International Chickpea and Pigeonpea Newsletter

No. 9 2002

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 ëmini reviewsí whenever possible.
- Personal news (new appointments, awards, promotions, change of address, etc.)

How to format contributions?

- Keep the items briefóremember, 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 (e.g., use tons instead of kg). Every table should fit within the normal typewritten area of a standard upright page (not a ëlandscapeí page).
- Black-and-white photographs and drawings (prepared in dense black ink on a white card or a heavy-duty tracing paper) are welcomeó 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 shortónot 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. Please send a file, which should match the printout, on a double-sided/high density IBM-compatible disk using **Microsoft Applications**.
- Contact the Editor for detailed guidelines on how to format text and diskettes.
- **Include the full address with telephone, fax, and email numbers of all authors.**

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

Contributions should be sent before 31 March to:

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Contents

Pathology

Biotechnology

Utilization

Pigeonpea

Breeding

Pathology

Entomology

Biotechnology

Publications

From the Editor

Although this issue of the International Chickpea and Pigeonpea Newsletter (ICPN) contains many articles from Asia, it also includes some articles from developed countries, indicating the growing importance of these crops. A substantial research on these crops is being carried out in Africa and ICPN can be a good informal vehicle to bring this research to wider readership. High proportion of our research results remain unpublished or are published in vernacular publications, thus depriving a large section of the scientific community, the outcome of scientific efforts. I urge scientists to share their research results with the readers of ICPN. Let us publish more in ICPN and share knowledge through this newsletter.

To reduce time in acceptance of papers for publication in the ICPN, I request authors to follow ICPN guidelines for format and length of contributions.

I would like to acknowledge Y S Chauhan, P K Gaur, C L L Gowda, N Kameswara Rao, R V Kumar, J V D K Kumar Rao, N Mallikarjuna, S Pande, R Folkertsma, O P Rupela, K B Saxena, N P Saxena, H C Sharma, K K Sharma, S D Singh, R P Thakur, and F Waliyar as reviewers of contributions to this issue of ICPN, and the Learning Systems Unit at ICRISAT for compiling the SATCRIS listings and verifying the references cited in this issue.

My predecessors, Drs S N Silim and R B Jones, have laid the solid foundation and set high standards for ICPN. I assure you that with cooperation from contributors and readers, we will try our best to ensure that ICPN continues to maintain high standards in disseminating information efficiently and effectively among chickpea and pigeonpea workers.

H D Upadhyaya

News

About Scientists

Jagdish Kumar, Principal Scientist (Chickpea Breeding and Genomics), ICRISAT, Patancheru, India is on secondment to the Government of Canada as a Research Scientist with Agriculture and Agri-Food Canada, Delhi, Ontario, Canada. He was awarded the International Pulse Improvement Award by the North American Pulse Improvement Association in recognition of his contributions to chickpea research and development.

P M Gaur, Senior Scientist (Chickpea Breeding), Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh, India joined as Visiting Scientist in Genetic Resources and Enhancement Program, ICRISAT, Patancheru, India on 16 August 2001 for a period of one year to work in place of Dr Jagdish Kumar, Principal Scientist (Chickpea Breeding and Genomics) who is presently in Canada.

ICRISAT Scientist Honored with China's Highest National Scientific Award

K B Saxena, Senior Pigeonpea Breeder at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was selected by the State Council of People's Republic of China for the **'2001 Friendship Award'**. Saxena and his wife, Suman, were invited to attend the award-giving ceremony on 29 September 2001

in Beijing during the celebrations of the 52nd anniversary of the People's Republic of China.

The Friendship Award, instituted in 1991, is the highest recognition given by the Chinese Government to foreign experts. Saxena received this award in appreciation of his outstanding contributions and dedication to China's social development and economic, scientific, technological, educational, and cultural construction.

Saxena had earlier been honored with **'Golden Love Ball Award'** from the Guangxi Province of China for his scientific contribution to the development of the province.

The mountain slopes in southern China have serious soil erosion and low productivity problems. Saxena successfully introduced ICRISAT-bred short-duration pigeonpea varieties in this region to minimize the soil erosion and provide fodder/feed to animals and fuelwood for domestic use. He was also instrumental in training a number of Chinese researchers and technicians. Large areas in southern China are now being sown with pigeonpea (along with soybean or maize as intercrops, and walnut trees) to reduce soil erosion and conserve soil. Pigeonpea leaves are fed to rabbits, goats, buffaloes, and pigs. Farmers are happy as their net income by raising rabbits with pigeonpea leaves is around 600 Yuan per Mu (about (US$ 1000 ha^{-1}). "It is heartening to see pigeonpea being cultivated on a large-scale in the hilly areas successfully" said William Dar, Director General, ICRISAT in his concluding remarks when he visited these areas.

Chickpea Technology Workshop in Pakistan

A one-day workshop on Chickpea Technology was organized on April 9, 2002 at the Arid Zone Research Institute (AZRI), Bhakkar under Pulses Programme, National Agricultural Research Centre (NARC) with the close cooperation of Federal and Provincial Research Institutes and Department of Agriculture Extension, Punjab, Pakistan. This workshop was a part of Pulses Programme activities to disseminate pulses production technology among the pulses/chickpea growers and to create awareness among the extension workers working with farmers in chickpea-growing areas. Bhakkar district ranks high in chickpea production in Thal area; therefore, the venue of the workshop was AZRI, Bhakkar. AZRI, Bhakkar is also one of the Pulses Cooperating Units of Pakistan Agricultural Research Council (PARC), Pulses Programme. The objective of the workshop was to familiarize farmers and agriculture extension agents with latest research development about advanced technology on chickpea, and get feedback from them about the constraints to prioritize future research strategies.

Thal is a major chickpea production area in Punjab and contributes significantly towards national pulses production. The agroecological conditions of the region are harsh and unsuitable for cultivation of other field crops because of sandy soils, extremely high temperature, and low and erratic rainfall. But chickpea is well adapted in Thal region and economy of the farmers mainly depends on chickpea production and its better market price. The crop generally suffers from moisture deficit (drought), nutrient deficiency, diseases (ascochyta blight and fusarium wilt), and insect attack (mainly pod borer *Helicoverpa armigera*). Among these constraints, drought and chickpea pod borer attack are the main issues to be addressed through research and development. Until 1985, ascochyta blight was a major problem of chickpea, but due to introduction of blight resistant varieties under the Cooperative Research Programme on Pulses, blight is no more a serious problem in Thal. However, wilt/root rot complex is emerging as a serious problem due to drought stress.

Under the Pulses Programme, NARC, this workshop was organized with the cooperation of Ayub Agriculture Research Institute (AARI), Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Agriculture Research Institute (ARI), D I Khan, AZRI, Bhakkar and Department of Agriculture Extension. The chickpea growers and Agriculture Officers (Extension) from districts Mianwali, Bhakkar, Khushab, Layyaha, and Jhang were invited through the Executive District Officer (Agriculture) for participation. Ten to 15 growers and Agriculture Extension Officers from tehsils Kalurot, Bhakkar, Jhang, Layyaha, Mankera, Piplan, Mianwali, and Noorpur Thal participated in the workshop. The research scientists working on chickpea and other pulses were invited from AARI, NIAB, Faisalabad, ARI, D I Khan, AZRI, Bhakkar, and NARC, Islamabad to deliver lectures on various aspects of chickpea production technology to boost up production in the country. Muhammad Bashir, Coordinator (Pulses), NARC discussed the role of PARC in strengthening pulses research at Federal and Provincial levels and enhancing pulses production in the country. Breeders from various institutes discussed and emphasized the role of improved varieties and certified seed to boost chickpea production. The recommendations were prepared by the researchers and published in Urdu. The publication was distributed among the growers and extension staff. The progressive growers (Lt. Col. (Retd.) Muhammad Iqbal, Malik Zafar Mehdi, Muhammad Nawaz) were given

sufficient time to express and discuss their problems related to crop production. The representatives from Extension Department (Ch. Gulzar Ahmad District Officer, Bhakkar; Rana Muhammad Idress, Deputy District Officer, Noorpur Thal; and Muhammad Umar) discussed the role of extension department in Thal area to introduce chickpea technology among farmers. The progressive grower Lt. Col. (Retd.) Muhammad Iqbal represented the farmers' community and appreciated the role of PARC in conducting this workshop. Farmers requested that such a workshop should also be held on mung bean before its sowing time (last week of June 2002). The major issues of chickpea growers in Thal area are:

- Drought stress
- Chickpea pod borer
- Root rot/wilt complex
- Non-availability of certified seed of improved varieties of chickpea
- Defective marketing system (practically non-existing support price system)
- Lack of farmer training programs and technology dissemination system
- Lack of credit incentives to pulses growers
- Construction of Greater Thal Canal (This will affect chickpea cultivation in Thal area. However, there is a great scope of chickpea in sugarcane-based and cotton-based systems, provided funds for research are allocated to explore its possibility.)

At the end, a special session of questions and answers was held. Every farmer and extension agent actively participated in this session and benefited from the interaction with experts. Prolonged drought stress and chickpea pod borer were burning issues in Thal and the insecticides partially used by the farmers were not effective in controlling the insect. About 30–35% less chickpea production is expected in Thal of Punjab. All the participants showed great interest and demanded such type of workshops at the start of each season [rabi (postrainy season) and kharif (rainy season)]. At the concluding session, Muhammad Bashir, thanked the growers/participants of the workshop, host institution (AZRI), and experts from various institutes, and pointed out the importance of linkages among researchers, educationists, extensionists, and end users. The future research program on chickpea will be based on present burning issues. He also thanked PARC administration and the Cereals and Legumes Asia Network (CLAN), ICRISAT for financial and moral support to organize this workshop.

Expenditure statement

Total funds received from ICRISAT was US$1000 (Pak. Rs 61,000). This amount was spent with the approval of the Chairman, PARC under the following heads:

Item	Amount (in Pak. Rs)
TA/DA to Officers/Staff	6400
POL charges for vehicles	8000
Stationery	5000
Printing of pamphlets in Urdu (Chickpea Technology)	10000
Miscellaneous (Photocopy, etc.)	1600
Repair and maintenance of vehicle	10000
Trainer charges	11000
Entertainment charges	9000
Total	61000

Contributed by: Muhammad Bashir, Crop Sciences Institute, NARC, Islamabad, Pakistan.

Research Reports

Chickpea

Breeding

PKV Kabuli 2: An Extra Bold Kabuli Chickpea Variety

W N Zope[1], K B Wanjari[1], Jagdish Kumar[2], H A van Rheenen[3], and **B V Rao[2]** (1. Pulses Research Unit, Dr Panjabrao Deshmukh Krishi Vidyapeeth, Akola 444 104, Maharashtra, India; 2. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India; 3. Crop Production & Seed Technology Department, Moi University, PO Box 1125, Eldoret, Kenya)

Traditionally kabuli chickpeas (*Cicer arietinum*) were not grown in Maharashtra state of India because the then available genotypes were late in maturity and susceptible to fusarium wilt (Kumar et al. 1985). Earliness and wilt resistance have been introgressed from desi types through systematic breeding efforts. The first ever early-maturing kabuli genotype ICCV 2 was released for cultivation in Andhra Pradesh state of India in 1989 and in Maharashtra in 1992 (Kumar et al. 2001) and notified by the Central Variety Release Committee (CVRC) of Government of India in 1992. Thereafter it became popular in Maharashtra and was grown in more than 50,000 ha during 1999/2000. Better market price for kabuli chickpea was one of the major considerations for the growers of ICCV 2. However, during the last few years the price of ICCV 2 has been reduced in Indian market due to its relatively smaller seed, while the extra bold kabuli chickpeas are being sold at a much higher price. The bold kabuli types possess 100-seed mass of more than 40 g. Indian markets get such bold types mostly through import.

An advanced breeding line, ICCV 92311 [ICCX-870026-BP-BP-14P-BP-BP [(ICCV 2 × Surutato 77) × ICC 7344]] from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India was received by Dr Panjabrao Deshmukh Krishi Vidyapeeth (PKV), Akola, Maharashtra during 1992. This line was tested at various locations in Maharashtra during 1993/94 to 1997/98 and in the All India Coordinated trials as KAK 2 in the extra bold kabuli group during 1996/97 to 1998/99. It was later named as PKV Kabuli 2. The main features of PKV Kabuli 2 are listed below:

- Growth habit is semi-spreading with 4–5 basal branches.
- Flowers are white.
- Stem normally remains green without pigment and turns yellow at senescence.
- It has bold pods and seeds.
- It matures in 102 days; however, the duration may vary depending on temperature and moisture.
- It is resistant to fusarium wilt.

The multilocational yield trial data available from Maharashtra indicated that PKV Kabuli 2 has high yield potential of 1.72 t ha^{-1} which is comparable to the check ICCV 2 (Table 1). The increase in seed yield of PKV Kabuli 2 was 6.8% over ICCV 2 (kabuli check), 13.2% over L 550 (kabuli check), and 16.2% over Chaffa (desi check). It also performed well in All India Coordinated Trials in the extra bold kabuli group conducted in Central Zone (Table 2). The important agronomic characters of PKV Kabuli 2 are presented in Table 3.

The All India Coordinated Research Project on Chickpea identified this variety for cultivation in Central Zone in 1999/2000. Later, it was released by PKV, Akola for cultivation in Maharashtra. This variety is expected to act as a substitute for extra bold kabuli being imported in the Indian market.

Table 1. Seed yield of chickpea cultivar PKV Kabuli 2 and checks at various locations in Maharashtra, India during 1993/94 to 1997/98.

Year/Location	Seed yield (t ha^{-1}) PKV Kabuli 2	ICCV 2 (Kabuli check)	L 550 (Kabuli check)	Chaffa (Desi check)	CD (0.05)	CV (%)
1993/94						
Akola	1.99	1.92	1.19	1.54	0.16	7.3
Nagpur	0.71	1.22	0.92	1.33	0.13	9.6
Sakoli	1.31	0.99	1.17	1.39	0.14	7.5
Mean	1.34	1.38	1.09	1.42	0.36	17.4
1994/95						
Akola	2.40	1.93	1.11	1.54	0.39	15.9
Nagpur	3.19	3.46	3.12	3.01	0.24	5.6
Sakoli	0.49	0.59	1.08	0.38	0.12	15.8
Mean	2.03	1.99	1.77	1.64	NS[1]	17.0
1995/96						
Akola	0.68	0.75	0.83	0.72	0.12	10.7
Nagpur	2.65	2.82	2.87	2.64	0.26	6.6
Mean	1.67	1.79	1.85	1.68	NS	17.3
1996/97						
Akola	1.99	1.41	1.30	NA[2]	0.14	5.0
Sakoli	0.54	0.38	0.58	0.44	0.15	21.2
Mean	1.27	0.90	0.94	0.44	NS	43.5
1997/98						
Akola	2.95	2.28	2.55	1.81	0.15	11.4
Weighted Mean	1.72	1.61	1.52	1.48	0.19	

1. NS = Not significant.
2. NA = Data not available.

Table 2. Seed yield of PKV Kabuli 2 in extra bold kabuli coordinated trials in Central Zone, India during 1996/97, 1997/98, and 1998/99.

Year	Locations	Seed yield (t ha^{-1}) PKV Kabuli 2	L 550	ICCV 2
1996/97	2	1.73	1.29	NA[1]
1997/98	3	1.86	1.69	1.46
1998/99	1	1.80	1.23	NA
Weighted Mean		1.81	1.48	1.46

1. NA = Data not available.

Table 3. Agronomic characteristics of PKV Kabuli 2 at different locations in Maharashtra, India during 1997/ 98.

Description	PKV Kabuli 2	ICCV 2 (Check)	L 550 (Check)
Days to 50% flowering			
Range	38–57	37–54	51–68
Mean	45.8±5.68	45.2±5.44	58.5±5.77
Days to maturity			
Range	95–113	85–113	94–132
Mean	102.1±6.52	100.6±8.13	111.9±11.73
100-seed mass (g)			
Range	38.1–43.0	21.7–28.0	22.7–23.4
Mean	40.3±1.76	24.5±2.05	23.0±1.79

References

Kumar, J., Haware, M.P., and **Smithson, J.B.** 1985. Registration of four short-duration Fusarium wilt resistant kabuli chickpea germplasms. Crop Science 25:576–577.

Kumar, J., Satyanarayana, A., Rao, B.V., Wanjari, K.B., and **Pandey, R.L.** 2001. A superior kabuli gram variety for peninsular and central India. Indian Farming 50(12):16–17.

PDG4: A New Multiple Disease Resistant Desi Chickpea Variety for Punjab in India

Sarvjeet Singh[1], R K Gumber[2], J S Sandhu[1], T S Bains[1], P S Sidhu[1], Inderjit Singh[1], and **Kuldip Singh[2]** (1. Department of Plant Breeding, Punjab Agricultural University, Ludhiana 141 004, Punjab, India; 2. Punjab Agricultural University, Regional Research Station, Faridkot 151 203, Punjab, India)

The area under chickpea (*Cicer arietinum*) is declining in Punjab, India due to tough competition from other rabi (postrainy season) crops particularly wheat (*Triticum aestivum*). Moreover, the susceptibility of chickpea to many diseases and insect pests makes it a risky crop that results in poor yield. Chemical control measures that are available to control some of the diseases are uneconomical and not completely perfect. The Pulse Improvement Program at the Punjab Agricultural University (PAU), Regional Research Station, Faridkot, Punjab made systematic efforts to combine high yield and disease resistance. These efforts resulted in the development of an outstanding variety of desi chickpea, PDG4. This variety possesses high yield, medium bold seeds, and multiple resistance to major diseases. It has been released for general cultivation under rainfed conditions in Punjab in 2000. It has also been tested, under the name FG703, in All India Coordinated Varietal Trials for three years in plant breeding and pathological trials. It is identified as a resistant donor for fusarium wilt, stunt, and collar rot.

The yield performance of PDG4 in different varietal trials, agronomy trials, microbiology trial, and adaptive trials conducted in the state from 1995/96 to 1999/2000 is given in Table 1. In 46 trials, the average seed yield of PDG4 was 1965 kg ha^{-1} as compared to 1728 kg ha^{-1} of check cultivar PDG3, with an increase of 13.71%. In 32 adaptive trials conducted in farmers' fields, the new variety gave an average seed yield of 1820 kg ha^{-1} as compared to 1640 kg ha^{-1} of PDG3 with 10.97% superiority. The performance of PDG4 in All India Coordinated Varietal Trials conducted in North-Western Plain Zone (including states of Punjab, Haryana, Delhi, Rajasthan, parts of Uttar Pradesh, and Jammu) from 1997/98 to 1999/2000 is given in Table 2. In 20 trials, the average seed yield of PDG4 was 1906 kg ha^{-1} as compared to 1707 kg ha^{-1} of check cultivar H 208 with 11.6% superiority. In 14 trials, the average seed yield of PDG4 was 1799 kg ha^{-1} as compared to 1483 kg ha^{-1} of

Table 1. Performance of chickpea cultivar PDG4 compared with check cultivar PDG3 in various trials in Punjab, India from 1995 to 2000.

Trials	Year	Number of trials	Yield (kg ha^{-1})		Yield increase (%) over PDG3
			PDG4	PDG3	
Research trials					
Varietal trials	1995/96 to 1999/2000	9	2326	1901	22.35
Agronomy trials	1998/99 to 1999/2000	4	2344	2072	13.12
Microbiology trial	1999/2000	1	1844	1613	14.32
Mean		14	2297	1929	19.07
Adaptive trials					
Farm Advisory Services (PAU)	1999/2000	21	1830	1670	9.58
Department of Agriculture, Punjab	1999/2000	11	1800	1583	13.72
Mean		32	1820	1640	10.97
Overall mean		46	1965	1728	13.71

Table 2. Mean performance of chickpea cultivar PDG4 and check cultivars in All India Coordinated Varietal Trials, North-Western Plain Zone, India from 1997 to 2000.

Year	Trial[1]	Number of trials	Yield (kg ha^{-1})			
			PDG4	H 208	Phule G5	RSG 143-1
1997/98	IVT-DTT	6	2156	1776	–	–
1998/99	AVT-1-DTT	7	2063	1718	1550	1985
1999/2000	AVT-2-DTT	7	1535	1636	1415	1584
Overall mean		20	1906	1707	–	–
		14	1799	–	1483	1785
Yield increase (%) of PDG4 over check cultivars				11.6	21.3	0.8

1. IVT = Initial Varietal Trial; DTT = Drought Tolerance Trial; AVT = Advance Varietal Trial.

Table 3. Disease incidence in chickpea cultivar PDG4 and check cultivars at Ludhiana and Faridkot in Punjab, India under artificially augmented conditions from 1996 to 2000[1].

Year	Ascochyta blight (score)[2]			Fusarium wilt (%)			Foot rot (%)			Dry root rot (%)		
	PDG4	PDG3	L 550	PDG4	PDG3	JG 62	PDG4	PDG3	JG 62	PDG4	PDG3	JG 62
1996/97	4.0	6.0	9.0	2.0	3.9	100.0	5.0	10.5	100.0	3.5	3.9	100.0
1997/98	–	–	–	5.2	20.0	100.0	1.3	13.9	100.0	0.0	12.4	100.0
1998/99	3.0	6.0	9.0	8.3	40.4	100.0	1.9	12.9	100.0	1.9	6.0	100.0
1999/2000	4.5	6.5	9.0	0.0	0.0	100.0	4.3	2.5	100.0	0.0	2.0	100.0
Mean	3.8	6.2	9.0	3.9	16.1	100.0	3.1	9.9	100.0	1.3	6.1	100.0

1. Susceptible check cultivars: L 550 for ascochyta blight; and JG 62 for fusarium wilt, foot rot, and dry root rot.
2. Rated on 1–9 scale, where 9 = susceptible.

Phule G5 and 1785 kg ha^{-1} of RSG 143-1 with an increase of 21.3% and 0.8% respectively.

The mean disease reaction of PDG4 and check cultivars to ascochyta blight, fusarium wilt, foot rot, and dry root rot in different trials conducted from 1996/97 to 1999/2000 is given in Table 3. The average incidence of ascochyta blight in PDG4 was 3.8 (on 1–9 rating scale) as compared to 6.2 and 9.0 in susceptible checks PDG3 and L 550, respectively. The average incidences of fusarium wilt, foot rot, and dry root rot were 3.9%, 3.1% and 1.3% in PDG4 as compared to 16.1%, 9.9%, and 6.1% in PDG3. There was 100% mortality in the susceptible check cultivar JG 62 against the three soilborne diseases. The disease reactions indicate that the new cultivar PDG4 is resistant to fusarium wilt, foot rot, and dry root rot and

Table 4. Seed quality parameters of improved chickpea cultivar PDG4 and check cultivar PDG3[1].

Parameter	PDG4	PDG3
Protein (%)	22.0	20.0
100-seed mass (g)	16.8	13.0
100-seed volume (cc)	12.0	9.5
Water absorption (%) (overnight soaking)	59.0	54.2
Volume expansion (%) (overnight soaking)	87.9	101.1
Hard grains (%)	0.0	0.0
Cooking time (min)	76.0	80.0
Water uptake (%) at cooking time	108.3	109.0
Volume expansion (%) at cooking time	165.9	159.3

1. Data are averages of 1998/99 and 1999/2000.

moderately resistant to ascochyta blight. The reaction of PDG4 against collar rot and stunt was observed in All India Coordinated Trials at different locations during 1999/2000 and 2000/01. Collar rot incidence was 14.3% at Kanpur and 2.7% at Jabalpur while stunt incidence was 10.1–20.0% at Junagadh. The nutritional quality parameters were in the normal range with 22.0% protein and the seed size was acceptable with 100-seed mass of 16.8 g (Table 4).

