1	26.5	74.6	46.3
ĆD (P = 0.05)	0.7	9.2	121	148	0.4	t0:0	SN	SZ	NN N	4.8	5 T	SN

Table 2. Moisture content (v/v) in soil as influenced by I x F interaction

		Moisture co	ontent (v/v)	
Treatment		vering stage DAS)	At pod dev stage (90	
Irrigation (1)	$F_0$	$F_1$	F <sub>o</sub>	$F_1$
$l_0$ $l_1$ 70.3	62.2	74.2 77.2	35.6 51.5	42.0 54.9
CD (P =	0.05)	3.4		1.3

(40 kg S ha<sup>-1</sup>). Application of sulphur @ 40 kg ha<sup>-1</sup> recorded higher protein and sulphur contents than in grain with 20 and 0 kg S ha<sup>-1</sup> (Table1). The differences in postharvest available nutrients such as nitrogen, phosphorus and potassium, were not observed due to sulphur application, while, the postharvest S content was higher under 40 kg S ha<sup>-1</sup> than in 20 and 0 kg S ha<sup>-1</sup>. Further, moisture content in soil at flowering stage (50 DAS) was higher in plots at 40 kg S ha<sup>-1</sup>, while, at pod development stage (90 DAS), it was unaffected due to sulphur application (Ram Hari and Dwivedi 1992).

As regards to effect of irrigation x FYM interaction (Table 2) with respect to moisture content in soil at pod development stage (90 DAS), the combination  $I_1F_1$  showed higher moisture content than in the other treatment combinations, while, at flowering stage (50 DAS), moisture content remained at par due to with or without application of irrigation in presence of FYM. The application of FYM improved the moisture content at flowering (50 DAS) and pod development stages (90 DAS) even in the absence or presence of irrigation. This might be due to organic manure's (FYM) role to improve the physical condition of the soil and increase the waterholding capacity of the soil.

#### Conclusion

It could be inferred from the present study that application of two irrigations (one at pre-sowing and second at flowering stage) with the application of FYM @ 10 tonnes and sulphur 20 kg ha<sup>-1</sup> can increase the yield of chickpea.

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# Effect of Soaking Seeds on Polyphenols of Chickpea

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Chickpea (Cicer arietinum L.) occupies a unique position in pulse crops due to its seed protein content and wide adaptability as a food grain. India contributes to about 80% of the total world production of chickpea. Although, a valuable source of protein, chickpea is known to synthesize certain anti-nutritional factors such as polyphenols, which cause damage to intestinal tract and lowers feed efficiency in animals. However, these polyphenols can be wholly or partly removed by processing (Singh 1988). Soaking seeds is one such method of processing and this note is intended towards it. Seeds of varieties of chickpea, viz., BGD 237, SAKI 93130 and ICC 11320, were procured from the Pulse Research Station, Aland, Gulbarga, Karnataka, India. The seeds were soaked in distilled water for 6, 12 and 18 h, and in 2% solution of citric acid, sodium bicarbonate and mixed salt solution (MSS) of 1.5% sodium bicarbonate, 0.5% sodium carbonate and 0.75% citric acid for 18 h at room temperature. Polyphenols were determined in triplicate of all treated seed samples by method of Folin and Denis (1915).

Soaking of chickpea seeds resulted in significant loss of polyphenols in all the three varieties. Greater losses were observed when the seeds were soaked in MSS (Table 1). Deshpande and Cheryan (1985) have reported similar losses in polyphenols for dry bean (*Phaseolus vulgaris*) seeds when soaked in distilled water, sodium bicarbonate and MSS. Nearly 50% reduction in polyphenols of chickpea due to overnight soaking in water is reported by Rao and Deosthale (1982). The losses resulting from soaking may be due to leaching out of polyphenols into the soaking media. The phenolic compounds have been detected in leacheates of chickpea seeds by Rajkumar et

	Table 1.	Effect of soaki	ng seeds on	loss of polyp	ohenols of chickpea.
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		Polyphenols (mg/100 g)	
Treatment	BGD 237	SAKI 93130	ICC 11320
Raw untreated seeds	$29.33 \pm 0.57$	$51.68 \pm 0.54$	$46.00 \pm 0.86$
Soaked Seeds			
DH <sub>2</sub> O	$26.70 \pm 0.61$	$44.53 \pm 0.84$	41.58 ±0.55
6 h	(9.01)	(13.8)	(9.6)
12 h	$18.32 \pm 0.63$	$34.88 \pm 0.98$	$34.38~\pm~1.73$
	(37.5)	(32.6)	(25.3)
18 h	$16.00 \pm 0.10$	$25.50 \pm 0.57$	$27.35 \pm 1.30$
	(45.4)	(50.7)	(40.6)
2% Citric acid	$14.00 \pm 1.02$	$24.33 \pm 1.75$	$25.25 \pm 0.26$
18 h	(52.3)	(52.9)	(45.2)
2% Sodium bicarbonate	$13.70 \pm 0.61$	$22.16 \pm 0.57$	$19.00 \pm 1.02$
18 h	(53.3)	(57.2)	(58.7)
2% MSS	$11.27 \pm 1.09$	$20.25 \pm 0.43$	$15.92 \pm 1.13$
18 h	(61.6)	(60.9)	(65.4)

Values are mean ± standard deviation of triplicate determination. Values in the parentheses denote percent reduction over control.

al. (1979). The greater losses observed as a result of soaking in MSS or sodium bicarbonate may be due to the effect of these chemicals in creating an ionic environment. Under such conditions, changes in seed coat permeability may be much greater and rapid thus allowing higher losses.

It may be concluded from this attempt that soaking chickpea seeds is the most simple and inexpensive method for bringing significant reduction in polyphenols.

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#### Increased Chickpea Yield and Economic Benefits by Improved Crop Production Technology in Rainfed Areas of Kurnool District of Andhra Pradesh, India

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Chickpea, *Cicer arietinum* L., is a drought tolerant leguminous crop used in various foods in several developing countries, particularly in India as a source of dietary protein. There is a big gap between the yield realized in experimental station (2200 kg ha<sup>-1</sup>) and the farm yield (1274 kg ha<sup>-1</sup>) in Andhra Pradesh. The major constraints responsible for this untapped yield potential are inappropriate production practices, viz, usage of low yielding and non-responsive genotypes, pest and disease problems, lack of stress-resistant high-yielding genotypes, lack of improved soil and crop management practices and lack of appropriate institutional support.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Government of Andhra Pradesh have initiated the Andhra Pradesh Rural Livelihood Project (APRLP) to help reduce rural poverty by increasing agricultural productivity and improving livelihood opportunities through technical backstopping and convergence through a consortium of institutions. Watersheds were used as an entry point for research and development activities.

Nandavaram and Jillella villages of Banaganapalle mandal in Kurnool district of Andhra Pradesh were selected for undertaking on-farm research. Systematically collected soil samples from thirty farmers' fields in Nandavaram and Jillella watersheds on a toposequence were analyzed for physical and biological parameters and various nutrients. The soil analysis indicated that the fields in the two watersheds were low in N (496 and 333 mg kg<sup>-1</sup> soil), low to medium in available P (5.71 and 2.72 mg kg<sup>-1</sup> soil) (Olsen's P), high in exchangeable K (223 and 178 mg kg <sup>1</sup> soil), and low in available Zn (0.39 and 0.24 mg kg<sup>-1</sup> soil), S (7.52 and 4.09 mg kg<sup>-1</sup> soil) and B (0.5 and 0.45 mg kg<sup>-1</sup> soil). This critical information aided in identifying better options to improve the chickpea yield levels and for sustaining natural resources.

Sixteen on-farm trials in 2002 and nine trials in 2003 were conducted during the postrainy season with the objective to demonstrate the beneficial effects of improved production technologies over farmers practice. Improved production technology was compared with the farmers' method in an area of 1000 m<sup>2</sup> in each of the farmers' fields. The improved technology package included improved cultivar (ICCC 37), a seed rate of 60 kg ha<sup>-1</sup>, seed treatment with thiram (3 g kg<sup>-1</sup> seed), inoculation with rhizobium, a fertilizer dose of 20 kg N and 50 kg  $P_2O_5$  ha<sup>-1</sup>, basal application of micro-nutrient mixture of 5 kg borax (0.5 kg B ha<sup>-1</sup>), 50 kg zinc sulphate (10 kg Zn ha<sup>-1</sup>) and 200 kg gypsum (30 kg S ha<sup>-1</sup>) per hectare together with need-based pest and disease control measures. Two inter-cultivations at 25 and 50 days after sowing to control weeds was taken up. One insecticide spray was given at pod formation stage to control pod borers. The fanners' method included a local variety, a seed rate of 50 kg ha<sup>-1</sup> and a fertilizer dose of 14 kg N and 35 kg  $P_2O_5$  ha<sup>-1</sup>. Entire dose of N and P was applied as basal. The amount of rainfall from June to December was 708 mm during 2002 and 504 mm during 2003. The data was analyzed separately for both years considering farmers as replications using one-way ANOVA with randomized blocks on GenStat. Subsequently, pooled analysis of two year's data was carried out using two-way ANOVA. The analysis of variance indicated that

ž Table 1. Yield and economics of chickpen is on-farm trials, Naadavaram and Jillella watersheds, Kurnool district, Andhra Pradesh, postrainy season 2002 and 6.7 5.1 Benefit Cost Ratio 2003 С. С 2.6 200 8 7 2 (US\$\$30) Pooled (US\$340) 15287 23862 38 Net return (Rs ha<sup>-t</sup>) (US\$541) (EEE\$SD) 14996 24330 2003 836 (DS\$\$524) US\$343 15450 2002 23598 924 18.9 US\$184) **USS128**] Pooled 5761 8271 2 8 8 F Cost of cultivation (Rs harl) (US\$182 (US\$127 2003 8200 5706 2 US\$185) US\$129) 2002 8311 5792 4 Pooled 2.09 5 0.04 Gmin yield (t ha<sup>1</sup>) 2003 2.03 1.29 0.03 2002 0.06 14.0 0.19 2.13 1.42 inproved Production Cultivation method fermers' practice ochnology SE ± 2

949

2786

58

6.0

12.8 0.13

9.6

LSD(5%) ¥ 2

5

12.8 2726

Cultivation method	Total dry matter (t ha <sup>-1</sup> )			100 grain weight (g)			Harvest Index		
	2002	2003	Pooled	2002	2003	Pooled	2002	2003	Pooled
Improved Production technology	3.76	3.85	3.80	18.93	19.41	19.10	0.57	0.53	0.55
Farmers' practice	2.83	2.74	2.80	17.22	17.74	17.41	0.50	0.47	0.49
SE+	0.12	0.08	0.08	0.34	0.34	0.25	0.01	0.01	0.01
C V %	14.6	7.5	12.6	7.5	5.4	6.8	8.9	8.0	8.6
LSD(5%)	0.36	0.27	0.24	1.02	1.10	0.73	0.04	0.04	0.03

Table 2. Yield components of chickpea in on-farm trials, Nandavaram and Jillella watersheds, Kurnool district, Andhra Pradesh, postrainy season 2002 and 2003.

management practices (improved crop production technology and farmers practice) differed significantly in both years (P = < 0.001 - 0.008), as well as in the combined analysis (P = < 0.001). The year and year x management were non-significant (data not given).

