Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Influence of pod maturity and level of domestication on biochemical components in wild and cultivated pigeonpea (*Cajanus cajan*)

R. Aruna[1,2], D. Manohar Rao[3], L.J. Reddy[1], S. Siva Ramakrishnan[4] & H.D. Upadhyaya[1]

1 Gene Bank, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India
2 Present address: Groundnut Breeding Unit, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India
3 Department of Genetics, Osmania University, Hyderabad, Andhra Pradesh, India
4 Department of Biotechnology, ANGRAU, Hyderabad, Andhra Pradesh, India

Keywords
Cajanus scarabaeoides; haemagglutination; lectin; pigeonpea; trypsin inhibitors.

Correspondence
R. Aruna, Groundnut Breeding, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India.
Email: a.rupakula@cgiar.org

Received: 8 September 2005; revised version accepted: 10 March 2007.

doi:10.1111/j.1744-7348.2007.00145.x

Abstract

Variations in the trypsin inhibitors and lectin content in the developing pods of thirty accessions of *Cajanus scarabaeoides*, a wild relative of pigeonpea, from wide geographical locations and six cultivated genotypes were estimated at juvenile, immature and mature stages of pod development. Genotypes differed at all three stages for these two biochemical components. Total protein and trypsin inhibitor contents were higher in the wild accessions than in the cultivated genotypes. Although lectin content in the juvenile stage of pod development in the wild accessions ICPW 138 and ICPW 98 was highest, it was absent in the mature pods in both the cultivated and the wild genotypes. Very high broad-sense heritability estimates indicated the possibility of involvement of few genes in the inheritance of these biochemical components, which could be easily introgressed into the elite agronomic background.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], also known as red gram, is the sixth most important grain legume crop grown in the semi-arid tropics of Asia, Africa and the Caribbean under a wide variety of cropping systems. Besides its main use as dhal (dry, dehulled, split seed used for cooking), pigeonpea's tender green seeds are used as a vegetable, while crushed dry seeds serve as animal feed, green leaves serve as fodder and stems are used for fuel wood and to make huts, baskets, etc. (Nene & Sheila, 1990).

In legumes, inhibitors of enzymes, such as trypsin, chymotrypsin and amylase, and flatulence-causing oligosaccharides such as stachyose, raffinose and verbascose, play important roles in contributing to insect pest resistance (Singh & Eggum, 1984). Proteinase inhibitors are potential tools of crop improvement targeting plant protection and human nutrition. This concept of plant protection is rapidly expanding and opens up diverse areas to harness the potential of these genes. The trypsin and chymotrypsin inhibitors are not only widely distributed among plant families and considered to be involved in regulating endogenous proteases and affording protection against insect and pathogen attack but also function as storage proteins (Liener & Kakade, 1980; Ryan, 1990; Harsulkar *et al.*, 1999). Lectins are a group of glycoproteins, have the ability to bind to specific sugars present on the surface of red blood cells of humans and animal species, resulting in the agglutination of the blood, and are widely distributed in many plants including the edible seeds of common legumes (Grant, 1991). High levels of both lectins and protease inhibitors in the edible parts of beans (Pusztai, 1966; Whitaker *et al.*,

1988; Moreno *et al.*, 1990) are highly toxic for some insects (Ishimoto & Kitamura, 1989) and are considered to be natural insecticidal proteins, which can be introduced into insect-susceptible crops to enhance their predator resistance (Altabella & Chrispeels, 1990).

