

# Chemical evaluation of wild under-exploited *Vigna* spp. seeds

Emanuele Marconi, Stefania Ruggeri & Emilia Carnovale\*

Istituto Nazionale della Nutrizione, Via Ardeatina 546, 00178, Rome, Italy

(Received 12 January 1996; accepted 21 February 1996)

Eight wild *Vigna* spp. (*Vigna vexillata*, *Vigna vexillata macrosperma*, *Vigna luteola*, *Vigna oblongifolia*, *Vigna unguiculata dekindtiana*, *Vigna racemosa*, *Vigna reticulata*, *Vigna ambacensis*) were analysed for chemical characteristics (protein, amino acid profile, starch digestibility) and for antimetabolic compounds, such as trypsin inhibitors, cysteine proteinase inhibitors, lectins, phytic acid and tannins, in order to find useful material for improving the resistance and nutritional aspects of cowpeas. *V. vexillata* showed a high protein content of up to 293 g kg<sup>-1</sup>, whereas all the accessions had high sulphur amino acids (2.05–3.63 g per 16 g N) and consequently a high chemical score. Moreover, the level of resistant starch was also high (64–75%). A wide variability was found in the trypsin inhibitors, tannins and lectins; *V. luteola* contained high levels of these compounds, whereas *V. unguiculata dekindtiana*, *V. reticulata* and *V. ambacensis* had very low levels. Significant positive correlations were found between bruchid resistance and trypsin inhibitor, tannin and resistant starch content. © 1997 Published by Elsevier Science Ltd. All rights reserved

## INTRODUCTION

Food legumes, particularly cowpeas (*Vigna unguiculata* L. Walp), are the most important sources of protein, carbohydrates and vitamins in the diet of many populations, especially in developing countries (Phillips & McWatters, 1991).

Despite the potential of cowpeas as a food, their optimal usage is somewhat limited due to pest infestation of seeds, the extended cooking time required and the low sulphur amino acid content. In order to resolve some of these problems, the wide genetic diversity of wild progenitors and primitive cultivars could be useful in providing genes to be transferred to the crop via interspecific hybridisation by conventional plant breeding or by genetic manipulation.

Preliminary investigations on the pest resistance of wild *Vigna* spp. were promising as regards their potential utilisation for creating enhanced ideotypes of cowpeas (Ng *et al.*, 1989).

Certain antimetabolites such as tannins, lectins, protease inhibitors and phytic acid may be related to cowpea resistance towards *Callosobruchus maculatus* F. (CM), the major insect pest (Gatehouse *et al.*, 1979, 1984; Piergiovanni *et al.*, 1991; Gatehouse & Boulter, 1983; Ng *et al.*, 1989; Xavier-Filho *et al.*, 1989; Murdock *et al.*, 1990).

It has been suggested that endogenous resistance to insect pests could be improved by manipulating primary gene products such as trypsin inhibitors, lectins and  $\alpha$ -amylase of the pulse crops (Foard *et al.*, 1983; Ng *et al.*, 1989). On the other hand, the high levels of antimetabolites may decrease the nutritional value of legumes, reducing protein and starch digestibility, and mineral bioavailability (Carnovale *et al.*, 1991; Bressani *et al.*, 1988; Desphande *et al.*, 1982; Reddy *et al.*, 1982; Licner, 1986; Sharon & Lis, 1989).

Trypsin and cystatin inhibitors and lectins are heat-labile compounds and their negative effects are therefore markedly reduced by cooking (Akinyele, 1989; Boufassac *et al.*, 1986; Hines *et al.*, 1991); however, tannins and phytic acid are heat-stable compounds that retain negative effects on mineral and protein bioavailability after cooking (Ogun *et al.*, 1989). A high variability in protein and sulphur amino acid content was found in the wild species (Carnovale *et al.*, 1991), which suggests that it should be possible to increase the nutritional quality of cowpeas through interspecific breeding programmes.

The aim of the project on cowpea germplasm conducted by the International Institute of Tropical Agriculture (IITA) in collaboration with several Italian institutes has therefore been to study germplasm variability in domestic *V. unguiculata unguiculata* and in wild *Vigna* spp. in order to transfer useful genes to cultivated

\*To whom correspondence should be addressed.

cowpeas or to use new species for making traditional products.

The present paper deals with the evaluation of protease inhibitor, lectin, phytic acid and tannin levels, as well as the protein content, amino acid composition and starch digestibility of wild *Vigna* spp., in order to characterise these under-exploited materials and to find possible sources of resistance for use in breeding programmes.

## MATERIALS AND METHODS

### Materials

Dry seeds of eight wild *Vigna* species—*V. vexillata* (five accessions), *V. vexillata macrosperma* (three accessions), *V. luteola* (three accessions), *V. oblongifolia* (three accessions), *V. unguiculata dekindtiana* (four accessions), *V. racemosa* (three accessions), *V. reticulata* (three accessions) and *V. ambacensis* (three accessions)—were obtained from the germplasm bank of the IITA, Ibadan, Nigeria. The subgenera were chosen on the basis of the genetic affinity to cowpea and of the feasibility of utilisation for tackling pests of cowpea (Ng *et al.*, 1989). The accessions differed in seed morphological characteristics (weight, size, colour), in seed bruchid resistance (BR) and bruchid susceptibility (BS) and in the use of vegetative and storage tissues (Table 1).