The improved production technology gave higher grain yields and recorded a mean yield of 2.09 t ha<sup>-1</sup> which was 53% higher than that obtained with farmers' practice yields of 1.37 t ha<sup>-1</sup> (Table 1). The increased grain yield with improved production technology was mainly because of increased total dry matter, higher 100grain weight and harvest index (Table 2). Yield increase in response to fertilizer recommendations was also reported by Tamboli et al. (1996).

The economic viability of improved technology over the traditional farmers' practice was calculated depending on prevailing prices of input and output costs. The additional cost of US\$56 ha<sup>-1</sup> (Table 1) incurred in the improved technology as compared to farmers practice was mainly due to balanced fertilization (micro-nutrients and additional N and P), additional seed cost, seed treatment, IPM and one additional inter-cultivation. However, the improved technology resulted in increased mean income of US\$190 with a cost-benefit ratio of 2.9 (Table 1). This additional income could substantially benefit the resource poor farmers and improve their livelihoods in the dry regions of Kurnool district of Andhra Pradesh. Thiyagarajan et al. (2003) reported that the use of sulphur and micronutrients (Zn, B, Mo and Fe) improved productivity of pulse crops considerably. Balanced nutrition is indispensable for achieving higher productivity. Sachdev et al. (1992) obtained increased grain yield and harvest index of chickpea due to balanced fertilization. Shinde and Mane (1996) reported that the balanced application of fertilizers based on soil testing improved the yield of chickpea by 47 percent and monetary returns by Rs 7676 (US\$171) per hectare over control. The results from the current study clearly brought out the potential of improved production technology in enhancing chickpea production and economic gains in the dry ecoregions of Andhra Pradesh.

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#### A method for germinating perennial *Cicer* species

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The germplasm collection at the USDA ARS National Plant Germplasm System includes chickpea that consists of cultivated Cicer arietinum accessions containing genetic diversity immediately accessible for breeding, and wild Cicer species that may be of importance in the future as sources of genes for resistance to biotic and abiotic stresses. The wild Cicer species in the collection include 113 accessions of seven annual wild Cicer species and 59 accessions of 13 perennial Cicer species (available online at www.ars-grin.gov/npgs). At USDA-ARS, located in Pullman, WA USA, in earlier years, seed aeration technique was used to promote germination in perennial Cicer species. N Kameswara Rao has used in vitro germination on water agar to uniformly germinate annual wild Cicer species (personal communication 2000).

In the present study, an *in vitro* germination method was examined as an alternative method to provide uniform germination of the perennial species with the goal of establishing a nursery for regeneration and evaluation of inter- and intra-accession genetic variability. Twenty-eight accessions of nine perennial species were surface disinfested, scarified, and cultured under sterile conditions on water agar. The average germination of 25 accessions of eight species was 82% in 2001 with a range of 43 to 100%. Two accessions of C. *montbretii* failed to germinate *in vitro*. In 2002, an additional 13 accessions were successfully germinated with the same method (data not shown) and three accessions of C. *montbretii* failed to germinate on water agar.

Seeds to be germinated were surface disinfested with a 30 sec dip in 95% ethanol followed by 10 min in 0.6% NaCIO (100% commercial bleach) with drops of the detergent Tween 80 (Sigma, St. Louis, MO USA). After surface disinfestations, the seeds were soaked in sterile water for 1-5 days, or until they soften enough to scarify using a sterile scalpel. Scarified seed were then placed

either on agar plus Murashige & Skoog (1962) Salt Mixture (M5524, Sigma, St Louis, MO USA) or on agar (Difco Bacto agar, Fisher Scientific, USA) medium alone. The culture vessels (Magenta GA-7-3, Sigma, St Louis, MO USA) were then placed under cool-white fluorescent lights until radicle emergence. Plantlets were left on agar until the shoots were between 2-5 cm long.

An experiment was conducted to test the effects of Murashige and Skoog (1962) mineral nutrients plus agar vs. unamended agar on germination. The percentage germination was similar; however, MS nutrient amended agar reduced root growth (data not shown).

The vessels containing the plantlets were then partially opened to begin a 2 or 3 day acclimation at lower relative humidity. Plantlets were then pulled from the agar and their roots dipped in a fungicidal slurry (Captan, Gustafson, Plano, TX USA) before transplanting to 18 cm flats (Rootrainers, Hummert, Earth City, MO USA) filled with soil-less planting mix (Sunshine Mix Aggregate Plus Blend #4, SunGro, Bellevue, WA USA) with added coarse perlite (#2 sieve, Therm-o-rock, Chandler, AZ USA). The planted flats were moved to a humidity-controlled chamber constructed on а greenhouse bench. Plastic sheeting, 67% light-reducing shade fabric (PAK Woven Shade Fabric, Hummert, Earth City, MO USA) and a humidifier (Model 500, Hummert, St. Louis, MO USA) were used to maintain a cooler atmosphere with constant relative humidity. Initial humidity settings were between 75-80% with a steady decline to approximately 50% over the course of 4 to 5 days. The seedlings were able to tolerate the ambient humidity after five days and were moved to an open greenhouse bench covered with shade fabric. The greenhouse conditions were 21°C day/15.5°C night temperature, no humidity control, and 16 h day length.

The *Cicer* plants were retained there for a few weeks and the plants grew robust enough to withstand the outdoor conditions. Later, the plants were moved to an outdoor lathe house to harden the seedlings for at least two weeks before planting in the field. The seedlings were hand-planted on either side of a central irrigation drip line with emitters next to each plant.

This procedure provided uniform germination of most of the perennial *Cicer* accessions, except *C. montbretii* (Table 1). Aseptic germination of perennial chickpea on water agar is a fast and efficient method to provide a uniform set of transplants for field regenerations, and also to offer sufficient uniform seedlings for replicated screenings to detect resistance to biotic and/or abiotic stresses. Once established, grafting may also be useful in supplying plants for resistance testing experiments (Chen

Accession Number	Genus	Species	Seed quantity	Germinated	% Germinated	Number Rotted <sup>1</sup>	% Rotted
PI 383626	Cicer	anatolicum	30	24	80.0%	4	13.3%
PI 561078	Cicer	anatolicum	30	14	46.7%	8	26.7%
PI 599087	Cicer	anatolicum	30	17	56.7%	3	10.0%
PI 557453	Cicer	canariense	30	13	43.3%	0	0.0%
PI 599079	Cicer	macracanthum	30	23	76.7%	2	6.7%
PI 599080	Cicer	macracanthum	30	28	93.3%	1	3.3%
PI 599081	Cicer	macracanthum	30	25	83.3%	2	6.7%
PI 532928	Cicer	microphyllum	30	27	90.0%	3	10.0%
PI 593718	Cicer	microphyllum	30	28	93.3%	0	0.0%
PI 593719	Cicer	microphyllum	30	27	90.0%	1	3.3%
PI 599061	Cicer	microphyllum	30	27	90.0%	1	3.3%
PI 599082	Cicer	microphyllum	30	29	96.7%	0	0.0%
PI 599083	Cicer	microphyllum	30	30	100.0%	0	0.0%
PI 599084	Cicer	microphyllum	30	27	90.0%	0	0.0%
PI 599088	Cicer	microphyllum	30	24	80.0%	2	6.7%
PI 599089	Cicer	microphyllum	30	29	96.7%	0	0.0%
PI 599093	Cicer	microphyllum	30	28	93.3%	0	0.0%
PI 599085	Cicer	multijugum	30	27	90.0%	0	0.0%
W6 11516	Cicer	multijugum	30	30	100.0%	0	0.0%
PI 561084	Cicer	oxyodon	30	27	90.0%	0	0.0%
PI 561103	Cicer	oxyodon	30	25	83.3%	1	3.3%
PI 599053	Cicer	songaricum	30	13	43.3%	0	0.0%
PI 504550	Cicer	vamashitae	30	28	93.3%	1	3.3%
PI 510657	Cicer	vamashitae	30	30	100.0%	0	0.0%
PI 510664	Cicer	vamashitae	30	21	70.0%	1	3.3%
PI 599090	Cicer	montbretii	30	0	0.0%	•	0.070
PI 599091	Cicer	monthretii	30	0	0.0%		

# Table 1. Results of aseptic germination of perennial *Cicer* species on water agar after seed surface disinfestation and scarification at USDA-ARS, Pullman, in 2001.

1. Seed was scored as rotted if contaminated in vitro with a micro-organism, usually fungal or bacterial in appearance.

et al. 2004). As reported by Kaiser et al. (1997), cold treatment followed by aeration of *C. montbretti* seed in fresh water failed to promote germination. At ICRISAT, Patancheru, India, seedlings of *C. montbretii* were routinely established following normal germination procedures. (Anonymous communication), and plants of *C. montbretti* were established at Pullman, WA USA, in the early 1990s using an unpublished germination procedure (Hellier, personal communication). Further research is needed to improve surface disinfestation of the seed to reduce losses from fungal and bacterial contamination (rotted seed), and compare this method with (1) aeration in fresh water and (2) ICRISAT procedures in germination efficiency and efficacy. A technique to achieve seed germination in *C. montbretii* has to be developed.

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#### **Pathology**

#### Antifungal Metabolites from *Arachniotus* sp for the Control of Wilt Disease of Chickpea

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More than 50 pathogens of chickpeas have been reported in different part of the world (Nene 1980), but the most important of them are ascochyta blight and fusarium wilt. Chickpea wilt caused by *Fusarium oxysporum* Schlecht. emnd Synd. & Hans. f. sp. *ciceris* (Padwick) Matuo and K Sato, (FOC), is reported from all areas of chickpea cultivation in the world. Both the diseases are reported to cause substantial yield losses to the crop (Halila et al. 1984) and are highly influenced by environmental conditions, being prevalent in warm and dry environments. In Pakistan, wilt is a major problem in Thal area where most of the chickpea crop is cultivated. Due to the absence of true resistance in chickpea against wilt disease and a continuous problem of the occurrence/ development of new pathogenic races (Jimenez-Diaz et al. 1989), it has become very difficult to overcome the yield losses. *Arachniotus* sp has successfully been used as bio-control agent for the control of wilt (Ansar et al. 1996a,b) and other diseases of chickpea in field conditions (Saleem et al. 2000). The bioactive metabolites from antagonistic micro-organisms can be successfully used to control the microbial diseases (Momose et al. 1998).

Fungal metabolites were produced by the procedure reported by Khan et al (2001). Arachniotus sp (white isolate) was grown on liquid minimal medium (100 mL) taken in roux bottles for 14 days in dark at 25°C. The culture filtrates (85 mL) was harvested by filtering and squeezing the contents of the bottles through muslin cloth (Alam and Khan 1996), pH of the culture filtrates was adjusted to 3.0 using dilute hydrochloric acid and then extracted in half the volume of ethyl acetate three times. Ethyl acetate phase was dried over anhydrous sodium sulphate and then concentrated on rotary evaporator to dryness and the contents were dissolved in 1.0 ml of ethanol (Alam and Strange 1992). Anti-fungal assay against a virulent wilt causing isolate of Fusarium oxysporum f. sp. ciceris, 2012 (Khan et al. 2002) was done by disc diffusion method as described by Jacoby and Archer (1991). Ethyl acetate extracts (50 ml) was poured in the metallic well (1.0 cm outer diameter) placed in the center of pre-inoculated PDA plates (inoculated with 50 ml of spore suspension of  $1 \times 10^4$ 

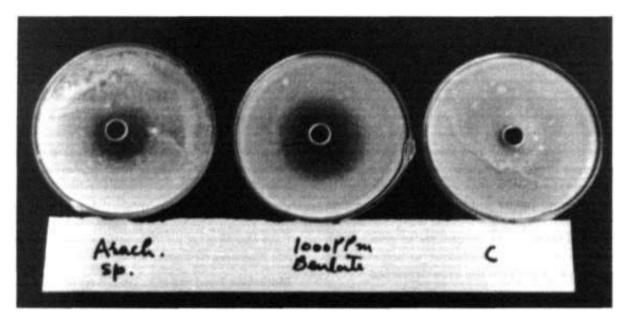


Figure 1. Inhibition zone produced by the ethyl acetate extract of Arachniotus sp and benlate against F. oxysporum f. sp. ciceris.

