Genotype × environment influence on cowpea (*Vigna unguiculata* (L) Walp) antinutritional factors: 1 – Trypsin inhibitors, tannins, phytic acid and haemagglutinin

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Abstract: The relative influence of genotype, environment and genotype × environment effects on four antinutritional factors ($g\,kg^{-1}$) of importance in cowpea were studied using 15 local and improved cowpea genotypes grown in 12 environments, comprising three locations over four seasons per location. The locations Ago-Iwoye (6°58′N4°00′E), Mokwa (9°17′N5°04E) and Kano (12°00′N8°31′E) were representatives of the major agroecological zones where cowpeas are produced. Genotypes effects were strongest in controlling trypsin inhibitor content, while the environment was the major source of variation for tannins, haemagglutinin and phytic acid contents. Thus, the variability in the levels of these antinutritional factors in cowpea seeds depends largely on the environment where they are grown. This implies that a cowpea genotype grown and consumed safely in an environment can be poisonous when grown and consumed in another environment. Genotype × environment effects were significant for tannins, haemagglutinins and trypsin inhibitor contents. Correlation coefficients (pooled data) from the three locations indicated that trypsin inhibitor was positively correlated to phytic acid ($r = 0.59$, $0.001 < P < 0.05$) and haemagglutinins ($r = 0.64$, $0.001 < P < 0.05$) but negatively correlated to tannin contents ($r = -0.79$, $0.001 < P < 0.05$).

Keywords: cowpea; *Vigna unguiculata* (L) Walp; trypsin inhibitors; tannins; phytic acid; haemagglutinins; environment; genetic; variation

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is extensively produced and consumed in tropical Africa as a major source of protein. It has the potentials to improve the protein quality of carbohydrate-based diets and is now being successfully used in child-feeding programmes.

However, cowpea contains certain antinutritional factors such as trypsin inhibitors, saponins, tannins, phytic acids and haemagglutinins.[1,2] Their presence in diets (both human and animal) has been associated with pancreatic enlargement, reduced digestibility, reduced absorption of amino acids and reduced bioavailability of essential minerals.[3–5]

Although cowpea seeds are usually heated before being consumed and heating has been reported to reduce the levels of antinutritional factors,[4,6] antinutritional factors vary in their thermal stability and the extent to which they are destroyed by heat, *in vivo*, is a function of several variables such as cultivar, particle size and moisture content.[7] Furthermore, the excessive amount of heating required to destroy the antinutritional factors can also lead to a decline in the overall biological value of the food due to the functional as well as nutritional damage to the protein. Some antinutritional factors such as phytic acid have been shown not to be heat labile.[8] On the other hand, the breeding of varieties or strains of cowpea with low levels of one or more of the antinutritional factors offers a more satisfactory long-term solution to this problem.[9]

Most breeding programmes of cowpea are aimed at either increasing yields or improving resistance to diseases and pests[10] and are not accompanied by nutritional evaluation of the improved varieties before their release to the farmers, even when reports[11–13] had indicated that breeding for pest and disease resistance inadvertently increases the content of antinutritional factors in cowpea seeds.

The nutritional significance of cowpea in human and animal diets suggests the needs for a genetic improvement programme to reduce the concentration of these antinutritional factors in cowpea seeds. However, information on the variability of these antinutritional factors in different varieties, advanced breeding lines and improved cultivars and the influence of environments on these traits is necessary for any effective improvement programme. Variability

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(*Received 27 June 1997; accepted 10 June 1998*)

among cowpea cultivars have been reported for tannins,[7] haemagglutinins, cathechin, polyphenol[2] and phytic acid,[14] but there is no information on the influence of genetic and environmental effects on these antinutritional factors in cowpea seeds. The purpose of this study was to determine the relative influence of genetic, environment and genetic × environment interactions on antinutritional factors in cowpea seeds.