BG 1053: A New Medium Bold-seeded Kabuli Chickpea Cultivar for Punjab in India

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Reduced returns from chickpea (*Cicer arietinum*) crop in comparison to wheat (*Triticum aestivum*) crop led to sharp decline in the area under this crop during the last decade in the states of Punjab and Haryana in India. Therefore, the economic returns from chickpea crop have been compared with wheat crop. The market trends of the past clearly indicated that kabuli chickpea fetches more price than desi chickpea, and bold-seeded kabuli type fetches high price. At present, recommended kabuli cultivars of the region are small seeded (L 550, L 551, and BG 267) and not stable in their performance due to susceptibility to diseases. Bold-seeded kabuli cultivars with yield potential of 2–2.5 t ha^{-1} are needed in order to match the profitability of this crop with other remunerative crops.

To fill this gap, research efforts have been made to develop kabuli chickpea cultivars with bold seed. Scientists at the Indian Agricultural Research Institute, New Delhi, India were able to develop a medium bold-seeded kabuli chickpea cultivar, BG 1053. It was developed through selection from a germplasm line ICC 3 from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The new variety possesses high yield potential, medium bold seeds (100-seed mass of 26 g), and resistance to wilt complex. It was released at national level for North-Western Plain Zone of India during 1999–2000. This variety was also extensively tested at different locations in Punjab and based on its merits in seed size and yield potential, it is now released for general cultivation in the state and included in the package of practices for rabi (postrainy season) crops during 2001/02.

Table 1. Performance of chickpea cultivars in various trials from 1995 to 2001, Punjab, India.

Trials[1]	Years	No. of trials	Yield (kg ha^{-1})				Yield increase (%) over L 550
			BG 1053	L 550	BG 267	L 551	
Varietal trials (research)	1995/96–2000/01	12	2057	1980	1963[2]	1557[3]	3.89
Agronomy trial (research)	2000/01	1	1412	1396	–[4]	–	1.14
Adaptive trials (Farm Advisory Services)	2000/01	23	2132	1999	–	–	6.65
Adaptive trials (Department of Agriculture, PAU)	2000/01	8	1678	1617	–	–	3.77
Mean of adaptive trials		31	2015	1900	–	–	6.00
Overall mean		44	2013	1911	–	–	5.33

1. PAU = Punjab Agricultural University, Ludhiana.
2. Mean of four research trials conducted during 1996/97 and 1997/98.
3. Mean of three research trials conducted during 1998/99 and 2000/01.
4. Not tested.

Table 2. Incidence of four diseases on chickpea cutlivars BG 1053 and L 550 at Ludhiana, Punjab, India under artificial augmented conditions from 1995 to 2001.

	Ascochyta blight[1] (score)		Fusarium wilt (%)		Foot rot (%)		Root rot (%)	
Year	BG 1053	L 550	BG 1053	L 550	BG 1053	L 550	BG 1053	L 550
1995/96	7.0	9.0	20.7	72.2	10.3	22.2	3.4	5.6
1996/97	5.0	7.0	33.3	45.3	22.9	12.3	10.9	4.5
1997/98	9.0	9.0	5.1	39.6	6.5	5.7	8.0	1.9
1998/99	9.0	9.0	25.1	50.0	8.8	31.7	5.7	15.5
2000/01	8.5	9.0	6.4	6.0	3.9	7.5	6.6	9.0
Mean	7.8	8.6	18.2	42.6	10.5	15.9	6.9	7.3

1. Disease rating on 1–9 scale, where 1 = highly resistant; and 9 = highly susceptible.

Table 3. Pod borer incidence on BG 1053, L 550, and infestor during 1998/99 and 2000/01.

	Pod borer incidence[1] (%)			
Year	BG 1053	L 550	Infestor[2]	CD at 5%
1998/99	47.3 (43.4)	62.7 (52.3)	62.0 (51.9)	6.34
2000/01	29.5 (32.8)	47.0 (43.3)	47.5 (43.5)	3.37
	28.3 (32.1)	41.7 (40.8)	52.0 (46.1)	5.24
Mean	35.0	50.5	53.8	–

1. Figures in parentheses are transformed values.
2. Mixture of susceptible chickpea genotypes.

The yield performance of BG 1053 from 1995/96 to 2000/01 in different trials conducted at different locations in the state is given in Table 1. In 44 trials including varietal trials, agronomy trial, and adaptive trials, the new cultivar recorded an average yield of 2013 kg ha^{-1} as compared to 1911 kg ha^{-1} of check L 550 with 5.3% superiority over the check cultivar. On farmers' fields, the new variety gave 2015 kg ha^{-1} seed yield as against 1900 kg ha^{-1} of check cultivar L 550 with 6.0% increase over the check cultivar (Table 1). BG 1053 was also tested against two other checks, BG 267 in four research trials and L 551 in three research trials during different years. In these trials, BG 1053 gave more grain yield than either of the checks.

Reaction of BG 1053 and check cultivar L 550 to four diseases from 1995/96 to 2000/01 is presented in Table 2. The average score of ascochyta blight was 7.8 in BG 1053 as compared to 8.6 in L 550 on a 1–9 disease rating scale, where 1 is resistant and 9 is susceptible. The average incidences of fusarium wilt, foot rot, and root rot were 18.2%, 10.5%, and 6.9% in BG 1053 as compared to 42.6%, 15.9%, and 7.3% in check cultivar L 550, respectively. It indicates that the new cultivar BG 1053 has better resistance to wilt complex than the check cultivar.

The average incidence of pod borer during 1998/99 and 2000/01 was 35.0% on BG 1053 as compared to 50.5% on L 550 and 53.8% on the infestor (mixture of susceptible chickpea genotypes) (Table 3). Besides bold seeds, the new variety also possesses other desirable quality traits. Its protein content is 23.3%. Thus, the new medium bold-seeded kabuli cultivar has a good scope for its cultivation in Punjab and may occupy a considerable area that can help in crop diversification in the state in the coming years.

Evaluation of Super Early Chickpea Genotypes for Vegetable Purpose as a Catch Crop

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It is an old tradition to use immature green seeds of chickpea (*Cicer arietinum*) as vegetable in northern India, particularly in the states of Punjab and Haryana. Immature green chickpea seeds, called *chhollia*, are generally available from the end of February to end of March from the crop grown in these states. However, of late green seeds of chickpea are being sold from mid-December to end of the crop season. Perhaps the chickpea grown in the warm climate of southern India is being sold in the region. This aroused our interest to work on early chickpea for vegetable purpose and to find the possibility to have a chickpea crop of 65–75 days as catch crop. The early rice (*Oryza sativa*) crop is generally harvested by mid-September and catch crop of chickpea may be taken after it. The catch crop will accrue double benefit. Firstly, the crop of chickpea will be highly remunerative for the farmers as the green seeds are sold at Rs 40–50 kg^{-1} in December and January. Secondly, a pulse crop will be introduced in cereal-based cropping system of the region and it will help to check further deterioration of soil health. It is fortunate to have two super early chickpea genotypes ICCV 96029 and ICCV 96030 developed at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India (Kumar and Rao 1996). Therefore, this investigation was undertaken to test the super early genotypes in northern India and evaluate their performance as a catch crop for green seed yield.

In 1999, super early genotype ICCV 96029, early cultivar ICCV 2 (kabuli type), and late flowering local check PBG 1 were sown on three sowing dates, October 12, October 22, and November 2, 1999 at the Punjab Agricultural University, Ludhiana, Punjab. The experiment was unreplicated due to limited quantity of seed of super early line ICCV 96029. Each genotype was sown in two rows of 4 m length. Observations were recorded for days to first flower, days to first pod appearance, and pods $plant^{-1}$ at 60 days after sowing (DAS). Preliminary observations of this experiment enthused us to conduct further experimentation on super early chickpea. Four genotypes, two super early genotypes ICCV 96029 and ICCV 96030, one early cultivar ICCV 2, and one local check (cultivar PBG 1) were included in the study during 2000. These genotypes were sown in sub-plots and the three sowing dates as main plots in split plot design with three replications. The sowing dates were September 20, September 30, and October 10, 2000. Each sub-plot had 4 rows of 3 m length with interrow spacing of 30 cm. Recommended dose of fertilizers (15 kg nitrogen ha^{-1} and 20 kg P_2O_5 ha^{-1}) and one ton farmyard manure ha^{-1} were applied before sowing. First irrigation was given at 20 DAS and subsequent irrigations were given at intervals of 10 days due to high temperature (30±5°C). Observations were recorded for days to first flower, days to first pod appearance, and at 70 DAS for biomass, green pod mass, and green seed yield. However, the last three parameters were not noted for cultivar PBG 1 because the pods did not develop even at 70 DAS.

The results of the preliminary experiment conducted in 1999 are given in Table 1. The super early genotype ICCV 96029 took 28 days for first flower appearance in the first two sowing dates while it flowered in 35 days in the third sowing date. The early cultivar ICCV 2 took 31, 34, and 40 days to flower in the three sowing dates, respectively. The local cultivar PBG 1 took more than

Table 1. Performance of chickpea genotypes at different sowing dates during 1999 at Ludhiana, Punjab, India[1].

	October 12			October 22			November 2		
Character	ICCV 96029	ICCV 2	PBG 1	ICCV 96029	ICCV 2	PBG 1	ICCV 96029	ICCV 2	PBG 1
Days to first flower	28	31	59	28	34	65	35	40	82
Days to first pod	36	40	122	40	55	125	45	105	125
Pods $plant^{-1}$	33 (38)	10 (32)	0	21 (37)	8 (38)	0	19 (31)	0	0

1. Data in parentheses indicate total number of plants scored for podding at 60 days after sowing.

twice the number of days to first flower than ICCV 96029 in the first two sowing dates and in the third sowing date it took 82 days to first flower. ICCV 96029 took less days to first pod appearance than the other two genotypes ICCV 2 and PBG 1. It had highest number of pods (33 pods plant^{-1}) in the first sowing date October 12 while in the other two sowing dates it had 21 and 19 pods plant^{-1}, respectively. The early genotype ICCV 2 produced few pods (10 and 8 plant^{-1}) in the first two sowing dates, while no effective pods were observed in the third sowing date. However, PBG 1 did not start podding in all the three sowing dates even at 60 DAS. These observations suggested that effective pod formation in the second and third sowing dates were adversely affected by low minimum temperature (3±2°C) prevailing in the end of December 1999 and early January 2000, irrespective of the genotypes. However, effect of low temperature on ICCV 2 was more pronounced than ICCV 96029. These observations further indicated that sowing dates can be advanced to escape from low minimum temperature generally prevailing from mid-December to January in the region.

In the following year 2000, a planned experiment was conducted and sowing dates were advanced. The results of this experiment are presented in Table 2. These results confirmed the observations recorded in 1999 that the super early genotypes ICCV 96029 and ICCV 96030 were early in flowering and first pod appearance than the other two cultivars ICCV 2 and PBG 1 (Fig. 1). Significant differences were observed for biomass, green pod yield,

Table 2. Performance of chickpea genotypes in early sowing during 2000 at Ludhiana, Punjab, India[1].

	September 20				September 30				October 10				CD at 5%		
Character	1	2	3	4	1	2	3	4	1	2	3	4	Date (D)	Genotype (G)	D×G
Days to first flower	24	26	31	57	24	27	33	58	25	28	35	60	1.11	0.69	NS[2]
Days to first pod	30	33	40	120	31	34	41	125	34	37	43	127	2.58	1.38	NS
Biomass (kg ha^{-1})	6052	4252	4951	–[3]	8444	6741	6926	–	8096	7489	7370	–	560	276	477
Green pod yield (kg ha^{-1})	2526	1944	2178	–	3622	2904	2296	–	2371	2141	1081	–	188	188	326
Green seed yield (kg ha^{-1})	1527	1152	919	–	3135	1709	800	–	2033	1403	840	–	121	87	151

1. Genotypes: 1 = ICCV 96029; 2 = ICCV 96030; 3 = ICCV 2; and 4 = PBG 1.
2. NS = Not significant.
3. – = Data for PBG 1 (late flowering local check) not recorded.

Figure 1. Podding (on 25 Nov 2000) in super early chickpea genotypes ICCV 96029 and ICCV 96030 sown on 30 September 2000 at Ludhiana, Punjab, India.

Figure 2. Podding in super early chickpea ICCV 96029 at about 60 days after sowing (on 25 Nov 2000) at Ludhiana, Punjab, India.

and green seed yield among the genotypes, sowing dates, and their interaction. ICCV 96029 produced the highest biomass of 8444 kg ha^{-1}, green pod yield of 3622 kg ha^{-1}, and green seed yield of 3135 kg ha^{-1} in the second sowing date (September 30) (Fig. 2). The other super early genotype ICCV 96030 yielded highest biomass yield of 7489 kg ha^{-1} in the third sowing date (October 10) while green pod yield of 2904 kg ha^{-1} and green seed yield 1709 kg ha^{-1} were highest in the second sowing date (September 30). These observations confirm the earliness of super early genotypes ICCV 96029 and ICCV 96030 at Ludhiana (31° N) as observed at ICRISAT, Patancheru (18° N) by Kumar and Rao (1996). In another study, Kumar et al. (2001) reported that the super early genotype ICCV 96029 had taken 43 days to flower and matured in 128 days at Hisar (29° N) in early November sown crop. They further indicated that ICCV 96029 might have some mechanism of cold tolerance and can set effective pods at low temperature. The kabuli cultivar ICCV 2 produced good amount of biomass but significantly poor green seed yield than both the super early genotypes. The super early lines produced profuse flowering in October and November which was as good as that seen in normal crop in March. The pods of a few plants of both the super early lines were not harvested deliberately and allowed to mature. It was noticed that pods attained physiological maturity in the end of December and in situ germination was noticed inside the pods. This may be due to high humidity and foggy conditions, generally prevailing in the region.

Based on two years' experimentation, it is concluded that super early chickpeas can be exploited as a catch crop for vegetable purpose (*chhollia*) after the harvest of rice crop. Further experimentation is being continued on super early chickpeas to generate more information to have successful catch crop for green seeds.

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Performance of Chickpea in Ilocos Norte, Philippines

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The Ilocos region in the Philippines is characterized by semi-arid tropical climate. Most of the agricultural land in the region depends on rainfall and is kept fallow during the dry season because of the high temperature, lack of water, and high evaporation. However, crops which have root system that can make use of the groundwater or residual moisture from rainfall can be grown under this climatic condition.

In the Philippines, chickpea (*Cicer arietinum*) seeds are consumed mainly as a vegetable. The "processed/ canned" chickpea seeds are among the ingredients of meat dishes, mostly imported from other countries thereby draining the foreign exchange reserves of the country. Yield of chickpea in the Philippines is very low (100–500 kg ha^{-1}) compared to yield in India (400–700 kg ha^{-1}).

Considering the importance of chickpea, the Mariano Marcos State University (MMSU), Dingras, Ilocos Norte, Philippines in collaboration with the Cereals and Legumes Asia Network (CLAN) based at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India and the Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD), Los Baños, Philippines is undertaking a research and development program on chickpea. Therefore, there is a need to evaluate the genetic diversity of chickpea in relation to various farming systems in the Philippines.

Fourteen desi chickpea lines acquired from ICRISAT were evaluated for their yield potential and other agronomic characters in 1997/98 and 1998/99 dry season (November–April) at MMSU, Dingras (18° 3' N, 120° 32' N, 18 m altitude). The lines were arranged in a randomized complete block design (RCBD) and replicated three times. Each entry was planted in a 4-row plot of 5 m length and an interrow distance of 50 cm. One seed was dibbled in furrows 10 cm apart. Optimum cultural requirements from planting to harvesting was followed to permit expression of genetic potential. Thirty kg nitrogen (N) ha^{-1}, 30 kg

Table 1. Seed yield, seed mass, and shelling percentage of chickpea lines grown in Ilocos Norte, Philippines, 1997–99.

	Seed yield (kg ha^{-1})			100-seed mass (g)			Shelling (%)		
Genotype	1997/98	1998/99	Mean	1997/98	1998/99	Mean	1997/98	1998/99	Mean
ICCV 92904	1832	1690	1761	34.4	40.8	37.6	78.5	84.5	81.5
ICCV 92925	1476	1965	1720	19.8	27.1	23.4	76.9	81.4	79.1
ICCV 92928	1384	1164	1274	16.3	24.1	20.0	77.4	81.4	79.4
ICCV 96007	1763	1241	1502	21.4	29.0	25.2	78.4	74.0	76.2
ICCV 96010	1795	1463	1629	22.8	28.3	25.5	79.1	84.2	81.6
ICCV 96012	1544	1640	1592	25.0	32.5	28.7	75.7	66.7	71.2
ICCV 96013	1391	1123	1257	17.6	22.5	20.0	79.7	80.1	79.9
ICCV 96017	1726	1551	1638	21.3	27.8	24.5	79.7	82.0	80.8
ICCV 96018	1711	1776	1743	21.8	28.6	25.2	76.8	69.6	73.2
ICCV 96021	1756	1540	1648	23.4	30.6	27.0	77.2	74.6	75.9
ICCV 96023	1856	1955	1905	23.8	30.1	26.9	76.6	77.2	76.9
ICCV 96025	1659	2181	1920	18.2	24.3	21.2	78.8	82.2	80.5
ICCV 96027	1760	1052	1406	22.1	26.4	24.2	74.2	78.9	76.5
Phule G8-1-1	1431	1331	1381	18.4	23.6	21.0	77.4	83.2	80.3
Mean	1650	1548	1598	21.9	28.3	25.0	77.6	78.6	78.0
CV (%)	11.72	21.19		8.34	4.70		3.18	4.21	
SE ±	136	268		1.29	1.76		1.74	10.90	

P_2O_5 ha^{-1}, and 30 kg K_2O ha^{-1} were applied in the field at planting time. Spraying of Lannate® and Thiodan® was done 30 and 45 days after planting (DAP), respectively against leaf defoliators. Decis 2.5 EC was sprayed at 60 DAP to control pod borer. Harvesting was done when about 95% of the pods were mature. All data were taken from the two inner rows of each plot.

There were highly significant differences (P <0.01) in seed yield, seed size, shelling percentage (Table1), plant height, days to flowering, and days to maturity among the genotypes evaluated under Ilocos Norte conditions. The highest mean seed yield was recorded in ICCV 96025 (1920 kg ha^{-1}) followed by ICCV 96023 (1905 kg ha^{-1}) while the lowest seed yield of 1257 kg ha^{-1} was recorded in ICCV 96013. ICCV 96018, ICCV 96012, and ICCV 96023 registered consistent seed yield for two seasons.

ICCV 92904 produced the largest seeds having 100-seed mass of 37.6 g. ICCV 96013 and ICCV 92928 had the smallest seeds with 100-seed mass of 20 g. High shelling percentage was recorded in ICCV 96010 (81.6%) and ICCV 92904 (81.5%) while ICCV 96012 had the lowest (71.2%) shelling percentage. ICCV 96007 grew tallest (45 cm) while ICCV 92928 and ICCV 96025 were the shortest (35.5 cm). Most of the genotypes flowered at 48–49 DAP and matured at 94–97 DAP.

Because of the importance, uses, and the demand of processed/canned chickpea in the Ilocos region, chickpea production should be given importance especially in areas where only one crop such as rice (*Oryza sativa*) is grown and rice-fallow is practiced. Results showed that chickpea production was feasible especially in the Ilocos region.

Response of Chickpea to Dates of Sowing in Ilocos Norte, Philippines

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In the Philippines, chickpea (*Cicer arietinum*) seeds are consumed chiefly as vegetables. The processed/canned seeds are among the ingredients of meat dishes, mostly imported from other countries thereby draining the foreign exchange reserves of the country. Chickpea is a new crop in this country and its yield potential is yet to be determined.

Ilocos region in the Philippines is characterized by semi-arid tropical climate. Most of the agricultural lands depend on rainfall and are kept fallow during the dry hot season (February–May) because of the high temperature, lack of water, and high evaporation. Crops that have deep root system can make use of the groundwater or residual moisture from rainfall and thus can be grown under this climatic condition.

Chickpea is able to extract moisture from deep layers of the soil profile and can survive with limited supply of water if planted at the right time. However, crop duration is a key factor in the productivity of chickpea (ICRISAT 1991). Considering the importance of chickpea as a drought-tolerant crop and its merits in the Filipino diet, this study was conducted to know the best time to sow chickpea and the variety adapted for commercial cultivation.

Two chickpea varieties, ICCV 2 and ICCV 5, from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India were planted on five sowing dates, 15 October, 15 November, 15 December, 15 January, and 15 February, in a randomized complete block design with three replications during the dry season (October–April) of 1998/99 at the experimental fields of Mariano Marcos State University (MMSU), Dingras, Ilocos Norte (18º3' N, 120º32' E, 18 m altitude). Each plot consisted of 5 rows of 5 m length with plant spacing of 50 cm × 10 cm and one seed per hill. Optimum cultural requirements from planting to harvesting were followed. A fertilizer at 30 kg nitrogen (N) ha^{-1}, 30 kg P_2O_5 ha^{-1}, and 30 kg K_2O ha^{-1} was applied at planting. Spraying of Lannate® and Thiodan® was done at 30 and 45 days after planting (DAP) respectively, against leaf defoliators. Decis 2.5 EC was sprayed at 60 and 75 DAP to control pod borer. Harvesting was done when about 95% of the pods matured. All data were taken from the three inner rows of each plot.

Seed yield of chickpea was affected significantly by date of sowing (Table 1). The mean seed yield of the two varieties was highest (1670 kg ha^{-1}) in November 15 planting followed by October 15 planting (1237 kg ha^{-1}) and December 15 planting (1144 kg ha^{-1}). January 15 and February 15 plantings gave significantly lower seed yield of 369 kg ha^{-1} and 247 kg ha^{-1}, respectively. The same trend was observed in the production of pods and branches $plant^{-1}$. The number of pods and branches $plant^{-1}$ and height of plant were comparable in October 15, November 15, and December 15 plantings while these parameters were lowest in February 15 planting. The maturity duration was less in chickpea sown late, e.g., 15 February (86 days) than that sown early, e.g., 15 October (104 days). However, the varieties did not differ significantly in seed yield, pod and branches $plant^{-1}$, growth, and maturity at different sowing dates.

The results showed that both ICCV 2 and ICCV 5 when planted on 15 November gave the highest seed yield, highest number of pods and branches $plant^{-1}$, better plant growth and development, and matured in 98 days. The normal sowing of chickpea at ICRISAT, India is from October 15 through November 15 (ICRISAT 1987). The crop grown from October to November can produce

Table 1. Yield and agronomic characteristics of two chickpea varieties grown at different dates of sowing in Ilocos Norte, Philippines, dry season 1998/99[1].

Variety	Date of sowing	Seed yield (kg ha^{-1})	Number of pods $plant^{-1}$	Number of branches $plant^{-1}$	Plant height (cm)	Days to maturity
ICCV 2	Oct 15	1134 a	27.8 a	4.7 a	45.1 a	103
	Nov 15	1690 a	29.9 a	4.5 a	44.3 a	98
	Dec 15	1059 a	25.7 a	4.4 a	44.1 a	96
	Jan 15	337 b	12.3 b	4.3 a	33.5 b	94
	Feb 15	241 c	9.8 c	2.5 b	27.1 c	86
ICCV 5	Oct 15	1340 a	28.1 a	4.7 a	45.4 a	105
	Nov 15	1650 a	30.0 a	4.6 a	44.7 a	98
	Dec 15	1230 a	26.2 a	4.5 a	44.6 a	95
	Jan 15	400 b	15.0 b	4.2 a	33.2 b	94
	Feb 15	252 c	9.7 c	2.3 b	26.7 c	85
CV (%)		11.3	22.1	7.4	2.9	

1. Figures in a column followed by the same letters do not differ significantly at 5% level of significance.

dry seed yield up to 5000 kg ha^{-1}. Likewise, if planted too late in the season, it undergoes "forced maturation" and yields suffer accordingly (Saxena et al. 1980). Planting chickpea during December through late March hastens growth and forces maturity and thus plants cannot attain their fullest growth (Saxena 1984).

