Research Journal of Agriculture and Biological Sciences, 6(4): 481-486, 2010

Assessment Level of Some Anti-Nutritional and Nutritional Factors In Some Egyptian Cultivated Soybean and Barley

[1]Gehan, A.E.El-Emery and [1]Ayman Y. Amin

[1]Institute of Efficient Productivity, Zagazig University., Egypt.
[2]Department of Plant Physiology, Faculty of Agriculture, Cairo University.

Abstract: As a main source of protein, soybeans (*Glycine max* (L) Merril) and barley (*Hordeum vulgare*) provide the human and animal nutrition essential amino acids that are required for healthy individuals. However, these crops contain secondary plant metabolites, considered as anti-nutritional factors, which display negative effects on nutritional qualities produced. Three genotypes of soybean Giza35 (G35), Giza21 (G21), and Giza83 (G83) and three genotypes of barley Giza134 (G134), Giza2006 (G2006), and Giza123 (G123) which together represent the crops harvested in Egypt, were analyzed for their nutritional value and the levels of antinutritional factors i.e. phytic acid, tannins, and total phenol. The ranges of anti-nutritional factors in soybean seeds were total phenols 18.9–35mg/g, tannins 16.23–18.33mg/g, and phytic acid 1.6–3.08%. On the other hand, the ranges of anti-nutritional factors in barley seeds were total phenols 21– 27.86mg/g, tannins 14.23–18mg/g, and phytic acid 1.2–1.41%. The significant differences among the two crops genotypes were in the total phenol content; it being the highest in both but significantly higher in soybean reaching 45 mg/g but with a larger deviation of values. Barley genotypes reached a highest content of 27.86 mg/g and a low of 21 mg/g. Phytic acid content was typically low in both. The other factors were similar in concentrations among different germplasms.

Key words: Soybean, barley, nutritional and anti nutritional factors, protein patterns, phytic acid, tannin, total phenol.

INTRODUCTION

Soybean (*Glycine max* (L) Merril) is one of the most important crops for human and animal consumption. The most important organic components of soybean seeds are proteins and oil (about 40% and 20% respectively)[3], and cultivated both for oil production and their rich protein content. Soybeans are considered by many industries, to be a source of complete protein; one that contains significant amounts of all essential amino acids that must be provided to the human body because of it is inability to synthesize them. In the same trend [18] mentioned that

Soy is an alternative source of protein for vegetarians, or for people who cannot afford meat. On the other hand, barely is one of the first cereals to be cultivated by man, as it is grown throughout the world. It is used commercially in animal nutrition. Among the secondary plant metabolites, phytic acid, tannin and vicine have attracted the attention of plant breeders, mainly because their negative effect on the nutritional quality produced .These products are important as anti-nutritional factors of seeds and basically determined by genetic factors. In general, the anti-nutritional factors of seeds vary widely among species and even among varieties[4]. Raboy, V. *et al*.,[17] believed that phytic acid, the major storage of pin seeds, had a negative impact on nutritional quality. Since breeding for low phytic acid has been proposed for several cereal and legumes, it is important to predict the effects of selection against phytic acid on other major grain components. Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6, -hexa phosphate) is widespread in plant seed grains and is regarded as the primary storage form of both phosphate and inositol[5]. Additionally, phytic acid is a strong chelating agent that can bind metal ions, reducing availability of Fe, Zn, and Mg[1]; therefore, reducing phytic acid content would increase nutritional value. Tannins are known to be present in food legumes and they inhibit the activities of trypsin, chemotrypsin, amylase, and lipse enzymes[9]. They may locate primarily in the hull, and classified as polyphenolic substances[11], which makes tannins the most important anti-nutritional factors in legumes. Similarly, Singh *et al*.,[14] were used SDS-PAGE technique to identify seed proteins of chickpea cultivars.