MATERIALS AND METHODS

Genetic material and experimental design

Fifteen cowpea cultivars were tested over four seasons in each of the three locations. The entries include advanced breeding lines and commonly grown local and improved cultivars obtained from three institutions involved in cowpea research in Nigeria (see Table 4 below). These genotypes were grown at the Ogun State University Research Farm, Ago-Iwoye ($Cu = 3.2–4.8\,mg\,kg^{-1}$; $Zn = 2.4–5.1\,mg\,kg^{-1}$; $Mn = 15.0–18.0\,mg\,kg^{-1}$; $Fe = 3.9–9.6\,mg\,kg^{-1}$) and experimental stations at Mokwa ($Cu = 0.2–1.6\,mg\,kg^{-1}$; $Zn = 1.2–1.8\,mg\,kg^{-1}$; $Mn = 25.0–30.0\,mg\,kg^{-1}$; $Fe = 1.3–5.1\,mg\,kg^{-1}$) and Kano ($Cu = 0.2–1.5\,mg\,kg^{-1}$; $Zn = 0.8–1.2\,mg\,kg^{-1}$; $Mn = 28.0–39.0\,mg\,kg^{-1}$; $Fe = 1.2–3.4\,mg\,kg^{-1}$) in the early and late planting seasons of 1993 and 1994 resulting in 12 environments (Table 1). The genotypes were evaluated in the field in a completely randomised block design with four replicates. The treatment plot consisted of four rows, each 6 m long and 0.75 m apart. Two seeds of cowpea were planted per hole with an inter-row spacing of 0.30 m. The gross plot size was $18\,m^2$ per treatment. The application of a mixture of Benomyl (Benlate) and Mancozeb (Dithane M45), at the product rate of $0.6\,kg + 2.5\,kg\,ha^{-1}$ three times at weekly intervals commencing from 4 weeks after planting was used to control fungal diseases. Cypermethrin (Cymbush) and Dimethoate (Perferkthion) were each applied fortnightly at the rate of 0.75 litre ha^{-1} as tank mixture beginning 6 weeks after sowing until harvest to control various categories of insect pests. The average annual rainfall in Ago-Iwoye, Mokwa and Kano were 1308, 1122 and 968 mm, respectively. The plots were irrigated at the three locations as requested. Seeds were collected from plants in the centre two rows of each genotype for subsequent analysis.

Sample preparation

From the bulk of each sample, 20 g subsamples of seeds were ground to pass through 1 mm mesh on a Microhammer mill to give a fine flour. All milled samples were kept in screw-capped plastic bottles at room temperatures until used. All analysis were carried out in triplicate and are reported on a dry matter basis.

Table 1. Edaphic characteristics of the 12 environments

Trial site and year	*Season*	*pH (H_2O)*	*Organic carbon (%)*	*Organic matter (%)*	*Clay (%)*	*N (%)*	*K ($meq\,kg^{-1}$)*	*Mg ($meq\,kg^{-1}$)*	*Ca ($meq\,kg^{-1}$)*	*Available P (ppm)*
Kano										
1993	Wet	4.81	0.12	0.35	10.24	0.32	1.01	3.23	7.81	7.83
	Dry	5.05	0.15	0.42	12.3	0.31	1.01	3.38	7.99	6.85
	Mean	4.93	0.14	0.39	11.27	0.32	1.01	3.31	7.9	7.34
1994	Wet	5.23	0.13	0.38	15.76	0.29	1.08	2.44	7.88	6.08
	Dry	5.38	0.13	0.33	10.82	0.33	1.01	3.54	7.96	5.21
	Mean	5.31	0.13	0.36	13.29	0.31	1.05	2.99	7.92	5.65
Mokwa										
1993	Wet	5.26	0.95	0.98	4.08	0.68	1.66	3.02	6.53	7.19
	Dry	5.71	0.96	0.73	4.89	0.93	1.28	4.06	6.74	5.68
	Mean	5.49	0.96	0.86	4.49	0.81	1.47	3.54	6.64	6.44
1994	Wet	5.98	0.97	0.87	6.06	0.71	1.43	2.41	6.82	6.03
	Dry	6.11	0.97	0.63	5.66	0.86	0.84	3.89	7.15	5.12
	Mean	6.05	0.97	0.75	5.86	0.79	1.14	3.2	6.99	5.58
Ago-Iwoye										
1993	Wet	6.05	1.06	2.88	17.01	0.18	0.72	33.26	13.52	10.39
	Dry	6.31	1.52	2.01	24.23	0.09	0.41	34.08	14.06	9.01
	Mean	6.18	1.29	2.45	20.62	0.14	0.57	33.67	13.79	9.7
1994	Wet	6.21	0.98	2.71	20.02	0.16	0.81	34.18	13.85	7.24
	Dry	6.37	1.43	1.81	22.05	0.1	0.43	34.97	14.04	6.01
	Mean	6.29	1.21	2.26	21.04	0.13	0.62	34.58	13.95	6.63