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Agronomy/Physiology

Effect of Osmo- and Hydropriming of Chickpea Seeds on Crop Performance in the Field

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Priming of seeds is an economical, simple, and safe technique for improving germination, seedling growth, and crop production. The beneficial effect of osmo-priming with mannitol and polyethylene glycol on germination has been reported in cucumber (*Cucumis sativus*) and tomato (*Lycopersicon lycopersicum*) seeds, respectively (Passam and Kakouriotis 1994, Ozbingol et al. 1998). We have carried out detailed study on the effect of osmo- and hydropriming (with 4% mannitol and water for 24 h) on seedling growth and enzymes of carbohydrate metabolism during germination under water deficit stress conditions (Kaur et al., in press). In 7-day-old seedlings, obtained from osmo- and hydroprimed chickpea (*Cicer arietinum*) seeds, three- to fourfold higher growth with respect to root and shoot lengths was observed in comparison to non-primed seedlings (Kaur et al., in press). The main aim of this investigation is to study the effect of priming of chickpea seeds (with 4% mannitol and water for 24 h) on the performance of the crop in the field, in comparison with crop raised from non-primed seeds so as to ascertain the practical utility of low cost technology of seed priming on the yield parameters of chickpea crop.

Seeds of chickpea cultivar GPF-2 were washed with water, dipped in 0.1% mercuric chloride for 5 min and then washed thoroughly with sterilized water. The washed seeds were divided into two lots; one was fully immersed in 4% mannitol and the second in water (1:2 w/v) and kept in an incubator at 25±1°C for 24 h. The seeds were then washed with distilled water and dried on filter paper at room temperature (27ºC) and the two lots were named as mannitol primed and water primed seeds. The seeds without any treatment were termed as non-primed. The crop was sown in three different plots under irrigated conditions. Pre-sowing irrigation and one irrigation in the end of January were given. Each plot had five rows. The interrow spacing was 30 cm and plant spacing within the row was 5 cm. Plot size was 3.4 m × 1.8 m. Urea at 32 kg ha^{-1} and superphosphate at 125 kg ha^{-1} were applied before sowing. The data from border rows was not taken. There were 55–60 plants per row. Sowing was done on 1 November 2000. Ten plants from each plot were taken out randomly at 50, 60, 70, 85, 100, 115, and 130 days after sowing (DAS). The various parameters such as length and dry biomass of roots and shoots, number of branches, flowers, and pods at different DAS, and yield were recorded (Tables 1 and 2). Shoot length and shoot biomass of water and mannitol primed plants were greater as compared to those from non-primed plants. At 130 DAS, the increase in length of shoots due to priming with water and mannitol was about 17% whereas increase in shoot biomass was twofold as compared to non-primed plants (Table 1). The increase in shoot biomass of primed seedlings was due to increased number of branches as the number of branches $plant^{-1}$ was more in water and mannitol primed plants at all stages of development (Table 1). At 100 DAS, the number of flowers $plant^{-1}$ was 8 in non-primed, 12 in water primed, and 11 in mannitol primed plants whereas at 115 DAS the corresponding values were 46, 69, and 74. At 130 DAS, number of pods $plant^{-1}$

was 17 in non-primed, 39 in water primed, and 38 in mannitol primed plants. The increased number of flowers and pods observed in primed plants could be correlated with the increased number of branches associated with priming. At maturity, at 160 DAS, all the plants from each field were taken out and the number of seeds plant^{-1} and remaining biomass of each plant after removing pods were noted (Table 2). In non-primed crop, the average seed yield plant^{-1} was 3.61 g. With water and mannitol priming, the average seed yields plant^{-1} were 5.05 and 5.94 g, respectively showing corresponding increase of 39% and 64%.

In on-farm trials in western India, overnight seed priming with water promoted seedling vigor, yield, and crop establishment of chickpea, maize (*Zea mays*), and rice (*Oryza sativa*) (Harris et al. 1999). Musa et al.

Table 1. Effect of priming (4% mannitol and water) of chickpea seeds on different plant parameters at different days after sowing.

	Parameter value[1]						
Treatment	50[2]	60	70	85	100	115	130
Shoot length plant^{-1} (cm)							
Non-primed	15.4±2.6	20.8±2.2	23.2±2.2	30.7±3.4	33.6±3.3	43.0±2.1	47.8±5.3
Water primed	16.6±1.2	25.2±2.0	30.7±1.7	34.2±2.9	37.1±1.9	53.1±7.1	56.2±4.8
Mannitol primed	16.4±1.9	24.5±1.9	28.1±1.6	32.6±2.7	40.3±3.1	52.8±3.5	56.4±7.0
Shoot dry biomass plant^{-1} (g)							
Non-primed	0.48±0.1	0.69±0.1	1.0±0.3	1.3±0.4	2.1±0.8	4.7±0.9	5.0±1.5
Water primed	0.67±0.1	1.12±0.16	1.7±0.25	2.6±0.94	4.0±0.6	8.1±1.7	10.9±1.0
Mannitol primed	0.72±0.1	1.21±0.2	1.3±0.3	1.6±0.3	2.8±0.56	7.8±2.3	10.3±2.0
Root length plant^{-1} (cm)							
Non-primed	10.8±0.1	11.3±1.9	10.2±2.2	11.4±1.2	12.9±1.8	13.0±1.6	12.8±2.8
Water primed	9.8±1.4	11.1±1.7	11.6±1.5	11.0±1.8	13.4±2.4	13.6±1.9	13.7±1.9
Mannitol primed	11.2±1.8	10.7±1.0	10.1±2.2	12.0±0.9	13.0±1.0	12.9±1.0	12.9±1.7
Root dry biomass plant^{-1} (g)							
Non-primed	0.11±0.03	0.13±0.02	0.23±0.08	0.28±0.08	0.33±0.08	0.38±0.10	0.53±0.13
Water primed	0.10±0.03	0.16±0.01	0.33±0.08	0.37±0.12	0.39±0.60	0.46±0.10	0.74±0.09
Mannitol primed	0.10±0.01	0.20±0.02	0.23±0.03	0.27±0.06	0.34±0.06	0.40±0.11	0.76±0.18
Number of branches plant^{-1}							
Non-primed	35±4	48±6	73±9	79±9	80±5	169±9	182±11
Water primed	47±6	61±4	93±6	100±7	144±9	199±10	280±18
Mannitol primed	46±4	74±8	80±5	91±9	102±10	253±14	224±15

1. Data represents the mean ± SD of ten plants from each treatment.
2. Days after sowing.

Table 2. Effect of priming of chickpea seeds (4% mannitol and water) on the performance of the crop in the field[1].

Priming treatment	Average plant biomass after removing pods (g)	Number of seeds plant^{-1}	Seed mass (g seed^{-1})	Seed yield plant^{-1} (g)	Yield (t ha^{-1})
Non-primed	2.76±0.36	19±1.0	0.190±0.03	3.61	1.7
Water primed	4.06±0.47	25±5.0	0.202±0.04	5.05	2.4
Mannitol primed	5.34±0.06	30±2.0	0.198±0.02	5.94	2.8

1. Values are mean ± SD of all the plants (60–80) from each treatment.

(1999) also reported that overnight priming of chickpea seeds with water resulted in an early emergence and enhanced plant height, number of pods, seed yield, and residue yield of chickpea crop grown in harsh conditions of high Barind Tract of Bangladesh. They reported an increase in seed yield of about 47%. The biochemical events responsible for higher seed yield are not yet known. However, during seedling growth of primed chickpea seeds, increased activities of amylases, invertases, sucrose synthase, and sucrose phosphate synthase were observed in the shoots of primed seedlings in comparison with non-primed seedlings (Kaur et al., in press). These enzymes could be responsible for better plant growth and yield.

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Pathology

First Occurrence of Foot Rot of Chickpea Caused by *Operculella padwickii* in Bangladesh and Nepal

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Diagnostic surveys were conducted in March 1999 crop growing season to determine the prevalence of diseases of chickpea (*Cicer arietinum*) in the rice (*Oryza sativa*)-wheat (*Triticum aestivum*) based cropping systems of Bangladesh and Nepal. A new disease of chickpea, foot rot, caused by the fungal pathogen *Operculella padwickii* was observed for the first time in the village Gwaliarpur on Faridpur-Rajbari road in Bangladesh. Later on foot rot was also observed in farmers' fields in the districts of Jessore, Jhenaidah, Magura, Faridpur, Rajbari, and in Barind area of district Rajshahi. The disease was also observed in chickpea trials at the Regional Agricultural Research Station (RARS), Ishurdi, Pabna district, Bangladesh. The disease incidence ranged between 1% and 10% across locations and sites surveyed.

Foot rot was also observed in both farmers' fields and research stations in the major chickpea-growing areas in Nepal. Several national and international on-station trials which included improved high-yielding cultivars at the National Grain Legumes Research Program (NGLRP), Rampur, RARS, Khajura, Banke, and RARS, Tarhara in Nepal had substantially high incidence of the disease (10–25%). The disease incidence varied from 1% to 25% in farmers' fields irrespective of chickpea cultivars sown.

Foot rot is often confused with fusarium wilt (*Fusarium oxysporum* f. sp *ciceris*), collar rot (*Sclerotium rolfsii*), and root rot (*Rhizoctonia solani*) but the symptoms are distinctly different from these diseases. Foot rot affects

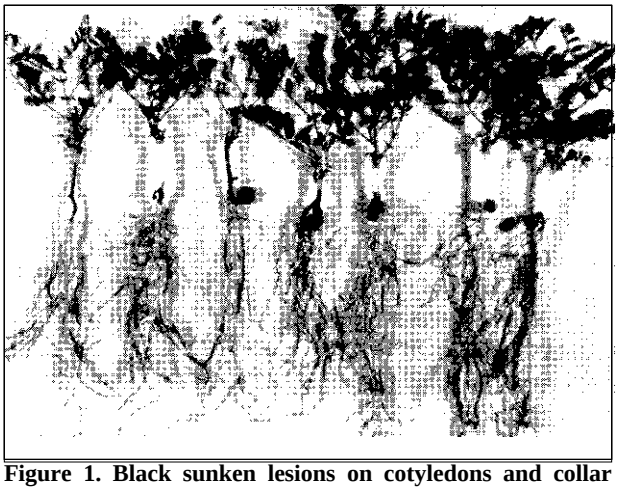

Figure 1. Black sunken lesions on cotyledons and collar region of chickpea plants.

the collar region and tap root of the plant. It produces dark brown to black sunken lesions on cotyledons and collar region of the plant (Fig. 1). Later the lesions enlarge, become sunken, dark brown to black, extending to the epicotyl and basal tap root of the plant. In advanced stages of disease development, a complete girdling of the plant in the collar region takes place (Fig. 2), resulting in wilting and death of the plants. The leaves of affected plants are pale green and finally become straw colored. There is no drooping of petioles and leaflets and vascular discoloration as in fusarium wilt; however, distinct browning of phloem takes place. The fungus produces white mycelium and pycnidia (270–810 µ in diameter). Conidiophores are of two kinds: short conidiophores, which are simple, appear as lining on the wall of pycnidium, and bear spores terminally; and long conidiophores which are branched, sometimes septate, and bear spores laterally as well as terminally. The spores are hyaline, irregular in shape, and yellowish-white measuring 7.4–16.6 µ × 5.5–11.1 µ. The pathogenicity was confirmed by planting seeds of chickpea variety Nabin in *O. padwickii*-sick plots. The pathogen *O. padwickii* was reisolated from the diseased plants. This is the first report of *O. padwickii* on chickpea in Bangladesh and Nepal; however, it was first reported from Karnal in Haryana state of India

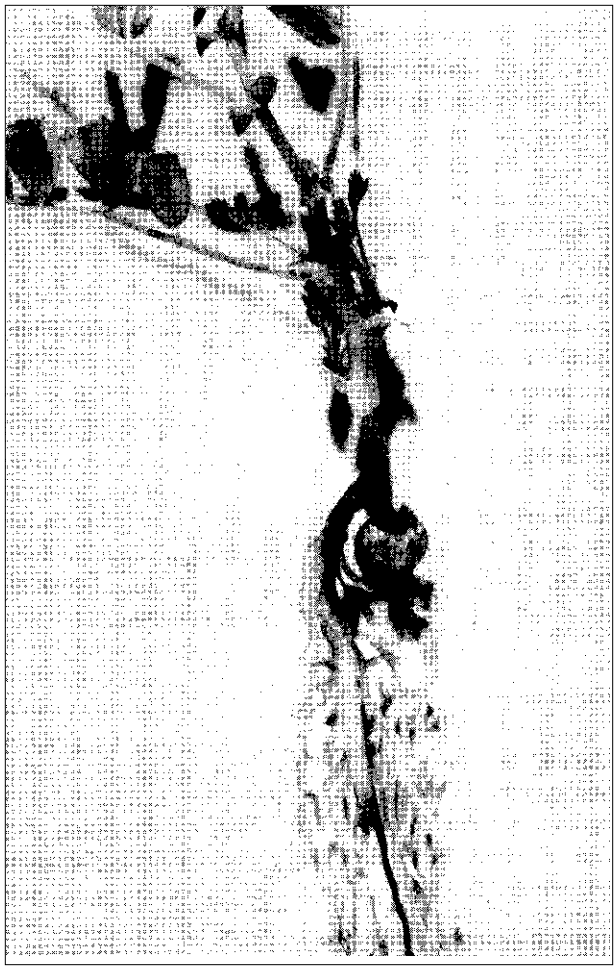

Figure 2. Girdling of chickpea plant in the collar region.

(Kheswalla 1941) and later on from Gurdaspur in Punjab, India causing 53–70% damage to chickpea crop in certain environments and fields.

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Selection for Resistance to Fusarium Wilt and its Relationship with Phenols in Chickpea

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Among the many fungal diseases, fusarium wilt caused by *Fusarium oxysporum* f. sp *ciceris* is the most devastating disease of chickpea resulting in 10–50% crop losses every year in Pakistan. A total of 40 advanced chickpea lines received from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria were tested for wilt resistance using the pot method as described by Nene et al. (1981) against a virulent strain of *F. oxysporum* f. sp *ciceris* (2012; isolated from diseased chickpea samples collected from Rangpur, Thal, Punjab, Pakistan, during a survey in 2000). The cultivars Aug-424/ILC 1929 (susceptible) and CM 98 (resistant) were used as checks. Four seeds of each test line and the checks were sown in separate plastic pots in three replications. Wilt incidence was recorded at 15, 20, 25, and 30 days after germination (DAG). The resistance/susceptibility of the test lines were determined by using the rating scale described by Iqbal et al. (1993) where 0–10% mortality = highly resistant, 11–20% = resistant, 21–30% = moderately resistant (tolerant), 31–50% = susceptible, and 51–100% = highly susceptible.

Two highly resistant and four susceptible representative test lines were sown in small plastic pots (10 cm × 10 cm) containing autoclaved soil. Total phenols in the roots of all the lines were estimated at 10 DAG by the procedure given by Simson and Ross (1971) to find out their relationship with wilt resistance.

The susceptible check (Aug-424/ILC 1929) completely wilted within 15 DAG and the resistant check (CM 98) wilted at 25–27 DAG. Flip 90-131C, Flip 96-152C, Flip 96-153C, Flip 96-155C, Flip 96-158C, and ICCV 95503 showed no wilt incidence up to 30 DAG and were classified as highly resistant (Table 1). Flip 85-29C, Flip 85-30C, and Flip 96-154C exhibited 16–17% wilt incidence and were considered resistant. Other lines were classified into susceptible and highly susceptible groups. The resistant lines identified in this study can be used as sources of wilt resistance in the chickpea breeding program.

Total phenols in the healthy roots of resistant/susceptible test lines did not show any correlation with wilt resistance (Table 2). The susceptible lines Flip 90-2C, Flip 93-28C,

Table 2. Estimation of phenols in roots of wilt resistant and susceptible chickpea lines.

Test line	Disease reaction	Total phenols (mg g^{-1} fresh roots)
Flip 90-2C	Susceptible	0.68
Flip 93-28C	Susceptible	0.77
Flip 90-155C	Susceptible	0.72
Flip 96-153C	Highly resistant	0.67
Flip 96-155C	Highly resistant	0.59
ILC 1929	Susceptible	0.51

Table 1. Reaction of chickpea genotypes to fusarium wilt by pot method at ICARDA, Syria.

Disease reaction	Wilt incidence[1] (%)	Genotypes[2]
Highly resistant	0	Flip 90-131C, Flip 96-152C, Flip 96-153C, Flip 96-155C, Flip 96-158C, ICCV 95503
Resistant	11–20	Flip 85-29C, Flip 85-30C, Flip 96-154C
Susceptible/Highly susceptible	31–100	Flip 85-7C, Flip 88-1C, Flip 89-14C, Flip 89-73C, Flip 89-126C, Flip 90-2C, Flip 90-74C, Flip 90-144C, Flip 90-155C, Flip 90-181C, Flip 91-20C, Flip 91-217C, Flip 92-16C, Flip 92- 48C, Flip 92-49C, Flip 92-75C, Flip 92-104C, Flip 92-113C, Flip 92-139C, Flip 92-148C, Flip 92-171C, Flip 93-22C, Flip 93-23C, Flip 93-28C, Flip 93-50C, Flip 93-52C, Flip 93-226C, Flip 96-157C, ICCV 95506, UC 15

1. At 30 days after germination;
2. UC 27 had poor germination.

and Flip 90-155C produced higher phenolic content as compared to the resistant lines Flip 96-153C and Flip 96-155C. The results are in agreement with that of Sahi et al. (2000), who reported that total phenolic content was higher in susceptible lentil (*Lens culinaris*) lines prior to the pathogen inoculation. The present data showed that there is no relationship between total phenols and wilt resistance. The qualitative production of highly specific antifungal compound(s) prior to fungal invasion or phytoalexin production after invasion might have a role for imparting resistance in chickpea.

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Effect of Fusaric Acid on In Vitro Pollen Germination and Tube Growth in Chickpea

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Among biotic factors that affect seed yield of chickpea (*Cicer arietinum*) in India and elsewhere, fusarium wilt caused by *Fusarium oxysporum* f. sp *ciceris* is considered to be most devastating. Efforts are being made to develop high-yielding wilt resistant varieties through conventional breeding. Conventional breeding requires screening of a large set of segregating populations or genotypes over several generations under uniform selection environment (sick plot). This obviously is tedious and imposes heavy cost and time and hence the progress is very slow. In view of these difficulties efforts have been made in recent years to seek alternate approaches. Pollen screening offers to be a simple but effective technique of testing the genotypes. Pollen screening and/or selection was identified in the early 1980s following realization that there is a high proportion of overlap of gene expression between gametophyte and sporophyte stage (Hormaza and Herrero 1996). Exposure of pollen to stress during its formation, germination, tube growth, and/or fertilization may lead to selection of tolerant pollen resulting in the selective accumulation of resistant alleles in the progeny (Chikkodi and Ravikumar 2000). An attempt has been made to study the effect of fusaric acid, a toxin from the wilt pathogen, on pollen germination and pollen tube growth of chickpea genotypes.

Chickpea pollen can be successfully grown in a liquid medium (Shivanna et al. 1997). A series of pollen germination medium (PGM) containing 0, 50, 100, 150 and 200 µg of commercially available fusaric acid (Sigma Cat. # F-6513) ml^{-1} of PGM were prepared. Two resistant chickpea genotypes, WR 315 with all the resistant genes (h_1, h_2, and h_3) and K 850 with two genes (h_2 and h_3), were chosen for this study (Tekeoglu et al. 2000). Pollen grains of the selected genotypes were placed in cavity slides containing 100 µl of PGM. Four cavities for each concentration of fusaric acid in PGM per genotype were used. The cavity slides with pollen were incubated at room temperature (25–26°C) for one hour in petri dishes maintained at relative humidity of 70–80%. Five randomly chosen fields per cavity were scored for pollen germination and tube length. The pollen grain with a tube length of more than twice its diameter was considered as germinated and the pollen tube length was measured on the graduated screen of projection microscope (1unit = 50 µm).

Pollen germination of both the genotypes was nearly 80% in the absence of fusaric acid in PGM. The pollen tube was also prominent in the controls (Fig. 1). In PGM with fusaric acid there was a reduction in pollen germination and tube growth. In PGM with fusaric acid at 50 µl ml^{-1} there was no drastic reduction in germination. With further increase in the fusaric acid concentration there was drastic reduction in pollen germination (Fig. 2). Such reduction in germination and tube growth with the addition of fungal culture filtrate has been reported in several crops (Hodgkin and MacDonald 1986). Fusaric

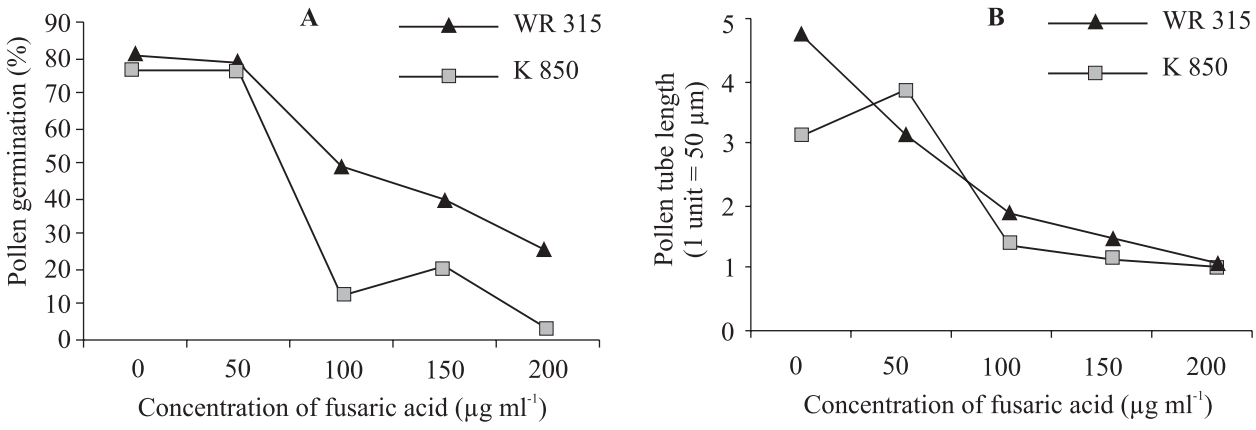

Figure 1. Effect of fusaric acid on in vitro pollen germination (A) and tube growth (B) in chickpea genotypes.