The seeds were finely milled (< 50 µm) in a Cyclotec 1093 Tecator laboratory mill.

### Methods

Protein content (N×6.25) was determined by AOAC (1980) methods. Amino acids were determined by single-column ion-exchange chromatography (Beckman amino acid analyzer, Model 120) after hydrolysis with 6 N HCl at 110°C for 24 h and 72 h in vacuum-sealed tubes. Performic acid oxidation, followed by acid hydrolysis, was used for cysteine and methionine determination (Schram *et al.*, 1954).

Trypsin inhibitors (TI) were determined by the method of Kakade *et al.* (1974), as modified by Hamerstrand *et al.* (1981), and were expressed as trypsin inhibitor units (TIU) mg<sup>-1</sup> protein.

Cysteine proteinase inhibitors were extracted in a phosphate buffer, 100 mM (5 ml g<sup>-1</sup> seed) at pH 7.5, after soaking in water for 30 min according to Rele *et al.* (1980). The extract was dialysed overnight in a phosphate buffer, 10 mM, and precipitated with ammonium sulphate at 90% saturation. The precipitate was dissolved in a phosphate buffer, pH 7.5, and dialysed as above. The thiol inhibitory activity was tested using Na benzoyl-DL-arginine *p*-nitroanilide (BAPNA) as a substrate and papain as a protease, and the *p*-nitroaniline liberated was measured spectrophotometrically at 410 nm (Arnon, 1970).

Haemagglutinating activity (HA) was analysed as described by Lis and Sharon (1972) with untrypsinated erythrocytes as reported by Marconi *et al.* (1993). HA was expressed as the reciprocal of the highest dilution (g ml<sup>-1</sup>) giving positive agglutination. Phytic acid (PA) was determined by the ion-exchange procedure of Harland and Oberleas (1986). Tannin content was evaluated by the method of Price *et al.* (1978). Total starch (TS), rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) fractions were determined according to Englyst *et al.* (1992).

All the determinations were carried out in triplicate. The data were statistically evaluated by one-way analysis of variance.

## RESULTS AND DISCUSSION

### Seed characteristics and utilisation

The 100 seed weight range was 1.3–5.7 g (Table 1), which is much lower than the range of 6.4–24 g for cultivated cowpeas (Kachare *et al.*, 1988; Omueti & Singh, 1987; Carnovale *et al.*, 1991). Most wild accessions examined are dark-coloured seeds, except for the cream-coloured seeds of the TVnu 136 and TVnu 306 accessions.

Plant tissues of wild *Vigna* spp. are used for human consumption (seeds and tubers), for animal feed (leaves, pods) and for their medicinal properties (roots) (Padulosi & Ng, 1990), as summarised in Table 1.

### Protein content and amino acid composition

The protein content of wild *Vigna* spp. (Table 2) varies from 208 to 292 g kg<sup>-1</sup>, a higher variability than that reported for cultivated cowpeas (Ologhobo & Fetuga, 1982; Omueti & Singh, 1987). *V. vexillata* is characterised by a significantly ( $P \leq 0.05$ ) higher protein content than the other species, whereas *V. luteola* and *V. reticulata* have the lowest values. Accessions of the same species, except for *V. reticulata*, show great uniformity in protein levels without significant difference between them.

The amino acid profile, the chemical score based on the FAO (1985) pattern and the relative limiting amino acids of *V. vexillata* (TVnu 71), *V. vexillata macrosperma* (TVnu 72, TVnu 73a), *V. luteola* (TVnu 24, TVnu 29), *V. oblongifolia* (TVnu 38), *V. unguiculata dekindtiana* (TVnu 278), *V. racemosa* (TVnu 105), *V. reticulata* (TVnu 225) and *V. ambacensis* (TVnu 306) are given in Table 3.

The trend and variability of the level of amino acids in the wild *Vigna* spp. are similar to those of the cultivated lines (Bressani, 1985; Kochhar *et al.*, 1988; Ene-Obong & Carnovale, 1992; Chan & Phillips, 1994), except for cystine (CV 30.12), which had very high values in two accessions (2.05 and 1.79 in TVnu 24 and TVnu 29,

respectively). Such high values have not previously been found in wild or cultivated *Vigna* spp. (Khalil & Khan, 1995; Rajaram & Janardhanan, 1990; Mohan & Janardhanan, 1993). The high variability range suggests that the cystine content of cultivated cowpeas could be increased by interspecific breeding. In addition, no correlations were found between protein and methionine and cystine content. Therefore the breeding programmes for improving the protein content of *Vigna* would not necessarily have a negative effect on the sulphur amino acid content.

