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ANTI-NUTRITIONAL FACTORS FOR GERMPLASMS OF FABA BEAN AND PEANUT SEEDS, AND WHEAT

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ABSTRACT

Anti-nutritional factors of faba bean (*Vicia faba* L.), peanut (*Arachis hypogaea* L.) and wheat (*Triticum aestivum* L.) germplasms were studied. Tannins, total phenols, vicin and trypsin inhibitors were determined for the above-mentioned plant germplasms. These parameters were significantly varied among the different germplasms of faba bean, peanut and wheat. In addition, such differences in the levels of the anti-nutritional factors can be used as means for varietal identification.

INTRODUCTION

The future problems in agricultural are not predictable since the world population increases year after year and new diseases and pests may arise and causes damage to food crops and changes may occur in the environment. Therefore, it is necessary to find out more crops from the collected germplasms which may have special advantages as new food crops. The first step in germplasm conservation work is to list characterization of plant materials.

Antinutritional factors of seeds are basically determined by genetic factors. In general, the anti-nutritional factors of seeds vary widely among species and even among varieties, Egberg *et al.* (1975). Through crossing and selection programmes plant breeders are able to manipulate the anti-nutritional factors of many domestic crops and increase their usefulness as food, and as sources for different raw materials.

Tannins are known to be present in food legumes and they inhibit the activities of trypsin, chemotrypsin, amylase and lipase enzymes (Griffiths, 1979 and Jumbunathan and Singh, 1980). Tannins cause growth depression in rats (Abbey *et al.*, 1979) and in poultry (Martin *et al.*, 1977). This effect may be due to reduction in the digestibility of dietary proteins and to a lesser extent that of available carbohydrates and lipid. Tannin in the diet of human and animals bind to protein and reduce the nutritional value of proteins (El-Shemy *et al.*, 2000) Tannins also interfere with dietary iron absorption.

Victor-Raboy *et al.* (1991) believed that phytic acid, the major storage of pin seeds, has a negative impact on nutritional quality. Therefore, breeding for low phytic acid has been proposed for several cereal and legumes. Its important to predict the effects of selection against phytic acid on other major grain components.

Filippetti and Azadegan (1994) elucidated the variability in protein and trypsin inhibitor content, 113 lines/varieties of faba bean (*Vicia faba* L.), originating from several countries and grown at Valenzano (Bari, Italy) in the 1992/93 season. There was no correlation between trypsin inhibitor level and protein content (r = -0.19). Numerous varieties are selected for high protein content (>30 %) and low trypsin inhibitor levels (<2000 TIU/g DM).

MATERIALS AND METHODS

Source of germplasms:

Seeds of various germplasms were obtained from the Genetic resources Research Section, Agricultural Research Center. The germplasms used in this study were faba bean (*Vicia faba* L.), wheat (*Triticum aestivum* L.) and peanut (*Arachis hybpogeae* L.). A random sample of seeds of each accession was subjected to the following chemical analysis.

Crude protein content:

A known weight of fine powdered seeds (ca 0.1 g) was digested using a micro Kjeldahl apparatus. The crude protein was calculated by multiplying the total nitrogen by 6.25 (A.O.A.C. 2000) for peanut and faba bean. While, the factor 5.75 was used for wheat.

Trypsin inhibitor content:

The method of Roy and Bhat (1974) was utilized in this study for determination trypsin inhibitor activity (TIA) in all samples under investigation.

A) Extraction of the sample:

Four grams of fine or homogenated defatted (by acetone) sample were treated with sodium phosphate buffer (40 ml, 0.05 M) adjusted at pH 7.0. The mixture was shaken for 3 hr at room temperature, kept overnight in a refrigerator at 3-4 °C then centrifuged at 2000 r.p.m for 30 min at 15 °C. The supernatant was filtered and 1.0 ml of the filtrate was diluted to 10 ml using distilled water.

B) Estimation of TIA:

Casein solution (2 %, w/v) in phosphate buffer (0.1 M, pH 7.6) was used as a substrate for trypsin (5 mg/ml). The incubation mixture was consisted of trypsin solution (0.5 ml), casein (2.0 ml, 2 %) phosphate buffer (1.0 ml), hydrochloric acid solution (0.4 ml, 0.001 M) and seed extract (0.1 ml). In all cases the total volume of the incubation mixture was kept at 4 ml. Incubation was carried out at 37 °C for 20 min. then trichloroaceteic acid (6.0 ml, 5 %) was added to stop the reaction. The mixture was filtered and the absorbance of the filtrate was measured using ultraviolet spectrophotometer at 280 nm. Two blanks were run concurrently, the first one was run by using distilled water instead of trypsin solution and the extract for adjusting the spectrophotometer to zero and 100. The

second blank was run as described in the determination of TIA using phosphate buffer (0.1 ml, pH 7.0) instead of the sample.