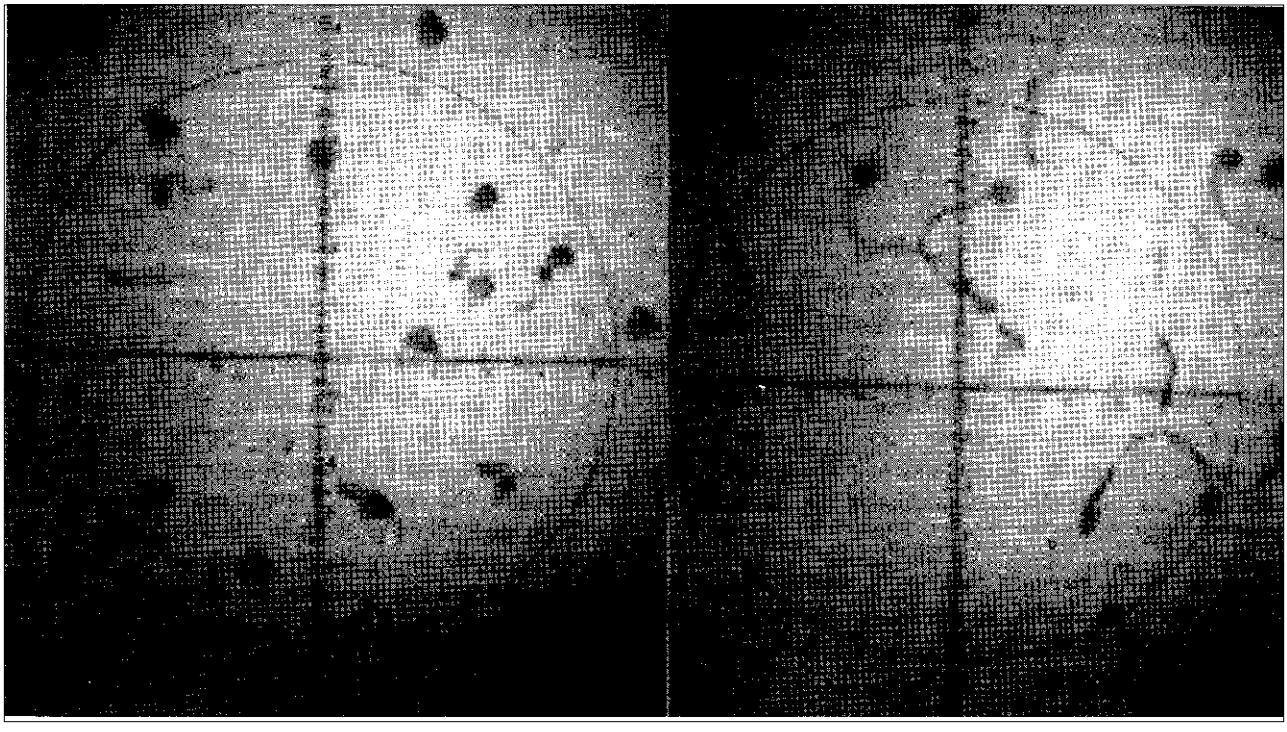

Figure 2. Effect of the toxin (T) fusaric acid on in vitro pollen germination in chickpea genotype K 850 (C = control).

acid also inhibited the pollen tube growth in both genotypes except at 50 µl ml^{-1} in K 850 (Fig. 2). The inhibition of pollen germination was not uniform in both genotypes. It was more in K 850 with two genes for resistance compared to WR 315 having all the three resistant genes. Apparently pollen from highly resistant genotype was less sensitive to fusaric acid than pollen from moderately resistant genotype. The differential sensitivity of pollen from different genotypes to pathotoxin has been reported in several plant species (Ravikumar and Chikkodi 1998). However, the study has to be extended over several genotypes involving both resistant and susceptible genotypes to determine the utility of this technique in screening genotypes for wilt resistance.

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Role of Pectic Enzymes in the Virulence of *Fusarium oxysporum* f. sp *ciceris*

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About 10–50% incidence of fusarium wilt caused by *Fusarium oxysporum* f. sp *ciceris* (FOC) has been reported on chickpea in the dry areas of Pakistan during the past several years. Two races of FOC, 0 and 5, produce two different vascular syndromes, i.e., yellowing and wilt respectively in the susceptible chickpea variety, and markedly differ in virulence although the underlying mechanisms that govern this difference are yet unknown. Pectic enzymes have been frequently implicated in the pathogenesis of wilt. FOC has been reported to produce multiple forms of pectic enzymes and production of pectin lyase (PL) and polygalacturonase (PG) activities were markedly different in race 0 and race 5. Endo-PG enzymes were found relevant for pathogenesis in producing the yellowing syndrome and not in the wilt complex (Artes and Tena 1990). The objective of our study was to find out the role of pectic enzymes in the pathogenicity/virulence of FOC strains of Pakistani origin.

FOC isolates of race 0 (7952) and race 5 (8012) were provided by Prof. R M Jimenez-Diaz (Department of Agronomy, ETSIA, University of Cordoba, Spain). Six local isolates, 2004, 2008, and 2014 (less virulent), and 2005, 2012, and 9718 (highly virulent) were collected from Thal area of Pakistan during a survey in March 2000. The fungal isolates were grown on minimal medium containing 1% citrus pectin (w/v) for the production of enzymes. PG and PL activities were assayed by the methods described by Nelson (1944) and Pitt (1988) respectively. Protein was determined by the method of Bradford (1976) with bovine serum albumin as a standard. Phytotoxicity of the culture filtrates was determined against cut seedlings. The phytotoxic activities of the heated culture filtrates (heated at 80°C in a waterbath for 5 min) of each isolate were also tested by this method.

The virulent and hypovirulent isolates of FOC produced PL and PG activities. The less virulent isolates 2004, 2008, 2014, and race 0 (7952; causing yellowing)

produced very high PL activities as compared to the highly virulent isolates 2005, 2012, 9718, and race 5 (8012; causing wilting) (Table 1). But in PG assay the results were almost contrary to PL assay. The highly virulent isolates 2005, 2012, 9718, race 5 as well as race 0 produced higher amounts of PG activities as compared to the less virulent isolates. Highest PG activity was produced by the most virulent isolate 9718.

Phytotoxicity of the culture filtrates on chickpea cuttings was rated on 0–3 scale based on symptoms produced (0 = healthy; 1 = burning of leaves; 2 = drooping; and 3 = wilting) (Fig. 1). The phytotoxicity assay revealed that the culture filtrates of the highly virulent isolates and race 0 produced symptoms of burning of leaves of chickpea cuttings, while the culture filtrates of the less virulent isolates 2004, 2008, and 2014 did not produce any symptom on chickpea cuttings 24 h after incubation. Most of the isolates produced maximum phytotoxicity (rating 3) at 6 days after incubation except the isolates 2004 and 2014. The phytotoxicity of the culture filtrates reduced after heating at 80°C for 5 min and reduction was more prominent in the heated culture filtrates of the less virulent isolates 2004 and 2008 (Fig. 1). With isolate 9718, a rating of '1' was observed at 6 days after incubation. Control treatments did not show toxicity.

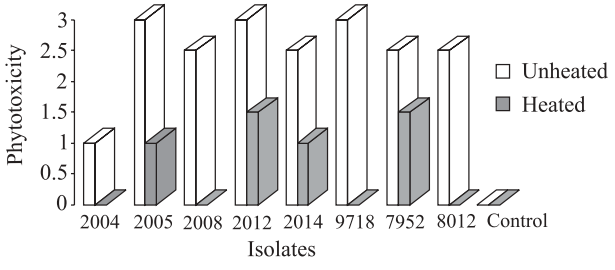

Figure 1. Phytotoxicity (rating) of the culture filtrates of isolates of *Fusarium oxysporum* f. sp *ciceris* on chickpea cuttings at four days after incubation.

Higher phytotoxicity of the culture filtrates has been correlated with the production of either phytotoxic metabolites (Alam and Khan 1996) or with the cell wall degrading enzymes including pectic enzymes and cutinase enzymes (Kollattukudy 1985, Artes Perez and Tena 1990). FOC has been reported to produce phytotoxin involved in wilt (Alam and Khan 1996). Artes and Tena (1990) found that race 0 of FOC has been reported to produce three forms of PG designated PG I_0, PG II_0, and PG III_0 and one PL form designated PL_0, whereas race 5 produced only one PG form designated PG_5 and two PL forms, designated PL I_5 and PL II_5. They concluded that the endo-PG enzymes could be relevant for pathogenesis in yellowing caused by race 0 of FOC and not in wilting. But in some other plant diseases endo-PL forms were found important for the pathogenesis based on the presence of higher amounts of PL enzymes than PG enzymes (Wijesundra et al. 1984). In our study local isolates of different virulence groups showed a clear difference in the production of PL and PG activities, which indicated that total PL activities may not have any role in the virulence of FOC, whereas the total PG activities may be responsible for the virulence in these isolates. Furthermore, the higher phytotoxicity of the

Table 1. Total pectin lyase (PL) and polygalacturonase (PG) activities of less virulent and highly virulent isolates of *Fusarium oxysporum* f. sp *ciceris*[1].

Isolate	Virulence	Protein (mg)	Total PL activity (IU) (mg ml^{-1})	Total PG activity (IU) (mg ml^{-1})
2004	Less virulent	0.92	57.50	3.00
2008	Less virulent	ND	25.65	2.58
2014	Less virulent	0.89	32.20	3.12
2005	Highly virulent	0.82	11.50	5.30
2012	Highly virulent	1.02	10.45	4.90
9718	Highly virulent	0.90	6.75	6.30
7952 (Race 0)	Yellowing	0.99	49.00	5.25
8012 (Race 5)	Wilting	0.93	4.35	5.75
Medium[2]	–	0.80	–	–

1. IU = International units; ND = Not detected.
2. Minimal medium containing 1% citrus pectin.

culture filtrates of virulent isolates may be due to PG activities if phytotoxins are not responsible for the total toxicity. Heating caused reduction in the toxicity of the culture filtrates, which may be due to the deactivation of the PL and PG enzymes whereas the remaining toxicity may be due to the phytotoxic metabolites produced by the pathogen. This showed that pectic enzymes especially PG enzymes have phytotoxic effects and may be the virulence factor in FOC. Further studies on the involvement of phytotoxins in the virulence of FOC may provide a better insight regarding the possible role of total PG activities.

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Plant Growth and Infestation by Root-knot Nematode *Meloidogyne incognita* in *Rhizobium*-treated Chickpea

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Chickpea (*Cicer arietinum*) is one of the major pulses in India. Root-knot nematodes are known to supress rhizobial nodulation in pulses (Veech and Dickson 1987) and cause severe yield losses in chickpea (Ahmed and Hussain 1988, Zaidi et al. 1988). Therefore, we evaluated *Rhizobium* strains that nodulated chickpea and improved plant growth even in the presence of the root-knot nematode *Meloidogyne incognita*. The efficacy of different *Rhizobium* strains in root-knot infested fields has not been reported in the literature.

Seeds of chickpea variety RSG-2 were treated with different rhizobial strains obtained from the Coordinator, All India Coordinated Pulses Improvement Project, using gum as adhesive. Treated seeds were dried in shade and then sown in microplots of 4 m × 2 m with plant to plant and row to row spacing of 30 cm (6 rows per plot of 4 m length). Culture plots had uniform infestation of pure population of *M. incognita* (2.67 larvae g^{-1} soil). A basal dose of 40 kg $P_2 0_5$ ha^{-1} was applied at the time of sowing. Application of 30 kg nitrogen (N) ha^{-1} and untreated seeds were kept for comparision.

Sixty days after sowing, 5 plants were uprooted from each plot to record the observations on intensity of inoculation. Nodulation was categorized from poor to excellent based on the color, size, and number of nodules per plant. Grain yield of chickpea per plot was recorded at harvest.

The presence of nematode decreased the average growth and yield of chickpea plants (Table 1). Plants from *Rhizobium*-treated seeds sown in nematode infested soil had greater yield and plant growth than control (untreated seeds). This indicates that rhizobial seed treatment enhances seed yield and plant growth even under nematode infestation. Maximum yield (548 kg ha^{-1}) was obtained when seeds were treated with G5-B1 strain of *Rhizobium* and the nodulation was also excellent. It was followed by the strains B 1, KG 61, and DWG 4 (520 kg ha^{-1} each) and IC 53 (500 kg ha^{-1}); also nodulation was excellent. Strains

Table 1. Effect of seed treatment with *Rhizobium* strains on growth of chickpea plants and development of root-knot nematode *Meloidogyne incognita*.

Bacterial strain	Galls (number plant^{-1})	Egg masses (number plant^{-1})	Root length (cm)	Shoot length (cm)	Plant mass (g)	Yield (kg ha^{-1})	Nodulation
IC 94	44.2	32.2	6.2	12.7	2.40	343	Poor
CM 1	39.6	29.4	6.7	15.1	1.96	322	Poor
IC 149	63.0	49.6	7.5	13.5	2.40	335	Poor
IC 126	12.8	8.2	6.8	13.7	1.80	500	Good
G 567	11.8	4.8	6.8	15.3	1.80	470	Good
IC 53	16.0	12.2	7.5	13.6	2.56	500	Excellent
IC 2018	21.0	18.0	6.3	13.1	3.00	447	Good
CBH 32	31.6	25.4	8.0	16.6	2.90	485	Good
G 10-80	22.2	14.2	7.9	14.7	2.80	364	Moderate
DWG 4	54.8	30.6	6.0	12.6	1.40	520	Excellent
KG 61	59.0	36.4	6.6	12.8	2.24	520	Excellent
B 1	13.4	10.2	6.6	16.0	3.10	520	Excellent
TAL 1748	61.6	48.4	6.1	13.0	2.62	468	Poor
G 5-B1	24.0	16.0	6.0	12.0	2.20	548	Excellent
G 37	61.0	32.8	7.4	17.8	3.60	475	Poor
KG 46	64.6	36.0	5.2	13.0	1.75	416	Poor
H 60	62.0	36.2	6.5	15.8	2.60	395	Poor
GB 2	41.8	22.0	6.7	15.4	2.70	427	Poor
Nitrogen (30 kg ha^{-1})	71.6	23.2	6.8	14.7	2.40	375	Poor
Control	60.0	44.2	6.1	11.1	1.80	312	Poor
SEm ±	1.22	0.99	0.13	0.30	0.09	18	
CD (5%)	3.42	2.79	0.36	0.84	0.27	54	

of *Rhizobium*, viz., IC 126, G 567, IC 53, IC 2018, CBH 32, B 1, and G 5-B1 provided excellent nodulation and the plants had fewer galls (12.8, 11.8, 16.0, 21.0, 31.6, 13.4, and 24.0 respectively) and egg masses (8.2, 4.8, 12.2, 18.0, 25.4, 10.2, and 16.0 respectively) per plant. In general, the plant growth and yield in all the mentioned treatments was statistically more than in treatments with other strains wherein the plants exhibited poor to moderate nodulation and greater nematode infestation on roots.

In the process of nodule formation, *Rhizobium* passes through a developmental sequence in a delicately balanced state and fixes atmospheric N in the soil resulting in better growth of plants. The presence of phytoparasitic nematodes pose a biotic stress, disturbing both the partners in gall and nodule formation. Differential tolerance of rhizobial and bradyrhizobial strains to biotic stresses have been observed (Singleton et al. 1982). However, performance of bacterial strains under nematode infestation need to be studied in much more detail.

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Use of *Pasteuria penetrans* with Nematicides in the Control of Root-knot Nematode *Meloidogyne javanica* on Chickpea

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Chickpea (*Cicer arietinum*), a major pulse crop in Pakistan, successfully grows in the vast sandy (rainfed) tracts of the Thal in Punjab and northern areas of Pakistan (Hussain and Malik 1997). Chickpea suffers from several biotic and abiotic stresses including nematodes. Among the plant parasitic nematodes, the root-knot nematodes *Meloidogyne incognita* and *M. javanica* have been encountered on over 50 hosts in Pakistan, including chickpea (Maqbool and Shahina 2001). The bacterium *Pasteuria penetrans* is a promising biological control agent against root-knot nematodes. The role of *P. penetrans* in suppressing plant parasitic nematodes has been tested on many crop plants under greenhouse conditions (Walia 1998). Compatibility of biocontrol agents with commercially available chemicals is essential to promote an integrated system for the control of plant parasitic nematodes and other plant diseases. An experiment was carried out to test the effect of nematicides on the efficacy of *P. penetrans* for the control of root-knot nematode on chickpea under greenhouse conditions.

Sandy loam soil (pH 8.1) was transferred into clay pots (12 cm diameter) at 800 g soil pot^{-1}. The soil was treated with carbofuran and Fertinemakil at 50 ppm pot^{-1} and 500 ppm pot^{-1}, respectively. *Pasteuria penetrans* spore powder (isolate UK 1) was incorporated at 0.5 mg kg^{-1} soil (15×10^3 spores ml^{-1}) separately and in combination with nematicides. Chickpea seeds at 6 seeds pot^{-1} were sown. The pots without *P. penetrans* and nematicides served as control. Pots were randomized in a complete block design with three replicates and kept in the greenhouse. Pots were watered daily. After germination, only three seedlings were maintained per pot and 3000 second stage larvae or juveniles (J2) of *M. javanica* were introduced near the root zone of chickpea. The experiment was terminated at 45 days after nematode inoculation.

Nematicides (at higher doses) in combination with the bacterial antagonist enhanced growth of chickpea crop compared to the nematicide or bacterial antagonist alone and control. Plant height and fresh shoot mass were greater when *P. penetrans* was used with carbofuran (at 500 ppm). When carbofuran was used at 500 ppm, there was maximum reduction in root mass over control (Fig. 1). Carbofuran (at 50 ppm and 500 ppm) and Fertinemakil (at 50 ppm and 500 ppm) were effective against the root-knot nematode *M. javanica* on chickpea compared to *P. penetrans* alone. Maximum reduction in root-knot index (0–10 scale, where 0 = no disease and 10 = maximum disease severity) and egg mass production was observed in pots treated with carbofuran (at 500 ppm) and *P. penetrans*. Nematode densities (g^{-1} root and $200g^{-1}$ soil) were reduced by individual applications of test nematicides and *P. penetrans* as well as the combined treatments over the untreated control. Maximum suppression in nematode invasion g^{-1} root was observed in pots with carbofuran (at 500 ppm) and *P. penetrans* (38%). Population of *M. javanica* in the soil in chickpea rhizosphere was also reduced by carbofuran (at 500 ppm) with *P. penetrans* (Fig. 2). Different doses of carbofuran (at 50 ppm and 500 ppm) and Fertinemakil (at 50 ppm and 500 ppm) improved chickpea growth (P = 0.001) and suppressed disease severity (P = 0.01) when applied with *P. penetrans*. Increased effectiveness of bacterial antagonist in the presence of commercial nematicides is attributed to their compatibility. Chemical pesticides used to control pests have a knock down effect and also affect non-target organisms (Griffiths 1981). Synthetic chemical protectants reduce competitive rhizosphere microorganisms and may provide a chance for microbial activity of biocontrol agents. Compatibility of biocontrol agents with commercially available chemical nematicides is important to integrate them for controlling plant parasitic nematodes and other soilborne pathogens (Crump and Kerry 1986). The results of this study suggest that the biological control agent *P. penetrans* integrates with other control measures and can play an important role in the development of an integrated control strategy for the control of nematode diseases.

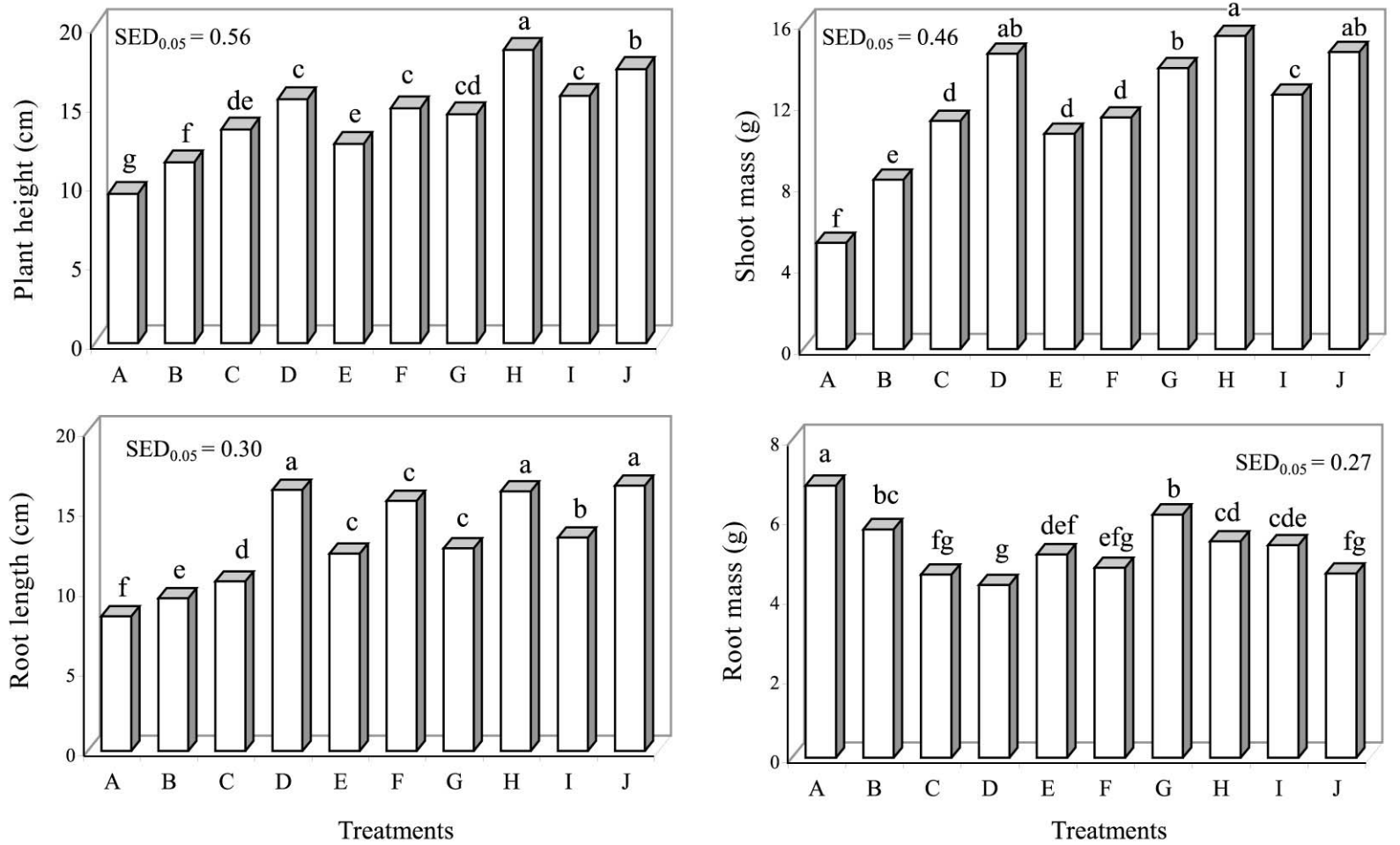

Figure 1. Effect of different doses of nematicides alone or in combination with *Pasteuria penetrans* on growth of chickpea.
[Note: Bars sharing similar letters are not significantly different from each other according to Duncanís Multiple Range Test (DMRT).
A = *Meloidogyne javanica* (3000 J2), B = *Pasteuria penetrans*, C = carbofuran (at 50 ppm), D = carbofuran (at 500 ppm), E = Fertinemakil (at 50 ppm), F = Fertinemakil (at 500 ppm), G = *P. penetrans* + carbofuran (at 50 ppm), H = *P. penetrans* + carbofuran (at 500 ppm), I = *P. penetrans* + Fertinemakil (at 50 ppm), J = *P. penetrans* + Fertinemakil (at 500 ppm).]