In the amino acid patterns of some accessions, two peaks of uncommon amino acids were found. One was characteristic of *V. vexillata* (TVnu 71) and *V. vexillata macrosperma* (TVnu 72 and TVnu 73a); the other was characteristic of *V. reticulata* (TVnu 225). In order to identify these peaks, the standards for the unusual amino acids previously found in legumes were tested, in particular L-DOPA (Rajaram & Janardhanan, 1990; Vijayakumari *et al.*, 1993), L-canavanine (Vangala & Menden, 1969; Cacho *et al.*, 1989), L-pipecolic acid

Table 1. Seed characteristics and utilisation of wild *Vigna* spp.

| Accessions                        | Seed coat colour | 100 seed weight (g) | Bruchid resistance <sup>a</sup> | Utilisation <sup>b</sup>   |
|-----------------------------------|------------------|---------------------|---------------------------------|--|
| <i>V. vexillata</i>               |                  |                     |                                 |  |
| TVnu 66                           | Brown-black      | 2.0                 | BR                              | Edible tuber, medicinal properties, cover crop, fertilizer plant |
| TVnu 71                           | Brown            | 2.2                 | BR                              |  |
| TVnu 73                           | Light brown      | 1.9                 | BR                              |  |
| TVnu 120                          | Brown, black     | 2.1                 | BR                              |  |
| TVnu 226                          | Brown, black     | 2.5                 | —                               |  |
| Mean                              |                  | 2.1                 |                                 |  |
| <i>V. vexillata macrosperma</i>   |                  |                     |                                 |  |
| TVnu 64                           | Green, brown     | 5.7                 | BR                              | Edible tuber, medicinal properties                               |
| TVnu 72                           | Green, brown     | 5.1                 | BR                              |  |
| TVnu 73A                          | Black            | 4.0                 | BR                              |  |
| Mean                              |                  | 4.9                 |                                 |  |
| <i>V. luteola</i>                 |                  |                     |                                 |  |
| TVnu 24                           | Brown            | 3.0                 | BR                              | Cover crop, excellent pasture plant, highly palatable            |
| TVnu 28                           | Brown mottled    | 2.3                 | BR                              |  |
| TVnu 29                           | Brown            | 2.4                 | BR                              |  |
| Mean                              |                  | 2.6                 |                                 |  |
| <i>V. oblongifolia</i>            |                  |                     |                                 |  |
| TVnu 38                           | Brown, black     | 2.2                 | BR                              | Cover crop with excellent leafy growth                           |
| TVnu 85                           | Brown            | 3.3                 | BS                              |  |
| TVnu 133                          | Brown            | 2.8                 | BS                              |  |
| Mean                              |                  | 2.8                 |                                 |  |
| <i>V. unguiculata dekindtiana</i> |                  |                     |                                 |  |
| TVnu 136                          | Light brown      | 3.0                 | —                               | Edible seeds, medicinal properties                               |
| TVnu 140                          | Brown            | 4.0                 | BS                              |  |
| TVnu 255                          | Cream            | 1.8                 | BS                              |  |
| TVnu 278                          | Brown            | 1.3                 | BS                              |  |
| Mean                              |                  | 2.5                 |                                 |  |
| <i>V. racemosa</i>                |                  |                     |                                 |  |
| TVnu 105                          | Brown mottled    | 1.3                 | BS                              |  |
| TVnu 163                          | Brown mottled    | 1.6                 | BS                              |  |
| TVnu 220                          | Light brown      | 1.5                 | BS                              |  |
| Mean                              |                  | 1.5                 |                                 |  |
| <i>V. reticulata</i>              |                  |                     |                                 |  |
| TVnu 137                          | Brown mottled    | 4.3                 | BR                              | Edible tuber   |
| TVnu 225                          | Light brown      | 3.4                 | BR                              |  |
| TVnu 323                          | Brown mottled    | 1.7                 | BR                              |  |
| Mean                              |                  | 3.1                 |                                 |  |
| <i>V. ambacensis</i>              |                  |                     |                                 |  |
| TVnu 306                          | Cream            | 4.6                 | BS                              | Edible tuber, forage and cover crop                              |
| TVnu 456                          | Brown            | 2.9                 | BS                              |  |
| TVnu 585                          | Brown mottled    | 2.8                 | BS                              |  |
| Mean                              |                  | 3.4                 |                                 |  |

<sup>a</sup>Ng *et al.* (1989)

<sup>b</sup>Padulosi & Ng (1990).

(Quemener *et al.*, 1986; Griffiths & Savage, 1991) and *S*-methyl-L-cysteine (Evans & Boulter, 1975; Otoul *et al.*, 1975; Boulter *et al.*, 1976).

*S*-Methyl-L-cysteine was identified as the uncommon amino acid of TVnu 225, which could explain why this accession had the lowest content of cysteine (0.71 g per 100 g protein). However, further studies are necessary to identify the other unusual amino acids in order to have more taxonomic and phylogenetic information on *Vigna* spp. (Quemener *et al.*, 1986).

The chemical score of all the accessions, calculated against the FAO (1985) pattern, is exceedingly good. In fact, it is better than those of cultivated *Vigna*

(Ologhobo & Fetuga, 1982; Carnovale *et al.*, 1991; Enc-Obong & Carnovale, 1992), with only four accessions scoring just under 1 with sulphur amino acids as the limiting amino acids.