Podborer (*Helicoverpa armigera*) of the lepidopteran family is a serious pest of many important crops and claims a major share in crop losses every year. It is a polyphagous pest of over 180 plant species. Larvae of *H. armigera* are voracious foliar feeders as early instars and later shift to the developing seeds, fruits or bolls, leading to drastic reductions in yield (Reed & Pawar, 1982). Exogenous chemical means to counteract *H. armigera* have become less feasible, mainly because of the development of pesticide resistance in insects and inherent possible environmental hazards (Armes *et al.*, 1996). Host plant resistance is the most economical and ecofriendly method of managing podborer. Several studies have focussed on identifying and characterising morphological and biochemical components that contribute to resistance in wild species of pigeonpea such as *Cajanus scarabaeoides* and *Cajanus platycarpus* (Pundir & Singh, 1987; Saxena *et al.*, 1990; Shanower *et al.*, 1997). Among morphological components, the role of trichomes and pod wall surface in conferring insect pest resistance has been investigated (Shanower *et al.*, 1997; Romies *et al.*, 1999; Stevenson *et al.*, 2002). However, studies on the role of biochemical components (trypsin inhibitors and lectins) in cultivated pigeonpea and its wild relatives are limited (Singh & Jambunathan, 1981; Mulimani & Paramjyothi, 1992; Pichare & Kachole, 1994). Considering that *H. armigera* must infest the pods to feed internally on grains, initial plant defence responses must be targeted from constituents present in the pods. However, there are few studies on the variation in pod biochemical constituents and their role in conferring podborer resistance in pigeonpea. Most of these studies are concentrated on the whole seed rather than on the pod. In the present study, we examined the variation of trypsin inhibitors and lectins among the cultivated pigeonpea and its wild relatives at different pod stages and investigated their possible role in conferring resistance against podborer.

Materials and methods

Plant materials and field design

Thirty accessions of *C. scarabaeoides* (ICPW numbers) and six cultivated genotypes of *C. cajan* (ICP numbers) were used. Wild accessions were selected from the world germplasm collection available at Rajendra S. Paroda GeneBank, maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Experiments were carried out in the rainy season of 2001 at ICRISAT at an altitude of 545 m, 17°N and 78°E. The entries were grown in deep alfisols. The experiment was laid out in a randomised complete block design, with three replications. Seeds of each entry were sown on a 4-m-long ridge with an inter-row spacing of 60 cm and interplant spacing of 10 cm. Recommended agronomic practices were followed to raise the crop. The crop was given protective irrigation and minimal quantities of insecticides were sprayed to reduce the crop damage, to capture the maximum diversity.

Extraction of proteins

Pods at different developmental stages, viz. juvenile or the milk stage, immature and the mature stages, were collected from five randomly selected plants during the morning for analysis of total protein, trypsin inhibitors and the lectins in pods. One-gram pod, along with seeds at three developmental stages, was ground separately in 8 mL of 0.025 M HCl. The ground paste was transferred to 25-mL centrifuge tubes for centrifugation at 18 000 *g* in Sorval centrifuge for 10 min. The supernatant was collected in 1.5-mL Eppendorf tubes and was used for the estimation of total protein, trypsin inhibitors and lectins. Protein content in the pods of the wild and cultivated pigeonpea was estimated using Lowry's method (Lowry *et al.*, 1951).

Estimation of trypsin inhibitor(s)

The supernatant of the extract was used for estimating the content of trypsin inhibitor. Trypsin activity was estimated using chromogenic substrates BA*p*NA (*N*-α-benzoyl-DL-arginine *p*-nitroanilide hydrochloride), which is a chromogenic trypsin substrate and dissolves only in dimethyl sulphoxide (DMSO) at room temperature (Erlanger *et al.*, 1964). The solutions used in the estimation were prepared freshly before the experiment. In 2 mL of DMSO, 60 mg of BA*p*NA was dissolved. The resulting solution was mixed in 20 mL of Tris–HCl buffer pH 8.0 and 4 mL of 1 M $CaCl_2$. The total volume was made up to 200 mL with sterile distilled water. Five grams of trypsin (Amersham Biosciences, Piscataway, NJ, USA) powder was dissolved in 100 mL of 0.0025 M HCl and stored at 4°C. Trypsin inhibitor activity was measured in 1:1 dilution (100 μL of supernatant with 100 μL of sterile distilled water). BA*p*NA assay was as described previously (Giri *et al.*, 1998).

Trypsin inhibitor (TI) activity was calculated with the following formula and the activity was expressed in the units of inhibition per gram of protein.