Location and year	*Season*	*Trypsin inhibitor*	*Tannins*	*Phytic acid*	*Haemagglutinins*
Kano					
1993	Wet	38.32 ± 2.51	5.63	20.78	14.78
			0.98	2.11	1.35
	Dry	29.08 ± 2.06	6.28	21.06	15.31
			1.05	1.97	1.31
1994	Wet	32.31 ± 2.09	6.07	22.96	15.65
			0.98	2.14	1.03
	Dry	24.67 ± 2.01	8.06	20.85	15.82
			1.01	3.01	1.19
Mokwa					
1993	Wet	29.85 ± 3.15	5.81	25.99	6.48
			0.97	1.89	0.85
	Dry	25.33 ± 2.07	6.08	25.08	6.51
			1.02	2.17	0.68
1994	Wet	31.88 ± 2.63	6.11	26.01	7.23
			1.02	1.94	0.77
	Dry	26.42 ± 1.89	7.38	25.92	6.87
			1.21	2.11	0.52
Ago-Iwoye					
1993	Wet	20.96 ± 3.14	6.09	18.06	10.24
			1.02	2.36	0.99
	Dry	20.08 ± 2.11	8.24	17.98	9.63
			1.11	2.13	1.01
1994	Wet	22.61 ± 1.97	6.31	17.23	8.89
			0.99	2.21	0.67
	Dry	19.08 ± 2.06	8.18	17.88	11.06
			1.14	2.11	1.03

Table 2. Means (±SD) of antinutritional factors (g kg^{-1}) in seeds of cowpea in 1993 and 1994

Trypsin inhibitor

A procedure described previously[15] was used to prepared trypsin inhibitor (TI) extracts using samples of 2.0 g of cowpea flour. TI activity was evaluated in terms of the extent to which a portion of the aliquots of cowpea flour extracts inhibited the enzymatic activity of trypsin on the synthetic substrate DL-benzoyl arginine-*p*-nitroanilide (Sigma Chemical Co Ltd).[16]

Tannins

Tannin contents were determined by extraction in 10 ml (0.01 M) acidified (HCl) methanol by a method described previously.[7] It was measured by a vanillin–HCl method.[17] Vanillin–HCl reagent was prepared by the method described in Ref 7. A votex mixer model S8223-1 (McGraw Park, II, USA), adjusted to position 6, mixed 1 ml extract supernatant with 5 ml 2% vanillin–HCl reagent for 30 min. After standing for 20 min, a Hitachi Spectrophotometer model 100-60 (Tokyo, Japan) measured absorbance at 500 nm.

Phytic acid

Phytic acid was determined by a combination of two methods. The extraction and precipitation of phytic acid were performed according to the method of Ref 18. Iron in the precipitate was measured by the method described in Ref 19. A 4 : 6 Fe/P atomic ratio was used to calculate the phytic acid content.

Haemagglutinin activity

Phytohaemagglutinin were extracted by the methods of Ref 20 with slight modifications. Haemagglutinin activity was determined by the photometric technique of Liener[21] as modified by Valdebouze *et al*[15] which measured the ability of haemagglutinin extracts to agglutinate rabbit erythrocytes. One haemagglutinin unit is defined as the smallest amount of sample necessary for agglutination under test condition..

Moisture level

Moisture measurements were determined on fresh and dried cowpea seeds by the AOAC vacuum oven procedure.[22]

Statistical analysis

The following formula was used to calculate the coefficient of variation of the data:[23]

$$V = \frac{S \times 100}{Y}$$

where S is standard deviation and Y is the mean.

Genotype	Origin[a]	Trypsin inhibitor	Tannins	Phytic acid	Haemagglutinins
Sel 4992	UI	27.28	5.65	16.05	7.86
Sel 4592	UI	29.63	7.21	24.06	15.31
Oolo-2	UI	32.03	6.38	22.48	5.63
TVx 3236	IITA	38.49	6.05	20.06	12.08
Ife Bimpe	IAR&T	38.62	6.22	22.01	4.63
Vita-3	IITA	32.54	8.74	23.06	16.08
IT81D – 994	IITA	39.63	6.01	17.61	11.61
Sel 2629	UI	26.59	8.06	19.91	14.23
IT86D-534	IITA	36.08	7.48	19.82	17.68
Ife Brown	IAR&T	30.82	7.23	25.07	9.21
Vita-5	IITA	26.82	5.63	16.36	10.08
Sel 3592	UI	27.08	5.82	23.42	7.29
Sel 5592	UI	29.73	5.78	21.38	6.98
Sel 2492	UI	45.63	6.96	24.21	13.17
Oolo-1	UI	36.48	5.61	20.61	5.28

Table 3. Summary of genotype antinutritional factor s contents (g kg^{-1} DM) in cowpea seeds grown in three locations in 1993 and 1994

[a] IAR&T, Institute of Agricultural Research and Training, Ibadan, Nigeria; IITA, International Institute of Tropical Agriculture, Ibadan, Nigeria; UI, University of Ibadan, Ibadan Nigeria.