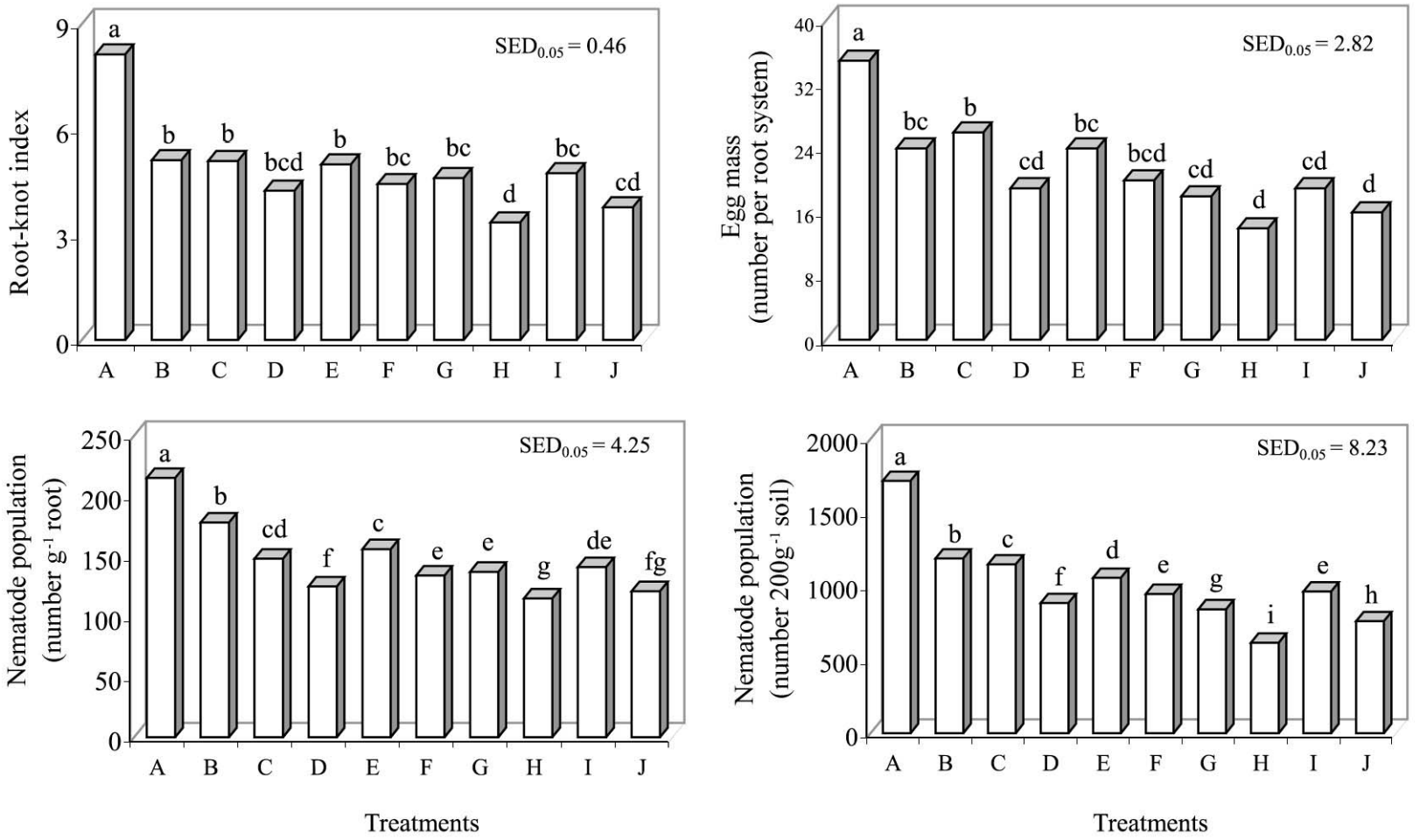

Figure 2. Effect of different doses of nematicides alone or in combination with *Pasteuria penetrans* on root-knot nematode development on chickpea.
[Note: Bars sharing similar letters are not significantly different from each other according to Duncanís Multiple Range Test (DMRT).
A = *Meloidogyne javanica* (3000 J2), B = *Pasteuria penetrans*, C = carbofuran (at 50 ppm), D = carbofuran (at 500 ppm), E = Fertinemakil (at 50 ppm), F = Fertinemakil (at 500 ppm), G = *P. penetrans* + carbofuran (at 50 ppm), H = *P. penetrans* + carbofuran (at 500 ppm), I = *P. penetrans* + Fertinemakil (at 50 ppm), J = *P. penetrans* + Fertinemakil (at 500 ppm).]

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Biotechnology

Construction of the First Bacterial Artificial Chromosome Library in Chickpea

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Chickpea (*Cicer arietinum*) (2n=2x=16) is an economically important food legume crop throughout the world (FAO 1996) and particularly in the semi-arid regions. Of the diseases that affect the crop, ascochyta blight caused by *Ascochyta rabiei* can cause up to 100% yield loss. Two major quantitative trait loci (QTLs) and a minor QTL which play a role in ascochyta blight resistance have been mapped in the chickpea genome using mapping populations developed from a cross between FLIP 84-92C (resistant) and PI 599072 (susceptible) (Santra et al. 2000). These locations in the genome provide information that can be used effectively for eventual isolation and cloning of the resistance genes. To facilitate this objective, we constructed a large insert Bacterial Artificial Chromosome (BAC) library from FLIP 84-92C, a resistant cultivar.

High molecular weight deoxyribonucleic acid (DNA) of chickpea was isolated using the nuclei method of Zhang et al. (1995) and then embedded in agarose plugs. A binary vector pCLD04541 was used for library construction as chickpea is a dicotyledonous plant and *Agrobacterium*-mediated transformation can be performed. The vector was isolated using cesium chloride or ethidium bromide equilibrium centrifugation to avoid any bacterial chromosomal contamination. The high molecular weight DNA agarose plugs were digested with *Hind* III restriction enzyme and run on 1% agarose under pulsed field. The digested DNA of the size 100kb, 150kb, and 200kb were chosen for the first size selection. These were placed on 1% low melting point agarose under pulsed field for second size selection. The size selected DNAs were not exposed to UV light or ethidium bromide to prevent shearing of high molecular weight DNA. After the second size selection, the DNA in the agarose was treated with agarase enzyme to digest the agarose.

After agarase treatment, the size selected chickpea genomic DNA and the *Hind* III digested vector were ligated in the presence of T4 DNA ligase at 16°C overnight. The ligated DNA and vector complex were transformed into ElectroMax™DH10B™ host strain by the electroporation method.

The recombinant white colonies and the non-recombinant blue colonies were selected in the presence of X-gal and isopropylthio-beta-D-galactoside (IPTG) using tetracycline as a selective antibiotic marker. The recombinant colonies were picked up by Flexys biorobot and arranged in 384 well plates. Randomly, 80 colonies were picked up and plasmid DNAs were isolated from individual clones. These were digested with *Not* I enzyme and run on Pulsed Field Gel Electrophoresis (PFGE) (Fig. 1). The library was screened with chloroplast specific probe that detected negligible amount of organellar DNA contamination. Based on the size of the inserts and the genome size of chickpea, we calculated our chickpea BAC library to have 3.8 times genome coverage

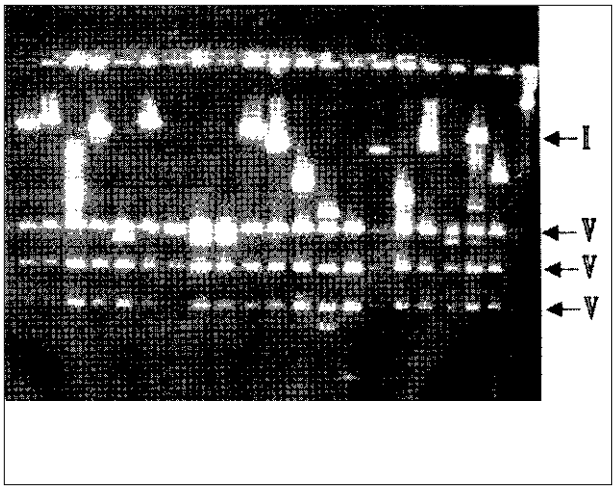

Figure 1. Insert size determination (M = Lambda ladder Pulsed Field Gel (PFG) marker; I = Insert; and V = Vector).

and 95% probability of finding any chickpea genomic fragment from the library.

This is the first chickpea BAC library constructed so far and the large insert library will make large-scale physical maps of genomic regions of chickpea easier to construct. The BAC library has wide application in the analysis of the arrangement and development of microsatellites in chickpea (Springer et al. 1994). Also the library can be used to study the structure and organization of multigene families and for cloning disease resistance genes. Genes underlying QTL or with related functions such as disease resistance are generally organized in clusters (Staskawicz et al. 1995). However, ascochyta blight resistance being governed by QTLs, BAC vector which can clone large fragments and may contain a gene cluster (Meksem et al. 2000), is the first step towards isolation of genes of interest.

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Use of Stem Cuttings to Generate Populations for QTL Mapping in Chickpea

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Quantitative trait locus (QTL) mapping is an effective method to identify genes controlling quantitative traits. The size of a mapping population is a critical factor for a mapping study, since population size determines the resolution of a genetic map and the ability to accurately determine marker order (Young 1994). Generally, framework genetic maps are constructed from 50 to 100 individuals (Paterson 1996). However, if high resolution mapping in specific genomic regions or mapping QTLs of smaller effects is required, then larger population sizes will be required.

The narrow genetic base of chickpea (*Cicer arietinum*) has prompted mapping research to focus on populations generated from interspecific crosses between chickpea and *C. reticulatum*, a wild relative and presumed progenitor of chickpea (Simon and Muehlbauer 1997, Santra et al.

2000, Winter et al. 2000). *Cicer echinospermum* is another wild relative of chickpea that can be crossed with chickpea to produce fertile interspecific hybrids. However, to date, populations derived from *C. arietinum* × *C. echinospermum* interspecific hybrids have not been used for mapping studies. Even though *C. reticulatum* and *C. echinospermum* are readily crossable with chickpea, obtaining adequate seed numbers for mapping from a single F_1 hybrid plant is not always simple. We report a simple method for using stem cuttings from an interspecific F_1 hybrid plant between chickpea and *C. echinospermum* to generate a sufficiently large F_2 population for QTL mapping.

Stem cuttings were taken from chickpea hybrid plants during the vegetative growth stage. A short (~15 cm) lateral branch was cut using scissors that was surface-sterilized by immersing in 100% ethanol. The lowest two leaves were removed and the ends of the stem cuttings were surface sterilized by immersing in 100% ethanol for five seconds. Once the ethanol had evaporated, the ends of stem cuttings were dipped into 8000 ppm indole butyric acid (IBA) (Kendon, Australia) in talc powder covering approximately 3 cm from the end of the stem cutting. The stem cuttings were placed in 10-cm diameter pots containing a mixture of autoclaved soil, sand, and perlite (1:1:1). The ends of the cuttings were inserted into holes made with a bamboo skewer; this ensured the IBA powder did not rub off while inserting into the soil mixture. To minimize stress caused by transpiration of the cuttings, pots were then placed in a misting chamber (~100% relative humidity) for 14 days until roots formed. Stem cuttings that had developed roots were transferred directly into 40-cm diameter pots containing soil and grown into mature plants in the glasshouse facility at the University of Melbourne, Victoria, Australia.

Stem cuttings were taken from two different interspecific F_1 hybrids (derived from crosses between chickpea cultivar Lasseter and *C. echinospermum* accession PI 527930) and the parental accessions. Stem cuttings were evaluated for rooting and survival (expressed as percentages) at 14 and 28 days respectively after treatment with IBA (Table 1). Percentage of rooting ranged from 48.6% to 70.8%. The percentages for rooting of stem cuttings from the F_1A and F_1B interspecific hybrid plants were significantly different, which may have been due to genetic differences between the interspecific F_1 plants or differences in plant health. Control stem cuttings that were not treated with IBA did not develop roots. The percentage of survival of stem cuttings ranged from 63.6% to 70.6%. All chickpea and wild *Cicer* accessions tested so far (data not shown) were amenable to propagation by stem cuttings, which is consistent with the previous studies (Rupela 1982, Bassiri et al. 1985). More than 1600 F_2 seeds were combined from the original F_1A plant and F_1A stem cuttings.

Table 1. Rooting and survival of stem cuttings of parents and interspecific hybrids of chickpea dipped in indole butyric acid powder.

Genotype	Rooting[1] (%)	Survival (%)
Lasseter (*Cicer arietinum*; chickpea)	66.7 a	68.8
PI 527930 (*C. echinospermum*)	62.5 ab	66.7
F_1A (*C. arietinum* × *C. echinospermum*)	70.8 a	70.6
F_1B (*C. arietinum* × *C. echinospermum*)	48.6 b	63.6

1. Figures followed by common letters do not differ significantly at 5% level of significance according to DNMRT (Duncan's New Multiple Range Test).

Previous reports of propagation of *Cicer* species by stem cuttings have utilized solution culture methods with or without IBA (Rupela and Dart 1981, Davis and Foster 1982, Bassiri et al. 1985). The solutions used in the previous studies had to be periodically checked for algal growth and to maintain a constant volume. Previous reports of propagation by stem cuttings in mung bean (*Vigna radiata*) and pea (*Pisum sativum*) also used solution culture methods; IBA was used in solution or in a modified nutrient solution and regularly renewed or replaced (Jarvis and Booth 1981, Eliasson and Areblad 1984). The study by Rupela and Dart (1981) used root hormone powder on wounded branches of chickpea. However, this method was difficult to perform because stem cuttings that were too deep or too shallow resulted in failure of rooting. The treatment of stem cuttings with IBA in powdered form is a much quicker and easier method compared to the use of nutrient solutions with or without IBA.

The success rates for rooting of stem cuttings were higher in two of the previous studies compared to the present study (Davis and Foster 1982, Rupela 1982). But different genotypes, stem cutting lengths, and ages of stock plants were used, and differences in rooting of stem cuttings were detected for different genotypes and length of stem cuttings. However, the simplicity and speed of the method described in this study suggests that this method could be more convenient for the vegetative propagation of chickpea and *Cicer* species. Furthermore, the efficiency of the present method could be improved by more thorough surface sterilization of stem cuttings and drenching the propagation medium with a fungicide, since fungal infection of stem cuttings was the usual

cause of rooting failure. The effects of other variables such as genotype, concentration of IBA, length of stem cuttings, and position of cutting could also be tested in order to increase the efficiency of the present method.

By taking stem cuttings from any F_1 hybrid plant, a sufficiently large F_2 or backcross (BC) population may by produced for QTL mapping. By producing an extremely large number of F_2 or BC seed (>500), high resolution QTL mapping may be undertaken in order to analyze specific genomic regions or detect QTLs with small effects. Furthermore, this method may also be used to rapidly maximize seed numbers for important genotypes such as parental lines or specific accessions that have resistance to biotic or abiotic stresses.

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Utilization

Traditional Medicinal Knowledge about Chickpea in India with Special Reference to Chhattisgarh

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Cicer arietinum, commonly known as Bengal gram or chickpea, is a much branched herb and native to Southwest Asia. It is now grown as a pulse crop all over India (Singh et al. 1996). In different Indian languages it is known as *chana*, *chania* (Gujarati); *boot*, *chana* (Hindi); *kari-kampukadale* (Canarese); *harbara* (Marathi); *balabhojya*, *chanaka*, *kanchuki*, and *vajibhakshya* (Sanskrit). Chickpea seed is consumed in various forms such as dal flour, boiled or parched, salted or sweet preparations, and green foliage as salad. Since ancient times chickpea has been known as a valuable medicinal plant. A preliminary survey of chickpea growers conducted by the author during 1998–99 in different districts of Chhattisgarh state of India revealed that most of the farmers are not aware of the valuable medicinal properties of chickpea. Only few traditional healers are aware of its therapeutic uses and there is a strong need to document this knowledge for the use of future generations (Oudhia et al. 1999). As the demand for medicinal and aromatic plant products (including chickpea) is growing at the rate of 7% per annum globally, it is essential to document and publicize the medicinal properties of chickpea mentioned in ancient Indian literature and also

the traditional medicinal knowledge of the common people (Oudhia 2001a).

Chickpea leaves and seeds, and acid exudation of the plants are commonly used as medicine. Chickpea holds a reputed position in Ayurvedic and Unani system of medicine. According to Ayurvedic philosophy, chickpea leaves are sour, astringent to bowels, and improve taste and appetite. Leaves are used to cure bronchitis specially the chronic bronchitis. The seed is used as tonic, stimulant, and aphrodisiac. Because of its aphrodisiac properties, it is referred as *vajibhakshya* in Sanskrit (Pandey 1993). The seed is used as an appetizer; it also has anthelmintic properties. It also cures thirst and burning. Seeds are mainly used for the treatment of bronchitis, leprosy, skin diseases, blood disorders, throat problems, and biliousness. According to Unani system of medicine, chickpea leaves are purgative and abortifacient. Leaves are used in treatment of cold, cough, and pains (Sastry and Kavathekar 1990). Seeds are mainly used for the treatment of diseases of liver and spleen. Seeds enrich the blood and cure skin diseases and inflammation of the ear (Caius 1989, Agharkar 1991, Warrier et al. 1995). Medicinal properties of weeds in chickpea fields (Oudhia 1999a) and of pod borer (*Helicoverpa armigera*), a major insect pest of chickpea, have also been reported (Oudhia 2001b).

The people of Chhattisgarh have rich traditional medicinal knowledge about plants (Oudhia and Tripathi 1998), insects (Oudhia 1998), and mites (Oudhia 1999b). Chickpea is one of the frequently used medicinal plants in Chhattisgarh. A survey was conducted during 1999–2000 in ten districts of Chhattisgarh to list the existing medicinal uses of chickpea. From each selected district, two blocks were selected and from each block, a random sample of four villages was taken to make a sample of 200 respondents. Information regarding existing uses was collected through personal interviews.

The survey revealed that chickpea is among frequently used medicinal plants in Chhattisgarh. It revealed that acid exudation from chickpea plants is most frequently used as compared to leaves and seeds during the crop season. The acid exudation is collected by spreading sheets of white cloth over the crop in the field at night and the next morning the dew mixed acid is collected and used as medicine. The natives use this "miracle potion" to cure common ailments like constipation and indigestion. It was also noted during the survey that many pharmaceutical companies are regularly purchasing this exudation at fair rates from the farmers. Many farmers have installed pH meters in their farms. The tribals of Chhattisgarh use this "miracle potion" to cure sunstroke. It is also believed to cure patients suffering from snake poisoning and dog bite. In many parts of India, the fresh plant is used for the treatment of dysmenorrhoea (i.e., painful menses). Many traditional healers of Chhattisgarh are also using chickpea plants for this purpose. Fresh chickpea leaves are styptic and farmers use these as first aid remedy to stop bleeding. The styptic properties of *Helicoverpa* pod borer have also been reported (Oudhia 2001b). The boiled leaves of chickpea (collected before flowering) are used as poultice to sprained and dislocated limbs.

The survey suggested that by contacting pharmaceutical companies and other potential buyers of acid exudation, leaves, and seeds, chickpea growers can be encouraged to earn extra profit from the crop. The survey also revealed that there is a strong need to prepare the list of potential buyers of medicinal chickpea plant parts and to recognize and promote traditional uses of chickpea.

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Pigeonpea

Breeding

Characterization of *Cajanus scarabaeoides* Growing in Yuanjiang County of Yunnan Province in China

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The known and unknown traits available in the wild relatives of the cultivated types are useful for dynamic crop improvement programs and therefore conservation and evaluation of secondary and tertiary gene pools assume great importance. At present the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India maintains a total of 213 accessions of 20 *Cajanus* species which can be used by breeders. In China limited attention has been paid to the collection, maintenance, and evaluation of pigeonpea (*Cajanus cajan* (L.) Millsp.) germplasm and its wild relatives. van der Maesen (1986) reviewed the taxa closely related to pigeonpea and listed six species from China; the same species are also recorded in "Reipublicae Popularis Sinicae" by Lee (1995). According to these records, *Cajanus crassus* (Prain ex King) van der Maesen is distributed in southern Yunnan, southwestern and southern Guangxi; *C. goensis* Dalz. in southern and northwestern Yunnan; *C. grandiflorus* (Benth. ex Bak) van der Maesen comb. nov. in Yunnan and Zhejiang; *C. mollis* (Benth.) van der Maesen comb. nov. in western and southern Yunnan; *C. niveus* (Benth.) van der Maesen comb. nov. in Yunnan; and *C. scarabaeoides* (L.) Thouars in Yunnan, Guizhou, Guangxi, Guangdong, Hainan, Fujian, and Taiwan (Lee 1995). In addition, Saxena (2000) found another wild species in Fengshan county of Guangxi province and based on its perennial habit, general morphology, leaf shape, and branching habit it was suspected to be *C. cajanifolius* (Haines) van der Maesen.

Cajanus scarabaeoides is the most widely spread wild species in Asia. In China it has two botanical varieties, i.e., var. *scarabaeoides* and var. *argyrophyllus* (W.T. Wei & Lee) Y.T. Wei & S. Lee. comb. nov. *Cajanus scarabaeoides* is called "Man Cao Chong Dou" in Mandarin Chinese, "Shui Kom Ts'o" in Guangdong dialect, and "Jia Yan Pi Guo" in Yunnan dialect. In Yunnan province it is endemic in Yang Tse Ferry near La Ka Triang between Yunnan and Huili of Shichuan while in Hainan province it has been found growing in Wanning. The species is widely distributed in Mojiang, Yongde, Jingdong, Gengma, Shuangjiang, and Changyuan counties in Yunnan (Lu Fuji, Chinese Academy of Agricultural Sciences, China, personal communication). Saxena (2000) found this species growing in the wastelands at 180 m elevation in Tiandong county of Guangxi province. In June 2000, we found a large population of *C. scarabaeoides* growing wildly in the dry slope hills of Yi Qun Yang mountain (23°36' N, 101°59' E, 450 m elevation) near Yuanjiang county town, located beside the Yuanjiang river.

To characterize this species, four sites with good plant population were identified and at each site 10 random plants were selected to record observations on various traits. The measurements were recorded according to the methodology suggested by Remanandan et al. (1988). The protein estimation in matured whole seed and fresh leaves was done using 751-GW spectrophotometer for colorimetric estimation.

Cajanus scarabaeoides was found in abundance in open grasslands and dry scrub vegetation on hill slopes and ridges between cultivated fields. It was also located along roadside, footpath, or convex ridges where reasonable amount of sunlight was available (Fig. 1). However, its population was low in the deep inland bushes and dark forests.

Cajanus scarabaeoides is a creeper-climber, supported by grass and small shrubs. Branches are straight or winding, quite woody at the base, up to 135 cm long; stem is white-pubescent with hair. Leaves are pinnately trifoliate, lower surface densely white-pubescent, upper surface white-pubescent; end leaflet obovate, 21–47 mm long, 10–30 mm wide, tip acute or obtuse, base cuneate; side leaflets obliquely obovate, 15–38 mm long, 7–24 mm wide. Flowering habit is indeterminate and sporadic and its duration ranged from early June to late November. Racemes are short with 1–4 flowers, peduncles 3–9 mm long, pedicels 3–5 mm long. The flowers are yellow or creamish yellow with dense sun-red veins. Calyx is densely pubescent with white hairs, tube 2–3 mm, 4 teeth, 4–8 mm long. Vexillum is obovate, 5–6 mm long, 2 mm wide, base clawed. Alae is elongate-obovate, 7–10 mm long, 2–5 mm wide, base auriculate. Keel petals are oblique, 8–11 mm long, ventrally adnate. Ovary is densely white-pubescent with hair, 5–6 mm long, 2 mm wide, ovules 4–6. Styles are 5–8 mm long, glabrous, the top 2–4 mm upcurved. Stamens are 9–13 mm long, with top 2–5 mm free and curved upwards; anthers mostly 9+1, but sometimes 8+1. Pods are oblong, 11–34 mm long, 6–10 mm wide, densely covered with golden brown long and short hairs (2–4 mm long), pods purple or dark purple with 1–7 seeds per pod (mostly 4–6). Seeds are rectangular-rounded, 2.4–4 mm long, 1.8–3 mm wide, 1–2 mm thick, black, and plain or speckled. Strophiole is divided, 1.9 × 0.7 mm, greenish or black. The 100-seed mass is 1.94 g. The mean protein content is 21.88% in the seed and 13.23% in the dried leaves.