In this assay, one trypsin unit (TU) was arbitrarily defined as an increase of 0.01 absorbance unit in 20 min for 10 ml of the reaction mixture. The trypsin inhibitor activity was calculated as the number of trypsin units inhibited by mg protein.

Determination of total phenols:

Total phenols were determined by using Folin-Denis reagent according to the method of Swain and Hilis (1959).

Determination of tannins:

Tannins were determined using vanillin hydrochloric acid (V.HCl) method as described by Burns (1971).

Determination of total vicine content:

Total vicine content, i.e., vicine and convicine were extracted from 1 g sample using 4 % m-phosphoric acid (Collier, 1976). The mixture was centrifuged then filtered to get a clear solution. The total vicine content was estimated using UV spectrocolrimeter spectronic 21 at wavelength 273 nm. The molar concentration of total vicine was determined according to the method described by Collier, 1976.

Statistical analysis:

The data for gross chemical composition were statistically analyzed according to Steel and Torrie (1980). L.S.D. values were used for comparison between means of the aforementioned parameters.

RESULTS AND DISCUSSION

Antinutritional factors

Soybean and faba bean seeds contain several antinutritional factors such as trypsin inhibitors, phytic acid and tannins (Kakade *et al.*, 1974).

It is well known that antinutritional factors such as vicine, trypsin inhibitor, tannin and total phenols play an important role in human and poultry nutrition and the qualitative and quantitative data of these components may help in identification of seeds of germplasms under study.

I- Faba bean germplasms:

1- Tannin contents

Table (1) shows the tannin contents of various faba bean germplasms under study. Faba bean seeds germplasms G.561 was characterized by the highest tannin content reaching 137.50 mg % based on dry seeds. Whereas, 880 germplasms had the lowest tannin content being 128.05 mg % based on dry seeds. The other studied germplasms had an intermediate tannin content.

Table (1): Tannin content, total phenols, vicine and trypisn inhibitor of faba bean germplasms.

Germplasms	Tannin (mg % dry weight)	Total phenol (mg % dry weight)	Vicine (mg/g dry weight)	Trypsin inhibitor activity (TIU/mg protein)
G.461	137.50	61.01	9.00	68.08
G.402	132.49	62.37	8.94	63.32
216	130.67	63.81	10.40	64.89
289	129.23	62.12	9.61	66.46
706	135.63	60.49	8.40	65.99
779	132.19	54.43	8.44	63.02
290	135.68	63.86	9.21	64.63
165	130.32	62.35	9.57	61.50
269	130.13	68.72	9.78	66.65
880	128.05	60.40	9.16	65.73
233	133.23	70.49	10.30	64.17
288	130.28	62.20	9.10	61.12
L.S.D. at 5%	0.94	1.86	0.98	3.07

2- Total phenols content

Table (1) illustrates the total phenols (mg %) of the different faba bean germplasms. The range of total phenols for the faba bean germplasms was 54.43-70.47mg %. This range indicates that these were varietal differences of studied faba bean germplasms.

3- Vicine content

Table (1) demonstrate the vicine content of the faba bean seeds germplasms. The germplasms 216 and 233 had the highest level of vicine being 10.40 and 10.30 mg %, respectively. The lowest vicine content was found in 706 and 799 faba bean germplasms. Such difference in the vicine content of the various germplasms can be used as mean in varietal identification.

4- Trypsin inhibitors

Table (1) shows that trypsin inhibitors are important factors that affect the nutritional value and protein digestibility in human. They cause pancreatic hypertrophy in rats and chicks fed in raw faba beans (Brik, 1974 and Youssef *et al.*, 1983).

The data of trypsin inhibitor activity (TIA) for the twelve studied faba bean germplasms are given in Table (1). The TIA was significantly varied among the different faba bean germplasms. The high significant TIA was found for faba bean germplasms of G.461, 269 and 289 being 68.08, 66.65 and 66.46 TIU/mg dry seed, respectively. The lowest TIA levels were present in the germplasms 288 and 165 (61.12 and 61.50 TIU/mg dry seed, respectively). These findings indicate clearly the low pancreatic hypertrophy effect of raw faba bean of 288 and 165 germplasms.