S.No.	Treatment *	k	Inhibition	2	zone	(cm)	Percent inhibition
I.	EtoAc Phase (50 µ	l) of	Arachniotus	sp	2.1±0.115		30%
2.	Benlate (1000 ppm)			-	$3.2 \pm 0.00$		46%
3.	Control				$0.0 \pm 0.00$		0.0 %

Table 1. Effect of ethyl acetate phase of Arachniotus sp. and benlate on the colony growth of F. oxysporum f. sp. ciceris.

spores/ml of FOC isolate) in three replicates. Ethanol (50 ml) was used as control, while benlate (1000 ppm) was also tested as reference fungicide. The plates were incubated at 25°C for 7 days and the activity was determined by measuring the diameter of inhibition zones produced.

The bioassay revealed that the ethyl acetate phase of Arachniotus sp produced inhibition zones against F. oxysporum f. sp. ciceris isolate (Fig.1). Benlate solution produced 3.2 cm average inhibitory zone (46% inhibition as compared to control) at 1000 ppm concentration, while 2.1 cm average zones (30% inhibition) were produced by the ethyl acetate extract of Arachniotus sp at 50 ml concentration, which was equivalent to 4.25 ml of culture filtrates, against the FOC isolate (Table1). On the other hand, the activity produced by 4.25 ml of culture filtrates of Arachniotus sp is equivalent to 625 ppm of benlate. No inhibition zones were produced by the control treatments. Results concluded that the metabolites produced by Arachniotus sp. were active against F. oxysporum f. sp. ciceris and may be used to manage wilt disease either by seed treatment or through soil treatments.

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# Management of root knot nematode and fusarium wilt disease complex by fungal bioagents, neem oilseed cake and/or VA-Mycorrhiza on chickpea

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Heavy infestation of both root-knot nematode and wilt fungus on common host chickpea has a synergistic effect in the farmers' fields around Allahabad. The present investigation was carried out under pot trial in a glasshouse with the objective to manage the disease complex through ecofriendly methods. The management components for this were fungal bioagents (Paecilomyces lilacinus and Trichoderma viride), VA-Mycorrhizal and neem oilseed cake under glasshouse condition. On uprooting the affected chickpea plants from the affected farmers' fields with heavy infection of root galls, the presence of wilt fungus, Fusarium oxysporum f. sp. ciceri, and galls of root-knot nematode, Meloidogyne incognita (Race-1) was confirmed. A preliminary pot trial with both M. incognita and F. oxysporum f. sp. ciceri on chickpea (cv H-108) confirmed synergistic effect on the host. This preliminary trial for establishment of disease complex with the above pathogens was done in 15-cm earthen pots filled with sandy loam soil in September 2003.

On establishment of the disease complex, the management experiment was carried out. The simultaneously inoculated treatment showing synergistic effect was taken as control with each of the treatments where management components were tried (Table1). The work was carried out with neem oilseed cake, fungal bioagents vesicular-arbuscular mycorrhizas and (Glomus fasciculatum). While the last component of the management trial, ie, V A M was isolated from the affected chickpea field; the two fungal bioagents and the neem oilseed cake were procured from fungal bioagents laboratory in the Division of Nematology. All the three management components were tested individually as well as collectively in 15-cm earthen pots filled with autoclaved sandy loam soil, to each of which both the pathogens (M incognita + Fusarium oxysporum f. sp. ciceri) were given prior treatment simultaneously as control (M. incognita + Fusarium oxysporum f. sp. ciceri, Tables1 and 2). A week before sowing of seeds, inoculations of M. incognita 2 larvae/g soil was done while about 100 chlamydospores of *G* fasciculatum were added to each of the Mycorrhizal treatments. Two weeks prior to sowing, the dosage of neem oilseed cake was 0.5% w/w. Both the fungal bioagents were applied to respective treatment in each pot along with sowing of chickpea seeds in talc based formulations with spore load of 12x108/g. Adequate control constituting all the components (Tables1 and 2) were also maintained.

The observations with respect to plant growth parameters, vield, number of nodules and also chlorophyll contents of each treatment were recorded after 90 days of final inoculation along with the population of root knot nematode, wilt percentage, mycorrhizal colonization percentage in root and also the population of V A M chlamydospores. Thus, in general, as is clear from Tables 1 and 2, there is a significant improvement of plant growth parameters and also the reduction in the disease incidence including suppression of *M*. incognita population in the treatments where more than one management component was used. The best performance, however, among the combined treatments was observed in the treatment constituting V A M, oilseed cake and both bioagents together followed by the treatment with V A M coupled with both bioagents. For years, attempts have been made to reduce the disease incidence through combination of either oil-seed cake and nematicide. References are available where either oilseed cake and nematicides (Singh 1965) or oilseed cake and fungal bioagents were applied, and the dual application of botanical antagonist with oil seed cake were noted. The present investigation focuses on more than two management components for two plant pathogens, i.e., root knot nematode and wilt fungus, both infecting common host chickpea. The cumulative effect of neem oilseed cake, G. fasciculatum and both fungal bioagents in reduced dose exhibited most promising results in reducing root knot nematode population and also the intensity of the wilt fungus (Table 1). The same treatment also revealed an outstanding improvement in plant vigor, which is significantly superior to oilseed cake treatment.

The discovery of an excellent recovery of chickpea plants from both *M. incognita* and *F. oxysporum* f. sp. *ciceri* is attributed to the joint reaction of neem oilseed cake, which possesses both fungicidal (Singh and Singh 1970) and nematicidal properties (Goswami and Swarup 1972). This is supplemented with the biopesticidal properties of both the fungal bioagents used with the growth hormonal character of *T. viride* (Chang et al. 1986). V A M, which in general occur abundantly around the rhizosphere of pulse crops (Allen 1991), is also

Table 1. Effect of *G. fasciculatum, T. viride, P. lilacinus,* neem oil-seed cake individually and together on disease complex caused by root-knot nematode and wilt fungus, with respect to plant growth characters, chlorophyll content, yield and test weight of chickpea.

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Chlorophyll contents (mg/g)	No of bacterial nodules	No of pods/ plant	100 seed weight (test weight)
Glomus fasciculatum + C (N+F)	25.8	21.8	18.4	10.8	26.7	42	31	17.5
T. viride + C	23.3	19.3	17.3	10.1	26.3	37	29	17.1
P. lilacinus + C	22.2	17.6	15.6	8.7	25.9	36	27	16.8
Neem oilseed cake + C	20.7	14.7	14.5	8.2	25.8	36	28	16.2
G. fasciculatum + T. viride + C	38.6	35.7	22.5	13.6	27.2	56	35	18.5
G. fasciculatum + P. lilacinus + C	37.5	32.1	21.7	13.2	27.1	52	34	18.3
<i>G. fasciculatum</i> + Neem oilseed cake + C	39.7	36.2	23.2	13.5	27.2	54	36	18.2
G. fasciculatum + T. viride + P. lilacinus + C	41.6	37.5	23.7	15.2	27.9	64	47	26.8
G fasciculatum + T. viride +	44.9	39.2	25.2	16.8	28.2	69	56	31.8
P. lilacinus + Neem oilseed cake + C								
Control (N + F)	16.5	11.5	12.5	6.5	20.7	16	18	11.8
CD at 5%	2.20	3.63	2.50	1.13	0.01	3.92	3.16	2.11

C = Control (M. incognita + Fusarium oxysporum f. sp. ciceri)

Table 2. Effect of *G. fasciculatum, T. viride, P. lilacinus,* neem oil-seed cake individually and together on disease complex caused by root-knot nematode and wilt fungus, with respect to nematode population, wilt percentage, and colonization and chlamydospores formation of VAM.

Treatment	No. of galls/ plants	Egg mass/ plant	Soil population/ 500g.	Wilt percentage	Colonization % o f VAM	Chlamydospores population of <i>G. fasciculatum/</i> 100 g soil
Glomus fasciculatum + C (N+F)	8	1.8	125.8(11.1)	23.8	46.7	417.8(20.4)
T. viride + C	11	1.9	147(12.1)	27.3	-	-
P. lilacinus + C	13	2.1	153(12.3)	29.6	-	-
Neem oilseed cake + C	16	2.6	167(12.9)	36.7	-	_
G. fasciculatum + T. viride + C	4	0.2	117(10.8)	21.1	53.6	369.5 (19.2)
G. fasciculatum + P. lilacinus + C	5	0.3	121(11)	22.6	57.2	348.6(18.6)
G. fasciculatum + Neem oilseed cake + C	4	0.2	120(10.9)	23.4	69.1	485.6 (22.0)
G. fasciculatum + T. viride + P. lilacinus + C	3	0.1	107(10.3)	8.6	72.1	450.5(21.2)
G. fasciculatum + T. viride + P. lilacinus +	-	-	73 (8.5)	-	79.6	528.7 (22.9)
Neem oilseed cake + C						
Control (N + F)	38	4.8	1575(39.6)	73.5	-	-
CD at 5%	2.35	0.12	3.72	4.82	6.73	1.23

Figures in parenthesis is the transformed value, C = Control (M, incognita + Fusarium oxysporum f. sp. ciceri)

considered as one of the management components (protecting the host from other root enemies by occupying cortical regions of the root). Bhagawati et al. (2000) have demonstrated that the combination of mustard oil-seed cake and VAM yielded better result. The results of this preliminary investigation will be confirmed under glasshouse and field conditions.

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## Screening of Chickpea Lines for Resistance to Ascochyta Blight

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Chickpea (*Cicer arietinum* L.) is an important winter grain legume sown under rainfed conditions in Pakistan. Average yield of chickpea in Pakistan is slightly above 500 kg ha<sup>-1</sup> (GOP 2003) that is lower than its actual yield potential (Haqqani et al. 2000). Ascochyta blight caused by *Ascochyta rabiei* (Pass) Lab is the most devastating disease of chickpea. The disease is widely prevalent in the chickpea growing areas of the world (Nene et al. 1996).

Resistant varieties offer an economical solution to combat this disease and reduce production losses. In this context, 495 promising chickpea lines received from the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria; National Agricultural Research Centre (NARC), Islamabad; Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad; Arid Zone Agricultural Research Institute (AZRI), Bhakkar; and Regional Agricultural Research Institute (RARI), Bahawalpur, Pakistan, were screened for resistance to ascochyta blight at NARC during the crop season of 2004-05.