This study was designed to examine seed extracts of different soybean and barley genotypes and identify those with higher phenolic content exhibiting increased

Corresponding Author: Gehan, A.E.El-Emery, Institute of Efficient Productivity, Zagazig University., Egypt.
E. mail: gehan.elemery@gmail.com.

antioxidant activity and assessment the level of essential amino acids, since this could provide important information for alimentary or pharmaceutical purposes. Thus, our main objective was to evaluate six cultivated Egyptian genotypes with regard to seed contents of amino acids, phytate, tannin, and phenol together with differences in protein patterns.

MATERIALS AND METHODS

Source of Genotypes: Seeds of six common Egyptian genotypes were obtained from the Institute of Agricultural Resources Center in Giza, Egypt. The genotypes used in this study were soybean (G35, G21, G83) and barley (G134, G2006, G123). A random sample of seeds of each genotype was subjected to the following chemical analysis, as described by Ivarson and Sowden,[8].

Determination of Tannins: Tannins were determined using vanillin hydrochloric acid (V. HCl) method as described by Price and Butler,[13]. A sample of seeds of various germplasms (2 gm) was shaken with 50 ml methanol containing 1%HCl for 24 hours at room temperature. 1 ml of the clear extract was treated with vanillin reagent and kept for 20 min. The absorbance was recorded at 500 nm. Pure catechin was used for standard curve.

Determination of Total Phenols: Total phenols were determined using the Folin–Ciocalteu reagent[15]. A sample of whole seeds (2 gm) were homogenized in 80% aqueous ethanol at room temperature and centrifuged in cold at 10,000 rpm for 15 min and the supernatant was preserved. The residue was re-extracted twice with 80% ethanol and supernatants were pooled and evaporated to dryness at room temperature. Residue was dissolved in 5.0ml of distilled water. 0.1 ml of this extract was diluted to 3.0 ml with water and 0.5ml of Folin–Ciocalteu reagent was added. After 3 min, 2.0ml of 20% of sodium carbonate was added and the contents were mixed thoroughly. The color was developed and absorbance measured at 650nm in an Uvikon 943 double beam UV/VIS ,spectrophotometer after 60 min using gallic acid as a standard.

Determination of Phytic Acid: The assay of phytic acid is based on precipitation of ferric phytate and measurement of iron remaining in the supernatant. About 0.5 g of grind seed sample was used for extraction of phytic acid in 25 ml 0.2 N HCl for 3 hr. The extract brought up to 50 ml with de-ionized water and centrifuged at 1000 rpm for 30 min, then 1 ml of supernatant was treated with 150 µl of 0.02% (w/v) Ferric solution ($NH_4Fe[SO_4]_2$. $12h_2O$), then placed in a boiling water bath for 30 min. After cooling, the samples were centrifuged and 1 ml of supernatant was treated with 150µl of 1% (w/v) 2, 2 bipyridine solution to measure Fe remaining in the supernatant calorimetrically at 519 nm. Authentic phytic acid (P-7660, Sigma) served as a standard[7].

Determination of Amino Acids: The composition of amino acids were detected by using amino acid analyzer LC 300 Ependorf model after acid hydrolysis of grind seed of barley and soybean genotypes G134, G2006, G123, G35, G21, and G83.

Detection of SDS-PAGE: One dimensional sodium dodecylsulfat polyacrylamide gel electrophoresis was carried out according to Laemmli,[10]. Protein markers or molecular weight standard of MW-SDS (Sigma) were used with bromophenol blue as the tracking dye with a range from 18.0 to 208 KDa. Electrophoresis was run at constant current 40mA or 35mA per 2 gels. Staining was done in 0.125 % coomassie brilliant blue R-250, 50% methanol, and 10% acetic acid for 4 hrs. De-staining was done in 50% methanol 10% acetic acid solution for 2 hrs, then continued de-staining in 5% methanol 7% acetic acid solution for 6 hrs. All assays were done at room temperature. Gels were stored in 7% glacial acetic acid solution until photograph was taken.