#### Starch and starch digestibility

The TS, RDS, SDS and RS values of one accession from each wild species (eight accessions) are shown in Table 4. A great variability is observed in TS with a range of 297.2–496.9 g kg<sup>-1</sup>. Ologhobo and Fetuga (1986) and Omuetti & Singh (1987) reported 370–480 g kg<sup>-1</sup> and 390–550 g kg<sup>-1</sup> for TS in cultivated

Table 2. Protein content and antinutritional factors of wild *Vigna* spp.

| Accessions                        | Protein (N×6.25)<br>(g kg <sup>-1</sup> FW <sup>a</sup> ) | Trypsin inhibitors<br>(TIU mg <sup>-1</sup> protein) | Lectin<br>(HA) <sup>b</sup> | Phytic acid<br>(g kg <sup>-1</sup> FW <sup>a</sup> ) | Tannin<br>(g kg <sup>-1</sup> FW <sup>a</sup> ) |
|-----------------------------------|---|--|-----------------------------|--|---|
| <i>V. vexillata</i>               |   |  |                             |  |   |
| TVnu 66                           | 282   | 154  | 60                          | 7.38   | 22.52   |
| TVnu 71                           | 292   | 105  | 200                         | 15.20  | 12.49   |
| TVnu 73                           | 279   | 112  | 150                         | 8.30   | 10.98   |
| TVnu 120                          | 258   | 132  | 2400                        | 13.01  | 25.14   |
| TVnu 226                          | 283   | 128  | 60                          | 6.58   | 30.12   |
| Mean <sup>c</sup>                 | 279a  | 126b   | 574b                        | 10.09a   | 20.25b  |
| <i>V. vexillata macrosperma</i>   |   |  |                             |  |   |
| TVnu 64                           | 249   | 158  | 90                          | 6.51   | 3.94  |
| TVnu 72                           | 257   | 182  | 60                          | 13.65  | 3.50  |
| TVnu 73A                          | 260   | 114  | 1400                        | 10.37  | 3.34  |
| Mean <sup>c</sup>                 | 255abc  | 151b   | 517b                        | 10.18a   | 3.59d   |
| <i>V. luteola</i>                 |   |  |                             |  |   |
| TVnu 24                           | 238   | 193  | 20000                       | 9.54   | 38.38   |
| TVnu 28                           | 221   | 230  | 12000                       | 8.62   | 30.00   |
| TVnu 29                           | 208   | 215  | 16000                       | 6.65   | 21.40   |
| Mean <sup>c</sup>                 | 222d  | 213a   | 16000a                      | 8.27a  | 29.93a  |
| <i>V. oblongifolia</i>            |   |  |                             |  |   |
| TVnu 38                           | 273   | 45   | 250                         | 14.70  | 14.87   |
| TVnu 85                           | 259   | 38   | 200                         | 8.54   | 9.50  |
| TVnu 133                          | 265   | 35   | 300                         | 8.85   | 7.81  |
| Mean <sup>c</sup>                 | 266ab   | 39cd   | 250b                        | 10.70a   | 10.73cd   |
| <i>V. unguiculata dekindtiana</i> |   |  |                             |  |   |
| TVnu 136                          | 260   | 70   | 125                         | 6.20   | 9.01  |
| TVnu 140                          | 256   | 91   | 250                         | 10.58  | 3.90  |
| TVnu 255                          | 266   | 63   | 250                         | 15.25  | 11.96   |
| TVnu 278                          | 279   | 37   | 300                         | 14.89  | 8.30  |
| Mean <sup>c</sup>                 | 265ab   | 65c  | 231b                        | 11.73a   | 8.29cd  |
| <i>V. racemosa</i>                |   |  |                             |  |   |
| TVnu 105                          | 223   | 63   | 2000                        | 8.58   | 13.21   |
| TVnu 163                          | 238   | 59   | 5500                        | 6.17   | 11.70   |
| TVnu 220                          | 244   | 53   | 2800                        | 8.60   | 11.54   |
| Mean <sup>c</sup>                 | 235bcd  | 58cd   | 3433b                       | 7.78a  | 12.15bcd  |
| <i>V. reticulata</i>              |   |  |                             |  |   |
| TVnu 137                          | 215   | 28   | 600                         | 9.38   | 10.69   |
| TVnu 225                          | 210   | 28   | 14400                       | 4.75   | 25.84   |
| TVnu 323                          | 268   | 32   | 700                         | 16.23  | 12.36   |
| Mean <sup>c</sup>                 | 231cd   | 29d  | 5233b                       | 10.12a   | 16.30bc   |
| <i>V. ambacensis</i>              |   |  |                             |  |   |
| TVnu 306                          | 235   | 21   | 300                         | 4.30   | 8.98  |
| TVnu 456                          | 245   | 26   | 1800                        | 5.60   | 10.74   |
| TVnu 585                          | 214   | 35   | 800                         | 7.03   | 8.98  |
| Mean <sup>c</sup>                 | 231cd   | 27d  | 967b                        | 5.64a  | 9.57cd  |
| LSD (0.05)                        | 8.3   | 9.3  |                             | 0.31   | 1.04  |

<sup>a</sup>Moisture was standardised for all accessions at 10% (FW, fresh weight).