Combined analysis of variance (ANOVA) was carried out for each trait, with partitioning of the environment into location, season and location × season effects and the genotype × environment (g × e) component into genotype × location, genotype × season and genotype × location × season. The following formula was used:

$$Y_{ikjn} = u + G_i + S_j + L_k + LS_{jk} + R_{n(\mathrm{jk})} + (GS)_{ij} + (GL)_{ik} + (GSL)_{ijk} + e_{ijkn}$$

where u is the parametric mean of the population; Y_{ijkn} is the value of the character for the ith genotype in the nth replicate in the jth season in the kth location; G_i is the effect of the ith genotype; S_j is the effect of the jth season; L_k is the effect of the kth location; LS_{jk} is the effect of the interaction between the kth location and the jth season; $R_{n(jk)}$ is the effect of the nth replicate in the jth season and kth location; $(GS)_{ij}$ is the effect of the interaction between the ith genotype and the jth season; $(GL)_{ik}$ is the effect of the interaction between the ith genotype and the kth location; $(GSL)_{ijk}$ is the effect of the interaction between the ith genotype, the jth season and the kth location; and e_{ijkn} is the error associated with the ith genotype in the nth replicate in the jth season in the kth location.

Environments was considered as fixed and genotype as random factor in order to obtain estimates of the causal components of the variance. Genetic variance (Vg) was estimated as the variance between the genotypes and the environmental variance (Vm) as the sum of the causal components of the variance corresponding to localities and error.

$$\text{Total variance } Vt = Vg + Vm + Vi$$

$$Vi = \text{interaction variance}$$

$$\text{Broad sense heritability} = Vg/Vt$$

Correlation coefficients were calculated as described in Ref 23. The method described in Ref 24 was used when the interactions were not significant in order to pool their mean squares with those of error.

RESULTS AND DISCUSSION

The means and standard deviations of antinutritional factors in seeds of cowpea grown in the three locations over four seasons are shown in Table 2. The results indicated that the TI activity of cowpea seeds grown at Kano are higher than those obtained from seeds grown at Mokwa and Ago-Iwoye. The values for these TI activities were also higher than those reported in cowpea seeds[9] and were also higher than in soybean,[16] lima bean[9] and peas.[25] The levels of tannins obtained in cowpea seeds from the three locations were similar, although the highest quantity of 8.24 g kg^{-1} DM was obtained at Ago-Iwoye. Phytic acid contents of cowpea seeds grown at Mokwa are higher than those grown at Kano and Ago-Iwoye, while the haemagglutinin levels were higher in seeds grown at Kano. The levels of tannins, phytic acid and haemagglutinins in cowpea seeds used in this study were also higher than those previously reported for cowpea seeds.[1,9] The higher levels obtained in this study may be due to varieties that were released from recent breeding programmes, particularly those breeding programmes aimed at improving resistance to pest and diseases. This suggestion is further corroborated previous reports[11,12] which correlated increases in resistance to pest with increases in some antinutritional factors in seeds of some cowpea varieties. The levels of TI were higher in cowpea seeds grown in the wet seasons than those grown in the dry seasons in the three locations suggesting that soil moisture may increase the uptake of

TI. The levels of tannins were higher in cowpea seeds grown in the dry seasons suggesting that high temperature (air and/or soil) and evaporation may favour bioaccumulation of tannins in cowpea seeds.