Statistical Analysis: The data were statistically analyzed according to Snedecor and Cochran,[16]. L.S.D values were used for comparison between means of the aforementioned parameters.

RESULTS AND DISCUSSION

It is well know that nutritional factors such as proteins, lipids, carbohydrates, fibers, amino acids and total phenols, as well as anti-nutritional factors such as phytic acid, and tannins are playing an important role in human and animal nutrition, such qualitative and quantitative data of these components may help in program breeding.

Phytic Acid, Tannin and Total Phenol Analysis: Six genotypes soybean and barley seeds were analyzed for their constituents of phytic acid, tannin, and total phenol. The obtained data are given in Tables 1. The results indicated that the phytic acid, tannin, and total phenol were varied among barley and soybean. In barley genotypes (G134, G2006, G123), phytic acid was recorded 1.41 %, 1.2%, and 1.3% respectively (Figure 1); while in soybean (G35, G21, G83) genotypes recorded 1.6%, 3.08%, and 2.79% respectively (Figure 2). The amount of phytic acid in

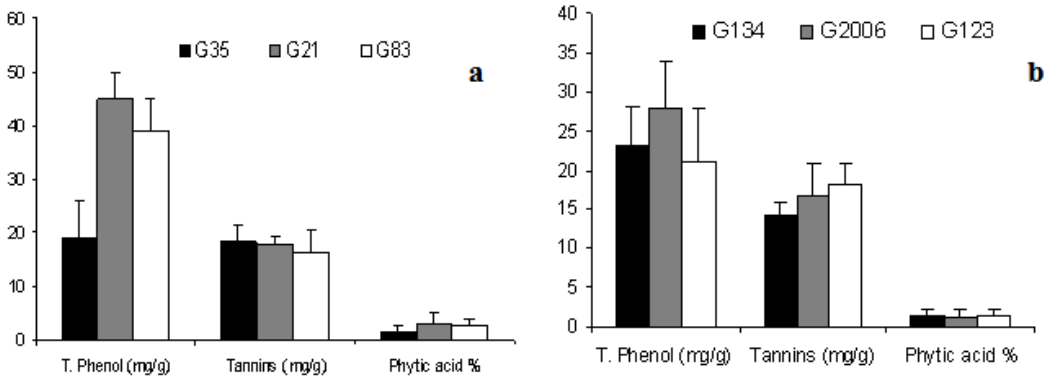

Fig. 1: Phenol, tannin, and phytic acid content in a) soybean (G35, G21, and G83) and b) barley (G134, G2006, and G123) genotypes.