<sup>b</sup>HA, haemagglutinating activity is expressed as the reciprocal of the highest dilution (g ml<sup>-1</sup> giving positive agglutination).

<sup>c</sup>The same letters in the same column indicate no significant differences ( $P \leq 0.05$ ).

cowpeas, respectively. The low starch content of wild species is related to their smaller seed size, confirmed by the significant correlation between seed weight and TS (Table 5).

The percentages of RDS and SDS are essentially quite low; in fact RS is very high (about 70%) compared with cultivated accessions (about 20%) (Ruggeri, unpublished results) and other cultivated legumes (Lintas *et al.*, 1992), whereas RS varies between 21% to 44%. Such low starch digestibility may be due to the high lectin and tannin contents (Deshpande & Salunkhe, 1982; Thompson & Gabon, 1987). Fish and Thompson (1991) demonstrated that the reduction of  $\alpha$ -amylase activity by lectins and tannins is a result of the interaction of the lectins or polyphenols with either the enzyme itself or with its substrate. In addition, the relatively high amylose and  $\alpha$ -amylase inhibitors may further decrease *in vitro* digestibility of starch (Srinivasa & Rao, 1976; Dreher & Dreher, 1984).

Gatehouse *et al.* (1987) reported that the heteropolysaccharide, high in arabinose and fucose content, isolated from a wild line of *Phaseolus vulgaris*, accounted for the resistance to *Acanthoscelides obtectus*. Whether this was a consequence of the structure of the carbohydrates or their composition was not clear. For this reason, the relationship between different fractions of starch and BR was investigated. In this study, a significant correlation between BR and RS was found (Table 5). This relationship would have to be confirmed by carefully assessing a larger number of accessions.

### Trypsin inhibitors

Trypsin inhibitors (Table 2) have a greater range of variability (from 20 TIU mg<sup>-1</sup> protein in TVnu 306 to 230 TIU mg<sup>-1</sup> protein in TVnu 28) than in cultivated

cowpeas where TI activity ranges from 27.1 to 66.2 TIU mg<sup>-1</sup> protein (Della Gatta *et al.*, 1989).

With regard to the other antinutritional factors, TI appears to be specific to each species, allowing a classification of the species by their TI content: high TI (*V. luteola*, *V. vexillata*, *V. vexillata macrosperma*), medium TI (*V. oblongifolia*, *V. unguiculata dekindtiana*, *V. racemosa*) and low TI (*V. reticulata*, *V. ambacensis*). A characteristic of *V. luteola* is that it has a significantly higher ( $P \leq 0.05$ ) TI activity than the other species.

No correlation was found between TI and protein content (Table 5), including the cultivated accessions of cowpeas (Marconi *et al.*, 1993). TI and cystine content are significantly correlated ( $r = 0.817$ ;  $P \leq 0.01$ ) as a consequence of the high disulphide bridge content of TI (Pusztai, 1968).

There is a significant correlation ( $r = 0.62$ ) ( $P \leq 0.01$ ) between TI and resistance to CM (Table 5), confirming earlier results. This has been used for a gene construct, containing a coding sequence for cowpea TI in the production of transgenic plants with enhanced insect resistance (Hilder *et al.*, 1987).

Trypsin inhibitors in legumes are responsible for damaging pancreatic metabolism and decreasing growth rate in animals (Pusztai *et al.*, 1992); since TI activity is considerably reduced by cooking or processing, accessions high in TI content could be used to hybridise cultivated cowpeas.

### Cysteine proteinase inhibitors

In contrast to the great amount of information on trypsin protease inhibitors, there are few reports on cysteine proteinase inhibitors in *V. unguiculata* (Rele *et al.*, 1980; Hines *et al.*, 1991).

Table 3. Amino acid profile of different wild *Vigna* spp. (g per 16 g N)

