EVALUATION OF PROTEIN AND AMINO ACID CONTENTS OF THE SEED IN THREE GROUPS OF BREAD WHEAT (*Triticum Aestivum* L.) MUTANTS

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ABSTRACT

The seeds of three groups of bread wheat (Triticum aestivum L.) mutants from parent cultivar (Inia-66) were tested according to FAO/WHO recommendations to determine level the amino acids. The analysis of variance revealed highly significant differences ($p \leq 0.05$) among varieties for all the mutant. The ZC115 and ZB76 were high for percentages of moisture, ash, protein, and total amino acids, (essential and non essential amino acids). The genotypic coefficients of the groups for most of the characters were almost equal to its phenotypic coefficient of variation. The predominance of genetic components that was further supported by high protein content, total amino acids (essential and non essential amino acids). A significant reduction in most of the amino acids was observed in the seeds of the mutants: ZA67 and ZB76 as compared with the parent Inia-66.

INTRODUCTION

Wheat is the number one food crop in the world based on area under cultivation and total production. The popularity of wheat is based on diverse uses, resistance to many pathogens and pests, low cost of production, rapid growth, genetic flexibility and adaptability to different climates (3). Bread wheat (Triticum aestivum L.) is most important cereal crop for the food security. As a staple food, it has got diversified domestic and industrial uses. Wheat provides nearly 55 percent of the carbohydrates and 20 percent of the food calories consumed globally (27). The wheat seed consists of an outer layer (epidermis) inside of which is contained a starchy endosperm which is the material from which white flour is made, it comprises starch granules embedded in a matrix of proteins (6).The proteins consist of albumins, globulins, gliadins and glutenins. The combination of gliadins and glutenins is referred to as the "gluten complex" or "gluten" and is regarded as storage protein. The protein fraction in wheat is crucial to the development of the gluten structure of dough and how well flour performs in processing (2). The interaction between high, intermediate and low molecular weight gluten proteins ensure strong, cohesive dough with excellent rising properties and texture. Various wheat varieties have different protein content and therefore different baking qualities(25).Water-insoluble wheat protein is isolated and treated under acidic conditions resulting in solubilisation of the protein due to conversion of the natural amino acids, glutamine and asparagine, to their non-amidated derivatives, glutamic acid and aspartic acid that are also naturally occurring amino acids. The partially or wholly deamidated protein product is recovered after several steps of purification as Isolated Wheat Protein in the form of powder (19). The Isolated Wheat Protein may be used directly as a functional food ingredient in certain food formulations to confer physical attributes to the final food form; alternatively, it may be

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Re-solubilised prior to use using one or more of a range of substances that facilitate its functional performance prior to use the contents of almost all the amino acids increased as the protein of the wheat and flour samples increased (15). Only cystine and methionine in the wheat, and lysine, cystine, and methionine in the flour failed to follow this pattern. Milling wheat to flour lowered (in decreasing order) concentrations of lysine, arginine, aspartic acid, glycine, alanine, tyrosine, histidine, threonine, and valine. The concentrations of glutamic acid, proline, and phenylalanine were higher in flour than in wheat. The concentrations in protein of basic amino acids, glutamic acid, and threonine were significantly correlated with certain rheological properties or bread-making potentialities of the flours (18). A promising three mutant lines: ZB76, ZA67and ZC115 of bread wheat Triticum aestivum L originated from irradiated stored seeds by gamma ray of Inia-66 cultivar, and characterized by high yielding compared with their parent, have been analysed for essential and non essential amino acids and protein contents.

MATERIAL AND METHODS

The present investigation was carried out through 2014-2015 at the farm and Genetic Engineering and biotechnology laboratories of Baghdad University. The three mutants (ZC115, ZB76 and ZA67) were chosen to their contents of amino acids (essential and non essential) and protein. There were taken by irradiated in nuclear Physics laboratory/ Tunisian atomic energy institute and stored seeds of the bread wheat cultivar Inia-66 (9). Properties of the seeds and their flour were taken. All samples and flour are marked in figures as series. The types of flour are the most produced in our mill industry of all other types.