Summary of genotype levels of trypsin inhibitors, tannins, phytic acid and haemagglutinin in cowpea seeds are shown in Table 3. The mean TI values ranged from 26.59 for Sel 2629 to 45.63 g kg^{-1} DM for Sel 2492. In contrast to a previous report,[9] varietal differences were evident in the genotypes mean TI activity. The cowpea genotypes could be classified into low (26.09 to 29.73 g kg^{-1} DM), medium (30.82 to 36.48 g kg^{-1} DM) and high (38.49 to 45.63 g kg^{-1} DM) on the basis of their TI activity. The mean tannin contents gave a range of 5.61 for Oolo-1 to 8.74 g kg^{-1} DM for Vita-3. Similarly, the mean values for phytic acid composition of these cowpea seeds expressed in g kg^{-1} DM ranged between 16.05 for Sel 4992 to 25.07 for Ife Brown while haemagglutinating activity gave a range of 4.63 for Ife Bimpe to 17.68 g kg^{-1} for IT86D-534. These results indicated that it was possible to classify the cowpea genotypes into low (4.0–9.0 g kg^{-1} DM), medium (10.0–14.0 g kg^{-1} DM) and high (15.0–18.0 g kg^{-1}) on the basis of their haemagglutinating activity. Previous workers had reported similar classifications in soybean[16] and cowpea.[9] On the other hand, genotype differences were not evident in the mean values for phytic acid contents of cowpea seeds. Apart from Sel 4992 and Vita-5, all other genotypes showed close similarities in phytic acid composition. Generally, this data suggests the existence of sufficient variability in TI activity, tannins and haemagglutinatin activity in this cowpea genotypes and a high possibility of lowering their levels in cowpea seeds in a carefully designed genetic improvement programme. The data on phytic acid contents suggest little possibility for lowering its levels in seeds through selections from this genotypes, but Sel 4992 appeared promising in a well-designed genetic improvement programme.

A test of homogeneity of the error mean squares among individual environments were conducted prior to conducting a combined analysis of data. The X^2 values for all the antinutritional factors were significant indicating that the intra-environmental error variances were not homogenous. Contrasting soil types, temperature (air and/or soil) and rainfall distribution patterns may be responsible for the heterogeneous error variances among individual variances. Table 4 presents the combined analysis of variances of TI, tannins, phytic acid and haemagglutinin activity contents of cowpea seeds grown in three locations. Environment and genotype × environmental interactions were highly significant for all the traits. This indicates that the relative contents of the antinutritional factors in a cowpea genotype was highly dependent on the environment and the interaction between the genotype and the environment being considered. Generally, environmental factors were much greater than genotypic effects except for TI which recorded a larger genotypic effect. When environmental effects were further partitioned into components, season effects were detected for tannins and TI contents, suggesting that selection for low tannins and TI in cowpea seeds should be conducted over more than one season in order to ensure a reasonable stability to seasonal influence, mainly rainfall and temperature. These results also suggest that location × season (L × S) effects made a strong contribution to the variability recorded within the four antinutritional factors. Similar findings have been

Table 4. Results of combined ANOVA over three locations and four seasons per location on trypsin inhibitor, tannins phytic acid and haemagglutinins contents of cowpea seeds

Source of variation	*df*	*Mean squares*			
		Trypsin inhibitor	*Tannins*	*Phytic acid*	*Haemagglutinin*
Genotype (G)	14	31.37**	28.78**	33.41**	35.36**
Environment (E)	11	26.22**	29.42**	46.29**	87.16**
Location (L)	2	15.98*	8.06	25.44	12.86
Season (S)	3	18.96*	11.65*	10.82	13.09
L × S	6	16.28*	10.67*	27.33*	35.26**
Replicate within environment	24	6.09	10.68*	2.21	3.92
G × E	154	15.28*	25.89**	12.98*	16.11*
G × L	28	6.91	6.37	1.64	10.17
G × S	42	10.83*	9.88	3.08	15.02
G × L × S	84	4.36	16.14*	12.39*	11.26
Error	336	3.82	4.83	2.41	6.08

Significance: * $P \leq 0.05$; ** $P \leq 0.01$.

Constituents[b]	Trypsin inhibitor	Tannins	Phytic acid	Haemagglitinin
Vg	19.82**	16.04**	18.25**	14.82*
Vm	14.98**	38.16**	19.81**	29.08**
Vi (G × E)	5.03	12.69	3.67	8.05
Vi (G × L)	11.16**	22.07**	10.22*	28.01**
Vi (G × S)	10.83*	10.65	5.84	4.61
Vi (G × L × S)	4.06	15.85**	10.78*	5.26
Total variance (Vt)	65.88	125.46	68.57	89.83
Heritability (bs)	0.3	0.21	0.27	0.16

Table 5. Components of variation[a]

[a] Significance: * $P \leqslant 0.05$; ** $P \leqslant 0.01$.
[b] Vg, genetic variance; Vi, Interaction variance; Vm, Environmental variance; bs, broad sense.

reported in carrots.[26] In this study, the L × S effects were significant and large particularly for haemagglutinin.