Ann Appl Biol **151** (2007) 25–32

$$\%\,\mathrm{TI} = [(\text{OD value of sample with trypsin} - \text{OD value of the sample without trypsin})/1.038] \times 100$$

$$\text{No. of trypsin units inhibited} = \%\ \text{trypsin inhibited} \times 0.0166$$

Estimation of lectin by haemagglutination

For the measurement of haemagluttinating activity, rabbit red blood cells were used, which were collected in an equal volume of Alsever solution (20.5 g glucose + 0.80 g Na-citrate + 0.42 g NaCl in 100 mL distilled water and adjusted to pH 7.2 with 10% citric acid, containing two drops of heparin 5 mL^{-1} Alsever). Cells were collected by centrifugation (2000 *g* for 15 min), washed five times with saline buffer pH 6.5 (0.9% NaCl containing 0.02 % Na azide) to give 4% (v/v) suspension. The lectin samples were serially diluted (twofold) in a microtitre plate. To each dilution, 0.05 mL of the rabbit erythrocyte suspension was added. After 2 h, the end point of the titration was estimated visually as the lowest dilution which showed the agglutination (titre). The lectin content is expressed in the form of specific haemagglutination units (HAU) which is calculated as follows:

$$\mathrm{HAU} = (\text{agglutination titre/protein content}) \times \text{dilution factor}$$

Data on these three biochemical components at different pod maturity stages were subjected to statistical analysis. Data were analysed using the analysis of variance on Genstat V, release 7.0 (VSN International Ltd, Hemel Hempstead, UK), where pod development stage and their levels of domestication (cultivated and wild pigeonpea genotypes) were taken as treatment structure. Heritability was estimated as the ratio of calculated genotypic variance to phenotypic variances (Falconer, 1960). Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation were calculated for all the 36 genotypes.

Results and discussion

Significant differences between cultivated pigeonpea and its wild relative *C. scarabaeoides* were evident for pod protein and trypsin inhibitors (Table 1). However, neither levels of domestication nor its interaction with pod development stage had any influence on lectin content. Lectins may therefore not be associated with reproductive fitness, a trait critical for natural survival or with agronomic traits for which selections were made by the farmer/breeder interventions during the process of domestication and genetic improvement. Despite significant differences in lectin contents between wild and cultivated pigeonpea genotypes in the initial pod development stages, the gradual disappearance of lectins with the advance in pod development stage in both wild and cultivated types would provide sufficient support to this hypothesis (Table 2).

Protein content

The protein content increased with stage of pod development, although the magnitude of increase was marginal in wild pigeonpea genotypes. In the cultivated genotypes, the protein content was similar in both juvenile and immature pods but decreased marginally in mature

Table 1 Analysis of variance to study the interaction between level of domestication and pod development stages for biochemical constituents in wild and cultivated pigeonpea

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Sum of Squares	Variance Ratio	*F* Probability
Protein content					
LD	1	11.119	11.119	3179.47	<0.001
PDS	2	316.7	158.3	1.33	0.252
Wild × PDS	58	22.7	0.4	0.00	1.000
Cultivated × PDS	10	7190.3	719.0	6.05	<0.001
Trypsin inhibitor content					
LD	1				
PDS	2	0.346	0.173	7.61	<0.001
Wild × PDS	58	4.945	0.085	3.75	<0.001
Cultivated × PDS	10	1.082	1.1082	39.16	<0.001
Lectin content					
LD	1				
PDS	2	1.68×10^{-7}	8.40×10^{-6}	1568.12	<0.001
Wild × PDS	58	9.88×10^{-6}	1.703×10^{-5}	3178.95	<0.001
Cultivated × PDS	10	6.106×10^{-5}	6.106×10^{-4}	1139.53	<0.001

LD, level of domestication; PDS, pod development stage; variance ratio ≡ F.

Table 2 Content of protein, trypsin inhibitor and lectins at three maturity stages (juvenile, immature and mature) of *Cajanus scarabaeoides* and *Cajanus cajan*