Cajanus scarabaeoides is reportedly a useful but unimpressive species in grasslands for fodder (Dabadghao and Shankarnarayan 1973). Kirtikar and Basu (1933) reported that *C. scarabaeoides* is effective against diarrhea in cattle. Leaves are used in traditional Chinese medicine to improve indigestion and diuresis (Lee 1995). Insects such as podborers (*Helicoverpa armigera*) and podfly (*Melanagromyza obtusa*) also attack wild *Cajanus* spp, but in a few species including *C. scarabaeoides* some degree of antibiosis is observed (van der Maesen 1986). In lac production areas such as Jingdong county in Yunnan province of China, the lac insect *Kerria lacca* was found occasionally growing on branches of *C. scarabaeoides* and lac secretion was observed (Lu Fuji, Chinese Academy of Agricultural Sciences, China, personal communication).

According to Lee (1995), stem and leaf traits such as white hairs, broad elliptical, obovate, or near round leaflet, and veins on upper side prominently concave make var. *argyrophyllus* distinct from var. *scarabaeoides*. Thus, based on the available information, we suspect the species found in Yuanjiang county to be var. *argyrophyllus*. This variety is widely distributed in Guangxi, Yunnan, and Sichuan provinces. Since only *C. scarabaeoides* and *C. cajanifolius* among the wild species so far reported in China can be crossed easily with pigeonpea, it is necessary that the economic traits of these two species should be further evaluated in China for their use in breeding programs.

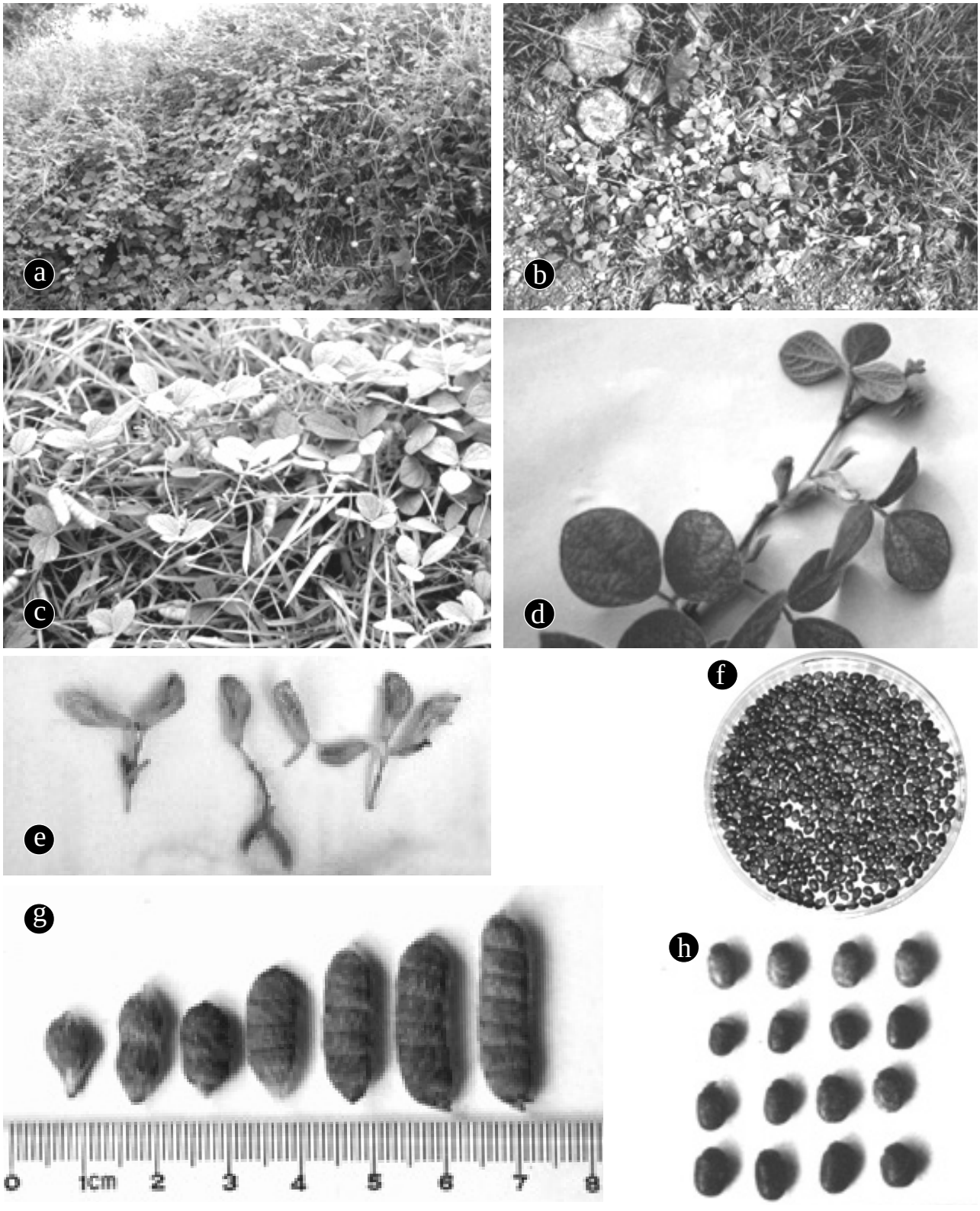

Figure 1. ***Cajanus scarabaeoides*** **in Yunnan, China: (a) Growing in a ridge; (b) Growing in barren soil; (c) Mature plants; (d) Racemes; (e) Flowers; (f) Seeds showing color diversity; (g) Pods with 1–7 seeds; and (h) Seeds with strophioles.**

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First Report of Natural Outcrossing in Pigeonpea from China

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Natural outcrossing in pigeonpea (*Cajanus cajan*) is primarily responsible for the deterioration of purity of cultivars and genetic stocks. Several insects are responsible for transferring pollen from one plant to another within and across the fields. The major pollinating insects identified are *Apis mellifera*, *A. dorsata*, *Megachile lanata*, *Ceratina binghami*, and *Xylocop* spp. The populations of these pollinating insects and local environmental factors that assist in their movement determine the extent of natural outcrossing at a particular location. Natural outcrossing in pigeonpea has been reported from India, Kenya, Australia, Hawaii (USA), and Sri Lanka (Saxena et al. 1990, 1994).

Pigeonpea was introduced into China in the 6th century from India and since then it was cultivated sporadically in the southern provinces. In the 1950s, Chinese scientists in Yunnan province identified pigeonpea as a favorable host for lac insect (*Kerria lacca*) because it was found to have useful traits such as easy establishment, fast growth, and high yield of quality lac. Recently, under the crop diversification program in China several other uses of pigeonpea have emerged, e.g., for soil conservation; and as fodder, feed, and fresh vegetable (Saxena 2000). Specific cultivars for precise purposes have been developed from breeding materials received from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. To sustain pigeonpea productivity it has now become mandatory to maintain the genetic purity of these cultivars. Therefore, an experiment was conducted to determine the extent of natural outcrossing in China.

Pigeonpea variety ICPL 87091 with a distinct recessive trait of 'determinate growth habit' was selected for this study. The experiment was conducted in 1999 at Nanning in Guangxi province in China. Due to lack of experimental facilities 20 single-row plots of ICPL 87091 were sown in a field planted with breeding materials having dominant genetic marker of indeterminate growth habit and matching flowering time. These plots, measuring 5 m in length, were scattered at different places in the field. At least 10 pollinator rows on either side of each ICPL 87091 plot were ensured. The inter- and intra-row spacing was 100 cm and 50 cm respectively. Only two insecticide sprays were done at early flowering stage to control the pod borer *Maruca vitrata*. From each plot five individual plants were randomly harvested. The progenies of 66 selections with sufficient quantity of open-pollinated seed were sown in the subsequent rainy season. Since indeterminate growth habit is dominant over determinate growth habit, counts were recorded in each plant-progeny row for the self (determinate) and hybrid (indeterminate) plants. The frequency of natural outcrossing in each row was estimated as percentage of the observed number of hybrid plants.

In spite of two insecticide sprays a lot of insect activity was observed during flowering stage in the entire field. Also, several insect species were active but their identification was not feasible. However, among these, honeybees (*Apis* spp) were predominant. The data from the single plant progenies of ICPL 87091 revealed a large plant-to-plant variation (Fig. 1) for natural outcrossing in the preceding generation with a mean of 24.6%. In two progenies no outcrossed hybrid plant was recorded while in four progenies more than 40% hybrid plants were observed. In one progeny 60% natural outcrossing was

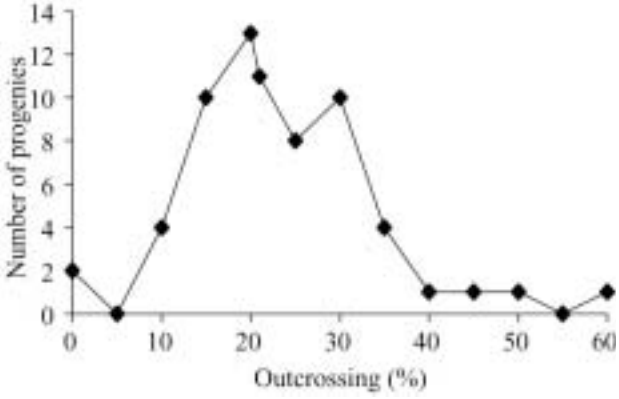

Figure 1. Frequency distribution of natural outcrossing in the open-pollinated progenies of pigeonpea ICPL 87091.

recorded. Of the 66 progenies evaluated, 52 exhibited 15–30% natural outcrossing. The inter-plant variation for natural outcrossing observed at Nanning may be due to variation in plant growth or shading from neighboring tall plants due to which the pollinating insects were relatively less active on such plants and the insects preferred to land on the well grown tall plants.

The results show that as in other countries, in China too the active pollinating insects are present in large numbers and they are a potential danger to the maintenance of genetic purity in pigeonpea. Since pigeonpea is being promoted in a range of environments, the extent of natural outcrossing is likely to vary from place to place. Therefore, it is advisable to determine the extent of natural outcrossing in the representative locations before undertaking the seed production of more than one genotype in one locality. For small-scale pure seed production in the breeding programs the use of mosquito nets is recommended while for large-scale seed production the plots should be isolated at a safe isolation distance. Since in a particular environment the population of pollinating insects and other physical factors are important, effective isolation distances for major pigeonpea-growing areas need to be estimated. Pending such study, the sowings in isolated blocks separated by at least 200 m, as recommended by the Food and Agriculture Organization of the United Nations (Ariyanayagam 1976) should be used. Besides seed production, there are two other contrasting implications of natural outcrossing in pigeonpea breeding. On one hand, it is detrimental to pedigree breeding that involves evaluation and selection of pure genotypes. On the other hand, the phenomenon of natural outcrossing can be used for the genetic improvement of breeding populations (Khan 1973) and hybrid breeding (Saxena et al. 1996). At ICRISAT a considerable progress has been made in hybrid breeding and the world's first commercial hybrid was released in 1991 (Saxena et al. 1996). Since pigeonpea research program in China is evolving the information on the extent of natural outcrossing is essential to develop both long- and short-term research and development strategies.

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Field Studies on Genetic Variation for Frost Injury in Pigeonpea

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Pigeonpea (*Cajanus cajan*) cultivation in China is being revived primarily for soil conservation and fodder production. Experiments show that in certain areas freezing temperatures (<0°C) cause considerable damage to the foliage of the crop. Considering the potential of pigeonpea in China, this study was conducted to understand the nature and magnitude of damage caused by freezing temperatures and to assess the feasibility of identifying freezing tolerant genotypes.

Three genotypes (ICPL 151, ICP 8863, ICP 11298) bred by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India and one local landrace were evaluated. Four test sites were selected in different agroecological zones in Yunnan province in China. At each location, about 500 plants of each genotype were grown in June 1999 in an unreplicated block. The crop was grown with recommended cultural practices. In September/October, 30 competitive plants of each genotype were tagged randomly during the vegetative stage and in January 2000, these plants were scored for frost injury on five-point scale as: 0 = resistant, no visible symptom of damage; 1 = tolerant, up to 10% leaves killed; 2 = moderately tolerant, only terminal branches and tender leaves killed; 3 = moderately susceptible, upper-half of plant canopy killed; and 4 = susceptible, entire plant killed. In March 2000, when the temperatures for pigeonpea growth were conducive, 40 moderately susceptible (score 3) plants were tagged randomly in each block for visual assessment for their regeneration capability. Mean frost injury grade (ã) and average frost injury index (δ) were estimated for each genotype using the formulae given by Wang (1987):

$$\tilde{a} = \frac{\Sigma (a \times n)}{N} \qquad \delta = \frac{\Sigma (a \times n)}{a_{max} \times N}$$

where a = frost injury score; n = index in certain grade; and N = total number of plants.

The minimum, maximum, and average temperatures were recorded daily at each location to correlate frost injury with the prevailing temperatures of the coolest period (December 21 to 31). The minimum temperatures in Jingdong (range −0.3 to −3.0°C) and Yongren (range −1.3 to −4.1°C) remained below zero for nine consecutive days (Fig. 1) and killed the entire population of all the four lines. In Binchuan, on the other hand, the sub-zero temperatures persisted only for seven days and distinct varietal differences in response to freezing tolerance were observed. Both ICP 11298 (δ = 0.333) and the landrace (δ = 0.225) suffered least mortality. In these lines about 50% plants recorded no damage. In ICP 8863, over 90% plants died while in ICPL 151, there were no survivors (Table 1). It appears that both the temperature as well as its tenure (duration) are responsible for causing frost injury in pigeonpea. The results suggested that some genotypes such as ICP 11298 and local landrace could tolerate temperatures up to −4°C for a maximum period of seven days. In Yunxian, where the temperature persisted at −1°C for about a week, no plant mortality was recorded in any genotype suggesting that in such areas pigeonpeas of all durations could be grown successfully.

The study on regeneration of the plants partially killed due to freezing temperature (score 3) in Binchuan revealed significant variation among the genotypes. The local landrace recorded highest revival (82.5%) followed by ICP 11298 (65.0%). The regeneration rates in ICPL 151 (12.5%) and ICP 8863 (20.0%) were low. In comparison to the long-duration types, the early-maturing pigeonpea lines are known to have weak canopy and less food reserves and thus produce relatively less regenerated growth even under conducive environments. Of the test genotypes, ICPL 151, the most susceptible line to freezing temperature, is the earliest to mature (120 days); it has relatively less biomass and food reserves. On the contrary, the plants of the local landrace were of long-duration (>250 days) with high biomass and food reserves. The stress of freezing causes ice formation inside the plant and the most common symptoms are wilting and death of the whole plant. According to Wery et al. (1993) the intra-cellular ice, created around small particles inside the cell, is responsible for cell dehydration and, later, for cell membrane destruction due to freeze-thaw cycle which forces water back into the cell too rapidly. The extra-cellular ice produces a matrix around the plant cell causing mechanical damage to it and this results in the development of necrotic zones on the plants. Cold tolerance or winter hardiness has also been reported to be positively correlated with sugar content of cell and osmotic potential in chickpea (*Cicer arietinum*) (Malhotra and Saxena 1993). The osmotic adjustment promotes accumulation of solutes within cells and thereby helps in

lowering the osmotic potential to maintain turgor, which consequently imparts tolerance to dehydration (Ludlow and Muchow 1988).

Pigeonpea is a unique plant with its ability to survive through various stresses. It is intrinsically perennial and this quality helps in retaining a sufficient supply of assimilates and other nutrients essential to maintain the primary functioning of roots, to tide over unfavorable conditions, and in providing reserves for new growth. Also, during stress periods the deep root system of pigeonpea helps in maintaining optimum water status within the plant. Therefore, the ability of pigeonpea plants to withstand extra-cellular ice formation, as observed in this study, could be attributed to the avoidance of cell dehydration. Although this study was conducted with limited number of genotypes, it provides some understanding about the

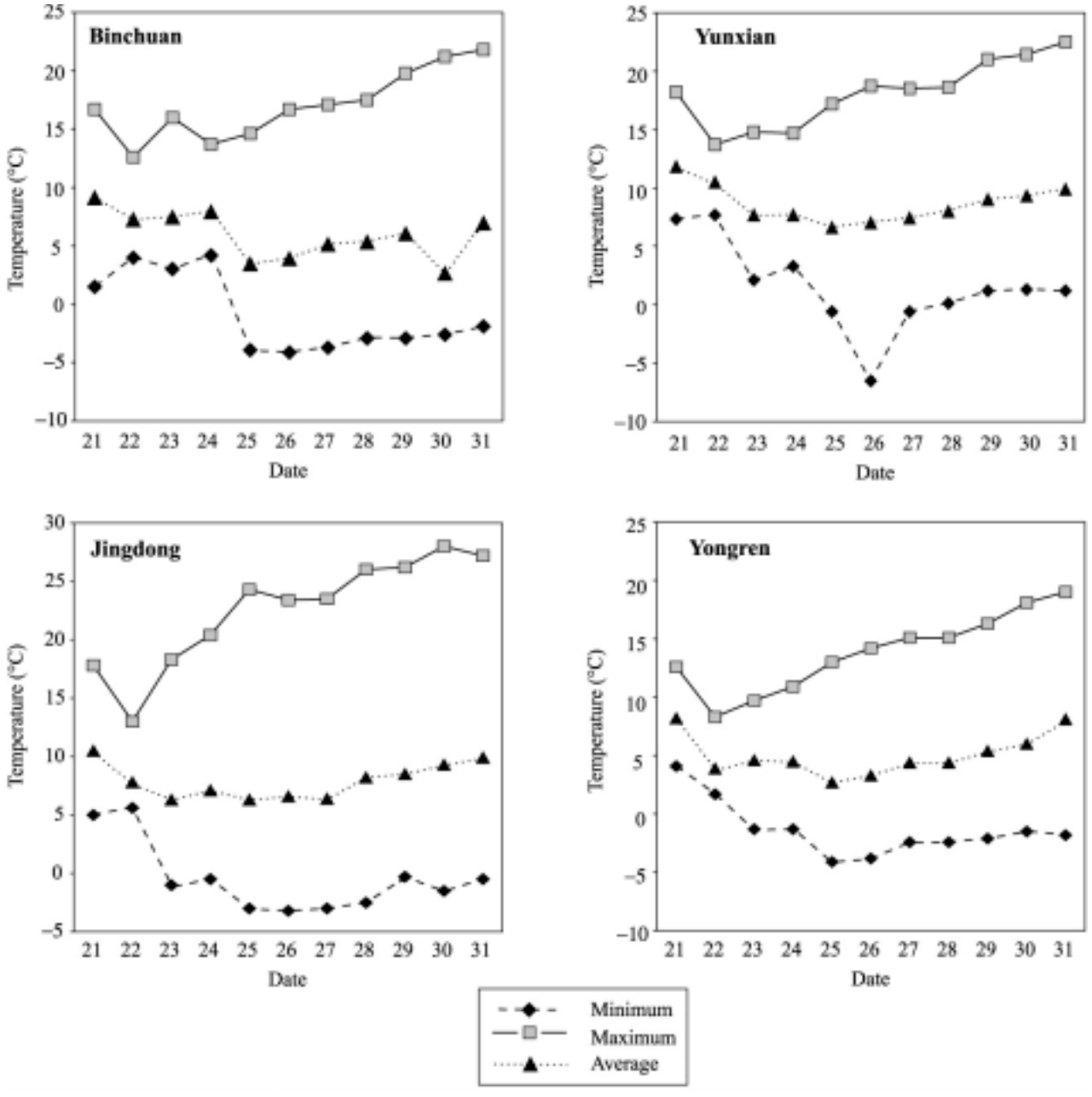

Figure 1. Temperatures at four locations in Yunnan province of China during 21–31 December 1999.

Table 1. Frequency distribution of pigeonpea genotypes to frost injury at four locations in Yunnan province of China during 1999.

Location/Genotype[1]	Number of plants with freezing injury score					Frost injury grade (ã)	Frost injury index (δ)
	0	1	2	3	4		
Binchuan							
ICPL 151	0	0	0	0	30	4.0	1.000
ICP 8863	0	0	0	3	27	3.9	0.975
ICP 11298	15	3	3	5	4	1.3	0.333
Local landrace	15	6	6	3	0	0.9	0.225
Yunxian							
ICPL 151	30	0	0	0	0	0.0	0.000
ICP 8863	30	0	0	0	0	0.0	0.000
ICP 11298	30	0	0	0	0	0.0	0.000
Local landrace	30	0	0	0	0	0.0	0.000
Jingdong							
ICPL 151	0	0	0	0	30	4.0	1.000
ICP 8863	0	0	0	0	30	4.0	1.000
ICP 11298	0	0	0	0	30	4.0	1.000
Local landrace	0	0	0	0	30	4.0	1.000
Yongren							
ICPL 151	0	0	0	0	30	4.0	1.000
ICP 8863	0	0	0	0	30	4.0	1.000
ICP 11298	0	0	0	0	30	4.0	1.000
Local landrace	0	0	0	0	30	4.0	1.000

1. Total number of plants observed in each genotype is 30.

nature and extent of damage caused by freezing temperatures to pigeonpea. However, precise experiments under field and controlled environments are necessary to understand various aspects of frost injury. Also, its quantification in different agroecological zones is essential before a systematic screening of germplasm could be undertaken. Since the problems of soil erosion and shortage of fodder are widespread, the development of high-yielding frost tolerant varieties will help in promoting pigeonpea in China. According to Blum (1988) the genotypes with smaller cells having better osmotic adjustment and less intra-cellular water are likely to survive freezing temperatures. To breed such varieties, the genetic variation available within and among the landraces and other germplasm need to be exploited for identifying parental lines with high survival and revival rates. Alternatively, the tolerant selections from local landraces can be improved for yield and various organoleptic traits. The genetic variation observed in this study leads to an optimism for successful breeding of frost tolerant pigeonpeas in the near future.

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Pathology

Evaluation of Pigeonpea Genotypes for Resistance to Phytophthora Blight

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Phytophthora blight of pigeonpea (*Cajanus cajan*), caused by *Phytophthora drechsleri* f. sp *cajani*, appears from seedling to maturity stages of plant growth and causes damage to the crop during heavy and frequent rain, particularly in areas that are low lying and have poor field drainage. The management of disease through fungicidal spray is ineffective due to dilution or washing away of chemicals from the plant surface. The most effective, economical, and safe way to control phytophthora blight would be the development of resistant cultivars.

A large number of pigeonpea genotypes have been screened and resistant sources identified to phytophthora blight by several workers (Pal et al. 1970, Kannaiyan et al. 1981, Singh et al. 1985, Amin et al. 1993). However, these genotypes have become susceptible perhaps due to evolution of a new pathotype during the past few years. This study was carried out to evaluate some pigeonpea genotypes for resistance to phytophthora blight.

One hundred and twenty one genotypes of pigeonpea were evaluated against phytophthora blight during two consecutive years, 1998/99 and 1999/2000. Fifty seeds of each test line were sown in 5-m rows with spacing of 60 cm × 10 cm in phytophthora sick field at the research farm of the Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The genotype ICP 7119 was grown as a susceptible check and sown after every two test rows of pigeonpea. The trials were conducted in a randomized block design with three replications.

When the crop was 2.5 months old, the plants that escaped natural infection were artificially inoculated by the knife-cut method (Nene et al. 1981). A mycelial disc (4 mm × 2 mm) of *P. drechsleri* f. sp *cajani* grown on potato dextrose agar medium for a week was inserted below the bark of the 'I'-shaped cut on the stem and banded with cellophane tape to retain moisture. Plant mortality after natural and artificial infection was recorded 15 days after artificial inoculation. Percent disease incidence was calculated and pigeonpea genotypes were categorized as resistant (0–10%), moderately resistant (10.1–20.0%), moderately susceptible (20.1–40.0%), and susceptible (40.1–100%).