Each entry was planted in a single row of 4 m long with 30 cm row-to-row and 10 cm plant-to-plant spacing. A highly susceptible cultivar AUG 424 was sown as a disease spreader and indicator after every five test entries. The genotypes were artificially inoculated with diseased crop debris collected form the previous year. Additionally crop was inoculated with spore suspension  $(5 \times 10^4 \text{ spores mL}^{-1})$ . Inoculations were done in the evening hours on cloudy days, at the preflowering stage. High humidity which is a prerequisite for disease epidemic was naturally created by the continuous rains during the crop season. Final disease observations were recorded on a 1-9 disease rating scale (Singh et al. 1981) in mid-March.

Test genotypes varied for disease reaction and three genotypes (FLIP 97-132C, FLIP 98-226C and FLIP 98-231C) were resistant (score 2-3) while eleven genotypes -FLIP97-120C, FLIP97-221C, FLIP97-229C, FLIP98-33C, FLIP 98-54C, FLIP 98-206C, FLIP 00-20C, FLIP 02-28C, FLIP 02-45C, ILC 1929 and ICC 12004 - were moderately resistant (score 4-5). The potential resistant material identified in the study was originated at ICARDA (Table 1). Several sources of resistance to ascochyta blight have been reported at ICARDA (Reddy and Singh 1984; Singh et al. 1984). Some of the lines, eg, ILC 72 and ILC 3279 that showed high level of resistance in several other countries were not found highly resistant in Pakistan (Iqbal et al. 1994). Therefore, resistant genotypes originated from ICARDA need to be re-tested with aggressive pathotypes of Pakistan before their use in the breeding program. Our data indicates that 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 through breeding blight resistant varieties of chickpea.

Source	Total	Resistant	Moderately resistant	Susceptible
ICARDA, Aleppo, Syria	164	3*	11**	150
NARC, Islamabad, Pakistan	132	-	-	132
NIAB, Faisalabad, Pakistan	99	-	-	99
AZRI, Bhakkar, Pakistan	90	-	-	90
RARI, Bahawalpur, Pakistan	10	-	-	10
Total	495	3	11	481

\* 2 - 3 score on 1-9 rating scale.

\*\* 4 - 5 score on 1-9 rating scale.

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#### Entomology

First-instar *Helicoverpa punctigera* larvae: feeding responses and survival on desi chickpea and the wild relative *Cicer bijugum* 

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Lepidopterous pod borers in the genus Helicoverpa are major constraints to chickpea (Cicer arietinum) production in the Indian subcontinent [especially H. armigera (Hubner)], Australia [especially H. punctigera (Wallengren)], and in many other parts of the world (Lateef 1985; Clement et al. 2000). Conventional insecticides are often used to control pod borers on chickpea and many other crops. However, intensive insecticide use on a wide variety of crops has led to widespread development of insecticide-resistant populations off H. armigera in India (Amies et al. 1996). Development of insect resistance to insecticides and the possible adverse effects of insecticides on humans and environment have stimulated interest in other methods such as resistant genotypes to manage pod borers (Lateef 1985). Screenings of Cicer arietinum germplasm stocks showed that *H. armigera* larvae reared on 'less susceptible' genotypes were lighter in weight and took longer to develop than those reared on 'more susceptible' genotypes (Srivastava and Srivastava 1989; Yoshida et al.

1995). Likewise, Sharma et al. (2002) recorded low weights for larvae of *H. armigera* and *H. punctigera* reared on some wild annual *Cicer* species, indicating that wild relatives of chickpea could be sources of resistance to *Helicoverpa*.

Although detailed observations of neonate lepidopteran larvae commencing their feeding on test plants have been used for evaluating resistance in crop plants (Zalucki et al. 2002). This approach has not been used to identify Cicer genotypes with varying levels of resistance and susceptibility to *H. punctigera*. Previously, > 5 day trials, albeit without detailed observations of the host acceptance and feeding behavior of first-instar larvae, have been used to identify Cicer genotypes with varying levels of susceptibility to both H. armigera and H. punctigera. We employed 48 h trials to observe and quantify the onset of feeding and survival of neonate H. punctigera on Cicer genotypes to assess the usefulness of short-term trials so as to identify resistant germplasm and possible mechanisms of resistance (antibiosis and antifeedant effects) in this pest.

The trials were carried out at the Entomology Laboratory, Commonwealth Scientific and Industrial Research Organization (CSIRO), Centre for Mediterranean Agricultural Research, Western Australia. A H. punctigera culture at the Entomology Laboratory provided larvae for experiments, and the experimental plant material was obtained from potted plants grown in a glasshouse (natural light, 15 to 26℃). Neonate larvae were expose d to test material from pre-flowering plants of two C. arietinum genotypes (Annigeri-susceptible; and ICC 506-resistant) and two accessions of annual wild species of C. bijugum (ILWC 260, ILWC 7, both resistant), which exhibited a range of susceptibility to H. armigera and H. punctigera in > 5 day trials (Sharma et al. 2002, Ridsdill-Smith TJ unpublished data). Test material consisted of a main stem (with two branching stems and leaves) embedded into water-agar (10 g Bacto agar/l water) in a 35 ml plastic cup using forceps. There were three trials, each involving two Cicer genotype or species combinations (Table1). The experimental design was a completely randomized design with three replicates per

			% larvae			
Trial	Genotypes	1 h	4 h	24 h	48 h	% mortality at 48 h <sup>2</sup>
1.	Annigeri	61.1	94.3	94.3	94.3	5.6a
	ICC 506	39.0	78.0	83.3	83.3	16.7a
	ANOVA		F	Р		
	Genotype (G)		2.78	0.17		
	Time (T)		19.48	<0.01		
	G x T		0.40	0.76		
2.	ILWC 7	27.7	66.7	100.0	94.3	5.6a
	ICC 506	44.3	66.7	78.0	66.7	33.3b
	ANOVA		F	Р		
	Genotype (G)		1.15	0.34		
	Time (T)		42.11	<0.01		
	G x T		8.11	<0.01		
3.	ILWC 260	66.7	94.3	88.7	77.7	22.2a
	ICC 506	44.3	72.3	78.0	78.0	22.2a
	ANOVA		F	Р		
	Genotype (G)		2.86	0.17		
	Time (T)		15.96	<0.01		
	G x T		2.72	0.09		

Table 1. Comparison of feeding and mortality rates of first-instar larvae of *Helicoverpa punctigera* on selected *Cicer arietinum* (Annigeri and ICC 506) and *C. bijugum* (ILWC 7and ILWC 260) genotypes (Perth, Australia).

1. Means are based on three replications of 6 larvae per replication.

2. Means followed by the same letters do not differ significantly (P = 0.05). Data transformed ( $\log_{10} (x + 1)$ ) to meet assumptions of A N O V A. Untransformed means reported here.

Cicer genotype. One potted plant provided all of the test material for a replication, which consisted of six larvae (one per plastic cup). After a 2 h starvation period, a neonate larva was transferred with a camel-hair brush to the basal part of test plant material and its movements were observed with the aid of a stereoscopic microscope for 2 minutes at 1,4, 24 and 48 h intervals. At each reading, we recorded if a larva had established a feeding site and was feeding or if it had not commenced feeding. The number of dead larvae was also recorded. Cups were randomly distributed on a laboratory ( $= 22^{\circ}$ C) bench near a window for natural light and redistributed after each reading. From these observations, the percentage of larvae feeding on the plant per replication was calculated.

The analysis of variance [completely randomized design with one-way treatment structure (genotypes) with repeated measures] showed that larval feeding rates were not affected by genotype, but time significantly affected feeding with the lowest rates at 1 h and higher rates (irrespective of plant genotype) recorded from 4 h onwards in all trials. There was a significant genotype x time interaction in trial 2, indicating that the effect of time on feeding rates on 1LWC 7 and ICC 506 was different. In all trials, the onset of feeding by neonate H. punctigera larvae was consistently delayed on ICC 506 and larval mortality was relatively high (16.7-33.3%) on this desi chickpea (Table1). The leaf chemistry of this genotype may influence the feeding and survival of neonate and first-instar H. punctigera, as was suggested for H. armigera (Lateef 1985; Yoshida et al. 1995). Also, the results of trial 1 confirmed the susceptibility of Annigeri to H. punctigera. Contrary to Sharma et al. (2002), who detected //. punctigera resistance in ILWC 7 and ILWC 260 after 5 day feeding assays, our 48 h trials did not reveal the existence of strong resistance (compared to ICC 506) in the C. bijugum genotypes (Table1).

This study detected *H. punctigera* resistance and susceptibility in ICC 506 and Annigeri, respectively, but failed to confirm resistance in C. *bijugum* as previously found after 5-day feeding trials (Sharma et al. 2002). More investigations are required, because this study shows that interactions between first-instar larvae of *H. punctigera* and species and genotypes of *Cicer* are variable, with the possibility that different plant resistance factors are involved.

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# Screening of Chickpea for Resistance to Pod Borer *Helicoverpa armigera* (Hubner) at Rahuri, Maharashtra, India

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Gram pod borer, *Helicoverpa armigern* (Hubner) is a key pest and with its regular **OCCUITENCE** in the state of **Mahamshtrn** early vegetative to podding stage **GRASSING** losses (Puri et al. 1998) in chickpea. It ls economically significant. In North India, Sehgal and Ujagir (1990) reported 90% pod damage by *Helicoverpa*. Management strategies for gram pod borer relied heavily on chemical insecticides. However, concerning chemical insecticides, the farmers' reluctance to use it, the nonavailability, high cost, development of resistance and environmental pollution (Armes et al. 1996), have opened up avenues for the identification and adoption of chickpea genotypes resistant/tolerant to *Helicoverpa*. The genotype is the best/preferred component of integrated pest management.

Twenty-five promising chickpea genotypes from the International Chickpea *Helicoverpa* Resistant Nursery (ICHRN) were screened under pesticide-free field conditions during *Rabi* 2002-03 and 2003-04 seasons in a randomized block design in three replications at Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra, India (532 m altitude with longitude of 19°44' to 19°57' N and latitude of 74°82' to 74°91' E).

The genotypes were sown in two row plots of 2 m length with the spacing of 30 x 10 cm on 28 October 2002 and 2003. All the recommended agronomic practices were adopted for raising the chickpea crop.

The observations on pod damage were recorded on five randomly sampled plants at maturity by counting the total number of healthy and damaged pods from which percent pod damage of each entry was calculated and compared with that of resistant check, ICC 506EB. The percentage data was converted to pest susceptibility rating on a scale of 1-9 as suggested by Lateef and Reed (1983).

The mean pod damage among the test entries (Table1) ranged from 20.37% in ICCL 87311 to 34.27% in ICC 12492

Table 1. Performance of chickpea ger	otypes against Helicoverpa	armigera at Rahuri, Mahara	shtra, India, <i>Rabi</i> 2002-03 and
2003-04.			