	Protein Content (g)			Trypsin Inhibitor (units g^{-1} protein)			Specific Haemagglutination Activity (HAU g^{-1} protein)		
Accession	Juvenile	Immature	Mature	Juvenile	Immature	Mature	Juvenile	Immature	Mature[$]
C. scarabaeoides accessions									
ICPW 82	2.59	2.52	2.79	1.46	1.46	1.51	988	127	0
ICPW 83	2.39	3.08	2.79	1.65	1.68	1.71	536	13	0
ICPW 86	3.54	4.18	3.78	1.72	1.62	1.54	362	77	0
ICPW 90	3.74	3.62	4	1.71	1.66	1.65	343	23	0
ICPW 94	3.81	3.81	4.25	1.68	1.61	1.57	672	168	0
ICPW 95	2.13	2.32	2.58	1.5	1.44	1.48	601	138	0
ICPW 96	1.68	1.89	2.38	1.34	1.46	1.56	762	170	0
ICPW 98	2.1	3.08	3.22	1.49	1.54	1.48	1220	0	0
ICPW 101	2.47	2.6	2.54	1.54	1.59	1.54	260	16	0
ICPW 111	2.14	2.59	3.66	0.39	1.03	1.44	300	31	0
ICPW 115	2.47	3.04	3.08	1.53	1.56	1.47	519	0	0
ICPW 116	2.51	2.79	2.91	1.59	1.72	1.7	1020	115	0
ICPW 119	2.11	2.33	2.41	1.71	1.71	1.77	304	0	0
ICPW 122	2.24	2.9	2.78	1.79	1.7	1.67	143	0	0
ICPW 125	2	1.79	2.58	1.76	1.76	1.8	320	0	0
ICPW 130	2.44	2.57	2.91	1.74	1.74	1.7	525	250	0
ICPW 132	2.19	2.48	2.38	1.7	1.74	1.69	293	0	0
ICPW 137	2.28	3.73	4.66	1.71	1.85	1.79	561	43	0
ICPW 138	2.49	2.42	2.35	1.57	1.61	1.64	2057	67	0
ICPW 141	2.63	2.72	3.29	1.43	1.51	1.51	487	59	0
ICPW 147	4.14	3.53	3.28	1.61	1.65	1.75	309	91	0
ICPW 152	1.78	2.72	2.77	1.73	1.77	1.86	719	0	0
ICPW 278	2.44	2.71	2.58	1.76	1.57	1.66	525	31	0
ICPW 280	2.62	2.84	2.42	1.51	1.41	1.58	31	0	0
ICPW 281	3.57	4.04	5.15	1.69	1.59	1.66	12	0	0
ICPW 302	4.04	4.95	4.27	1.79	1.79	1.72	159	33	0
ICPW 305	4	4.34	3.59	1.66	1.75	1.73	640	148	0
ICPW 308	2.54	2.56	2.83	1.77	1.85	1.78	32	0	0
ICPW 310	4.09	4.34	3.7	1.71	1.66	1.67	157	37	0
ICPW 315	3.69	3.95	3.62	1.75	1.61	1.58	174	21	0
Mean	2.76	3.08	3.19	1.6	1.62	1.64	501.14	53.98	0
SE±	0.765	0.786	0.74	0.258	0.162	0.113	419.44	66.944	0
C. cajan genotypes									
ICP 26	1.74	1.98	1.47	1.57	1.07	1.09	736	324	0
ICP 28	1.37	1.7	1.38	1.54	1.16	1.04	934	188	0
ICP 8518	1.43	1.38	1.62	1.12	1.11	1.06	224	58	0
ICP 8863	2.71	2.41	2.91	1.4	1.24	1.04	473	266	0
ICP 14722	2.38	1.97	1.71	1.11	1.17	1.25	538	163	0
ICP 14770	1.75	1.92	1.71	1.13	1.11	0.97	732	84	0

Table 2 Continued

Accession	Protein Content (g)			Trypsin Inhibitor (units g^{-1} protein)			Specific Haemagglutination Activity (HAU g^{-1} protein)		
	Juvenile	Immature	Mature	Juvenile	Immature	Mature	Juvenile	Immature	Mature[$]
Mean	1.89	1.89	1.8	1.31	1.14	1.08	605.88	180.13	0
SE±	0.536	0.341	0.56	0.218	0.06	0.094	248.57	102.419	0
Overall mean	2.62	2.88	2.95	1.55	1.54	1.55	518.6	75	0
SEM±	0.13	0.14	0.15	0.05	0.04	0.04	65.86	14.42	0
SED	0.8	0.85	0.88	0.27	0.23	0.24	395.18	86.52	0
Range	1.37–4.14	1.38–4.95	1.38–5.15	0.39–1.75	1.03–1.85	0.97–1.86	11.00–2056.00	0.00–323.00	—
F probability	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	—
LSD (5%)	0.31	0.24	0.23	0.29	0.26	0.30	20.28	7.10	—
PCV	29.28	30.34	38.58	19.12	14.04	15.85	76.29	113.62	—
GVC	29.01	29.87	42.65	17.21	12.17	13.63	76.25	113.01	—
Heritability (%)	99.30	97.32	95.45	74.56	89.46	87.32	99.52	99.53	—