II- Peanut germplasms:

1- Total phenols content

Total phenol values of peanut germplasms are shown in Table (2), and with regard to this character, 11035, 11034 germplasms contained the highest values, whereas 8332 and 1561 germplasms had the lowest values. The values for the rest of the germplasms ranged between these two limits.

Table (2): Total phenols, vicine and trypsin inhibitor of peanut germplasms.

Germplasms	Total phenol (mg % dry weight)	Total Vicine (mg/g dry weight)	Trypsin inhibitor activity (TIU/mg protein)
G.4	44.16	3.58	27.98
G.5	44.26	3.99	30.42
10999	44.86	4.20	30.96
8332	38.89	4.08	34.98
1561	40.83	3.63	29.52
11034	53.79	3.51	30.17
11035	52.30	3.78	34.07
5815	49.49	4.23	33.38
11933	44.40	4.42	36.11
5996	46.95	4.16	30.36
5943	44.99	4.91	34.97
11011	41.44	4.54	33.50
L.S.D. at 5%	1.74	0.16	4.09

2- Vicine content

Meanwhile, the total vicine content was measured and the data are shown in Table (2). The total vicine range for the peanut germplasms under study was 3.08-4.94 mg/g. one has to point out that the germplasms 8332 and 5943 had the highest and lowest values, respectively. Other germplasms contained intermediate values.

3- Trypsin inhibitor

The activity of trypsin was determined for the peanut germplasms under study and the values are shown in Table (2). This investigation revealed that 11933 germplasms contained the highest value, followed by 8332, 5943 and 11035 germplasms.

III- Wheat germplasms

1- Total phenols content

The total phenol contents of wheat germplasms were shown in Table (3). The highest and lowest total phenol contents are present in Egy 44-4-2 and Egy16-1, respectively. All the studied germplasms significantly differed in their amount of total phenol content ranging from 12.73 to 21.78 mg %.

2- Total vicine content

The data of total vicine content are shown in Table (3). The concentration of the total vicine was generally low, ranging between 0.84 to 1.67

mg/g dry weeds. Wheat germplasms Egy22-1 was characterized by the highest value of 1.67 mg/g dry seed. Whereas, the lowest value is shown in wheat germplasm Egy5-2-1.

Table (3): Total phenols, vicine and trypisn inhibitor of wheat germplasms.

Germplasms	Total phenol (mg % dry weight)	Vicine (mg/g dry weight)	Trypsin inhibitor activity (TIU/mg protein)
Sids 1	19.67	1.45	12.98
Sakha 69	20.44	0.86	17.43
Egy5-2-1	18.62	0.84	12.13
Egy 5-2-2	14.82	1.29	16.28
Egy 16-1	12.73	1.00	17.26
Egy 22-1	16.73	1.67	13.65
Egy 22-4	19.90	1.62	15.51
Egy 23-8	18.54	1.02	13.20
Egy 44-4-2	21.78	1.37	17.10
Egy 46-3	14.37	0.86	12.73
Egy 51-3	18.79	1.57	18.06
Egy 52-2-1	15.74	0.86	13.57
L.S.D. at 5%	1.06	0.07	1.69

3- Trypsin inhibitor

The trypsin inhibitor activity of wheat germplasms is shown in Table (3). The TI activity ranged from 12.13-18.06 depending upon the wheat germplasms. This range indicates significant variation of TI activity between the wheat germplasms under study.

In general, the values of total phenols, vicine and TI demonstrates that faba bean germplasms contained the highest values followed by peanut and wheat germplasms. On the other hand, tannin content was found in faba bean germplasms only.

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العوامل المضادة للتغذية لبعض الأصول الوراثية لبذور الفول البلدى والفول السودانى والقمح

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** قسم بحوث تكنولوجيا البذور-معهد بحوث المحاصيل الحقلية-مركز البحوث الزراعية-الجيزة

تـم تقدير بعض العوامل المضادة للتغذية على بعض الأصول الوراثية لبذور الفول البلدى (*Vicia faba* L.) والفول السودانى (*Arachis hypogaea* L.) والقمح (*Triticum aestivum* L.) حيث تم تقدير التانينات والفينولات الكلية والفيسين وأيضا مثبطات إنزيم التربسين لكل من الأصول الوراثية المستخدمة فى الدراسة.

أظهرت النتائج وجود اختلافات معنوية فى العوامل المضادة للتغذية للأصول الوراثية المستخدمة لبذور الفول البلدى-الفول السودانى-القمح وبالتالى يمكن استخدام هذه الاختلافات فى العوامل المضادة للتغذية فى التعرف على الأصول الوراثية وتوصيفها لمختلف محاصيل الفول البلدى والفول السودانى والقمح.