		Pod dam	Grain yield (kg ha <sup>-1</sup> )				
Entries	2002-03	2003-04	Mean	PSR*	2002-03	2003-04	Mear
ICCL 79033	32.63	14.27	23.45	6	733	1650	1191
ICC 13	37.19	15.10	26.14	7	1250	616	933
ICC 14	32.10	19.54	25.82	6	583	1100	841
ICCX 730041	35.03	20.40	27.71	7	750	1392	1071
ICCL 80129	36.30	16.78	21.56	6	1275	1083	1179
ICC 11509	32.85	17.87	25.22	6	1133	1058	1095
ICC 9854	33.64	20.00	26.86	7	900	1883	1391
ICC 926	38.64	21.18	29.91	8	700	1500	789
ICC 5800	34.42	19.50	26.96	7	900	775	837
ICC 12476	28.33	17.81	23.07	6	800	1450	1125
ICC 12479	24.03	19.45	21.74	6	966	1266	1116
ICC 12480	26.32	19.23	22.77	6	791	1558	1174
ICC 12493	38.08	19.60	28.84	7	800	750	775
ICC 12492	40.82	27.73	34.27	8	858	1341	1100
ICC 12490	31.82	20.64	26.23	7	916	2050	1483
ICC 87220	45.59	21.95	33.77	8	841	1375	1108
ICC 87311	19.55	21.20	20.37	5	1041	1558	1033
ICC 87314	22.01	23.07	22.54	6	1191	592	891
Vijay	30.27	18.72	24.49	6	666	1608	1137
JG 362	26.63	19.23	22.94	6	916	1300	1108
ICCV 2	21.05	19.73	20.39	5	833	392	612
ICC 37	40.49	19.01	29.75	8	675	1350	1012
Annigeri	26.35	20.80	23.57	6	1066	1508	1287
ICCV 10	32.39	19.46	25.92	6	425	2124	1247
ICC 50 EB (ch.)	28.82	18.57	23.69	_	1125	1650	1387
Mean	31.94	19.47	25.51		885	1329	1088
SD	6.23	2.07	3.61		205	416	209

\*-PSR= Pest susceptibility rating. Ch. = Resistant check.

with the mean damage of  $25.51 \pm 3.61\%$ . Of the 25 genotypes screened, ICCL 87311 recorded the lowest damage (20.37%), which was significantly less than 14 genotypes. From the pest susceptibility ratings (on a scale of 1-9), it was noticed that ICCL 87311 and ICCV 2 were scored at 5 and were most promising, whereas eight other genotypes ICCL 79033, ICCL 80129, ICCL 12746, ICC 12479, ICC 12480, ICCL 87314, IG 362 and Annigeri, were at 6 and suffered less damage than the resistant check ICC 506EB. Genotypes ICC 13, ICCX 730014, ICC 9854, ICC 5800 and ICC 12493, with a rating of 7, and ICC 926, ICC 12492 and ICCL 87220, with a rating of 8, were susceptible to *Helicoverpa* damage.

Genotypes ICC 9854 and ICC 12490 had grain yield of 1391 and 1483 kg ha<sup>-1</sup>, respectively, and were superior over resistant check, ICC 506EB. Despite, recording higher pod damage (26.86% and 26.23%), they recorded higher grain yield indicating their tolerance to *Helicoverpa* damage.

Thus, the genotypes ICCL 87311, ICCV 2, ICCL 12490 and ICC 9854, showed fairly good resistance/ tolerance against pod borer, and they derive an attention for *per se* cultivation by the farmers.

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## Preliminary evaluation of chickpea genotypes for resistance to pod borer and wilt complex

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Chickpea (*Cicer arietinum* L.) production in India (4.33 million tonnes in 1980 and 5.12 million tonnes in 2000) has stagnated in the last two decades. The major limiting factor has been the susceptibility of cultivars to several biotic and abiotic stresses that affects yield adversely (Singh et al. 1994). Most of the existing varieties are susceptible to fusarium wilt, ascochyta blight and podborer (*Helicoverpa armigera*), which are the major bottlenecks in increasing production potential of chickpea (Kalia and Dawa 1988; Singh et al. 1994). The parents which are resistant to pod borer and fusarium wilt are not yet available. In the present studies, one hundred and eighty four genotypes of chickpea were evaluated, during 2002-2003 at PAU, Regional Station, Faridkot, to find a donor for pod borer and wilt resistance, together.

The genotypes were sown in multiple disease sick plot with susceptible variety JG 62 as a check, in two replications. Each genotype has row length of 4 m and 30 cm apart with plant-to-plant spacing of 15 cm and recommended package of practices were followed. One hundred and eleven genotypes having less than 25% (moderately resistant reaction) combined score for wilt complex (wilt/foot rot/root rot complex) were selected for further entomological study. Pod borer infestation was recorded as percent bored pods of total pods at the end of harvesting. The data was subjected to analysis of variance to compare their relative performance (resistance) and the genotypes were categorized as per the method given by the All India Research Project on Soybean (1995) and used by Aditya Pratap et al. (2002) for chickpea.

The results on pod borer infestation are given in the Table 1. There was a large variation (30.87-70.65%) in pod damage among all the entries screened. Pod damage was highest in PBG 126 (70.65%) and lowest in IPC 96-3 (30.87%). Out of the 111 genotypes, 64 showed very high insect infestation and fell under lowly resistant group with infestation range of 52.15-70.65%. Forty-five genotypes were moderately resistant with infestation range of 34.05-51.65%. Only two genotypes IPC 96-3 and FG 1235 with mean infestation of 30.85% and

Table 1. Reaction of different genotypes of chickpea according to percent pod borer infestation.

S.No.	Type of resistance	Name of varieties
1 2	R = Resistant MR = Moderately resistant	FG 1235, IPC - 96 - 3 BG 1087, BG 1088, BG 1087, BG 1088, BG 1106, BGD 110, BGD 112, C53-104, CSJ 9807,FG 559, FG 711, FG 712, FG 908, FG 1044, FG 1100, FG 1121, FG 1184, FG 1186, FG 1204, FG 1206, FG 1212, FG 1221, FG 1222, FG 1228, FGK 848, FGK 1220, GL 1267, GL 20010, GL 940022, GLK 90079, GNG 469, GPF 2, H 82-2, IPC - 99 - 1, IPC 99-4, IPC 2000-1, PBG 195, PBG 233, PG 95424, PG 97403, RSG 902, RSG 906, WCG-3, WCG 98-1
3	LR = Lowly resistant	BCP 1002, BG 1053, BG 1067, BG 1080, BG 1103, BG 1108, BGD 32, C 235, CL 99033, CSJ 195, CSJ 253, CSJ 8962, FG 694, FG 702, FG 897, FG 974, FG 1056, FG 1197, FG 1205, FG 1210, FG 1217, FG 1224, FG 1225, FG 1227, FG 1231, FG 1232, FG 1238, FG 1268, FG 1292, FGK 1085, FGK 1133, FGK 1141, FGK 1170, FGK 1199, FGK 1218, GCP 9516, GG 1267, GL 769, GL 20035, GL 20081, GL 98014, GL 99103, H 87-23, H 97-23, H- 97-47, H 98-155, IPC 95-2, IPC 97-1, IPC 97-7, IPC 98-2, IPC 99-38, JG 1100, PBG 126, PBG 161, PBG 168, PBG 204, PBGK 220, PDG 3, PDG 4, PG 96005, PG 97121, PG 97128, WCG 97-16, WCG 9737

30.95% were found to be disease resistant (with 2.6 and 4.2% disease incidence, respectively).

Out of 45 moderately pod borer resistant genotypes 16 were having less than 5% disease incidence and 29 were having more than 5% incidence of disease. The important varieties such as GPF 2 and GNG 469 also fall in moderately resistant group with mean infestation of 43.25 and 41.8%. The genotypes, ie, BG 189, BG 373, BGD 110, FG 559, FG 711, FG 712, FG 908, FG 1184, FG 1206, GLK 90079, GPF 2, PBG 195, PBG 233, RSG 902, RSG 906 and WCG 98-1, reflected promising reaction by having less than 5% disease incidence and moderate resistance to pod borer.

Aditya Pratap et al. (2002) while evaluating the chickpea against pod borer, also reported wide variation (29.33 to 63.44%) in pest infestation among the varieties.

The study revealed that genotypes IPC 96-3 and FG-1235 were resistant to both wilt complex and pod

borer infestation and thus they can serve as potential donors for insect pests/disease resistance, in chickpea breeding.

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#### Genetics/Breeding/Biotechnology

## Identification of Dwarf and Extra-early Mutant of Pigeonpea [*Cajanus cajan* (L.) Millsp.]

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Plant height and maturity contribute significantly for pigeonpea cultivation under different cropping systems. Among the various constraints for higher pigeonpea production and productivity, Helicoverpa armigera (gram pod borer) is a major constraint (Shanower et al. 1999). Farmers mainly rely on insecticides to manage this borer. Traditional pigeonpea genotypes are tall (3-4 meters) and farmers have serious practical problems to use insecticides to manage pod borer through spray operations. Dwarf(1 meter) and high-yielding pigeonpea types are then an obvious choice to control the menace of the pod borer. Moreover, the adoption of pigeonpea cultivation by farmers on a large scale should accommodate crop rotations. Extra-short-duration pigeonpea genotypes could contribute to higher productivity of pigeonpeawheat rotation system (Dahiya et al. 2002). Even the existing pigeonpea short duration (140-150 days) types have been observed to delay the normal sowings of wheat crop.

Table 1. Distinguishing characters of the dwarf mutan	t
(HDM04-1) and the parental line (ICPL 88039).	

Characters	HDM 04-1	ICPL 88039
Days to 50% flowering	49±4.50	<u>9</u> 0 + 5.90
Days to maturity	90+5.50	<u>1</u> 35 + 6.20
Plant height (cm)	$\underline{1}03.20 + 10.20$	$\underline{2}71 + 11.32$
Fruiting branches/plant	<u>9</u> .10 + 3.21	12 + 2.96
Internode length (cm)	$\underline{3.40} + 0.70$	5.00 + 0.35
Pods/plant	$\underline{1}02.80 + 37.11$	132 + 25.48
Pod length (cm)	3.97 + 0.26	<u>5</u> .00 + 0.35
Seeds/pod	3.70 + 0.45	4.1 + 0.70
100 Seed weight (g)	8.84 + 0.80	<u>8</u> .17 + 0.75

To solve such problems, efforts were made to identify dwarf and extra-early genotypes through induced mutations. Besides dwarfness and earlyness, such genotypes should possess comparable yield levels to commercial types. Pigeonpea genotypes ICPL 88039 and 'Manak' were used for gamma rays irradiations. Five hundred, 1000 and 1500 dry, healthy and uniform seeds of each of the two genotypes were treated with 10,20 and 30 kR of gamma rays, respectively. The treated seeds were sown immediately in the field during rainy season 2001 to raise M<sub>1</sub> generation. In M<sub>1</sub> generation of ICPL 88039 population, one dwarfand extra-early mutant was obtained from 10 kR dose in ICPL 88039. The generation of this mutant was advanced to M<sub>4</sub> generations (2004) to obtain uniform progenies. The dwarf and extraearly M<sub>4</sub> progeny of this mutant is named as H D M 04 -1 and evaluated along with ICPL 88039 in field conditions during rainy season 2004. Five rows of the parent and the mutant genotypes were sown in 4 meter rows 45 cm apart. The plant-to-plant distance was kept at 10 cm. All the recommended cultural practices for pigeonpea were followed. Data were recorded on 50 random plants of HDM 04-1 and ICPL 88039 for morphological characters, viz, days to 50% flowering, days to maturity, plant height (cm), fruiting branches/plant, internode length (cm), pods per plant, seeds/pod and 100-seed weight (g). The data representing mean of 50 plants is presented in Table 1. The mutant possess 103.20 cm height with shorter internodes compared to ICPL 88039 (271 cm). It matures in 90 days as compared to 135 days of parental genotype. Its seed weight (8.84 g/100 seeds) is also higher than ICPL 88039 (8.17 g/100 seeds). The commercial cultivars normally possesses 6.0-7.0 g/100 seeds. Its yield levels are at par with the ICPL 88039. However, yield level are yet to be confirmed through large-scale trials against checks and with varying spacings and fertility regimes.