GCV, genotypic coefficient of variance; LSD, least significant difference; PCV, phenotypic coefficient of variance; SE, standard error; SEM, standard error of mean; SED, standard error of difference; $, mature pods were devoid of any lectin; variance ratio ≡ *F*.

pods. The protein content in general was higher in wild accessions in all the three pod development stages compared with that in cultivated types (Table 2). The protein content increased in both wild and cultivated genotypes stepwise from juvenile pod, immature to mature pod stage. In the juvenile stage, protein content was highest in ICPW 147 (4.14 g) and the least in ICPW 96 (1.68 g). The mature pods of ICPW 281 had the highest protein (5.15 g) and ICPW 138 (2.35 g) the least (Table 2). However, in *C. cajan*, protein content was equal in both the juvenile and immature pod stages with a mean of 1.90 g. *C. scarabaeoides* accessions, because of their high protein content, have been used as donor parents for transferring high-protein genes to pigeonpea (Saxena *et al.*, 1987, 1990).

Trypsin inhibitor(s)

The trypsin inhibitors were higher in wild types in all pod stages compared with those in cultivated types (Table 2). Individually, the *C. scarabaeoides* accessions and pigeonpea genotypes differed significantly ($P < 0.005$) for trypsin inhibitor contents at all the three stages (Table 2). While the content of trypsin inhibitors did not vary with pod development stages in wild types, it decreased with the pod development stages, although marginally, in cultivated types. Juvenile pods of both the cultivated and the wild accessions had higher trypsin inhibitor levels than the immature and mature pods. The wild accessions ICPW 302 and ICPW 122 had the highest content in the juvenile pod stage (1.79 units g^{-1}). However, ICPW 308 and ICPW 137 with 1.85 units g^{-1} and ICPW 152 with 1.86 units g^{-1} had the highest trypsin inhibitor content in the immature and mature stages, respectively (Table 2). The late maturing accessions ICPW 302 and ICPW 308 also showed high levels of resistance to podborer (R. Aruna, unpublished data), which could be attributed to these high levels of protease inhibitors, in addition to other physical factors. The juvenile pods of the cultivated genotype ICP 26 had higher levels of trypsin inhibitors (1.57 units g^{-1}), but its content decreased in the immature pods (1.07 units g^{-1}). Mature pods of ICP 14722 had the highest trypsin inhibitor (1.25 units g^{-1}) and ICP 14770 (0.97 units g^{-1}) had the lowest (Table 2). As the trypsin inhibitors are know to cause reduced digestion and mainly are antinutritional (Rackis, 1981) and insecticidal (Johnston *et al.*, 1991), their presence in the pod from juvenile to mature stages could be an important component of the biochemical basis of resistance to podborer. The levels of these antinutritional factors have been determined in pigeonpea and chickpea whole seed (Singh & Jambunathan, 1981; Singh *et al.*, 1989). However, examination of these inhibitors in

soybean, the most thoroughly studied of all legume species, showed that they are antinutritional and that their residual activities, even in processed human foods, are a cause of concern to human health (Broadway & Duffey, 1986; Gumbmann *et al.*, 1986; Liener, 1986; Brandon *et al.*, 1991), but in pigeonpea, they can be reduced by cooking, germination or fermentation (Singh & Eggum, 1984; Singh, 1988). Interestingly, there are no differences in the electrophoretic forms of these protease inhibitors in the wild and cultivated species, and no significant differences in the trypsin and chymotrypsin levels in *H. armigera*-tolerant and susceptible varieties (Pichare & Kachole, 1994). However, these reports are limited to three genotypes only and cannot be generalised. The conservative nature of these proteins has also been demonstrated and used as markers in biosystematic studies of several plant species (Kollipara *et al.*, 1991; Weder, 1991).