The following parameters of seeds and flour are tested: wet protein content (Glutamatic, AACC method 38-12) (4). Because proteins are made up of chains of amino acids joined by peptide bonds, they can be hydrolysed to their component amino acids, which can then be measured by Glutamatic test apparatus (ICC Standard No.155 and No.158 (AACC) method 38-12) and Farinograph (ICC Standard No. II5/ I Iso 5530-1 AACC standard No. 54-21.

The sum of the amino acids then represents the protein content (by weight) of the food. Amino acids content was analysed by method of acidolisys. The bounded amino acids are lose from proteinic string at first. The following chemicals may be used in the manufacture of Isolated Wheat Protein, hydrochloric acid. Sodium hydroxide as food approved bases. The salt acid as hydrogenation agent is used (17). The analysis continue at AAA 400 apparatus based on liquid chromatography.

The amino acid content in flour has been converted into amino acid content per 100gm protein. In subsequence have been counted chemical score, based on the 10 (1985) pattern.

Moisture content based on (Motomco Moisture, AACC1976 model 919). Softening of wheat flour dough and its blends were determined according to 1 (1987) methods using a Farinograph type (PL) (Barbensder Farinograph, Germany).

Ash content (Muffle furnance, AACC). All results were expressed as percentage. 100 g of wheat was ground and mixed it in original glass test tube. Glass test tube was filled to the top, closed and put in device for determination. The above mentioned parameters of wheat and flour were determined by different advices and methods.

RESULTS AND DISCUSSION

The content of the seed protein, ash, moisture, amino acids content were studies in three mutant samples and origin bread wheat (Triticum aestivum L.) cultivar (Inia-66). According to the results table (1) were found differences in the parameters in the mutant bread wheat (ZA67, ZB76 and ZC115) samples compression with that in the parent sample (Inia-66). The protein fraction is the great important for flour and wheat seed. It is also important to determine it. These results were in accordance with the results of 4 (2013), of the popularity of wheat is based on diverse uses, resistance to many pathogens and pests, low cost of production, rapid growth, genetic flexibility and adaptability to different climates. The unique breadmaking quality is related to the type and quantity of gluten proteins, especially the high molecular weight gluten in subunits that are synthesized and stored in the seed endosperm (22).

Table 1: Average percentage of amino acids and protein in the three mutants and their parent cultivar (Inia-66).

Mutants	Moisture (Moisture)	Ashes	protein	Amino acids (total)	*Essential amino acids
ZB76	11	1.5	11.54	6.75	2.92
ZA67	11.5	1.5	12.62	11.38	4.84
ZC115	12	2	11.16	9.67	3.97
parent cultivar	11	1	12.98	11.28	4.86
LSD at 5%level	3.7	0.9	2.3	1.85	2.5

*Except tryptophan.

Sometimes the seed protein referred to as a "true protein". The advantage of this approach is that it requires no assumptions about, or knowledge of, either the non-protein nitrogen (NPN) content of the food or the relative proportions of specific amino acids - For removing the two problems by using of total Nx a conversion factor. Its disadvantage is that it requires more sophisticated equipment than the Kjeldahl method, and thus may be beyond the capacity of many laboratories, especially those that carry out only intermittent analyses (5). In addition, experience with the method is important; some amino acids (e.g. the sulphur-containing amino acids and tryptophan) are more difficult to determine than others. Despite the complexities of amino acid analysis, in general there has been reasonably good agreement among laboratories and methods (16).

Moisture is a term used to describe the water content within the seed. During the plant growth stage moisture is important for starch development as well as ensuring the filling out of the endosperm resulting in optimum milling yield. Post harvest low moisture content is expected, which is necessary for the storage life of the seed prior to being milled for its intended use (12). Excess moisture is detrimental as it can lead to mould growth, toxin formation, insect infestation and sprouting in storage. The assessment of moisture content in seeds is an important parameter as it influences the seed quality and storage life of the seed. Excessive rain during the final plant growth phase can result in high moisture content which will have the effect of reducing the protein content as a percentage of total weight. High moisture content above 12% may cause rejection at receivable point to prevent the risk of mould and insect growth during storage. Seed with excessively low moisture will result in a hard seed with low flour yield (13). The Increase or decrease moisture can efficiency affect of representation and vitality of the plant and then increase vegetation stress and works to improve the plant's resistance to disease, increase shoots and activate