The naturally infected plants of pigeonpea showing purple brown to brown lesions on stems toppled over and dried out. The symptoms on artificially inoculated plants appeared as brown to dark brown discoloration around the inoculation site and plants died within 10–12 days after inoculation. Of the 12 pigeonpea genotypes screened only AKT 9726 was resistant and six lines (C 11, MAL 13, KPBR 80-2-1, KA 32-2, 286-96 RSW-1, 337-97-20) were moderately resitant in both years. The remaining test lines were moderately susceptible (26 in 1998/99; 27 in 1999/2000) or susceptible (88 in 1998/99; 87 in 1999/2000).

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Efficacy of Entomopathogenic Nematode against *Helicoverpa armigera* on Pigeonpea

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Pigeopea (*Cajanus cajan*), commonly known as *arhar*, is one of the important pulse crops of India and widely grown in Central Gujarat. Amongst various insect pests attacking pigeonpea, *Helicoverpa armigera* and *Melanagromyza obtusa* are of major importance. Long-term use of insecticides has eliminated the natural enemies, and also raised the danger of insecticide residues in the seed. Amongst various natural enemies, entomopathogenic nematodes (EPNs) are widely used for the management of insect pests in many countries in the West. A field study was carried out in 1999/2000 to test the efficacy of native EPN *Heterorhabditis* sp (GAU EPN 16) against *H. armigera* infecting pigeonpea at the Gujarat Agricultural University (GAU), Anand, Gujarat, India.

Pigeonpea variety GT 100 was sown on 30 July 1999 at 75 cm × 20 cm spacing in 3 m × 6 m plots in 5 rows at the Agronomy Farm of GAU, Anand. Four treatments were tested in a completely randomized block design, and replicated six times. Plants were selected randomly in each plot, and tagged for recording *H. armigera* population and pod damage. *Helicoverpa armigera* population was monitored at weekly intervals after onset of flowering to schedule EPN sprays (mass produced in vitro on egg yolk agar) based on economic thresholds. The population was also recorded before EPN spraying and 7 days after spraying. At the time of harvesting overall pod damage was recorded on the tagged plants. Yield of pigeonpea was also recorded.

Treatments included: (1) GAU EPN 16 alone at 100,000 infective juveniles (IJs) m^{-2}; (2) GAU EPN 16 with adjuvants (5% starch + gum arabic) at 100,000 IJs m^{-2}; (3) adoptable integrated pest management (IPM) module [spray of profenophos at 2 L ha^{-1} in 4th week of November, spray of *H. armigera* nuclear polyhedro virus (HaNPV) at 250 larval equivalent (LE) ha^{-1} in 1st week of December, fenvalrate at 0.02% in 4th week of December, chlorpyriphos at 0.04% in 1st week of January, and quinalphos at 0.05% in 3rd week of January]; and (4) control (untreated).

EPN significantly reduced *H. armigera* population (Table 1). EPN alone and with adjuvants reduced the larval population by 16.7% and 28.5% respectively over the initial population, and resulted in 66.9% and 97.3% higher grain yield over the untreated control. However, IPM treatment provided the highest control of *H. armigera*. Pod damage in EPN alone and with adjuvants was reduced to 31.7% and 46.8% respectively as compared to untreated control. IPM-treated plots had the maximum grain yield, which may be due to the reduction of other pests along with *H. armigera*. Thus native EPN has good scope for management of *H. armigera*. Tahir et al. (1995) investigated the susceptibility of *H. armigera* to EPNs, and reported that it was highly susceptible to *Steinernema riobravis*, *S. carpocapse*, and *Heterorhabditis* sp. Patel

Table 1. Bio-efficacy of the entomopathogenic nematode (EPN) *Heterorhabditis* sp against *Helicoverpa armigera* on pigeonpea.

Treatment	*H. armigera* population plant^{-1}		Pod damage at harvest[2] (%)	Yield[2] (kg ha^{-1})
	Before spraying	7 days after spraying[1]		
EPN 16	7.00	5.83 (16.7)	31.45 (−31.7)	635.19 (+ 66.9)
EPN 16 + adjuvants	5.83	4.17 (28.5)	24.53 (−46.8)	750.80 (+ 97.3)
IPM module	7.00	3.50 (50.0)	13.60 (−70.3)	979.48 (+157.4)
Control	6.33	8.17	46.00	380.47
SEm	0.61	0.53	1.83	36.71
CD at 0.05%	NS[3]	1.59	5.52	110.63
CV (%)	22.66	23.91	15.50	13.09

1. Figures in parentheses indicate percent reduction over initial population.
2. Figures in parentheses indicate percent increase (+) or decrease (−) over control.
3. NS = Not significant.

and Vyas (1995) have also reported efficacy of *S. glaseri* against *H. armigera* on chickpea (*Cicer arietinum*) in India.

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A New Graft Inoculation Method for Screening for Resistance to Pigeonpea Sterility Mosaic Virus

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Sterility mosaic disease (SMD) is a serious threat to pigeonpea (*Cajanus cajan*) production in the Indian subcontinent (Ghanekar et al. 1992) and can cause yield reduction up to 90%. The SMD-affected plants show severe stunting and mosaic symptoms on leaves, with complete or partial cessation of flowering. Cultivating SMD-resistant genotypes is the most viable way to manage this serious disease of pigeonpea.

Several methods have been used to identify pigeonpea accessions with useful levels of resistance to SMD. However, progress in developing broad-based SMD resistant material has been hindered by the lack of information on the causal agent and the absence of diagnostic tools. The SMD agent, transmitted by the eriophyid mite, *Aceria cajani* (Acari: Arthropoda), is not transmissible to pigeonpea by mechanical inoculation of plant sap. Therefore, previous efforts for resistance screening have used viruliferous mite vectors using either the 'leaf-stapling' or 'infector row' methods (Ghanekar et al. 1992). Selection of SMD-resistant genotypes was based solely on visual symptoms. Evaluation of genotypes as resistant (no symptoms), tolerant (ring spots or mild mosaic), or susceptible (severe mosaic symptoms) to SMD (Ghanekar et al. 1992) did not provide information on mechanisms governing resistance. Furthermore, screening of wild *Cajanus* accessions, which have been suggested to contain useful resistance genes for diseases and pests (Remanandan 1981), was difficult because susceptible wild accessions seldom showed visible symptoms.

Recently, the SMD causal agent was identified as a distinct virus and named pigeonpea sterility mosaic virus (PPSMV) (Kumar et al. 2001). Polyclonal antiserum to PPSMV was produced and was found very effective in detecting PPSMV in plant tissues by double antibody sandwich (DAS)-enzyme-linked immunosorbent assay (ELISA) (Kumar et al. 2000). Using this assay, a system was developed for rapid screening of cultivated and wild pigeonpea genotypes, and for identification of resistance to the virus or to the vector or to both.

Transmission of PPSMV by vector mites (leaf-stapling method) occurs if the test accession is susceptible to mites as well as to the virus. Failure of virus transmission suggests that the test accession could possess resistance to vector or to virus, or to both. To confirm this precisely, it is essential to test the accessions by graft inoculation, which facilitates reliable testing for virus resistance. Previously the 'tissue implant grafting' method was used for establishing SMD, but this method resulted in a very low level (about 12%) of virus transmission (Ghanekar et al. 1992).

In this study, three different graft transmission methods were evaluated to identify an efficient method suitable for PPSMV transmission. These were 'chip grafting', 'cleft grafting', and 'petiole (leaflet) grafting' (Fig. 1) as described by Jones (1993). Pigeonpea genotype ICP 8863, highly susceptible to SMD, was used for all graft transmission experiments. Fourteen- to 16-day-old plants raised in growth chambers were grafted using scion from PPSMV-infected plants (Patancheru isolate), which were rendered free from mites by spraying with Kelthane (Dicofol) (Indophil Chemicals Ltd., India). Infected leaflet tissue (scion) was used in petiole grafting while infected stem tissue (scion) was used for cleft or chip grafting. For petiole grafting, the terminal end of a test plant was excised and an incision of about 5–10 mm down the center of the stem was made with a clean scalpel blade

(Fig. 1). A leaflet (scion) from the SMD source plant was collected and its petiole was trimmed into a wedge shape and inserted into the stem slit of the stock plant (Fig. 1). The grafted portion was tightly bound with cellophane tape, ensuring that contact surfaces between grafted parts fitted neatly and closely. Excess scion tissue was removed with a scissors. All graft-inoculated plants were covered with polythene bags to maintain high humidity for 7 days. To avoid contamination by mites, grafted plants were maintained in mite-proof growth cabinets or away from the known sources of SMD-affected plants. They were also sprayed with Kelthane at weekly intervals. Test plants were assayed for PPSMV by DAS-ELISA as described by Kumar et al. (2000), at 14, 20, and 35 days after grafting.

Of the three graft-transmission methods, maximum virus infection (>80%) occurred by petiole grafting (Table 1). Virus transmission by chip and cleft grafting was low (Table 1). It is likely that virus concentration was high in leaf tissues compared to stem and due to the establishment of good union between the scion and the stock (scion tissue remained fresh for a longer time in petiole grafts), high virus transmission was observed in petiole grafting. A PPSMV-susceptible genotype tested positive in DAS-ELISA, 15–20 days after grafting. Petiole grafting is convenient and simple to perform and, like leaf-stapling, allows testing of plants at the seedling stage, and the virus can be detected in plants within two weeks of grafting. On susceptible genotypes virus transmission levels in petiole grafts and plants with vector-mites are similar. This improved SMD screening method is now being used routinely for the identification of broad-based SMD resistant pigeonpea genotypes.

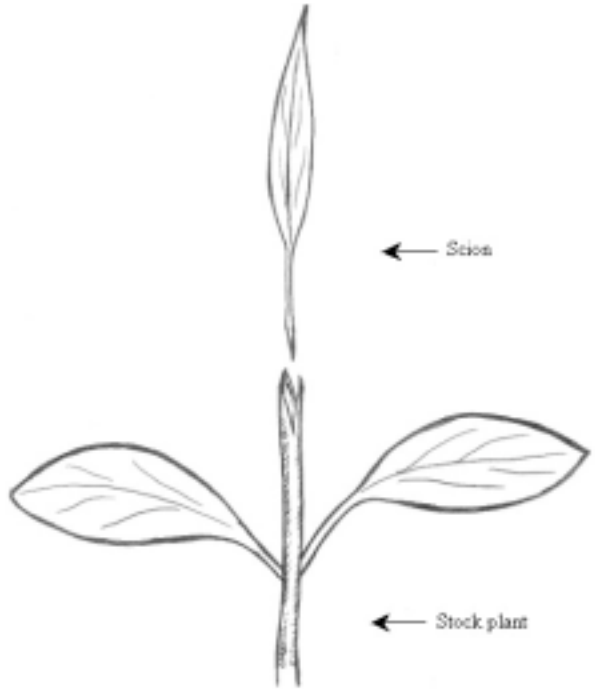

Figure 1. Petiole grafting for pigeonpea sterility mosaic virus transmission.

Table 1. Efficiency of various grafting methods in pigeonpea sterility mosaic virus transmission.

Grafting method	No. of plants tested	No. of plants infected	Infection (%)
Chip grafting	15	2	13.3
Cleft grafting	17	4	23.5
Petiole grafting	15	13	86.6

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Entomology

Validation of Integrated Pest Management of Pigeonpea Pod Borer *Helicoverpa armigera*

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Among several species of pod borers, gram pod borer *Helicoverpa armigera* is a major constraint in enhancing the production and productivity of pigeonpea (*Cajanus cajan*) (Shanower et al. 1999). In the last decade, three outbreaks of this pest were recorded, the latest being in 1997 in Gulbarga, which is known as the pulse bowl of Karnataka state of India. On an average, the pod borer incidence caused 90–100% yield loss in 1992/93 and 1997/98 (Yelshetty and Sidde Gowda 1998).

Farmers mainly rely on insecticides for the management of *H. armigera* (Sachan 1992). However, attempts have been made to develop an integrated pest management (IPM) package for this pest (Sachan and Lal 1997). Studies were conducted at the Agricultural Research Station, Gulbarga to develop an IPM module for pod borer. Based on the results, a sound viable and effective IPM module has been developed. The module for pigeonpea includes various practices as given below:

- Plowing in summer soon after harvest.
- Sowing sorghum (*Sorghum bicolor*) at 250 g ha^{-1} as live bird perches or erecting branched twigs of *Leucaena* sp or *Acacia* sp (5 twigs ha^{-1}).
- Monitoring the pest using pheromone traps (5 traps ha^{-1}).
- First spray with ovicides such as thiodicarb 75 WP at 0.6 g L^{-1} water or profenophos 50 EC at 2 ml L^{-1} water or methomyl 40 SP at 0.6 g L^{-1} water.
- Second spray with 5% neem (*Azadirachta indica*) seed kernel extract or 2 ml L^{-1} of commercial neem formulation at 1500 ppm.
- Third spray with *H. armigera* nuclear polyhedro virus (HaNPV) at 250 larval equivalent (LE) ha^{-1}.
- Fourth spray with indoxycarb 14.5 SC at 0.3 ml L^{-1} water or chlorpyriphos 20 EC at 2.5 ml L^{-1} water or quinalphos 25 EC at 2 ml L^{-1} water or endosulfan 35 EC at 2 ml L^{-1} water.
- Fifth spray with alphamethrin 5 EC at 0.5 ml L^{-1} water.
- In case of water scarcity, dust with quinalphos 1.5% followed by malathion 5% and endosulfan 4% at 25 kg ha^{-1}.
- Hand collection of full grown larvae by shaking the plants.

The IPM module for pigeonpea pod borer developed by the Agricultural Research Station, Gulbarga was demonstrated during 1998/99 to eight farmers in Pattan village in Gulbarga. The pigeonpea cultivar ICP 8863 was sown during the second week of June 1998. All the agronomic practices were followed as per recommended package of practices. With the onset of flower bud initiation, regular monitoring of pest population was undertaken at weekly intervals on 50 plants per 0.4 ha at random and imposition of treatments was taken up whenever the pest population reached the economic threshold level, i.e., two eggs or one larva per plant. The pest population was monitored on a total of 1000 plants in 8 ha.

Further, the non-IPM fields were identified in the nearby areas of the demonstration plot. The agronomic and the pod borer management practices followed by the farmers were recorded from time to time. The observations on pod damage and yield data were recorded for both IPM and non-IPM fields. The benefit-cost ratio was worked out taking into consideration the cost of IPM and non-IPM practices, yield, and market price.

The average number of good pods per plant did not vary among IPM (117.15) and non-IPM fields (109.63). However, the number of pods damaged by *H. armigera* in IPM fields was less compared to non-IPM fields. This was reflected in pod damage. IPM fields recorded 7.8% pod damage compared to 16.4% in non-IPM fields. Seed yield was 985 kg ha^{-1} in IPM fields and 500 kg ha^{-1} in non-IPM fields (Table 1). The difference is mainly due to constant monitoring of the pest and timely application of pesticides in IPM fields.

Table 1. Pod damage of pigeonpea and economics of production in IPM and non-IPM fields.

Parameters	IPM (range)	Non-IPM (range)
Pod damage		
No. of good pods plant^{-1}	117.15 (85.7–203.5)	109.63 (102.5–116.8)
No. of damaged pods plant^{-1}	10.18 (7.2–17.5)	21.56 (20.4–22.7)
Total no. of pods plant^{-1}	127.32 (92.9–212.0)	131.18 (125.1–137.2)
Pod damage (%)	7.99 (4.0–14.0)	16.44 (14.9–18.1)
Economics		
Cost of plant protection (Rs ha^{-1})	1400 (1320–1770)	2140 (1916–2375)
Cost of agronomic practices (Rs ha^{-1})	4763 (3888–5034)	4490 (4342–4622)
Total cost of cultivation (Rs ha^{-1})	6163 (4506–6354)	6630 (6558–6717)
Seed yield (kg ha^{-1})	985 (500–2250)	500 (458–542)
Gross income (Rs ha^{-1})	19908 (10250–46125)	10250 (9396–11104)
Net profit (Rs ha^{-1})	13745 (4093–39543)	3620 (2838–4387)
Profit over non-IPM field (Rs ha^{-1})	10125	

The cost of plant protection was Rs 1400 ha^{-1} in IPM fields and Rs 2140 ha^{-1} in non-IPM fields. The net returns were Rs 13,745 ha^{-1} in IPM fields and Rs 3620 ha^{-1} in non-IPM fields. Thus, an additional income of Rs 10,125 ha^{-1} was obtained due to implementation of the IPM module (Table 1).

There are no studies on the large-scale demonstration of IPM in pigeonpea, except frontline demonstrations conducted through All India Coordinated Pulse Improvement Project trials. However, demonstrations conducted on this pest in cotton (*Gossypium* sp) have given spectacular results (Patil and Bhemanna 1998). The IPM demonstration clearly indicated the importance of monitoring the pest and timely application of pesticides in the management of pod borer.

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Insect Pest Problems of Pigeonpea in Guangxi and Hainan Provinces of China

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Pigeonpea (*Cajanus cajan*) crop in China is relatively new in the extensive diversified system. After the introduction of pigeonpea materials from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India into China in 1985, several trials were organized in the country to prove its potential as soil conservation agent, food, fodder, and fuel. Although the crop attracted the attention of the farming community for several years, in recent years its importance increased rapidly due to its fodder value in Guangxi province and as soil conservation agent in Yunnan province. Intensive work on this crop gained momentum during 1997 after the introduction of advanced breeding material of different maturity groups from ICRISAT and by 2000 the pigeonpea area extended to about 3000 ha in Yunnan, Guangxi, and Jiangxi provinces (Zong Xuxiao et al. 2001). Among various constraints of pigeonpea production, insect pests were recognized as the prime factor by various researchers (Yang Shiying et al. 2001). During October 2001, detailed crop monitoring was undertaken by a team of ICRISAT and Chinese researchers to quantify the importance of insect pests in Nanning, Tiandeng, Dahua, Fengcheng, and Longan counties in Guangxi and Hainan provinces.

Insect Pest Observations

During the visit, the team had the opportunity to inspect pigeonpea crops ranging from vegetative to maturity stages at different places. In Longan county it was preferred for livestock fodder particularly for goats fed in stalls.

The crop adapted well for ratooning and the health of the goats improved after feeding with pigeonpea fodder at flowering stage, 2–3 times a day. Fodder is of immense value in this area and continuous cutting of the crop for feeding goats has resulted in the shortage of seed for further plantings. The plants are attacked by *Maruca vitrata*, podfly, and the webber *Lamprosema* sp. As the reduction in fodder quantity through insect attacks is not significant, one need not worry about any plant protection measures; however, one must plan to protect the seed for future sowings.

At the Guangxi Academy of Agricultural Sciences (GAAS) research station, in the trials in which the crops were in the vegetative phase, plants were severely attacked by *M. vitrata* (10–12 webs plant^{-1}). It was severe particularly in determinate types. Though podfy and aphids were noticed in early-maturing types, they were of secondary importance.

At Tiandeng pigeonpeas (ICP 7035 and ICPL 87091) sown on hill slopes (about 200 m above village) were at peak reproductive phase and were loaded with pod borers (*M. vitrata* and *Helicoverpa armigera*) (*Maruca*: mean of 10 larvae plant^{-1}; and *Helicoverpa*: 3–4 larvae plant^{-1} with overlapping stages). Other insects such as blister beetles (*Mylabris* spp), *Euproctis* sp, and *Lamprosema* sp were of minor importance. Farmers applied one spray of contact insecticide for controlling pod borers but it was ineffective due to rains in the preceding week.

In Taipin village of Wuming county, the ratoon crop was excellent in growth with more than 500 pods plant^{-1}. Though insects like mealy bugs and podfly were seen they were of no economic importance (<5% damage). A new homopteran pest was found feeding on the tender branches and the reproductive parts of pigeonpea. Adults of this pest were collected and preserved for further identification. Since the crop was close to harvest this new sucking pest was not of much economic importance. The farmer applied two insecticidal sprays in the first crop but did not apply any spray in the ratoon crop and he was happy with the bumper ratoon yields without any inputs on pest management.

At Manjiang village pigeonpea was mainly cultivated as intercrop with maize (*Zea mays*) for fodder. During our visit, pigeonpea (after maize harvest) was in podding phase. Since the crop was meant for fodder and was about to be cut, the presence of insects such as *Maruca*, semilooper, plume moth (*Exelastis atomosa*), *Helicoverpa*, and sap-sucking bugs (*Clavigralla* sp) were of less economic importance.

The seed production plots organized in collaboration with Dawang Seed Company Ltd. at Pinguang of Fengcheng county were in excellent condition at preflowering phase but the pod borers such as *Maruca* and *Helicoverpa* started infesting the plants. In anticipation of the pest problem the organizers were cautioned to take up appropriate plant protection measures within a fortnight.

At Fushan village of Hainan province the germplasm evaluation plots were severely infected with yellow mosaic and sterility mosaic viral diseases. Though the

Table 1. Insect pests on pigeonpea at different locations in China during October–November 2001.

Location (County/Province)	Insect pests observed	Damage levels (%)
Guangxi Academy of Agricultural Sciences (GAAS) (Nanning, Guangxi)	*Maruca*, podfly, aphids	20
Longan (goat farm) (Longan, Guangxi)	*Maruca*, podfly, *Nezara*, *Lamprosema*, semiloopers	1–5
Tiandeng (Guangxi)	*Maruca*, *Euproctis*, *Helicoverpa*, *Mylabris*, *Lamprosema*	30
Taipin (Wuming, Guangxi)	Podfly, sucking pests (bugs), blue butterflies, hairy caterpillars	<5
Manjiang (Guangxi)	*Helicoverpa*, *Maruca*, plume moth, podsucking bugs, semiloopers	<5
Pinguang (Fengcheng, Guangxi) (vegetative phase)	*Maruca*, *Helicoverpa*	<1
Qi Bailong (Dahua, Guangxi)	Plume moth, podfly, *Maruca*	10
Fushan (Deugmai, Hainan)	*Maruca*, *Helicoverpa*, grasshoppers (*Oxya* spp), termites	25

crop was sprayed twice against insect pests particularly grasshoppers (*Oxya* spp), *Maruca*, and *Helicoverpa*, the crop growth was not optimum. *Maruca* was more serious in determinate types of pigeonpea and also infested the neighboring winged bean (*Psophocarpus tetragonolobus*) crop. The farmers in this area recognize grasshoppers and *Maruca* as the prominent yield reducers in all legume crops. The details of location-wise pest occurrence and damage levels is presented in Table 1.

Conclusions

The cultivation aspects of pigeonpea were well understood by the researchers and farmers in all counties. However, the utilization and plant protection measures needs to be better adopted for the successful establishment of the crop. In areas where the crop is used as fodder the seed supply need to be backed with appropriate insect management. The involvement of private seed sector which has better pest management skills than the farmers to meet the seed demand would be of immense value for the rapid establishment of the crop. Pigeonpea is prone to insect attack which has remained a severe threat for crop productivity in several countries. Hence, countries like China, where this legume has been newly introduced, need to be very cautious in promoting this crop.

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Efficacy of *Tephrosia vogelii* Crude Leaf Extract on Insects Feeding on Pigeonpea in Kenya

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Pigeonpea (*Cajanus cajan*) is an important source of dietary protein, and is consumed as green peas, whole grain, or split seeds. Yields of pigeonpea vary across locations, seasons, and cropping systems. In most areas, insect pests are an important constraint in pigeonpea. The most important flower- and pod-feeding Lepidoptera in eastern and southern Africa are *Helicoverpa armigera*, *Maruca vitrata* (= *testulalis*), *Etiella zinckenella*, and *Lampides* spp and they account for 5–35% loss in grain yield (Minja 2001). The pod-sucking Hemiptera [dominated by *Clavigralla* (= *Acanthomia*) spp] cause 30–70% loss in yield (Minja 2001). The common seed-feeding Diptera is *Melanagromyza chalcosoma*, which accounts for 4–45% yield loss in mid- to high-altitude elevations.