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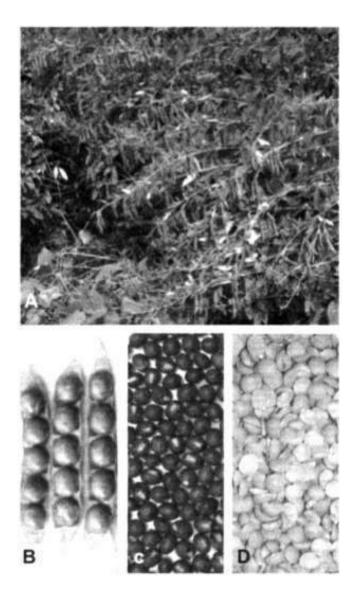
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# ICP 7035 - A Sterility Mosaic Resistant Vegetable and Grain Purpose Pigeonpea Variety

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Pigeonpea (Cajanus cajan) is an important pulse crop in Karnataka, India. Sterility mosaic disease (SMD), caused by the pigeonpea sterility mosaic virus (PPSMV) and transmitted by an eriophyid mite, Aceria cajani, is a major problem of pigeonpea (Jones et al. 2004). Pigeonpea yields have been declining due to heavy and recurring occurrence of the SMD in southern Karnataka. Most of the pigeonpea genotypes available for farmers are highly susceptible to the SMD. This was more so because of the PPSMV isolate prevalent in southern Karnataka-the Bangalore (B) isolate-is highly virulent and host-plant resistance to it are scarce. ICP 7035, a landrace collected in 1973 from Bedaghat (near Jabalpur) Madhya Pradesh state, India (Sharma and Reddy, unpublished), was found to be consistently resistant to PPSMV-B isolate. ICP 7035 was evaluated against ten PPSMV isolates at several locations in India, and the genotype was found resistant to all these isolates (Reddy et al. 1993; Kumar et al., unpublished).

ICP 7035 was evaluated, along with the two local varieties, TTB7 and Hy3C, in SMD and wilt nursery at



**Figure 1.** ICP 7035: Pod bearing plant (A), vegetable pods (B), dried whole seed (C) and dried decorticated split seeds - *dhal* (D).

Year	% SMD			Green	pod yield (kg	ha <sup>-1</sup> ) <sup>1</sup>	Grain yield (kg ha <sup>-1</sup> ) <sup>1</sup>			
	ICP7035	Hy3C	TTB7	ICP7035	НуЗС	TTB7	ICP7035	Hy 3C	TTB7	
1999	0	15.5	60.5	5085	4521	1785	_2	-	-	
2000	0	11.0	82.0	3551	2958	101	-	-	-	
2001	0	18.2	75.5	4268	3658	1210	1905	1825	2357	
2002	0	23.0	90.3	6107	5189	521	1349	1312	1706	
2003	0	<2.0	-	7153	7101	-	1824	1736		
Mean	0	16.93	77.08	5232.8	4685.4	904.25	1692.6	1624.3	2031.5	

Table 1. Green pod and grain yield of three pigeonpea genotypes at Bangalore.

1. Green pod and grain yields are from separate trials

2. '-' not tested

the Gandhi Krishi Vignana Kendra (GKVK), Bangalore; and also under natural conditions in the State Agriculture Research Stations and farmers' fields in Bangalore Rural, Tumkur and Kolar districts of Karnataka, during 1999-2004 rainy seasons. ICP 7035 produced a mean vegetable pod yield of 5232.8 kg ha<sup>-1</sup> and dry seed yield of 1692.6 kg ha<sup>-1</sup> as compared to 4685.4 kg ha<sup>-1</sup> mean vegetable pod yield and 1624.3 kg ha<sup>-1</sup> of dry seed yield for Hy3C (Table 1). Average SMD incidence in susceptible cultivars ranged from less than 2.0 to 90.3% during various years, but ICP 7035 remained free from SMD (Table 1). Stability of SMD resistance in ICP 7035 was verified by exposing test plants to high dose of PPSMV-B inoculum using viruliferous *A. cajani* by following the

Character	ICP7035	Hy3C	TTB7
Plant characters			
Plant height (cm) <sup>1</sup>	160-180	160-170	160-180
Stem colour	Green	Purple	Green
Flower arrangement	Intermediate	Clusters	Clusters
Flower colour	Yellow purple	Red	Yellow
Pod colour	Purple with dark green streaks	Green with black streaks	Green with black streaks
Seed coat colour (fresh)	Light purple and mottled	Light green and plain	Light green and plain
Seed colour (fresh)	Plain green	Plain green	Plain green
Seed coat colour (dry)	Brown and mottled	Dull white	Brown
Seed (dhal) colour (dry)	Yellow	Dull white	Yellow
Days to 50% flowering	<b>75-8</b> 0 <sup>2</sup>	80-90 <sup>2</sup>	90-100 <sup>2</sup>
Days to maturity	160-170 <sup>2</sup>	170-180 <sup>2</sup>	180-200 <sup>2</sup>
Pods per plant <sup>3</sup>	90-110	70-80	90-110
Seeds per pod	5	4-5	4-5
Pod length (cm)	7.5	_4	
100 fresh seed weight (g)	39.6	20.5	17.21
100 dry seed weight (g)	19.2	16.1	10.5
100 fresh pod weight (g)	254.2	-	79.63
Post harvest qualities of dried seed <sup>5</sup>	5		
Good quality split seed (dhal) (%)	85.8	86.47	85.53
Broken split seeds (%)	1.72	0.78	2.62
Recovery of husk (%)	14.52	14.74	13.54
Nutritional factors in dhal <sup>6</sup>			
Cooking time of vegetable seed (min)	) 35.62	35.25	35.33
Cooking time of dhal (min)	47.7	42.3	36.8
Water absorption (%)	102.06	104.12	102.54
Solids in the aqueous extract (%)	10.63	12.21	11.46
Moisture (%)	11	10.8	8.1
Protein in dried seeds (%)	19.6	22.14	23.6
Soluble sugars (%)	5.3	3.7	-
Fat (%)	2.4	2.3	-
Methionine (mg g <sup>-1</sup> of seed)	1.99	2.07	-
Methionine (mg $g^{-1}$ of protein)	8.82	9.35	-
Cystine (mg $g^{-1}$ of seed)	1.80	1.87	_
Cystine (mg g <sup>-1</sup> of protein)	7.98	8.45	_

Table 2. Morphological, cooking and nutritional characters of three pigeonpea genotypes.

1. At the time of pod maturity (around 170 days; plant can grow up to 2 m).

2. In Bangalore region.

3. First pod picking at maturity (around 170 days).

4. '-' Not tested.

5. Determined with mechanical 'dhal' mill.

6. Estimated at Pristine Laboratories, Bangalore.

leaf-stapling technique. Plants were monitored for PPSMV by EL1SA method as described in Kumar et al. (2002). All inoculated plants remained symptom free and tested negative to PPSMV, and no vector multiplication observed on these plants. To determine whether the observed resistance was against virus and/or due to vector non-preference, the genotype was tested by petiole graft inoculation as described in Kumar et al. (2002). All graft-inoculated ICP 7035 remained uninfected, indicating that plants were resistant to the virus. ICP 7035 was also evaluated for fusarium wilt and alternaria blight resistance at GKVK, Bangalore. The genotype showed moderate resistance to both these fungal diseases (<10% incidence), whereas TTB-7 is highly susceptible to wilt and blight, and Hy3C is moderately resistant to wilt (<10% incidence), but it was not tested against blight. Up to 35.7% H. armigera incidence was observed on ICP 7035, whereas on TTB7 and Hy3C, it was 55.3% and 28.75%, respectively.

ICP 7035 is a medium duration, non-determinate variety. Plants mature in 170-200 days (in south-central regions of India) and at this stage it reaches to an average height of 120-140 cm (Fig 1). Each plant produced around 100 pods and each pod contained 5 seeds, which are nutritionally rich and contain highest percent of digestible carbohydrates, vitamins and micronutrients (Table 2). Fresh seeds are large (9-11 mm diameter) with purple seed coat and green cotyledons, and suitable for consumption as vegetable (Table 2). Fresh seed contains 8.6% protein, 12% fibre and 45.7% carbohydrate and starch. The pinkish-purple colour of pod and seed coats was due to high anthocyanin content, which adds to health benefits as dietary antioxidants. In addition, sweetness of the pigeonpea seed is a preferred trait for vegetable purpose. While normal sugar levels in most pigeonpea varieties is about 5%, sugar content in ICP 7035 seeds is 8.8% (Paris et al. 1987). Decorticated dried split seeds measures 5-6 mm in diameter and 100 dried seeds weigh 19.2 g (Table 2). It contains 19.6% protein, 27.4% dietary fibre, 33% starch, and 67% carbohydrate. It is also rich in copper, calcium, magnesium, phosphorous, and has good dhal making quality.

SMD resistance in ICP 7035 has positive impact on yield as a result of negligible crop loss in endemic areas contributing to the revenue gains to the farmers at no additional cost. Under no disease situation, the crop yields are on par with the local varieties. ICP 7035 does not alter input requirements from existing practice. Cultivation of ICP 7035 prevents buildup of SMD inoculum during the cropping and off-season and controls the disease spread in the fields. Recently, provisional approval was given for the release of this variety in SMD endemic areas of southern Karnataka.

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# Effect of Improved Crop Production Technology on Pigeonpea Yield in Resource Poor Rainfed Areas

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Pigeonpea (*Cajanus cajan* (L.) Millspaugh) is a deeprooted and drought-tolerant (Troedson et al. 1990) leguminous food crop used in several countries particularly in India as a source of dietary protein. India accounts for about 80% of the total world pigeonpea production. It is one of the principal dryland crops in Andhra Pradesh with a very low productivity (450 kg ha<sup>-1</sup>). The production is constrained by the use of less productive land, water logging or dry spells during critical stages of crop growth, pest and disease problems, and lack of drought-resistant, high-yielding genotypes, and appropriate agronomic management.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Government of Andhra Pradesh have initiated the Andhra Pradesh Rural Livelihoods Project (APRLP) in the drought prone districts of Andhra Pradesh state of India, viz, Kurnool, Mahabubnagar, Nalgonda, Anantpur and Prakasam, to help reduce poverty by increased agricultural productivity and improved livelihood opportunities through technical backstopping and convergence through a consortium of institutions. Watersheds are used as an entry point for these activities.

Nandavaram village of Banaganapalle mandal in Kurnool district was selected as a representative watershed site based on the extent of rainfed area in the district, current crop productivity, and willingness of the community to participate in the on-farm research activities. Systematically collected soil samples from thirty farmers' fields in the Nandavaram watershed on a toposequence were analyzed for physical and biological parameters and various nutrients. The soil analysis indicated that all the fields are low in N (496 mg kg<sup>-1</sup> soil), low to medium in available P (5.71 mg kg<sup>-1</sup> soil) (Olsen's P), high in exchangeable K (223 mg kg<sup>-1</sup> soil), and low in available Zn (0.39 mg kg<sup>-1</sup> soil), S (7.52 mg kg<sup>-1</sup> soil) and B (0.5 mg kg<sup>-1</sup> soil). The information from soil analysis along with historical rainfall, and minimum and maximum temperature data enabled to calculate the length of growing period (LGP). This critical information assisted in identifying better options for pigeonpea cultivation to improve the productivity levels and for sustaining the natural resources.