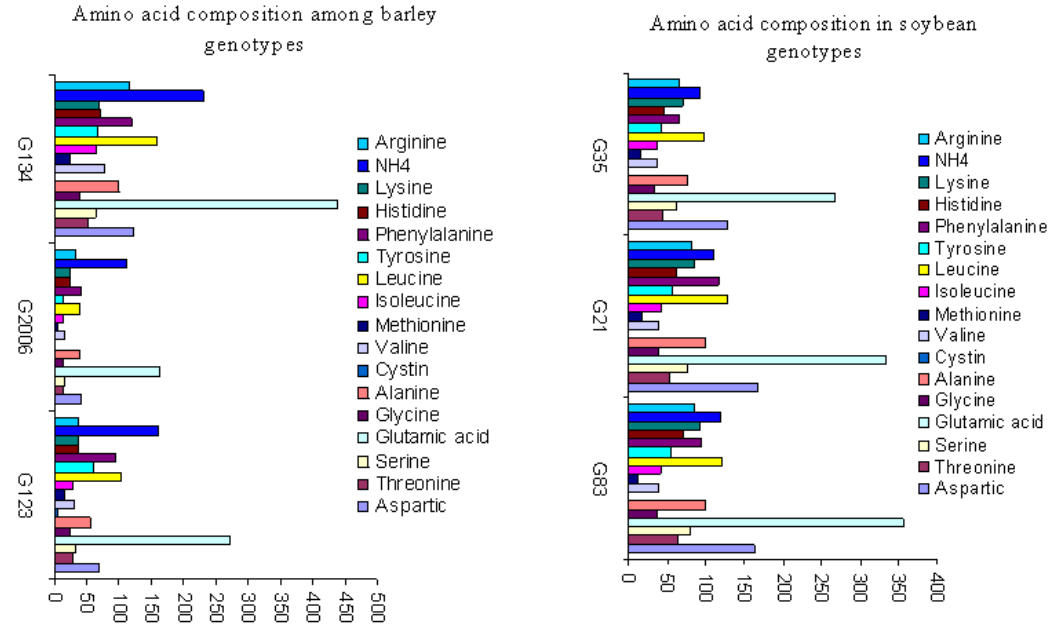

Fig. 2: Amino acids composition among 1- barley genotypes (G123, G2006, and G134) 2- soybean genotypes (G83, G21, and G35).

G21, G83) was more than doubled to those reported by Miranda *et al*.,[12] which was ranged from 0.56% to 1.20% (phytic acid) in soybean cultivars, however, G35 was found to have similar phytic acid content. High concentrations of phytic acid usually found in legumes to be used as alert, considering that phytic acid can act as a chelating agent on some important nutritional ions i.e. zinc, calcium, magnesium, and iron[5]. On the other hand, tannin levels in soybean and barley showed almost the same values (Figure 2). Tannins might vary within a plant species but the origin of this variation seems to be not well studied as reported by Hattenschwiler and Vitousek,[6]. In Table 1, the total phenol of various among the barley genotypes, results were showed barley seeds genotype G2006 was characterized by the highest total phenol reaching 27.86mg/g whereas, G123 germplasms had the lowest total phenol 21mg/g. In comparison, the total phenol in the soybean seeds germplasms in table 2 showed that G21 were characterized by the highest total phenol content reaching 45mg/g whereas, G35 had the lowest total phenol content 18.9mg/g.

Amino Acids Analysis: Table 2 and Figure 2 demonstrated the type of individual amino acids content in seeds of barley and soybean. In barley seeds genotype G134 showed the highest level compared to G2006 and G123. On the other hand, G134, G123, and G2006 genotypes showed high levels in glutamic acid (439.22, 271.12, and 164.72), NH_4 (229.34, 162.18, and

Table 1: Total phenols, tannin, and phytic acid of different barley and soybean genotypes

Crop	Germplasms	T. Phenol (mg/g)	Tannins (mg/g)	Phytic acid %
Barley	G134	23.13 ± 0.61	14.23 ± 0.4	1.41 ± 0.028
	G2006	27.86 ± 0.71	16.63 ± 0.6	1.2 ± 0.015
	G123	21 ± 0.8	18 ± 0.12	1.3 ± 0.03
	LSD 5%	1.42	0.9	0.05
Soybean	G35	18.9 ± 1.3	18.33 ± 0.15	1.6 ± 0.025
	G21	45 ± 1.3	17.7 ±0.17	3.08 ± 0.11
	G83	39 ± 0.6	16.23 ± 0.35	2.79 ± 0.35
	LSD 5%	2.19	0.48	0.136