The majority of pigeonpea-growing farmers are poor rural women, who cannot afford the high costs for insect pest control using commercial insecticides. *Tephrosia*

vogelii, commonly referred as fish poison bean, has been widely used in the tropics to kill fish and in treatment of various animal ailments. It has a potential in eastern and southern Africa for biocontrol. The work reported here aimed at determining the effectiveness of *Tephrosia* in field insect pest control on pigeonpea.

The short-duration pigeonpea ICPL 87091 was planted at the Kenya Agricultural Research Institute (KARI) station at Kiboko, Kenya during 1998/99 short rain season (November/February). Field plots measuring 10 m × 20 m were used and seeds were sown at 30 cm × 10 cm spacing. *Tephrosia* plants were established in a separate plot during the long rains of 1998. There were eight treatments replicated three times in a randomized complete block design.

Tephrosia crude leaf extract was prepared by picking mature leaves, pounding them in a mortar, and soaking them in the appropriate amount of water (50, 100, and 200 leaves L^{-1} of water) for 10–12 hours under ambient conditions. Two liters of spray fluid was used in these experiments. The following morning or late afternoon the leaf extract was filtered through muslin cloth. The filtrate was mixed with the detergent Teepol® (2–3 ml L^{-1}) to assist in dispersion of the spray on the plant surface. The resulting mixture was sprayed on pigeonpea plants in the field. The first spray was applied at flower bud expansion stage, and subsequent sprays at 10- to 15-day intervals. Dimethoate (Rogor® E40) was used as a standard commercial insecticide. All plots were weeded by hand hoeing, and supplementary irrigation was given when needed.

Damage assessment was carried out at early podding (25% of pods with expanded seed), late podding (about 50% of pods with expanded seed), and maturity (about 75% of pods are mature but not dry) stages. Pods from 5 randomly tagged plants in the middle of each plot were sampled destructively. Each pod was later examined in the laboratory to determine the number of seeds damaged by different insect pests. The total number of damaged seeds was expressed as a proportion of total number of seeds $plot^{-1}$. Grain yields were recorded at harvest by harvesting all pods (excluding the outer row and one meter band at the edges of each plot). The pods were dried and shelled, and the grain separated into clean (usable) and unclean (unusable) seeds. The clean and unclean seeds were weighed separately. Yield gains in sprayed plots were based on the yield differences between sprayed and unsprayed plots, and expressed as proportion of the seed yield in unsprayed plots. All data was subjected to analysis of variance using Genstat 5.

Plots sprayed with Dimethoate and *Tephrosia* leaf extract showed significant reduction in seed damage compared to untreated control (Table 1). Differences in seed damage in plots with three and four sprays of *Tephrosia* were not significant. Although yield differences were not significant between sprays with 100 and 200 *Tephrosia* leaves, the latter gave higher yields. Seed mass was slightly improved by all the sprays.

The results indicated that plots sprayed with extracts from 200 leaves of *Tephrosia* applied three to four times had acceptable levels of insect control. Similar observations have earlier been reported from Uganda (Kyamanywa et al.

Table1. Seed damage by insect pests at three pod developmental stages and seed characteristics of the short-duration pigeonpea variety ICPL 87091 sprayed with *Tephrosia* extracts at Kiboko, Kenya (1998/99 short rains).

Treatment	Seed damage (%)			100-seed mass (g)	Total seed yield (kg ha^{-1})	Usable seed (kg ha^{-1})	Usable seed gain (%)
	Early podding	Late podding	Pod maturity				
Tephrosia 50 leaves, 3 sprays	8.9	15.3	18.2	11.8	1767	1620	19.6
Tephrosia 50 leaves, 4 sprays	8.1	14.0	13.0	11.6	1868	1769	30.5
Tephrosia 100 leaves, 3 sprays	5.1	12.5	8.8	11.7	2079	1858	37.1
Tephrosia 100 leaves, 4 sprays	6.1	11.0	15.6	11.9	1950	1816	34.0
Tephrosia 200 leaves, 3 sprays	4.4	13.3	12.1	11.5	2229	2123	56.7
Tephrosia 200 leaves, 4 sprays	4.6	9.9	8.9	11.5	2287	2188	61.5
Dimethoate at 0.05% ai[1]	2.2	8.4	6.2	11.2	2120	2030	49.8
Untreated control	18.9	16.6	26.9	10.8	1455	1355	0.0
Mean	7.3	12.6	13.7	11.5	1970	1845	
SE ±	2.45	2.52	3.15	0.28	48.63	63.31	

1. ai = Active ingredient.

2001). Anti-feeding effects of *Tephrosia* have also been reported on spotted cereal stem borer (*Chilo partellus*) (Machocho 1992). There were significant (P = 0.05) increases in grain yield in the sprayed plots and a concomitant improvement in grain quality. Kyamanywa et al. (2001) observed similar yield increases through the application of *Tephrosia* leaf extract in Uganda. Application of *Tephrosia* leaf extract has shown beneficial effects on grain yield and quality when used appropriately. These applications have to be effected either very early in the morning or late in the evening to avoid degradation of this bio-pesticide due to exposure to light and air. Farmers are now being encouraged to establish their own plants for quick accessibility when they need the leaves. These farmers can prepare their own crude extracts and apply them in their fields.

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Biotechnology

Efeect of Feeding Legume Proteinase Inhibitors on *Helicoverpa armigera* Gut Proteinase Activity

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Legumes rank second after cereals as a source of human and animal feed. Pigeonpea (*Cajanus cajan*), chickpea (*Cicer arietinum*), mung bean (*Vigna radiata*), and soybean (*Glycine max*) are the most common legumes grown in India. They are a valuable source of proteins, minerals, and vitamins. However, they also contain some antinutritional factors such as oligosaccharides, proteinase inhibitors, and phenols (Singh 1988). Proteinase inhibitors are common natural products in plants and have been studied as phytochemical resistance factors against herbivorous insects (Broadway 1996). The legume pod borer *Helicoverpa armigera* is a major pest of several legume crops. The larva is a voracious feeder and damages buds, flowers, pods, and seeds. The objective of this study was to examine the effect of proteinase inhibitors extracted from different legumes on *H. armigera* gut proteinase (HGP) activity.

The seeds were procured from the Agricultural Research Station, Gulbarga, Karnataka, India. The chemicals N-α-benzoyl-DL-arginine-p-nitroanilide (BApNA), tosyllysinechloromethylketone (TLCK), TLCK-treated chymotrypsin, bovine serum albumin, pheynylmethylsulfonylfluoride (PMSF), and tosyl-phenylalaninechloromethylketone (TPCK) were purchased from Sigma Chemical Co., St. Louis, MO, USA. All other chemicals were of analytical grade.

The flour of seeds of various legumes was defatted by washing it with acetone (thrice) and hexane (twice), and air-dried. The defatted flour was stirred with 50 ml of 0.1 M sodium phosphate buffer (pH 7.1) for 4 h at room temperature (28°C). The resulting mixture was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was dialyzed with distilled water and used as an inhibitor source. Protein concentration was assayed by Bradford method (Bradford 1976). The proteinase inhibitory activity and HGP activity were assayed by incubating the seed extract/gut extract with 15 µg of trypsin at room temperature (28°C). One ml of 1 mM BApNA solution was added and incubated at 37°C for 10 min. The

reaction was arrested by adding 200 µl of 30% acetic acid. The liberated p-nitroaniline was measured at 410 nm in a spectrophotometer. One unit of proteinase acitivity is defined as the amount of enzyme that caused an increase of 1 optical density (OD) unit. One proteinase inhibitory unit is defined as the amount of inhibitor that inhibited 1 unit of proteinase activity.

The 4th and 5th instars of *H. armigera* were used in these studies. The larvae were reared on a basal diet/ supplemented diet of inhibitor mixture. The mid-guts were dissected and stored at −40°C. The gut tissue was mixed with 3 volumes of 0.2 M glycine-sodium hydroxide buffer (pH 10.0) and allowed to stand for 1 h. The mixture was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was used as HGP source. The protein concentration and HGP inhibitory activity was determined as described earlier.

Bioassays were conducted by feeding *H. armigera* on a diet containing synthetic inhibitors and legume inhibitors. The composition of the diet (per 100 ml) is: 15 g seed flour (contains 2 proteinase inhibitor unit mg^{-1} flour), 1.2 g yeast extract, 250 µl formalin, 4.5 g ascorbic acid, 1.5 g sorbic acid, and 1 tablet tetracycline. The synthetic inhibitor diet contained above ingredients + 100 µl of PMSF/TPCK/TLCK (10 mM). Also 4 g of agar was added. The mixture was boiled in 100 ml of distilled water and mixed thoroughly. The diet was then poured into small trays and each larva was left to feed for 24 h. The feeding was continued for 72 h or until pupation. The experiment was repeated thrice.

Table 1. Effect of feeding legume inhibitors and synthetic inhibitors on HGP activity[1].

Legume/synthetic inhibitor	Residual activity (%)
Pigeonpea	68 ± 0.72
Chickpea	70 ± 0.98
Mung bean	76 ± 0.32
Soybean	65 ± 0.72
TLCK	44 ± 0.72
TPCK	42 ± 0.74
PMSF	28 ± 0.98

1. HGP = *Helicoverpa armigera* gut proteinase; TLCK = tosyllysine-chloromethylketone; TPCK = tosylphenylalaninechloromethylketone; PMSF = pheynylmethylsulfonylfluoride.

Table 2. Effect of feeding legume proteinase inhibitors on growth of *Helicoverpa armigera* larvae.

Legume inhibitor	Average mass (mg)
Pigeonpea	630 ± 10.0
Soybean	675 ± 22.5
Mung bean	325 ± 10.0
Chickpea	310 ± 12.5

The diet comprising pigeonpea and soybean inhibitors did not inhibit HGP activity significantly while mung bean and chickpea inhibitors showed moderate inhibition (Table 1). Ten mM PMSF in diet inhibited HGP significantly. TPCK and TLCK diets also inhibited the HGP to a moderate extent. The proteinase inhibitors of soybean and pigeonpea did not inhibit the larval growth, but chickpea and mung bean inhibitors significantly reduced the larval growth (Table 2). The larvae failed to pupate and showed stunted growth. In vitro analysis of HGP activity also confirmed these results (Table1).

Proteinase inhibitors present in the leaves and storage tissue are induced upon wounding, thereby significantly reducing the insect attack (Green and Ryan 1972, Howe et al. 1996). The serine proteinase inhibitors in plants function as defensive agents against herbivores and pathogens. Pigeonpea and soybean proteinase inhibitors did not exhibit any inhibitory activity against *H. armigera* because the gut proteinases also contain some other types of proteinases such as cysteine and aspartic proteinases. Herbivorous insects can overcome the activity of these proteainses by secreting 'inhibitor-resisitant' enzymes. The insect mid-gut contains a number of different proteins with trypsin-like activity and this allows the insect to digest dietary protein in the presence of proteinase inhibitors (Broadway 1996). Harsulkar et al. (1999) reported the use of non-host proteinase inhibitors to study the interaction on HGP activity. Non-host plant proteinase inhibitors such as groundnut (*Arachis hypogaea*) and winged bean (*Psophocarpus tetragonolobus*) have the potential to inhibit HGP activity.

Ten mM PMSF mixed with proteinase inhibitors in the diet inhibited the HGP activity. This may be due to PMSF being a selective inhibitor of serine proteinases and the gut proteinases may contain large amounts of serine proteinases. TPCK and TLCK also inhibited HGP to a moderate extent. Johnston et al. (1993) reported that soybean trypsin inhibitor (SBTI) and soybean bowmanbirk inhibitor (SBBI) do not inhibit the growth of the larvae. Continuous feeding of larvae on SBTI diet reduces the trypsin-like activities found in the gut and long-term feeding of SBTI diet killed the larvae abruptly. The studies indicated that *H. armigera* is a polyphagous pest.

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Screening of Wild Species of Pigeonpea against *Helicoverpa armigera* Gut Proteinases

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Pigeonpea (*Cajanus cajan*) is a rich source of proteins and minerals. It is also a staple diet in most parts of India, eastern and southern Africa, and the Caribbean. Pigeonpea is also known to contain some antinutritional factors such as proteinase inhibitors, oligosaccharides, phenols, tannins, and phytic acid (Singh 1988). The pod borer (*Helicoverpa armigera*) is an economically important pest of this crop. It attacks the pods during seed development, thereby reducing the grain yield. It also causes huge losses in other legumes such as chickpea (*Cicer arietinium*) and mung bean (*Vigna radiata)*. So far, pest control strategies have relied on chemical insecticides only. Some varieties of pigeonpea with resistance to the pod borer ICPL 332 and PPE 45-2 have been developed. The proteinase inhibitors of legumes can be used to fight this pest by altering its gut proteinase activity. The proteinase inhibitors are now being extensively studied as a possible defense against pests. This study reports the screening of some wild varieties of pigeonpea against *H. armigera* gut proteinases.

The seeds of wild varieties of pigeonpea were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The local pigeonpea varieties were purchased from local market. N-α-benzoyl-DL-arginine-p-nitroanilide (BApNA), trypsin, bovine serum albumin, and soybean trypsin inhibitor (SBTI) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Acetone, hexane, tris-buffer, and sodium hydroxide (NaOH) were purchased from Sisco Research Laboratory, Mumbai, India. All other reagents used were of analytical grade. The 4th and 5th instar larvae of *H. armigera* were collected from pigeonpea fields at the Agricultural Research Station, Gulbarga, India and were used in these studies.

The seed powder was first defatted by washing it with chilled acetone and hexane (3 to 4 washes). The proteinase inhibitors were then extracted with 1% polyvinylpyrrolidine in distilled water to coagulate the phenols. The resulting mixture was then centrifuged at 12,000 rpm for 20 min at 4°C. The clear supernatant obtained was dialyzed extensively against distilled water, and was used as inhibitor source.

The larvae (4th and 5th instars) were dissected and the mid-guts were frozen at −40°C. The gut tissue was homogenized in 40 ml of 0.2 M glycine-NaOH buffer, pH 10.0 and incubated at 8°C for 1 h. The suspension was centrifuged at 10,000 rpm for 20 min at 4°C. The clear supernatant obtained was used as a source of *H. armigera* gut proteinase (HGP). The protein concentration was determined by Bradford method (Bradford 1976).

To determine trypsin inhibitory activity and HGP inhibitory activity, appropriate volumes of pigeonpea seed extract, which gave 40–60% inhibition of trypsin (40% in case of HGP) were mixed with 15 µg of trypsin or an equivalent amount of HGP and allowed to stand for 15 min at 30°C. The residual proteinase activity was measured by incubating the seed extract with synthetic substrate BApNA for 10 min at 37°C. One unit of proteinase activity is defined as the amount of enzyme that caused an increase of 1 optical density (OD) unit at 410 nm due to the release of p-nitroaniline. One proteinase inhibitor unit is defined as the amount of inhibitor that inhibited 1 unit of proteinase activity.

Of the 14 genotypes of *Cajanus* species screened (12 wild accessions + 2 local pigeonpea varieties), seven genotypes did not exhibit much inhibition against HGP. *Cajanus albicans* (ICPW 014 and ICPW 024), *C. lineatus* (ICPW 042), and *C. sericeus* (ICPW 162) showed moderate inhibitory activity against HGP; *C. sericeus* (ICPW 160) showed very good inhibitory activity against HGP (Table 1). The two local pigeonpea varieties showed some inhibitory activity against HGP; the inhibitor extracted from local variety 2 was more effective than that of local variety 1.

Table 1. Inhibition of *Helicoverpa armigera* gut proteinase (HGP) activity by proteinase inhibitors of pigeonpea and its wild relatives.

Accession number	*Cajanus* species	Source	Inhibition of HGP[1] (%)
Local variety 1	*C. cajan* (pigeonpea)	ARS	34 ± 0.81
Local variety 2	*C. cajan* (pigeonpea)	ARS	38 ± 0.47
ICPW 014	*C. albicans*	ICRISAT	36 ± 0.47
ICPW 024	*C. albicans*	ICRISAT	35 ± 1.24
ICPW 030	*C. cajanifolius*	ICRISAT	16 ± 0.00
ICPW 031	*C. cajanifolius*	ICRISAT	12 ± 0.94
ICPW 041	*C. lineatus*	ICRISAT	7 ± 0.81
ICPW 042	*C. lineatus*	ICRISAT	32 ± 0.94
ICPW 082	*C. scarabaeoides*	ICRISAT	23 ± 0.48
ICPW 092	*C. scarabaeoides*	ICRISAT	19 ± 0.47
ICPW 160	*C. sericeus*	ICRISAT	46 ± 0.94
ICPW 162	*C. sericeus*	ICRISAT	37 ± 0.82
ICPW 169	*C. crassus*	ICRISAT	14 ± 0.00
ICPW 172	*C. crassus*	ICRISAT	24 ± 1.24

1. Average of three replications.

Table 2. Inhibition of *Helicoverpa armigera* gut proteinases by proteinase inhibitors of *Cajanus* species mixed with 10 mM soybean trypsin inhibitor.

Accession number	*Cajanus* species	Source	Inhibition of HGP[1] (%)
Local variety 1	*C. cajan* (pigeonpea)	ARS	37 ± 0.47
Local variety 2	C. cajan (pigeonpea)	ARS	40 ± 0.94
ICPW 014	*C. albicans*	ICRISAT	42 ± 0.00
ICPW 024	*C. albicans*	ICRISAT	45 ± 0.81
ICPW 042	*C. lineatus*	ICRISAT	45 ± 0.81
ICPW 160	*C. sericeus*	ICRISAT	64 ± 0.47
ICPW 162	*C. sericeus*	ICRISAT	46 ± 1.24

1. Average of three replications.

The commercially available SBTI in combination with 5 wild accessions and 2 local varieties showed moderate inhibition towards HGP. When inhibitor extract of *C. albicans* (ICPW 014 and ICPW 024), *C. lineatus*, and *C. sericeus* was mixed with 10 mM SBTI solution, a moderate change in inhibitory activity was observed (Table 2). Also the two local varieties showed more inhibition towards HGP when SBTI was mixed with the inhibitor. There was a significant amount of inhibition when SBTI was mixed with the proteinase inhibitor of *C. sericeus*. A purified inhibitor of pigeonpea has been reported to have very low affinity towards HGP when compared to other inhibitors (Godbole et al. 1994). Giri et al. (1998) reported that the proteinases of *H. armigera* degraded the trypsin inhibitors of chickpea, thus making it completely defenseless. Patankar et al. (1999) reported the screening of wild relatives of chickpea against HGP. Harsulkar et al. (1999) reported that the proteinase inhibitors from non-host plants such as groundnut (*Arachis hypogaea*) and winged bean (*Psophocarpus tetragonolobus*) have the potential to inhibit HGP and larval growth, thus making it possible for successive use of these inhibitors in developing *H. armigera* resistant transgenic plants. Nandi et al. (1999) reported that even high-level expression of SBTI gene cloned in transgenic tobacco plants have failed to confer resistance against *H. armigera*. This may be due to the fact that HGPs get accustomed to the host proteinases and they can easily digest the inhibitor by secreting proteolytic enzymes. It has been reported earlier that the mid-guts of Lepidoptera and Diptera also contain other proteinases like cysteine (thiol) and aspartic proteinases (carboxyl) besides serine proteinases. Plants are usually rich in serine proteinase inhibitors, so this may not lead to effective inhibition of HGP (Jongsma and Bolter 1997).

Our studies indicate that *C. serieus* in combination with SBTI inhibited HGP significantly. The co-evolution of proteinase inhibitors of plants and proteinases of insects provides an interesting point for ecological, physiological, and biochemical research for developing resistant varieties against *H. armigera*.

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Pande, S., Singh, G., Narayana Rao, J., Bakr, M.A., Chaurasia, P.C.P., Joshi, S., Johansen, C., Singh, S.D., Kumar, J., Rahman, M.M., and **Gowda, C.L.L.** 2002. Integrated management of botrytis gray mold of chickpea. Information Bulletin no. 61. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 32 pp. ISBN 92-9066-434-7. Order code IBE 061. HDC $36.00. LDC $12.00. India Rs 579.00.

Botrytis gray mold (BGM) caused by *Botrytis cinerea* is an important disease of chickpea worldwide. Its origin, distribution, losses, symptoms, causal organism and its variability, epidemiology, and host range are briefly reviewed. Information on histopathology and host-pathogen interaction with respect to chickpea as a host is not available. Therefore, efforts were made to supplement this part of the literature from other hosts of *B. cinerea.* Attempts have also been made to assemble the information on integrated disease management (IDM) of BGM. The IDM components reviewed are host-plant resistance, agronomic and cultural practices including effects of sowing date (escape), row spacing, plant type, and intercropping; management by chemicals which include seed treatment and foliar sprays; and management with biological agents. Integrated management of BGM in chickpea involves use of BGM-resistant cultivars with improved agronomic and cultural practices including economical use of fungicides, but these practices are not yet sufficiently refined to be adapted by resource-poor farmers. Therefore, farmersí participatory on-farm research is needed to devise appropriate packages of these strategies for BGM endemic areas.

Joshi, P.K., Parthasarathy Rao, P., Gowda, C.L.L., Jones, R.B., Silim, S.N., Saxena, K.B., and **Jagdish Kumar.** 2001. The world chickpea and pigeonpea economies: facts, trends, and outlook. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 68 pp. ISBN

92-9066-443-6. Order code BOE 030. HDC $49.50. LDC $16.50. India Rs 802.00.

Globally chickpea and pigeonpea are third and fifth most important pulse crops mainly grown in the developing countries by resource-poor farmers in drought prone areas and on degraded soils. Chickpea is traditionally grown in temperate areas while pigeonpea is mainly grown in the tropics. South Asia accounts for bulk of production of both these pulses. During the last 20 years there has been some diversification in area and production as reflected in the internationality index of these crops.

Considerable progress has been achieved in developing improved short- and medium-duration varieties of chickpea and pigeonpea that fit specific niches in the cropping pattern. Fallow areas were brought under chickpea cultivation as the crop could now escape terminal drought. Short- and medium-duration pigeonpea varieties resistant to diseases enabled double cropping leading to an increase in farm income. However, large-scale adoption could not be sustained due to several socioeconomic and technological constraints.

Low productivity growth of chickpea and pigeonpea has resulted in declining or stagnant per caput availability of these pulses in the major producing regions. An important policy question is whether the decline in per caput availability of pulses is a supply or demand constraint. In the short to medium term, supply would be more constrained than demand for both chickpea and pigeonpea. Population and income growth and positive income elasticity of demand would ensure present levels of consumption. In the long run demand would be more constrained due to changes in tastes, preferences, and urbanization.

Chickpea and pigeonpea complement cereals in production and consumption. Their overall benefits extend much beyond generating income to resource-poor farmers. For the long run sustainability of the system improvement in production through improved varieties resistant to pests and diseases and better agronomic management should continue in the future.

Ranga Rao, G.V., and **Shanower, T.G.** 2001. Identification and management of pigeonpea and chickpea insect pests in Asia. Information Bulletin no. 57. (In Kannada.) Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 96 pp. ISBN 92-9066-430-0. Order code IBK-057. HDC $37.50. LDC $13.50. India Rs 520.00.

Pigeonpea (*Cajanus cajan* (L.) Millspaugh) and chickpea (*Cicer arietinum* L.) are important grain legumes in Asia. These crops are often heavily damaged by insect pests. Farmers in many areas apply insecticides in an attempt to manage these pests. This bulletin provides descriptions of the most common species, their biology, distribution, and damage symptoms. Color photographs are provided for easy identification. Possible modes of control are also included with an emphasis on integrated pest management and reduced reliance on insecticides.

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Chickpea publications

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About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of Southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the worldís population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISATís mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the SAT. ICRISATís update mission is to conduct research that can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is cosponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Bank, and the United Nations Development Programme (UNDP).

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