The components of variation, total variation and heritability in the broad sense for TI, tannins, phytic acid and haemagglutinin activity contents of cowpea seeds are shown in Table 5. Environmental effects were generally stronger than the genetic effects except for TI which recorded a stronger genotypic effect. However, all the antinutritional factors showed a workable level of genetic variability. These results are in agreement with the analysis of variance in Table 4 and implies that the levels of these antinutritional factors will be influenced by planting in certain locations in certain seasons. Thus, the expansion of cowpea production into new areas must be undertaken with caution. Safe environments for production of cowpea with low levels of antinutritional factors in Nigeria and indeed in subtropical and tropical environments where cowpea are largely pro-

Parameter	Trypsin inhibitor	Tannins	Phytic acid	Haemagglutinin
Soil				
pH	−0.08	0.11	0.21	−0.06
Nitrogen	0.01	0.05	0.21	0.05
Phosphorus	−0.28*	0.39*	−0.27*	−0.19
Potassium	0.62**	0.58**	0.55**	0.68**
Calcium	0.36*	0.69**	0.39	0.25
Magnesium	0.38*	0.35*	0.78**	0.79**
Organic matter	−0.89**	−0.76**	−0.82**	0.55*
Organic carbon	0.16	−0.04	0.23	−0.08
Season				
Wet	0.61*	−0.73**	0.26	−0.09
Dry	−0.28	0.36	0.08	0.16

Table 6. Overall correlations between environmental parameters and cowpea antinutritional factors[a]

[a] Significance: * $P = 0.05$; ** $P = 0.01$.

	Trypsin inhibitor	Tannins	Phytic acid	Haemagglutinin
Kano, Kano State				
Tannins	0.62**			
Phytic acid	0.58**	0.86***		
Haemagglutinin	0.63**	0.08	0.01	
Mokwa, Niger State				
Tannins	0.62**			
Phytic acid	0.44	0.73***		
Haemagglutinin	0.41	− 0.11	0.06	
Ago-Iwoye, Ogun State				
Tannins	0.38*			
Phytic acid	0.43*	0.64**		
Haemagglutinin	0.32	0.07	0.03	
Pooled				
Tannins	0.79***			
Phytic acid	0.57**	0.75***		
Haemagglutinin	0.64**	0.06	0.05	

Table 7. Correlation coefficients among trypsin inhibitor, tannins, phytic acid and haemagglutinin composition in cowpea seeds[a]

[a] Significance: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P > 0.001$.

duced and consumed must be identified and recommended to cowpea producers.

Table 6 shows overall correlation between environmental parameters and cowpea antinutritional factors. Significant positive correlation were detected between TI activity, tannins, haemagglutinin activity, and calcium, potassium and magnesium while a negative correlation was detected between the four antinutritional factors and the soil organic matter content. Significant negative correlation was also detected between tannins and wet season. These results imply that potassium fertilisation may increase the contents of the four antinutritional factors in these cowpea seeds. Alternatively, the levels of the four antinutritional factors in cowpea seeds appeared to be reduced by an increase in the organic matter content of the soil. Negative correlation was also recorded between phosphorus and the antinutritional factors except tannins, suggesting that increasing the available phosphorus contents in the soil may reduce the levels of TI, phytic acid and haemagglutinin in cowpea seeds.

Correlation coefficients (r) between TI, tannins, phytic acid and haemagglutinin composition in cowpea seeds are shown in Table 7. TI was negatively correlated to tannins content and positively correlated to phytic acid and haemagglutinin contents of cowpea seeds in the three locations. Tannins content was negatively correlated to phytic acid content.