| Amino acid     | TVnu<br>71 | TVnu<br>72 | TVnu<br>73a | TVnu<br>24 | TVnu<br>29 | TVnu<br>38  | TVnu<br>278 | TVnu<br>105 | TVnu<br>225 | TVnu<br>306 | Mean | LSD<br>(0.05) | CV   |
|----------------|------------|------------|-------------|------------|------------|-------------|-------------|-------------|-------------|-------------|------|---------------|------|
| Threonine      | 3.71       | 3.52       | 3.45        | 3.65       | 3.66       | 3.86        | 3.61        | 3.83        | 3.79        | 3.77        | 3.69 | 0.384         | 3.62 |
| 1/2 Cystine    | 1.20       | 1.24       | 1.33        | 2.05       | 1.79       | 1.19        | 0.92        | 1.38        | 0.75        | 1.02        | 1.29 | 0.054         | 30.2 |
| Valine         | 5.00       | 4.99       | 5.32        | 4.88       | 5.06       | <u>5.10</u> | <u>5.40</u> | 5.17        | <u>5.23</u> | <u>5.34</u> | 5.15 | 0.157         | 3.34 |
| Methionine     | 1.30       | 1.36       | 1.57        | 1.58       | 1.18       | <u>1.23</u> | <u>1.40</u> | 1.38        | 1.30        | 1.37        | 1.37 | 0.056         | 9.46 |
| Isoleucine     | 4.08       | 4.22       | 4.18        | 4.39       | 4.58       | 4.40        | 4.36        | 4.20        | 4.35        | 4.35        | 4.31 | 0.101         | 3.31 |
| Leucine        | 7.34       | 7.50       | 7.28        | 7.05       | 7.10       | 7.61        | 7.33        | 7.50        | 7.37        | 8.04        | 7.41 | 0.339         | 3.79 |
| Tyrosine       | 3.23       | 3.46       | 3.62        | 3.12       | 3.31       | 3.26        | 3.42        | 3.50        | 3.25        | 3.89        | 3.41 | 0.263         | 6.64 |
| Phenylalanine  | 5.60       | 5.32       | 5.36        | 5.94       | 5.99       | 5.84        | 5.61        | 5.96        | 5.52        | 5.68        | 5.68 | 0.269         | 4.30 |
| Lysine         | 6.74       | 7.23       | 6.53        | 6.39       | 6.66       | 7.12        | 6.51        | 6.92        | 6.66        | 6.93        | 6.77 | 0.434         | 4.05 |
| Aspartic acid  | 11.8       | 11.7       | 11.5        | 12.3       | 12.6       | 11.6        | 11.9        | 12.0        | 12.0        | 11.8        | 11.9 | 0.618         | 2.74 |
| Serine         | 5.54       | 5.12       | 5.59        | 6.00       | 6.15       | 5.73        | 5.21        | 5.81        | 5.63        | 5.22        | 5.60 | 0.216         | 6.11 |
| Glutamic acid  | 17.3       | 18.0       | 17.6        | 16.3       | 16.6       | 16.1        | 17.5        | 16.3        | 18.7        | 16.8        | 17.1 | 0.672         | 4.97 |
| Proline        | 4.56       | 4.73       | 4.38        | 4.14       | 4.44       | 5.02        | 4.67        | 4.15        | 4.21        | 4.40        | 4.47 | 0.518         | 6.29 |
| Glycine        | 4.86       | 4.43       | 5.03        | 4.18       | 4.25       | 4.43        | 4.43        | 4.34        | 4.05        | 4.20        | 4.42 | 0.307         | 6.93 |
| Alanine        | 3.98       | 4.02       | 4.24        | 4.06       | 4.24       | 4.34        | 4.21        | 4.40        | 4.19        | 4.80        | 4.25 | 0.238         | 5.55 |
| Histidine      | 2.73       | 2.70       | 2.68        | 3.06       | 3.14       | 2.75        | 2.99        | 2.74        | 2.74        | 3.32        | 2.89 | 0.263         | 7.81 |
| Arginine       | 8.34       | 7.15       | 8.18        | 7.19       | 6.97       | 7.25        | 8.02        | 7.29        | 7.21        | 6.11        | 7.37 | 0.513         | 8.92 |
| Chemical score | 1.00       | >1.00      | >1.00       | >1.00      | >1.00      | 0.97        | 0.93        | >1.00       | 0.82        | 0.96        | —    | —             | —    |

Limiting amino acids are underlined.

LSD, least significant difference; CV, coefficient of variation.

The occurrence of thiol proteinase inhibitors was analysed in wild BR accessions (TVnu 72, TVnu 64, TVnu 28), in wild BS accessions (TVnu 140, TVnu 220, TVnu 105) and in cultivated BR cowpeas (TVnu 2027).

Hines *et al.* (1991) found that soybean cystatin inhibited the proteolytic activity of the crude extracts of several insects, CM in particular. In this study, no direct relationship was found between the extent of inhibitory activity and BR, since the wild resistant line (TVnu 72) had a lower inhibitory activity than the more susceptible ones (Fig. 1).

Therefore, both cysteine proteinase and TI alone cannot explain the resistance, but it is probably due to a combined effect of these factors (Piergiorganni *et al.*, 1991; Marconi *et al.*, 1993).

These inhibitors should not affect human digestive proteinases as they would be destroyed during cooking, as reported by Hines *et al.* (1991), who found no inhibitory activity with the soybean cystatin after 30 min of heating at 100°C.

### Lectins

*V. luteola* was found to be very high in lectin content, up to 20 000 HA; *V. vexillata* had only 60 HA, which was the lowest value (Table 2). Marconi *et al.* (1993) found a range of 80–1173 HA, whereas Gatehouse *et al.* (1984) reported that there were no lectins in cultivated *Vigna*. Contrary to TI, highly significant differences in lectin content were found between the accessions of the same species in all the *Vigna* spp.

Although seed lectins represent up to 11% of storage proteins (Liener, 1976; Osborn *et al.*, 1985; Sharon & Lis, 1989), a significant negative correlation between protein content and lectins was found (Table 5).

