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Protease inhibitors and lectins in cowpea

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Wild and cultivated accessions of cowpea were analysed for trypsin and chymotrypsin inhibitors, and lectins in order to assess: their variability; their influence in the mechanism of Bruchid resistance; their evolution during species domestication.

Wild *Vigna vexillata* showed significantly higher values ($P < 0.0001$) for trypsin and chymotrypsin inhibitors and significantly lower values ($P < 0.025$) for lectins as compared with cultivated accessions. This may imply that domestication operates an indirect selection for these characters.

High degrees of correlation between chymotrypsin inhibitors and trypsin inhibitors ($r = 0.959$) and significant correlation ($P < 0.01$) between trypsin inhibitors and protein content were found.

The high resistance to Bruchid and the high trypsin inhibitor content of *V. vexillata* suggest that, even where there is no direct relationship between Bruchid resistance and trypsin inhibitor content, protease inhibitors promote, or are a component of, the plant's defence mechanism.

INTRODUCTION

Protease inhibitors and lectins are frequently investigated with regard to: (a) the bioavailability of nutrients (Carnovale *et al.*, 1991); (b) the resistance to pests (Gatehouse & Boulter, 1983, 1984; Hilder *et al.*, 1987; Murdock *et al.*, 1990; Gatehouse *et al.*, 1991); (c) their inter-intraspecific variability (Weder, 1985; Lombardi-Boccia *et al.*, 1991).

Trypsin and chymotrypsin inhibitors and lectins were determined in a series of accessions of *Vigna unguiculata* L. Walp., in order to explore, first, their variability, second, any relationships which may exist between them and resistance to *Callosobruchus maculatus* (CM) and third, their evolution in the course of species domestication.

MATERIALS AND METHODS

Dry seeds of the wild species *Vigna vexillata* (TVnu 72, TVnu 66, TVnu 64, TVnu 226) and of 22 cultivated cowpea samples (*V. unguiculata*) were obtained from the germplasm bank of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The different accessions showed a wide range of variation as regards colour, size, form and structure of the seed coats, and resistance to pests.

From 50 g of each wild accession and 100 g of each cultivated accession, sub-samples of 25 g and 50 g, respectively, were finely ground to 50 μm in a Cyclotec 1093 Tecator laboratory mill and the resulting flour was then taken for analysis.

The protein content was determined by the Kjeldhal procedure (N × 6·25). The Trypsin Inhibitors (TI) were determined by the Kakade method as modified by Hamerstrand *et al.* (1981).

A portion (1 g) of flour was extracted in 50 ml NaOH (0·01 N) for 3 h. The filtered extract was assayed for Trypsin Inhibitor Activity (TIA) by measuring inhibition of trypsin catalysed hydrolysis of substrate BAPNA.

One trypsin unit is arbitrarily defined as an increase of 0·01 absorbance units at 410 nm. TIA is expressed in terms of Trypsin Inhibited Units (TIU/mg). Chymotrypsin Inhibitors (CI) were analysed according to the method described by Kress *et al.* (1968).

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The same extract of TIA was assayed for Chymotrypsin Inhibitory Activity (CIA) by measuring inhibition of the rate of chymotrypsin-catalysed hydrolysis of substrate BTEE.

Assays were performed in 10 mm quartz cuvettes at 25°C in a thermostatted spectrophotometer (Beckman DU-8). CIA was expressed in arbitrary Chymotrypsin Inhibitor Units (CIU): one unit is defined as that giving a decrease in absorbance of 0·01/min at 256 nm, compared to the rate of chymotrypsin standard activity in the absence of any inhibitor. Lectin activity was analysed according to the method described by Lis & Sharon (1972) with untrypsinated erythrocytes. The crude seed extract was prepared by shaking 1 g of ground seed flour in 10 ml saline solution (0·9% NaCl) for 2 h and filtering off the insoluble residue. Starting with 25 μl of extract, serial twofold dilutions were made with saline solution on microtitre V plates. Rabbit red blood cell suspension (25 μl) was then added to the extract. After 2 h, agglutination manifests itself by an evenly distributed layer of red blood cells over the entire bottom of the well. In the absence of agglutination, the red blood cells collect only at the apex of the well.