Table 8 shows the environmental and genetic correlation coefficients obtained from the analysis of covariance. TI showed a strong negative genetic correlation with tannin contents and a strong positive correlation with phytic acid and haemagglutinin activity at the environmental level. A simultaneous reduction in the levels of TI and tannins may not be easy. On the other hand, a simultaneous reduction in the levels of TI and phytic acid is feasible in a well-designed genetic improvement programme. These results may be of nutritional importance since it has been suggested[27] that tannins do not affect nutritional quality unless $> 10\%$ or more of the seeds dry weight. The highest tannin levels were $8.74\,g\,kg^{-1}$ DM for Vita-3 and a coefficient of variation of 10.38% which is less than 1% of cowpea dry matter content. On the other hand, phytic acid and TI are abundant constituents of these cowpea seeds. These results are in agreement with previous reports[6] and are of nutritional significance because TI and phytic acid are able to chelate phosphorus, calcium, magnesium, zinc and molybdenum thereby reducing their bioavailability in the body systems[4] and can also react with proteins, forming complex products, which reduce digestibility.[5] Thus, their levels in these seeds must be reduced because of the growing importance of cowpea in human and animal diets. Furthermore, phytic acid showed negative and positive correlation at the genetic and environmental levels respectively with haemagglutinins activity in these cowpea seeds.

These results suggests that a simultaneous reduction in the phytic acid and haemagglutinin activity levels in these cowpea seeds may not be easy in a conventional genetic improvement programme except if specific cowpea genotypes are grown in selected environments. Thus, more efforts are needed to identify these suitable environments for subsequent recommendation to cowpea producers.

CONCLUSIONS

Results of this study showed that the levels of TI, tannins, phytic acid and haemagglutinin activity in cowpea seeds are largely affected by the environmental and genetic × environmental interaction effects. Thus, the variability in the levels of these antinutritional factors in cowpea seeds depend largely on the environment where it is grown. This implies that a cowpea genotype grown and consumed safely in an environment can be poisonous when grown and consumed in another environment. Thus, it is suggested that a multi-environmental testing of the improved cowpea genotypes be conducted in order to target specific genotypes to specific environment where they could be grown for safe consumption. Results of previous studies on the influence of genetic, environment and genetic × environment effects on cowpea yield, protein, amino acid and lipid composition,[28] starch, fatty acids and essential mineral nutrients[29] and this study suggests that in a carefully designed genetic improvement programme

Table 8. Genetic and environmental correlation coefficients[a]

	Trypsin inhibitor	*Tannins*	*Phytic acid*	*Haemagglutinin*
Genetic correlation coefficients				
Tannins	−0.86***			
Phytic acid	0.53**	0.07		
Haemagglutinin	0.21	0.55**	0.86*	
Environmental correlation coefficients				
Tannins	0.18			
Phytic acid	0.47**	0.31*		
Haemagglutinin	0.96	0.02	0.93	

[a] Significance: * $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P > 0.001$.

using sufficiently large number of cowpea genotypes, it is possible to obtain a high yielding product with an adequate contents of protein, essential amino acids, fatty acids and essential mineral nutrients with reduced levels of important antinutritional factors. It must be emphasised that the use of organic fertilisers, which improve the organic matter contents of the soil, and mycorrhizal inoculation, which improves the available phosphorus level in the soil, should be encouraged in our farming systems as they have the potentials to reduce the levels of antinutritional factors in cowpea seeds.

REFERENCES

1 Akpapunam MA and Achinewhu SC, Effects of cooking, germination and fermentation on the chemical composition of Nigerian cowpea (*Vigna unguiculata*). *Qual Plant Foods Hum Nutr* **35**:353–358 (1985).

2 Bressani R, Nutritive value of cowpea, in *Cowpea Research, Production and Utilisation*, Ed by Singh SR and Rachie KO, John Wiley and Sons Ltd, Chichester, UK. pp 353–359 (1985).

3 Yavelov J, Finlay TH, Kennedy AR, Troll W, Bowman–Birk soybean protease inhibitor as an anti-carcinogen. *Cancer Res* **43**:2454–2459 (1983).

4 Liener IE and Kakade ML, Protease inhibitors, in *Toxic Constituents of Plant Foodstuffs*, Ed by Liener IE. Academic Press, New York, USA. pp 7–71 (1980).

5 Gatel F and Grosjean F, Composition and nutritive value of peas for pigs: a review of European results. *Livestock Prod Sci* **26**:155–175 (1990).

6 Ologhobo AD and Fetuga BL, Effect of processing on the trypsin inhibitor, haemagglutinin, tannic acid and phytic acid contents of seeds of ten cowpea varieties. *Trop Agric* **61**:261–264 (1984).

7 Chang MJ, Collins JL, Baily JW and Coffey DL, Cowpea tannins related to cultivar, maturity, dehulling and heating. *J Food Sci* **59**:1034–1036 (1994).

8 Akpapunam MA and Markakis P, Oligosaccharides of 13 American cultivars of cowpeas (*Vigna sinensis*). *J Food Sci* **44**:1317–1318 (1979).