In addition, no correlation was found between lectin content and resistance to CM (Table 5), confirming the findings reported by Xavier-Filho *et al.* (1989) in cultivated cowpea, but all the accessions with very

high lectin content >14 000 HA were BR (*V. luteola* and *V. reticulata* TVnu 225).

Therefore, on the basis of the studies of Gatehouse *et al.* (1991), it would be useful to purify lectins from lectin-rich seeds such as *V. luteola* and to verify if any of them could be toxic to CM. If such lectin activity were to be confirmed, when incorporated into an artificial diet, *V. luteola* would represent an additional genetic resource for cowpea breeding programmes.

Lectins produce highly toxic effects such as a reduction in protein digestibility and stunted growth (Liener, 1986), but their effects are markedly reduced by cooking (Boufassac *et al.*, 1986; Thompson *et al.*, 1983).

### Phytic acid

In wild accessions, phytic acid content ranges between 4.30 g kg<sup>-1</sup> for TVnu 306 and 16.23 g kg<sup>-1</sup> for TVnu 323 (Table 2). In cultivated *V. unguiculata*, Farinu and Ingrao (1991), Carnovale *et al.* (1990) and Ologhobo and Fetuga (1983) reported less variability, in the range of 4.24–10.27 g kg<sup>-1</sup>.

Table 2 shows that PA is not specific to different species; in fact, no significant differences were found between the mean values of each species.

Deshpande and Damodaran (1989) and Vaintraub and Bulmaga (1991) found that phytic acid inhibited  $\alpha$ -amylase and trypsin; therefore, a high PA content could be involved in resistance to pests, inhibiting insect proteases, but in this study no relationship was found between PA content and BR (Table 5).

PA may form strong complexes with minerals, such as Zn, Fe and Ca, reducing their bioavailability (Sandberg *et al.*, 1993; Ellis *et al.*, 1987), and with proteins, reducing their digestibility (Reddy *et al.*, 1982). The reduction of phytate levels after cooking is quite small compared with that of TI or lectins (Reddy *et al.*, 1988; Lombardi-Boccia *et al.*, 1995). On the other hand, PA influences the cooking quality of legumes because it

Table 4. Total starch and starch digestibility of wild *Vigna* spp.

|            | TS (g kg <sup>-1</sup> FW) | Starch fractions (%TS) |       |       |
|------------|----------------------------|------------------------|-------|-------|
|            |                            | RDS                    | SDS   | RS    |
| TVnu 66    | 297.2                      | 1.48                   | 25.20 | 73.35 |
| TVnu 72    | 416.2                      | 7.90                   | 22.06 | 70.04 |
| TVnu 24    | 289.2                      | 1.45                   | 24.34 | 74.24 |
| TVnu 38    | 332.4                      | 5.26                   | 16.67 | 78.07 |
| TVnu 140   | 413.9                      | 2.95                   | 32.13 | 64.92 |
| TVnu 105   | 337.2                      | 14.68                  | 12.90 | 72.42 |
| TVnu 137   | 496.9                      | 4.99                   | 20.04 | 74.76 |
| TVnu 306   | 468.7                      | 5.38                   | 30.72 | 63.90 |
| LSD (0.05) | 32.1                       |                        |       |       |

FW fresh weight.

TS total starch.

RDS rapidly digestible starch.

SDS slowly digestible starch.

RS resistant starch = TS - (RDS + SDS).

LSD least significant difference.

chelates divalent cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) and prevents their cross-linking with pectin, facilitating dissolution of the cell wall during cooking (Bhatty & Slinkard, 1989; Stanley & Aguilera, 1985; Vindiola *et al.*, 1986).

### Tannins

The tannin content in wild species (Table 2) is much higher (3.34–38.38 g kg<sup>-1</sup>) than in the cultivated accessions (Carnovale *et al.*, 1991; Kachare *et al.*, 1988; Chang *et al.*, 1994). The high content of tannins in wild species is due to their dark seed coat and small size; the darker-coloured seed accessions contain higher concentrations of tannins than the white-coloured seed accessions (Chang *et al.*, 1994) and, since tannins are located in the seed coat, the large seeds will have a lower percentage of tannins in the same amount of wholemeal (tegument + cotyledon).

*V. luteola* and *V. vexillata* are characterised by high tannin levels compared with other species, *V. vexillata macrosperma* having the lowest amount.