Most of the previous quantification studies were performed using whole seed extracts and did not concentrate on the developing pod stages that are more prone to podborer attack. Major protease inhibitors in the seed were found specific to mammalian serine proteases (Godbole *et al.*, 1994*a*). The podborer larval midgut has a serine type protease (Johnston *et al.*, 1991), and these inhibitors are potent and could effectively inhibit the protease from insect gut (Godbole *et al.*, 1994*b*).

Lectin

Overall, the three pod stages differed significantly for lectin content. The juvenile pods contained mean lectin content of 518.6 HAU g^{-1}, followed by immature pods with a mean value of 75.0 HAU g^{-1} (Table 2). The mature pods did not contain any lectin in any of the 36 genotypes, indicating a decrease in the lectin levels with increasing pod maturity stages. Similar results were obtained when the leaves and developing pods of *C. scarabaeoides* and *C. cajan* were assayed for lectin content (Sonali, 2001). The lectin content in the juvenile and immature pods of the cultivated varieties was higher than in the wild accessions (Table 2), and in 11 accessions, no lectin was detected in the immature pods. Among the cultivated genotypes, the highest content was observed in ICP 28 (934 HAU g^{-1}) followed by ICP 26 (736 HAU g^{-1}) in the juvenile stage. The mature seeds of pigeonpea are consumed in many parts of the world, without toxic effects because there are no lectins in the mature pods. Furthermore, if eaten by humans, the pods are cooked to inactivate these antinutritional factors (Grant *et al.*, 1982). Lectins are know to be heat labile and their activity can be decreased by heat treatment (Pusztai, 1991; Liener, 1994; Aregheore *et al.*, 1998). Hence, if pigeonpea pods are directly consumed even before the seeds reach maturity, there is always a possibility to inactivate the toxin content by heat treatment. Further studies are required to test the time and amount of heat treatment (moist heat/dry heat) required to inactivate the toxic lectins in pigeonpea.

To the authors' knowledge, the present study is first to assess the genetic variation in pod biochemical constituents associated with podborer resistance in cultivated pigeonpea in comparison with those in wild pigeonpea. These biochemical traits are highly heritable (range 75–99%), which indicates that these are governed by a few genes with major effects (Table 2). Hence, these genes could easily be transferred into the cultivated background by simple backcrossing through conventional breeding. The study provides an insight that both lectins and trypsin inhibitors could be potential contributors in conferring resistance to the accessions, the lectins at the juvenile pod stage and the trypsin inhibitors at the mature pod stage. The three accessions ICPW 138, ICPW 98 and ICPW 116 were high in both lectins and trypsin inhibitor content, were also tolerant to *Helicoverpa* damage (data not shown) and could therefore be used as parents in the hybridisation programme. *C. scarabaeoides* belongs to the primary gene pool and is cross-compatible with cultivated pigeonpea. Hybridisation attempts to introgress the useful genes into the cultivated background would broaden the genetic base, forming a valuable step towards breeding for resistance to podborer. Selection of resistant lines based on these biochemical components and subsequent introgression into high-yielding background is highly resource demanding. A suitable morphological marker closely associated with these biochemical constituents would be useful in screening large breeding populations. Further investigations are required to study the environmental influences on these biochemical components.

Acknowledgements

The authors gratefully acknowledge Drs S.N. Nigam, Anjaiah Vanamala and S. Ramesh for their critical comments on the manuscript. The senior author is thankful to the Andhra Pradesh, India – Netherlands, Biotechnology Programme for extending their financial support. Sincere thanks are due to Narsi Reddy for help during biochemical analysis.

References

Altabella T., Chrispeels M.J. (1990) Tobacco plants transformed with the bean αAI gene express an inhibitor of insect α-amylase in their seeds. *Plant Physiology*, **93**, 805–810.

Aregheore E.M., Makkar H.P.S., Becker K. (1998) Assessment of lectin activity in a toxic and non-toxic variety of *Jatropha curcas* using latex agglutination and haemagglutination methods and inactivation of lectin by heat treatments. *Journal of the Science of Food and Agriculture*, **77**, 349–352.

Armes N.J., Jadhav D.R., DeSouza K.R. (1996) A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bulletin of Entomological Research*, **86**, 499–514.

Brandon D.L., Bates A.H., Friedman M. (1991) ELISA analysis of soybean trypsin inhibitors in processed foods. In *Nutritional and Toxicological Consequences of Food Processing*, pp. 321–337. Ed. M. Friedman. New York, USA: Plenum Press.