Twelve on-farm trials were conducted during the 2002/03 rainy season with the objective to demonstrate the effect of improved production technologies over farmers' practice. Improved production technology was compared with the farmers' method in an area of 1000 m<sup>2</sup> in each of the farmers' fields. The improved technology package included medium duration high-yielding variety (ICPL 87119) resistant to fusarium wilt and sterility mosaic diseases; a seed rate of 12 kg ha<sup>-1</sup>; seed treatment with thiram (3 g kg<sup>-1</sup> seed); inoculation with *rhizobium;* a fertilizer dose of 20 kg N and 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>; basal application of micro-nutrient mixture of 5 kg borax (0.5 kg B ha<sup>-1</sup>), 50 kg zinc sulphate (10 kg Zn ha<sup>-1</sup>) and 200 kg gypsum (30 kg S ha<sup>-1</sup>) per hectare together with

Table 1. Yield and economics of pigeonpea in on-farm trials (average of 12 trials), Nandavaram nucleus watershed, Kurnool
district, Andhra Pradesh, rainy season 2002.

Cultivation method	Grain yield (t ha <sup>-1</sup> )	Stalk yield (t ha <sup>-1</sup> )	Cost of cultivation (Rs ha <sup>-1</sup> )	Net return (Rs ha <sup>-1</sup> )	Benefit cost ratio
Improved production technology	1.61	2.93	6838	16476	2.4
			(US\$152)	(US\$366)	
Farmers' practice	0.53	1.10	4260	3437	0.8
			(US\$95)	(US\$76)	
SE±	0.096	0.202	14.2	1393.8	
CV%	31.2	34.7	0.9	48.5	
LSD (5%)	0.30	0.63	44.3	4338.3	

Cultivation method	Total dry matter (t ha <sup>-1</sup> )	Pod weight (tha <sup>-1</sup> )	Shelling (%)	100 grain weight (g)	Harvest index
Improved production technology	5.26	2.33	69.1	10.3	0.31
Farmers' practice	1.92	0.82	65.6	9.0	0.28
SE±	0.321	0.132	0.93	0.31	0.009
C V %	31.0	29.0	4.8	11.1	10.3
LSD (5%)	1.00	0.41	2.89	0.96	0.027

 Table 2. Yield components of pigeonpea in on-farm trials (average of 12 trials), Nandavaram nucleus watershed, Kurnool district, Andhra Pradesh, rainy season 2002.

appropriate need-based pest and disease control measures. Two inter-cultivations at 25 and 50 days after sowing to control weeds were taken up. One insecticide spray was given at pod formation stage to control pod borers. The farmers' method included a seed rate of 10 kg ha<sup>-1</sup> and a fertilizer dose of 12 kg N and 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Entire dose of N and P was applied as basal. The seasonal rainfall was 695 mm. The data was analyzed considering farmers as replications using analysis of variance (ANOVA) with randomized blocks on GENSTAT. A NOVA indicated that management practices (improved crop production technology and farmers practice) differed significantly for all the parameters presented in Tables 1 and 2.

The improved production technologies gave higher yields and recorded a mean grain yield of 1.61 t ha<sup>-1</sup> which was 204% higher than that obtained with the farmers' practice yields of 0.53 t ha<sup>-1</sup> (Table 1). In addition to increased grain yields, improved technology also resulted in higher stalk yield of 2.93 t ha<sup>-1</sup> compared to 1.10 t ha<sup>-1</sup> of farmers' practice. The increased grain and stalk yields with improved production practice were mainly because of increased total dry matter, increased pod weight, higher shelling percentage, higher 100-grain weight and harvest index (Table 2). Yield increase in response to recommended fertilizers and rhizobium inoculation were also reported by Jain et al. (1988).

The economic viability of improved technology over the farmers' practice was calculated depending on prevailing prices of inputs and outputs. The additional cost of US\$57 ha<sup>-1</sup> (Table1) incurred due to the improved technology as compared to farmers' practice was mainly due to balanced fertilization (micro-nutrients and additional N and P), additional seed cost, seed treatment, 1PM and one additional inter-cultivation. However, the improved technology resulted in an increased mean income of US\$290 with a cost-benefit ratio of 2.4 (Table1). This additional income could substantially benefit the resource poor farmers and improve their livelihoods in the dry regions of the district. Puste and Jana (1995) reported that the yield attributes and seed yield of pigeonpea varieties were significantly influenced by phosphorus and zinc application with a maximum benefit-cost ratio of 4.12. Yadav et al. (1997) reported that with the application of 100% recommended fertilizer, sole pigeonpea gave a grain yield of 2.12 tha<sup>-1</sup> with net returns of Rs 12,491 per hectare and a benefit-cost ratio of 2.94. The results from the current study indicate the potential benefits of improved production technology in enhancing pigeonpea yields and net returns in the dry regions of Andhra Pradesh.

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## **Pathology**

## Occurrence of *Urentius hystricellus* (Richt.) on Pigeonpea in the Net-House

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The lace bug Urentius hystricellus (Richt.) (U. echinus Distant) (Tingidae: Hemiptera) was first reported the eggplant, occurring on Solanum melongena Linnaeus, in different parts of India by Fletcher (1914). Since then, it has been reported from time to time as a specific pest of eggplant (Pillai 1921; Jepson 1924; Patel and Kulkarni 1955), with a degree of varietal preference in India. Recently, Chaudhury et al. (2001) recorded its presence on tomato crop in the tarai region of West Bengal. Besides India, it has also been reported from Ghana (Frempong and Buahin 1977) and Thailand (Tigvattn 1990). Nymphs and the adults of the lace bug suck sap from lower surface of leaves causing its yellowing and can be seen congregating. Affected leaves are covered with exuviae and excreta.

A total of 15 genotypes of pigeonpea, Cajanus cajan (L.) Millsp., viz., Pusa-33,991,992,2001,2001-1,2002-1, 2002-2, 2003-2, 2004-1, 2004-2, AK 2000-3 N 3, AK 2000-60 N 85, H 89-9x 85024 1 DT SP 2, MS Pusa 33x H 88-45, and RG 02-47 N were potted in the net-house, Division of Nematology, Indian Agricultural Research Institute, New Delhi 12, on 22 June 2004. After one month of sowing, 50-60 percent leaves of all the plants were infested with U. hystricellus, irrespective of genotypes. Observations revealed that pigeonpea is a new host record.

The lace bug infestation was also observed on another unrecorded host (*Abutilon theophrastii*), which is a weed of the wet season. It can be inferred that, although this insect spp is known to inflict injuries mainly to the eggplant, its spread on the other plant species in the vicinity cannot be ignored. Hence, the cultivation of eggplant away from the pigeonpea crop is suggested. With regards to curative measures against the infested plants, further studies indicated that spray application of karate 2.5 EC (lambda cyhalothrinI)@1mL/2L water provided satisfactory protection with in a week's time.

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# Response of Resistant Germplasm to Different Races/Populations of Pigeonpea Cyst Nematode, *Heterodera cajani*

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The existence of races (race A pigeonpea race and race B clusterbean race) in *Heterodera cajani* Koshy 1967, an important plant parasitic nematode of leguminous crops, has been reported by Walia and Bajaj (1986, 1988). Source of resistance against an unspecified population of *H. cajani* in *Cajanus platycarpus* accessions 1CPW 543, ICPW 544 and ICPW 545 (Elyas and Sharma 1997) and against Coimbatore population of *H. cajani* have been reported in *Phaseolus radiatus* L. cv TM 96-1 (Anon. 1998). Reaction of several populations of this species collected from different parts of India and belonging to two races against these resistant sources and also against *Glycine max* is discussed here under.

Table 1. Reaction of Cajan us platycarpus accessions to different races of pigeonpea cyst nematode, Heterodera cajani.

			Number			
		Race A			Race B	
Accessions	White (Female)	$J_2$	Male	White (Female)	$J_2$	Male
ICPW 543	0	0	0	6.0	5.0	1.3
ICPW 544	0	0	0	33.0	14.0	7.0
ICPW 545	0	0	0	8.0	3.7	2.3
Pigeonpea cv Manak (Control)	72.0	14.0	18.0	103.3	26.7	17.3
CD (P=0.05)	-	•	-	0.58	0.68	0.60

Populations of *H. cajani* race A and race B collected/ procured from various parts of India (Table1) and their pure cultures were maintained in isolation on their respective hosts under screenhouse conditions at the Department of Nematology, CCS Haryana Agricultural University, Hisar. The egg sacs of different races/ populations were incubated at room temperature (30  $\pm 2^{\circ}$ C) separately for collecting second stage juveniles of this species, when needed.

Seeds of C. platycarpus accessions ICPW 543, ICPW 544 and ICPW 545, and mung bean cv TM 96-1, and germplasm lines of soybean were procured from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad; the Indian Institute of Pulses Research (Kanpur), CCS and Haryana Agricultural University (Hisar), respectively. They were sown singly in 15 cm earthen pots of autoclaved sandy loam soil. Seeds of C. platycarpus accessions were, however, mechanically scarified before sowing. After 3 days of germination of seedlings, pots containing different plant species were inoculated @ 200 freshly hatched second stage juveniles/pot in the following manner:

- 1. *Cajanus platycarpus* accessions: Second stage juveniles of race A and race B
- Mung bean cv TM 96-1 and soybean cv PK. 564: Second stage juveniles of various populations (Table 2).
- 3. *Glycine max* germplasm lines: Second stage juveniles of race A and race B.

The plants were depotted after 50 days of inoculation and the soil was processed for white females, juveniles and males. Each treatment was replicated thrice.

Heterodera cajani race A failed to multiply on all the three accessions as reported by Elyas and Sharma (1997). However, race B reproduced on all the three accession lines though with different rate of multiplication (Table 2). Among these accessions multiplication of this race was significantly higher in ICPW 544 than ICPW 545 and ICPW 543. From the above studies it becomes clear that same cultivars show different reaction to different populations/races of H. cajani and therefore, one should be cautious while incorporating the resistance in the cultivars. Walia and Bajaj (1986, 1988) differentiated races of H. cajani on the basis of their reproduction on clusterbean and sun hemp. ICPW 544 can also be included as a differential host for discriminating races of H. cajani. Since race A failed to multiply on C. platycarpus accessions and race B multiplication was higher in ICPW 544.