the chlorophyll and increase grain size and consistency, activate the roots and root hairs of the plant growth, the early growth and activate buds growth by improving the hormonal balance and regulate the rate of flowers and grains install contract (26). The results of table (2) were showed differences in the parameters in the mutant bread wheat (ZA67, ZB76 and ZC115) samples compression with that in the parent sample (Inia-66), and the mutant bread wheat (ZC115) have largest quantity of total amino acids than the other bread wheat's samples. Amino acid analysis is a routine automated procedure for measuring the amount of each amino acid in a protein providing rapid reliable characterization of proteins, while many proteins have approximately similar proportions of particular amino acids; each protein has a unique amino acid composition and sequence. Certain proteins have very different amino acid compositions which cause them to be separately and specifically identified.

Table2: Average percentage of amino acids and protein in the three mutants and their parent (Inia-66)

Mutants	Essential amino acids	Non-Essential amino acids	Total
ZB76	3.14	6.90	10.04
ZA67	3.92	5.32	9.24
ZC115	4.57	6.97	11.54
parent cultivar	5.06	6.42	11.48

The discrepancy in the results of essential and non- essential amino acids here been found, and a higher results in CZ115 sample than other samples. Amino acids having nitrogen in their side chain moieties contribute a greater proportion of nitrogen. This group includes lysine, arginine, histidine, asparagine and glutamine. Many proteins have approximately similar proportions of amino acids which accounts for the similar relevant conversion factor of 6.25. Certain well defined protein materials are estimated using alternate conversion factors when these are known more accurately (21). The importance of amino acids of the plant where lies the importance of amino acids of the plant to contain consciousness carboxyl group and the total amin or more, this means ease of running out of free individual amino acids to the plant and then do a positive impact on the enzymatic activity of the plant, as well as the treatment of stress often exposed to the plant and the resulting inadequate conditions of the plant, such as cases of frost, and operates amino acids to increase plant immunity and vitality and resistance to changes in temperature phenomenon of pregnancy were use of amino acid compounds to reduces the occurrence of this phenomenon (23). The table (3) appear the results of essential amino acids (dry weight) in the seeds of three mutant originated from bread wheat compared with the parent cultivar (Inia-66). The ZC115 have higher average of essential amino acids than other samples except valine. Analysis of free amino acids in different wheat samples and wheat fractions showed that it was possible to analyse most of these, with their contents varying according to the wheat cultivar and the milling fraction.

Table 3: Average percentages of essential amino acids (dry weight) in the seeds of three mutants originated from bread wheat cultivar (Inia-66)

Essential amino acids	The mutant			Inia-66
	ZB76	ZA67	ZC115	
Methionine	0.07	0.09	0.18	0.12
Lysine	0.22	0.26	0.41	0.32
Tyrosine	0.15	0.20	0.3	0.25
Threonine	0.2	0.30	0.36	0.34
Valine	0.54	0.73	0.1	0.97
Leucine	0.5	0.72	0.93	0.83
Arginine	0.37	0.6	0.67	0.62
Phenyl alanine	0.38	0.5	0.63	0.59
Cystine	0.08	0.07	0.17	0.11
Histidine	0.19	0.23	0.38	0.29
Isoleucine	0.22	0.32	0.44	0.42

Activity of Isoleucine where increase shoot growth, and early in the crop, while the methionine accelerates ripening where it enters in the composition of ethylene cycle and has a role in the revitalization of the roots. The threonine increases the strength of the possibility of disease resistance in plants, while the valine play important role in the formation of radicals and the formation of seeds and the speed of plant growth speed (20). Cystine increases the speed of biological processes and organization within the plant and increases resistance to diseases. The Phenylalanine works to improve the plant cells and the formation of lignin. The arginine resistance to extreme conditions such as heat, cold, thirst, salinity - Its role is in the formation of chlorophyll and promotes the formation of roots and split cells but the lysine was increase vegetative growth and early crop. Hydroxyl lysine increase crop growth, and the histidine roles an increase in the growth, yield and early in the crop and improve the working efficiency of phosphorus in plant (14).