Phaseolus vulgaris lectin (SIGMA) was used as a standard. Haemagglutination Activity (HA) was expressed as the reciprocal of the highest dilution (g/ml) giving positive agglutination.

The data were statistically evaluated by Analysis of Variance calculating the Least Significant Difference (LSD) and the Coefficient of Variability (CV).

RESULTS AND DISCUSSION

The protein content varied between 20·8% for Vita 4 and 27·5% for TVu 13686 in the case of the cultivated accessions, and might reach as much as 28·2% for the wild species TVnu 66 (Table 1) (similar to that noted by Kochnar *et al.* (1988) and Omueti & Singh (1987)). The wide range of variability suggests that it should be possible to augment protein content through breeding programmes (Bliss & Hall, 1977).

The mean value for the cultivated species was 24·3%—a figure substantially in agreement with those reported by Della Gatta *et al.* (1989), Bressani (1985) and Omueti & Singh (1987).

Trypsin inhibitor content showed a minimum of 9·01 TIU/mg for TVu 9062 and a maximum of 25·9 TIU/mg for TVu 1, with a mean of 14·7 TIU/mg in the cultivated species (Table 1). Values higher than the 40 TIU/mg were found in *V. vexillata.*

Accession TVu 2027, with its inhibitor content of 20·0 TIU/mg, is characterized by its resistance to CM, so much so that the resistance gene (cowpea trypsin

Table 1. Trypsin and chymotrypsin inhibitors, haemagglutination activity and protein content in cowpea and *Vigna vexillata* accessions

Accessions	Resistance to Bruchid	Trypsin inhibitor (TIU/mg)	Chymotrypsin inhibitor (CIU/mg)	Haemagglutination activity (HA)	Protein content (N × 6·25) (%)
TVNu 64	BR	39·3	55·8	53	24·9
TVNu 66	BR	43·4	...	13	28·2
TVNu 72	BR	46·7	54·1	40	25·7
TVNu 226		36·1	—	27	27·1
TVu 13271		15·7	13·0	240	23·9
TVu 946	BS	14·1	16·5	147	25·5
TVu 1	BS	25·9	22·1	240	24·6
TVu 9062	BS	9·01	11·0	147	23·1
Ife Brown	BS	10·6	7·08	587	23·9
TVu 662	BS	18·5	17·5	587	24·6
TVu 13686		24·4	—	293	27·5
Vita 3	BS	9·45	9·54	200	24·2
TVu 2027	BR	20·0	16·3	120	22·3
TVu 3000	BS	14·5	15·6	400	23·5
IT84S 2231-15	BR	10·4	9·23	347	24·2
IT84EI 108	BS	14·5	11·5	240	25·4
IT82D 716	BR	9·56	8·48	213	22·9
Vita 4	BS	9·85	12·8	240	20·8
TVx 3236	BS	9·20	6·74	240	21·8
TVu 9357	BS	16·3	—	427	25·6
TVu 8331	BS	16·1	12·7	347	24·1
TVu 13000	BS	12·2	9·95	80	25·1
TVu 1087	BS	14·9	17·4	533	25·0
TVu 3451	BS	15·7	18·1	1173	25·7
TVu 22	BS	18·6	23·8	227	25·9
Mean		19·0	17·6	286	24·6
CV		58	75	86	7
LSD (95%)		5·44	1·85	129	0·53

inhibitor—CpTi) has been transferred to tobacco (Hilder *et al.*, 1987). It is important, here, to recall that some accessions with a TIU content similar to that of TVu 2027 (TVu 22 and TVu 662) or higher (TVu 1) are susceptible to CM, while accessions with a markedly lower inhibitor content than that of TVu 2027 (IT 82D 716 and IT 84S 2231-15) likewise exhibit CM resistance, as noted by Della Gatta *et al.* (1989) and Xavier-Filho *et al.* (1989). The activity of protease inhibitors against insects has been abundantly demonstrated but varies considerably, especially as governed by the type of inhibitor and the species of insect. In this connection, Gatehouse *et al.* (1991) found that trypsin inhibitor extracted from winged bean promotes CM larval growth, since the larvae are able to make use of the sulphurated amino acids.