All the populations belonging to race A as well as race B reproduced on Phaseolus radiatus L. cv TM 96-1 but with different rates of multiplication. Dharwar, Coimbatore, Ludhiana and Yamunanagar populations belonging to race A reproduced very less (1-10 cysts/ pot) and were statistically at par among each other and hence this cultivar can be designated as resistant to these populations. These results are in agreement with earlier findings (Anon. 1998). Multiplication of rest of the populations was moderate to high. Multiplication of Anand (Gujarat), Jaipur (Rajasthan) and Pusa (Bihar) was moderate in reproduction on TM 96-1 (11.60 cysts/ pot) and statistically at par. Multiplication of clusterbean, Hisar (Haryana), belonging to race B was moderate (60 cysts/pot) but differed significantly from the rest of the populations. Reproduction of New Delhi (Delhi), Pigeonpea, Hisar (Haryana) and Kanpur (U.P.) populations was maximum (>100 cysts/pot) and hence

Table 2. Reaction of Glycine max cv PK 564 and Phaseolus radiatus cv TM 96-1 to different populations of H. cajani

			Number			
	White (Female)	$J_2$	Male	White (Female)	$J_2$	Male
Populations	C	v PK 564			cv TM 96-1	
Race A						
Anand (Gujarat)	0	0	0	30.0	9.3	2.0
Coimbatore (Tamil Nadu)	0	0	0	8.3	2.0	1.0
Dharwar (Karnatka)	0	0	0	10.0	3.0	3.0
Hisar, Pigeonpea (Haryana)	0	0	0	102.0	27.7	14.0
Jaipur (Rajasthan)	0	0	0	30.0	11.7	4.0
Kanpur (U.P.)	0	0	0	117.7	27.3	8.0
Ludhiana (Punjab)	0	0	0	9.0	4.7	2.0
New Delhi (Delhi)	0	0	0	118.7	24.0	10.0
Pusa (Bihar)	0	0	0	32.3	8.3	3.0
Yamunanagar (Haryana)	0	0	0	7.0	5.0	2.0
Race B						
Hisar, Clusterbean (Haryana)	0	0	0	60.0	17.3	7.0
C.D. (P = 0.05)	-	-		12.6	6.3	0.3

mung bean cv TM 96-1 can be categorized as susceptible to these populations. Mung bean (TM 96-1) responds differently to different populations of *H. cajani* and therefore, it is essential to test the virulence of a particular population before using it as a source of resistance for incorporation.

No multiplication of any population of *H, cajani* was found in soybean cv PK 564 (Table 2). Also representative populations of both races of *H. cajani* failed to multiply on all the tested germplasm (AVT 1 PK 416, AVT 1 Pusa 16, MLT PK 416, MLT PK 471, MLT PK 472, SST 1 PB 1, SST 1 PK 472, SST 1 PS 1024). Koshy and Swarup (1973) found a very less reproduction of *H. cajani* on soybean cv Glycine 24, but no multiplication on cvs. Lee, Roanoke and IC 9620. From the above studies it can be speculated that soybean is either a no host or a very poor host for *H. cajani* unlike *H.* glycines Ichinohe, 1952.

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## Entomology

## Biological Activity of Lectins from Grain Legumes and Garlic against the Legume Pod Borer, *Helicoverpa armigera*

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Cotton bollworm/legurae pod borer, Helicoverpa armigera (Hubner), is one of the most devastating crop pests worldwide (Sharma 2001). It has a wide host range, and feeds on more than 300 plant species. Due to indiscriminate use of insecticides, it has developed high levels of resistance to conventional insecticides (Kranthi et al. 2002). Therefore, it is important to develop alternative methods of controlling this pest, including host plant resistance. However, the levels of resistance to H. armigera in the cultivated germplasm of several crops are low to moderate. Therefore, improving plant resistance to pests through genetic transformation, has raised hopes of using plant resistance as an effective weapon for pest management (Sharma et al. 2004). This includes incorporation of novel genes such as crystal protein from Bacillus thuringiensis (Bt-Cry genes), enzyme inhibitors (such as protease and alpha amylase inhibitors), vegetative insecticidal proteins (VIPs), small RNA viruses (SRVs), and secondary plant metabolites (SPMs). While the activity of Bt-Cry proteins has been investigated extensively, there is very little information on the biological activity of other insecticidal genes that can be used to confer resistance to insects in transgenic plants (Hilder and Boulter 1999). Therefore, we evaluated the biological activity of plant lectins as candidate genes for conferring resistance to H. armigera.

Lectins are carbohydrate-binding proteins (or glycoproteins) of non-immune nature, and bind reversibly to specific mono- or oligo-saccharides (Goldstein et al. 1980, Van Damme et al. 1998). They play an important role in the plant's defense against insect pests, and have been found to be toxic to viruses, bacteria, fungi, insects and higher animals. This paper reports the biological effects of plant lectins from field bean (*Phaseolus vulgaris*), pigeonpea (*Cajanus cajan*), chickpea (*Cicer arietinum*), and garlic (*Allium sativum*)

50 ICPN 12, 2005

along with snowdrop (Galanthus nivalis) lectin on the growth and development of H, armigera so as to identify the candidate genes for deployment through transgenic plants to control this pest.

Lectins extracted from chickpea, pigeonpea, garlic (garlic lectin I = from garlic leaves; garlic lectin II = from transgenic tobacco) and field bean were bio-assayed along with snowdrop lectin against the neonate larvae of H. armigera. The lectins were bio-assayed against the neonate larvae of H, armigera by treating the surface of the artificial diet (Armes et al. 1992) in a glass vial (2 cm diameter and 3.5 cm height) with 100 ml of different lectins. Each glass vial contained 5 ml diet. The lectin solutions were prepared in phosphate buffer (pH 6.8, molarity 0.2 M). The buffer was prepared by mixing 51.0 ml of A [0.2 M solution of mono-basic sodium phosphate (27.8 g in 1000 ml)] and 49.0 ml of B [0.2 M solution of dibasic sodium phosphate (53.65 g of Na<sub>2</sub>HPO<sub>4</sub>.7H<sub>2</sub>O or 71.7 g of Na<sub>2</sub>HPO<sub>4</sub>.12H<sub>2</sub>O in 1000 ml)] diluted to a total of 200 ml with distilled water. Lectins dissolved in phosphate buffer were spread uniformly over the diet surface with a micropipette, and allowed to dry under the table fan in the laboratory for 4 h. One neonate larva was released in each vial and observations were recorded on weight of the larvae five days after initiating the experiment, and larval, pupal, and total development period. Each treatment was replicated three times in a completely randomized design. There were 10 larvae in each treatment. Observations on larval weights were recorded 5 days later, while pupal weights were recorded one day after pupation. Data were also recorded on adult emergence. The data were subjected to analysis of variance.

The weights of the larvae at 5 days after initiating the experiment ranged from 16.54 mg on the artificial diet with buffer to 26.90 mg in diet treated with field bean lectin as compared to 22.68 mg in the untreated control diet (Table1). However, the differences in larval weights in diets with different lectins were not significant. The larval weights were also quite low in the diet treated with phosphate buffer only. This may be because of some effects of the buffer on the pH of artificial diet. However, no adverse effects of the buffer were observed on larval and pupal periods and the pupal weights. The weight of the pupae reared on diet containing garlic lectin II (from transgenic tobacco) was significantly lower (283.81 mg per larva) as compared to those fed on untreated control diet (325.00 mg per larva). None of the lectins tested showed any adverse effect on larval period. Pupal period of the insects reared on diet containing lectins from field bean, pigeonpea, chickpea and garlic, was significantly

Table 1. Bio-efficacy of lecting extracted from grain Patanchern 2002).	l frota graia le	gues and gar	lic on survival	legences and garlic on survival and development of legune pod borer, <i>Helicoverpa aradyre</i> s (ICRISAT	t of legume y	od borer, A	leticoverpa armi	ģere (ICRISAT
	Despe	Larvel wrinht (mo)	Pupal weinht	Larvai rerind	Pupai nerind	Pumbhian	Adult	Sex setio
Treatment	(mg per cm <sup>2</sup> )	(S DAI)	(2016) (2016)	(days)	(days)	(X)	(%)	(females:males)
Untreated control diet	1	22.68	325.00	14.101	15.633	76.67	46.67	1:1.18
Artificial diet with buffer	I	16.54	317.16	14.500	12.500	73.33	43.33	1:0.86
Field bean loctim	0.032	26.90	305.66	14.258	12.194	66.67	36.67	1:0.34
Pigeonpea lectin	0.032	22.02	312.00	14.222	716.11	60.00	33.33	95.0:1
Chickpes lectin (1.72 mg/ml)	0.032	22.65	306.20	14.333	11.711	80.00	43.33	1:1.56
Chickper lectin (in 60% (NH,),SO,)	0.032	22.25	304.29	14.222	11.083	60.00	36.67	1:0.75
Chickpea lectin (6 mg/ml)	0.032	24.48	315,46	14.073	11.744	66.67	40.00	1:1,26
Garlie lectin I (from garlie)	0.032	20.95	297.09	14.458	12.056	56.67	33.33	\$0.1:1
Garlie lectin [] (from transgenic tobacco)	0.032	22.83	283.81	14.535	12.622	60.00	33.33	1:1.14
Snowdrop lectin	0.032	24.12	308,57	14.151	12.322	70.00	40,00	1:1.44
Mean		22.54	307.52	14.29	I2.38	67.00	38.67	r
SE		±2.311	±11,96	±0.318	±],[44	±6.55	#6.95	I
LSD at 5%		6.87	35.55	NS	3.40	SN	NS	I
DAJ = Days after initiating the experiment. NS ~ Non-significat	<ul> <li>Non-significant</li> </ul>							

shorter than those reared on the untreated control diet.

The differences in percentage pupation and adult emergence were not significant. However, less than 60% pupation was recorded in diets treated with lectins from pigeonpea, chickpea in 60% ammonium sulphate solution, garlic, and garlic lectin extracted from transgenic plants as compared to 76.67% in untreated artificial diet. Adult emergence ranged from 33.33% in diets treated with pigeonpea and garlic lectin to 46.67% in untreated control diet. The sex ratio (males:females) was affected adversely in diets treated with lectins from field bean and pigeonpea.

Anti-insect properties of the plant lectins have earlier been reported against European corn borer, Ostrinia nubilalis (Czapla and Lang 1990). The snowdrop lectin (GNA) has previously been shown to be toxic to Homoptera (Rahbe et al. 1995; Powell et al. 1995,1998), Lepidoptera (Fitches et al. 1997), and Coleoptera (Gatehouse et al. 1995; Elden 2000). Snowdrop lectin (2%) inhibited feeding and reduced the weight of spotted pod borer, Maruca vitrata larvae (Machuka et al. 1999) and tomato moth (Lacanobia oleracea) (Fitches et al. 1997). Such effects of GNA were not observed in the present studies, possibly because of low concentrations used in the present studies.

Lectins have been reported to affect the survival and development of insect pests (Janzen et al. 1976; Shukle and Murdock 1983; Czapla and Lang 1990; Habibi et al. 1993; Gatehouse et al. 1993, 1995; Powell et al. 1995; Law and Kfir 1997). They bind to the glycan receptors present on the surface lining of the insect gut (Pusztai and Bardocz 1996), and interfere with the formation and integrity of the peritrophic membrane of the midgut (Harper et al. 1998), but how that affects the digestive physiology is unknown. Larval weights were slightly greater in diets treated with GNA, chickpea lectin, and field bean lectin. Similar effects of soybean lectin have earlier been reported in case of O. nubilalis (Czapla and Lang 1990). Percentage pupation was low (<60%) in diets treated with pigeonpea lectin, chickpea lectin in 60% ammonium sulphate solution, and garlic lectin, while adult emergence was low in diets treated with pigeonpea and garlic lectin. The garlic lectin had an adverse effect of the larval and pupal weights of H. armigera, but not on the duration of larval and pupal development. The lectins from garlic and pigeonpea can possibly be deployed in transgenic plants in combination with Bt genes to increase the levels of plant resistance to Н. armigera.

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Notes

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The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit, nonpolitical organization that does innovative agricultural research and capacity building for sustainable development with a wide array of partners across the globe. ICRISAT's mission is to help empower 600 million poor people to overcome hunger, poverty and a degraded environment in the dry tropics through better agriculture. ICRISAT belongs to the Alliance of Future Harvest Centers of the Consultative Group on International Agricultural Research (CGIAR).

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