Broadway R.M., Duffey S.S. (1986) Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology*, **32**, 827–833.

Erlanger B.F., Kokowsky N., Cohen W. (1964) The preparation and properties of two new chromogenic substrates of trypsin. *Archives of Biochemistry and Biophysics*, **95**, 271–278.

Falconer D.S. (1960) *Introduction to Quantitative Genetics*. Edinburgh, UK: Oliver and Boyd. 362 pp.

Giri A.P., Harsulkar A.M., Deshpande V.V., Sainani M.N., Gupta V.S., Ranjekar P.K. (1998) Chickpea defensive proteinase inhibitors can be inactivated by podborer gut proteinases. *Plant Physiology*, **116**, 393–401.

Godbole S.A., Krishna T.G., Bhatia C.R. (1994*a*) Purification and characterization of protease inhibitors from pigeonpea (*Cajanus cajan* (L.) Millsp) seeds. *Journal of the Science of Food and Agriculture*, **64**, 87–93.

Godbole S.A., Krishna T.G., Bhatia C.R. (1994*b*) Further characterization of protease inhibitors from pigeonpea (*Cajanus cajan* (L.) Millsp) seeds. *Journal of Science Food and Agriculture*, **64**, 331–335.

Grant G. (1991) Lectins. In *Toxic Substances in Crop Plants*, pp. 49–67. Eds J.P.F. D'Mello, C.M. Duffus and J.H. Duffus. Cambridge, UK: Royal Society of Chemistry.

Grant G., More L.J., Mckenzie N.H., Pusztai A. (1982) The effect of heating on the haemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *Journal of the Science of Food and Agriculture*, **33**, 1324–1326.

Gumbmann M.R., Spangler W.L., Dugan G.M., Rackis J.J. (1986) Safety of trypsin inhibitors in the diet: effects on the rat pancreas of long-term feeding of soy flour and soy protein isolate. In *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods*, pp. 33–79. Ed. M. Friedman. New York, USA: Plenum Press.

Harsulkar A.M., Giri A.P., Patankar A.G., Gupta V.S., Sainani M.N., Ranjekar P.K., Deshpande V.V. (1999) Successive use of non-host plant proteinase inhibitors for effective inhibition of *Helicoverpa armigera* gut proteins and larval growth. *Plant Physiology*, **121**, 497–506.

Ishimoto M., Kitamura K. (1989) Growth inhibitory effects of an α-amylase inhibitor from kidney bean, *Phaseolus vulgaris* (L) on three species of Bruchids (Coleoptera: Bruchidae). *Applied Entomology and Zoology*, **24**, 281–286.

Johnston K.A., Lea M.J., Gatehouse J.A., Anstee J.W. (1991) The partial purification and characterization of serine protease activity in midgut of larval *Helicoverpa armigera*. *Insect Biochemistry*, **21**, 389–397.

Kollipara K.P., Domagalski J.M., Hymowitz T. (1991) A quick method of resolving soybean Kunitz trypsin inhibitor using polyacrylamide gel electrophoresis. *Soybean Genetics Newsletter*, **18**, 234–236.

Liener I.E. (1986) Trypsin inhibitors: concern for human nutrition or not? *Journal of Nutrition*, **116**, 920–923.

Liener I.E. (1994) Implications of antinutritional components in soybean foods. *Critical Reviews in Food Science and Nutrition*, **34**, 31–67.

Liener I.E., Kakade M.L. (1980) Protease inhibitor. In *Toxic Constituents of Plant Foodstuffs*, pp. 7–71. Ed. I.E. Liener. New York, USA: Academic Press.

Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265–275.

Moreno J., Altabella T., Chrispeels M.J. (1990) Characterization of α-amylase inhibitor, lectins like protein in the seeds of *Phaseolus vulgaris*. *Plant Physiology*, **92**, 703–709.

Mulimani V.H., Paramjyothi S. (1992). Proteinase inhibitors of red gram (*Cajanus cajan* (L.)). *Journal of the Science of Food and Agriculture*, **59**, 273–275.