Table 5. Correlation coefficients (*r*)

| Parameter  | <i>r</i>           |
|--|--------------------|
| Seed weight to total starch (TS) ( <i>n</i> = 8)                   | 0.78 <sup>b</sup>  |
| Seed weight to tannin ( <i>n</i> = 27)                             | -0.33              |
| Seed weight to lectin (HA) ( <i>n</i> = 27)                        | -0.08              |
| Protein to trypsin inhibitor (TI) ( <i>n</i> = 27)                 | 0.12               |
| Protein to lectin (HA) ( <i>n</i> = 27)                            | -0.58 <sup>a</sup> |
| Trypsin inhibitor (TI) to tannin ( <i>n</i> = 27)                  | 0.43 <sup>b</sup>  |
| Tannin to lectin (HA) ( <i>n</i> = 27)                             | 0.70 <sup>a</sup>  |
| Bruchid resistance (BR) to trypsin inhibitor (TI) ( <i>n</i> = 25) | 0.61 <sup>a</sup>  |
| Bruchid resistance (BR) to lectin (HA) ( <i>n</i> = 25)            | 0.32               |
| Bruchid resistance (BR) to phytic acid (PA) ( <i>n</i> = 25)       | 0.19               |
| Bruchid resistance (BR) to tannin ( <i>n</i> = 25)                 | 0.41 <sup>b</sup>  |
| Bruchid resistance (BR) to resistant starch (RS) ( <i>n</i> = 25)  | 0.74 <sup>b</sup>  |

<sup>a</sup>significant at  $P \leq 0.01$ .

<sup>b</sup>significant at  $P \leq 0.05$ .

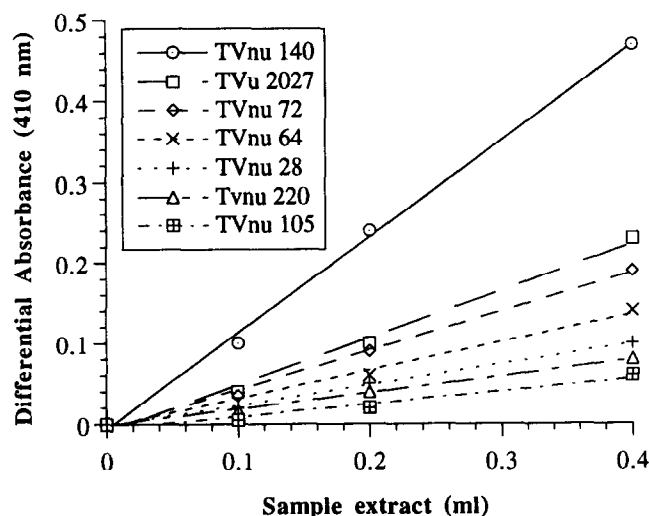


Fig. 1. Thiol proteinase levels in wild and cultivated *Vigna* spp.

There is significant correlation between tannin content and BR (Table 5), according to Hedin *et al.* (1988) and Lattanzio *et al.* (1990). The quantity of tannins may be the main factor to be considered when assessing the crop resistance to insects, but also the qualitative difference of phenolic compounds should limit the range of host plants for insects; in fact, Lattanzio *et al.* (1990) found different flavonoid patterns in extracts of wild *Vigna* leaves (*V. vexillata*, *V. marina*, *V. luteola*, *V. oblongifolia*).

As regards the significant correlation between tannins and TI activity (Table 5), several authors have reported that TI activity is due to two factors: the heat-labile protein factor, mainly present in the cotyledons (true TI), and the heat-resistant factor, located mainly in the seed coat and associated with tannins (Elias *et al.*, 1979; De Lumen & Salamat, 1980). Since wild *Vigna* seeds have a relatively high content of tannin (small, coloured seeds), a correlation between tannin and heat-resistant TI activity could be found. Fernandez *et al.* (1982) found a significant positive correlation between TI activity and tannin in the seed coat of beans, which was not observed for whole seeds or cotyledons. The significant correlation between HA and tannins (Table 5) may be explained by the fact that tannins are responsible, in part, for the HA activity of seed coats (Fernandez *et al.*, 1982).

The nutritional effects of tannins are mainly related to their interaction with proteins (Laurena *et al.*, 1984; Aw & Swanson, 1985). Tannin-protein complexes are insoluble and protein digestibility is decreased (Deshpande *et al.*, 1986; Carnovale *et al.*, 1991).

### CONCLUSIONS

The wild *Vigna* spp., which have a wider range of variability than the cultivated species in both nutrient and antinutrient content, may provide a valuable genetic source for improving cowpeas. This also implies that domestication could bring about an indirect selection for these characters. In particular, *V. luteola* showed far higher levels of antinutritional factors than cultivated cowpeas. Many of these inhibitors are rich in essential amino acids such as lysine and cysteine; when cooked they are inactivated and can be excellent food proteins (Filippone, 1993). However, there are some doubts as to the use of these legumes as a forage crop, in particular for monogastric animals, since the plant tissues (pods and leaves) would not be subject to any heating process (Hsu & Satter, 1995; Yu *et al.*, 1995).

As regards BR, significant correlations with TIU, tannin and RS were found. The latter BR-RS correlation should be confirmed by analysing a greater number of samples.

Considering that all the accessions with exceedingly high antimetabolic factors (*V. vexillata*, *V. luteola*) are BR, even where there is no direct relationship (in any

case, *V. reticulata*, which has few antimetabolic factors, proved to be just as resistant), all or part of the antimetabolic factors therefore promote the defence mechanism of the plants.

## ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr N. Q. Ng (IITA, Ibadan, Nigeria) for providing the wild *Vigna* seeds and for the helpful advice given on BR, and V. Vivanti (INN) for the technical assistance and advice given on amino acid determination.

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