9 Ologhobo AD and Fetuga BL, Investigations on the trypsin inhibitor, haemagglutinin, phytic acid and tannic acid contents of cowpea *Vigna unguiculata*. *Food Chem* **12**:249–255 (1983).

10 Singh SR, Jackai LEN, Thottappilly G, Cardwell KF and Myers GO, Status of research constraints to cowpea production, in *Biotechnology: Enhancing Research on Tropical Crops in Africa*, Ed by Thottappilly G, Monti L, Mohan Raj DR and Moorse AW, CTA/IITA Co-publication, IITA, Ibadan, Nigeria. pp 21–26 (1992)

11 Gatehouse AMR and Boulter D, Assessment of the antimetabolic effects of trypsin inhibitors from cowpea (*Vigna unguiculata*) and other legumes on the development of the bruchid beetle. *Callosobruchus maculatus*. *J Sci Food Agric* **34**:345–350 (1983).

12 Gatehouse A, Hilder V and Boulter D, Potential of plant derived genes in the genetic manipulation of crops for insect resistance, in *Plant Genetic Manipulation for Crop Protection*, Ed by Gatehouse AMR, Hilder VA and Boulter D, CAB International, Wallingford, UK. pp 155–181 (1992).

13 Hymowitz T, Genetics and breeding of soybeans lacking the Kunitz trypsin inhibitor. *Adv Exper Med Biol* **199**:291–298 (1986).

14 Farinu GO and Ingrao G, Gross composition, amino acid, phytic acid and trace elements contents of thirteen cowpea cultivars and their nutritional significance. *J Sci Food Agric* **55**:401–410 (1991).

15 Valdebouze P, Bergeron E, Gaborit T and Delot-Laval J, Content and distribution of trypsin inhibitors and haemagglutinins in some legume seeds. *Can J Plant Sci* **60**:695–701 (1980).

16 Kakade ML, Simons NR, Liener IE and Lambert JW, Biochemical and nutritional assessment of different varieties of soybean. *J Agric Food Chem* **20**:87–90 (1972).

17 Deshpande SS and Cheryan M, Evaluation of vanillin assay for tannin analysis of dry beans. *J Food Sci* **50**:905–910 (1985).

18 Wheeler EL and Ferrel RE, A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem* **48**:312–314 (1971).

19 Makower RU, Extraction and determination of phytic acid in beans *Phaseolus vulgaris*. *Cereal Chem* **47**:288–291 (1970).

20 Huprikar SV and Sohonie K, Haemagglutinin in Indian pulses. II. Purification and properties of haemagglutinin from white pea *Pisum sativum*. *Enzymologia* **28**:33–40 (1965).

21 Liener IE, The photometric determination of the haemagglutinating activity of soya and crude soybean extracts. *Arch Biochem Biophys* **54**:223–225 (1955).

22 AOAC, *Official Methods of Analysis* (15th edn). Association of Official Analytical Chemists, Washington, DC, USA (1990).

23 Sokal RR and Rohlf JF, *Introduction to Biostatistics* (2nd edn), WH Freeman Co, New York, USA. pp 43, 133–199, 267–286 (1987).

24 Ruiz M, *Metodos Estadistics de Investigation*, Presidecia del Gobierno, Instituto Nacional de Estadisca, Madrid, Spain (1977).

25 Bacon JR, Lambert N, Matthews P and Arthur AE, Genotype, environment and genotype × environment effects on trypsin inhibitor in peas. *J Sci Food Agric* **67**:101–108 (1995).

26 Dowker BD, Fennell JFM and Jackson JC, Variation studies in carrots as an aid to breeding. II. Effect of sites, years and densities on some quality characters. *J Hort Sci* **49**:311–321 (1974).

27 Chang SI and Fuller HL, Effect of tannin content of grain sorghums on their feeding value for growing chicks. *Poultry Sci* **43**:30–35 (1964).

28 Oluwatosin OB, Genetic and environmental variation for seed yield, protein, lipid and amino acid composition in cowpea (*Vigna unguiculata* (L) Walp). *J Sci Food Agric* **74**:107–116 (1997).

29 Oluwatosin OB, Genetic and environmental variability in starch, fatty acids and mineral nutrients composition in cowpea (*Vigna unguiculata* (L) (Walp). *J Sci Food Agric* **78**:1–11 (1998).