Nene Y.L., Sheila V.K. (1990) *The Pigeonpea*. India: International Crops Research Institute for Semi-Arid Tropics, CAB International Publications, ICRISAT.

Pichare K.P., Kachole T. (1994) Trypsin inhibitors of Pigeonpea. *International Pigeonpea Newsletter*, **16**, 20–22.

Pundir R.P.S., Singh R.B. (1987) Possibility of genetic improvement in pigeonpea utilizing the wild genetic resources. *Euphytica*, **36**, 33–37.

Pusztai A. (1966) The isolation of two proteins, glycoprotein I and a trypsin inhibitor, from the seeds of kidney bean (*Phaseolus vulgaris*). *Biochemical Journal*, **101**, 379–384.

Pusztai A. (1991) *Plant Lectins*. Cambridge, UK: Cambridge University Press.

Rackis J.J. (1981) Significance of soybean trypsin inhibitors in nutrition. *Journal of the American Oil Chemists' Society*, **58**, 495–501.

Reed W., Pawar C.S. (1982) Heliothis: a global problem. In *Proceedings of International Workshop on Heliothis Management*, pp. 9–14. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

Romies J., Shanower T.G., Peter A.J. (1999) Trichomes on pigeonpea [*Cajanus cajan* (L.) Millispaugh] in two wild *Cajanus* spp. *Crop Science*, **39**, 564–569.

Ryan C.A. (1990) Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *Annual Review of Phytopathology*, **28**, 425–449.

Saxena K.B., Kumar R.V., Faris D.G., Singh L. (1987) *Breeding for special traits. Report of work 1984-1986. Pigeonpea*

Breeding Progress Report 9. Patancheru, India: International Crops Research Institute for the Semi-Arid Tropics. pp. 36–89.

Saxena K.B., Singh L., Reddy M.V., Singh U., Lateef S.S., Sharma S.B., Remanandan P. (1990) Inter species variation in *Atylosia scarabaeoides* (L.) Benth., a wild relative of pigeonpea [*Cajanus cajan* (L.) Millsp.]. *Euphytica*, **49**, 185–191.

Shanower T.G., Yoshida M., Peter A.J. (1997) Survival, growth, fecundity, and behaviour of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on pigeonpea and two wild *Cajanus* species. *Journal of Economic Entomology*, **90**, 837–841.

Singh U. (1988) Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Quality of Plant Foods and Human Nutrition*, **38**, 251–254.

Singh U., Eggum O.B. (1984) Factors affecting the protein quality of pigeonpea (*Cajanus cajan* (L.)). *Quality of Plant Foods and Human Nutrition*, **34**, 273–283.

Singh U., Jambunathan R. (1981). Protease inhibitors and *in vitro* protein digestibility of pigeonpea (*Cajanus cajan* (L.) Millsp.) and its wild relatives. *Journal of the Science of Food and Technology*, **18**, 246.

Singh U., Jambunathan R., Saxena K.B., Subramaniam N. (1989) Nutritional evaluation of the newly developed high protein genotypes of pigeonpea (*Cajanus cajan* L.). *Quality of Plant Foods and Human Nutrition*, **34**, 273–283.

Sonali K. (2001) *Evaluation of bioactive compounds Bt., soybean trypsin inhibitors and plant lectins against* Helicoverpa armigera. M.Sc. Thesis. University of Agricultural Sciences, Dharwad.

Stevenson P.C., Green P.W., Simmonds M.S., Sharma H.C. (2002) Can larvae of the pod-borer, *Helicoverpa armigera* (Lepidoptera: Noctuidae), select between wild and cultivated pigeonpea *Cajanus* sp. (Fabaceae)? *Bulletin of Entomological Research*, **92**, 45–51.

Weder J.K.P. (1991) Plant protease inhibitors: chemical and kinetic aspects. In *Proceedings of the First Brazilian Congress on Proteins*, pp. 29–55. Eds B. Oliveira and V. Sgarberi. Brazil: Editoria Unicamp, Campine.

Whitaker J.R., Filho F.F., Lajolo F.M. (1988) Parameters involved in binding of porcine pancreatic α-amylase with black bean inhibitor: role of sulfhydryl groups, chloride, calcium, solvent composition and temperature. *Biochemistry*, **70**, 1153–1161.

Ann Appl Biol **151** (2007) 25–32