Table 4: Average percentages of non-essential amino acids (dry weight) in the seeds of three mutants originated from bread wheat cultivar (Inia-66)

Non-Essential amino acids	The mutant			Inia-66
	ZB76	ZA67	ZC115	
Glycine	0.36	0.50	0.58	0.52
Aspartic acid	0.33	0.46	0.60	0.58
Serine	0.30	0.42	0.54	0.52
Glutamic acid	1.73	2.39	3.42	3.09
Proline	0.89	1.25	1.4	1.31
Alanine	0.22	0.30	0.43	0.40

The roles of amino acid –glycine is to activate photosynthesis and raise its efficiency of chlorophyll formation and activates the vegetative growth and has a role in some of the elements interaction has linked with the process of pollination and fruit setting role. 15 (2012) reported that the glycine is an amino acid that enhances color formation in bakery products, whether added before dough fermentation or on the surface of the dough before baking. Decreasing in color at longer fermentation times might be due to the consumption of other amino acids and sugars, i.e. other main precursors for color formation. These results recommend the use of glycine along with amino acid reducing measures that tend to produce insufficiently colored products, e.g. when replacing reducing sugars with sucrose or when using lower temperatures for baking (11). Where the aspartic acids have roles in improves the plants resistance to disease and the

roles of serine in increases the probability of a plant's ability to resist disease and stimulate the formation of chlorophyll and has important role in hormonal balance within the plant role, while the glutamine have roles to increase shoot growth, and early in the crop but the Proline play important roles in resistance to harsh conditions and activate the germination of pollen. The hydroxyl-proline: resistance to the difficult conditions and extreme heat, thirst, cold, salinity (8). The alanine was affects plant growth speed and activate the formation of chlorophyll. Hydrolysis of glutamine and asparagine residues in gluten is achieved in suspension in dilute strong acid with application of heat. Acidic conditions and temperature are sufficiently moderate to result in minimal hydrolysis of peptide bonds and consequent polypeptide fragmentation, so the amino acid content of gluten is maintained and oxidized products are not generated (23). Each amino acid contributes a unique amount of nitrogen to the protein, with the exception of isomers such as leucine and isoleucine. Hence, depending on the relative proportions of each amino acid, the composite proportion of nitrogen differs for each protein (7). Wheat Protein, however, together with amino acid analysis. It provide a comparative characterization procedure to identify differences between products similar in amino acid composition, and Molecular size analysis allows discrimination between isolated wheat protein and wheat protein hydrolysates in which the polypeptide chains of gluten have been deliberately cleaved by hydrolysis of peptide bonds either enzymatically or under more vigorous chemical conditions (24). The conclusion of this research revealed significant differences among samples. The ZC115 and ZB76 were high for effective seeds, Moisture, Ash, protein, and total amino acids, (essential and non essential amino acids). The predominance of genetic components may be that was further supported by high protein content, total amino acids (essential and non essential amino acids). A significant reduction in most of the amino acids was observed in the seeds of the mutants: ZA67 and ZB76 as compared with the CZ115 and parent cultivar (Inia-66).

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تقييم المحتوى البروتيني والأحماض الأمينية لبذور ثلاثة ضروب لحنطة الخبز

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الملخص

أُختبرت بذور لثلاث مجاميع لحنطة خبز الطافرة من صنف أبوي (حنطة أينيا–66) حسب توصيات منظمة الأغذية العالمية ومنظمة الصحة العالمية (FAO/WHO) لتحديد مستوى الاحماض الأمينية. أظهر تحليل الضروب فروقاً معنوية عالية ($p\leq0.05$) بين الضروب الطافرة جميعها. كانت الضربات الطافرات ZC115 و ZB76 بنسبتين عاليتين للرطوبة، الرماد، البروتين والأحماض الأمينية الكلية (الأحماض الأمينية الأساسية وغير الاساسية). ان المعامل الوراثي للمجاميع الخاصة بمعظم الخصائص متساوية تقريباً لمعامل المظهري لها. ان المحتوى الجيني اكثر دعماً من خلال المحتوى العالي لنسبة البروتين، والأحماض الأمينية الكلية (الأحماض الأمينية الأساس وغير الاساس). لوحظ وجود انخفاض كبير في معظم الأحماض الأمينية في بذور الطافرة ZA67 وZB76 بالمقارنة مع صنف الأب (أينيا– 66).

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