Table 2: Amino acid composition (µg/ml) of soybean and barley

Amino acids(µg/ml)	Soybean			Barley		
	G35	G21	G83	G134	G2006	G123
Aspartic	127.33	169.03	163.67	122.01	42.73	70.25
Threonine	44.4	52.01	63.13	52.94	12.51	28.56
Serine	62	76.64	79.64	63.45	16.75	32.91
Glutamic acid	267.48	333.36	356.2	439.22	164.72	271.12
Glycine	33.12	40.68	36.79	38.38	14.6	23.83
Alanine	76.75	99.12	99.8	99.64	37.9	55.54
Cystin	---	---	---	---	2.34	6.52
Valine	37.5	40.58	39.54	77.32	16.99	30.53
Methionine	15.32	17.27	12.99	25.58	6.08	17.91
Isoleucine	37.04	42.36	42.05	64.66	15	29.08
Leucine	96.96	127.8	121.54	156.98	38.72	102.24
Tyrosine	41.05	56.56	56.28	66.2	12.61	59.86
Phenylalanine	65.42	116.27	94.52	119.26	42.12	95.5
Histidine	46.89	62.53	71.31	71.91	25.04	36.68
Lysine	71.38	87.01	93.29	69.07	24.28	35.12
NH4	93.26	111.05	120.37	229.34	112.24	162.18
Arginine	66.21	80.86	85.44	116.68	32.02	36.72

112.24), and Leucine (156.98, 102.24, and 38.72) respectively compared to other amino acids. All cultivars were low in Cystin and methionine.

Protein Electrophoresis: Expression of proteins in studied cultivars was significantly different in its number and intensity of protein bands as well as in appearance of new bands or disappearance of some bands, depending on the type of cultivar (Figure 3). The number of expressed protein bands recorded its minimum seven bands at lanes 2 and 3 (G2006, and G123 respectively). This number increased to a maximum of twelve bands at lanes 4, 5 and 6 (G35, G21, and G83 respectively). The common expressed protein bands recorded significant intensity; *viz,* the protein bands of molecular weights 92, 32 and 28 KDa produced the maximum intensity in lanes 4, 5 and 6 (G35, G21, and G83 respectively) but it produced the minimum intensity in lanes 1, 2 and 3 (G134, G2006, and G123 respectively). The protein band of molecular weight 196 KDa was newly expressed in lanes G35, G21, and G83 as well as protein band of molecular

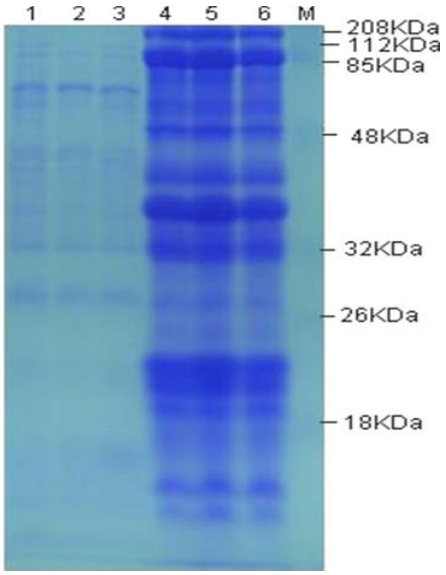

Fig. 3: SDS-PAGE protein patterns of three different barley cultivars lane1- G134, lane2-G2006, and lane-3 G123 and three soybean cultivars 4-G35, 5-G21 and 6-G83.

weight 119 KDa in lane 1 (G134). Concerning the G134, G2006, and G123 genotypes, four protein bands were expressed as common proteins having molecular weights 69, 45, 16 and 13 KDa. In lanes 4, 5 and 6, (G35, G21, and G83 respectively) the general protein bands having molecular weights 62, 51, 42, 37, 21, 19 and 17 KDa were present.

To Conclude: Some anti-nutritional factors were estimated from soybean and barley germplasms where the tannin, total phenols, and phytic acid were tested for each cultivar. These compounds have a positive effect in being antioxidants and negative effect as an anti-nutritional factors. In addition, Results obtained using amino acid analyzer were displayed a large difference in the amount and type of amino acid among different germplasms and also between the two species examined. It was found the highest percentage of amino acids was present in G134 for barley and also results showed the presence of anti-nutritional factors varies for germplasms of soybean and barley. Moreover, we can use these differences among the cultivated genotypes of barley and soybean in consideration during breeding programs in the future.

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