At the same time, it is to be noted that wild *V. vexillata*, with its markedly high TIU levels, is also characterized by its high resistance to CM and other insects (IITA, 1988; Ng *et al.*, 1989). *V. vexillata* can thus provide an important source for breeding programmes, even if there are still unsolved problems of interspecific incompatibility (Barone & Ng, 1990).

There is a high degree of correlation ($P < 0{\cdot}01$) between protein content and trypsin inhibitors (Table 2). This can be explained by the fact that such inhibitors are themselves protein: Gatehouse *et al.* (1980) found these inhibitors in cowpea seed at a level of 3 mg/g seed meal. No such correlation, on the other hand, was discerned by Singh & Jambunathan (1981) in the case of chickpeas, or by Griffiths (1984) with *Pisum* and *Vicia faba*.

Chymotrypsin inhibitors show values ranging from 6·74 CIU/mg for TVx 3236 to 23·8 CIU/mg for TVu 22, with peaks over 50 CIU/mg for wild species. The pattern followed in their case is similar to that of the TIU, there being a high degree of correlation between CIU and TIU ($r = 0{\cdot}959$), with a regression curve $y = 1{\cdot}304\,x - 4{\cdot}448$, where x = TIU and y = CIU.

That Singh & Jambunathan (1981) should have found this same relationship with chickpea, and Griffiths (1984) with *Pisum* and *V. faba*, can be explained by the fact that one of the two types of trypsin inhibitors also exhibits antichymotryptic activity (Filho & De Azevedo, 1978; Gatehouse *et al.*, 1980; Lombardi-Boccia *et al.*, 1991).

Trypsin and chymotrypsin inhibitors thus show a wide range of variability with very high values in the wild species. This may suggest that domestication has brought about an unintended diminution in these factors but has also led to a greater susceptibility to parasites, since wild *V. vexillata* and other wild *Vigna* show a broad range of parasite resistance (Ng *et al.*, 1989; IITA, 1988; Birch *et al.*, 1985).

Lectin content showed a minimum of 80 HA (TVu 13000) and a maximum of 1173 HA (TVu 3451) in the cultivated cowpea accessions, with a mean of 335 HA, whereas lower values (mean of 33 HA) are found in *V. vexillata*.

All these values are low compared to varieties of *Phaseolus vulgaris* (like Italian Cannellino, Borlotto) which have a value of 27 000 HA when raw and of higher than 2000 HA when cooked. The low lectin content noted in cowpea agrees with the findings of Gatehouse *et al.* (1984) (no lectin in their case), Grant *et al.* (1983), Elckowickz & Sosulski (1982) and Gupta (1988).

Again, no correlation was found between protein and lectin content (Table 2). This is because, even though these are both protein, the lectin contribution to total protein content is slight. Nor did any correlation emerge between lectin content and resistance to CM—a point also noted by Xavier-Filho *et al.* (1989).

Lectin from wheat germ (Murdock *et al.*, 1990) and from winged bean (Gatehouse *et al.*, 1991) offered a high toxicity for CM. A proposal has thus been made to transfer the gene to cowpea by genetic engineering.

Carnovale & Marconi (unpublished data) found that wild accessions of *V. luteola* have a high lectin content similar to that in *Phaseolus* with, at the same time, a high resistance to CM. If this relationship were to be confirmed it would represent an additional genetic resource for cowpea breeding programmes.

Table 2. Correlation coefficient (r)

	TIU	CIU	HA
Protein	0·613[a]	0·419	−0·036
TIU	—	0·959[a]	

[a] Significant at $P < 0{\cdot}01$.

CONCLUSIONS

The antinutritional factor content in cowpea is to be explained principally by trypsin inhibitors, while lectins are not present in great quantities.

Wild *V. vexillata* showed significantly higher values ($P < 0{\cdot}0001$) for trypsin and chymotrypsin inhibitors and significantly lower values ($P < 0{\cdot}025$) for lectins as compared with cultivated accessions. This suggests that domestication may operate an indirect selection for these characters (De la Vega & Sotelo, 1986; Broadway, 1989).

Further, the high resistance to CM and the high TI content in *V. vexillata* suggest that, even where there is no direct relationship between BR and TIU (in any case, cultivated accessions with a low TIU content prove to be likewise resistant), protease inhibitors promote (or are a component of) the plant's defence mechanisms.

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