PROSPECTS FOR IMPROVING GRAIN SORGHUM PROTEIN QUALITY

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ABSTRACT

Grain sorghum production of Egypt is around 0.8 million tons containing 83,403.0 tons of crude protein. This protein is of concern because, besides being low in the essential amino acid lysine, it has a lower digestibility than protein of other cereal grains. Therefore, the present study is a preliminary trail to induce frame-shift mutations projecting to modify sorghum storage protein, utilizing intercalating agents that insert or delete nucleotides in encoded DNA regions and consequently alter resulted polypeptides and proteins. Grains of the cultivated variety Dorado was treated with three doses of ethidium bromide (EtBr) and were planted with control (M_1 generation in 2007 summer season). The survived plants were monitored for four generations i.e., from M_1 to M_4 where they were evaluated. The germination percentages of treated seed (M_1) were very low compared with untreated ones (control), even the highest concentration of EtBr was completely toxic for sorghum seedlings. The low germination percentage of M_2 plants grown in green house in off season didn't surpass 40%, indicated that gene segregation caused homozygosity for lethal mutations and high mortality and consequently low germinations. Three mutants were identified according to their standardized performance that absolutely excess 2.6. The line 3 showed plant height of 205.9 cm with a standardized value of 4.6, the line 16 headed at standardized time of -3.3 (63.5 day) and the line 35 displayed wider panicle with standardized circumference of 4.7 (32.2 cm). A fourth mutant was in the line 12, which had been identified at M_3 because it`s panicle displayed aberrant kernels with an opaque appearance. The protein content of this line was 12.5% which exceeded the original variety Dorado (11.9 %, P=0.15). Seed storage protein was assessed for polymorphism between Dorado and Line 12 mutant in M_3 and M_4 generations. Protein electrophoresis and densitometric analysis of SDS-PAGE of water soluble extracted protein in Dorado and its derived mutant line 12 indicated that three bands in Dorado variety disappeared in the mutant line 12 (in both M_3 and M_4) and four bands appeared at low molecular weight compared with Dorado, suggesting an occurrence of frame-shift mutations due to the use of EtBr. This suggestion was supported by the grain sorghum protein constitution of amino acids for the variety Dorado and its derivative line 12. These results reveal that some amino acids conserved their percentages like isoleucine, leucine, tyrosine, phenylalanine, histidine, alanine and valine, while the amino acids aspartic acid, thrionine, Serine, glutamic acid and proline increased significantly. Unfortunately, the two amino acids, lysine and argnine proportionally decreased their percentage. It is concluded that the intercalating agent EtBr has a great potentiality to induce frame-shift mutations and consequently alter storage protein constitution of higher plants like grain sorghum.

INTRODUCTION

Grain sorghum [*Sorghum bicolor* (L.) Moench] is an important staple food in developing countries of the semiarid tropics and is used as an animal feed in both developed and developing countries. It is one of the oldest cereal crops. Although it is grown in the developed countries, primarily for livestock feeding, it is the world's third most important food grain that is exceeded only by rice and wheat, and is the staple cereal for human consumption in large areas of India, China, Manchuria, and Africa (Elliott 1969).

Grain sorghum grows in area ranging around 0.4 million feddans in Upper Egypt, so it ranks the fourth among cereal crops in Egypt after wheat, rice and maize in terms of acreage and production. Most of this area is concentrated in four governorates, i.e., Assiut, Sohag, Fayoum and Qena. However, the total grain sorghum production in 2009 was about 758,212 tons. The majority of grain sorghum in Egypt is used for animal and poultry rations. Assuming protein percentage about 11%, sorghum offers to Egypt 83,403.0 tons of protein.

The nutritional quality of sorghum protein is of concern because, besides being low in the essential amino acid lysine, it has lower digestibility than protein of other cereal grains. It is true that sorghum proteins are slightly less digestible than maize but as eaten in processed forms they are readily available and do not cause major problems. Where possible, white, tan plant straw color glumes and grain with a spherical medium to hard endosperm are required. Sorghum grains don't contain gluten and its slower hydrolysis makes them attractive to diabetics, celiacs and ethnic groups. Increasing the levels of lysine and trypotphan in sorghum is extremely valuable in terms of human and animal nutrition. Developing high yielding sorghums with improved levels of lysine and trypotphan would greatly enhance its value for both humans and animals (Rooney, 2007).

The proteins of the sorghum grain are classically divided, based on solubility in different solvents (Jambunathan *et al* 1975) into: albumins (water-soluble), globulins (salt-soluble), kafirins (prolamins, aqueous alcohol-soluble), cross-linked kafirins (aqueous alcohol reducing agent-soluble), cross-linked glutelins (detergent reducing agent alkaline pH-soluble) and un-extracted structural protein residue. A newer and more simplified classification scheme for sorghum proteins has been proposed that divides them into two groups, kafirins and non-kafirins. This scheme is based on the homogeneous nature and varied origin of the kafirin storage prolamins relative to the heterogeneous nature of the non-kafirin proteins (i.e., albumins, globulins and glutelins) that are involved in cellular functions (Hamaker and Bugusu, 2003).

Despite a high degree of sequence homology to maize zeins, sorghum storage proteins contain a higher proportion of cross-linked fractions and are more hydrophobic, explaining their greater propensity to form intermolecular disulfide-cross linkages and possibly additional protein aggregates that could facilitate the formation of more covalent bonds compared to maize zeins (Belton *et al* 2006 and Hamaker and Bugusu, 2003).

In the corneous endosperm, non-kafirins (albumins, globulins, glutelins) form around protein bodies, effectively "gluing" the bodies into a matrix surrounding the starch granules (Hamaker and Bugusu 2003). This protein matrix appears to act as a barrier to starch gelatinization and digestibility (Duodu *et al* 2002 and Ezeogu *et al* 2005 and 2008) due to cross-linking between α- and β kafirins and matrix proteins (Duodu *et al* 2001 and Hamaker and Bugusu 2003). Cooking reduces digestibility by effecting a conformational change in proteins that could facilitate formation of disulfide-linked polymers (Duodu *et al* 2002 2003). The negative impact of cooking on protein digestibility was mitigated by addition of 2-mercaptoethanol (ME) or other reducing agents (Elkhalifa *et al* 1999). Sorghum grains rich in kafirin-containing protein bodies also have a lower capacity for starch gelatinization (Ezeogu *et al* 2005 2008).

Sorghum grains contain inadequate levels of some essential amino acids, particularly lysine, threonine, tryptophan and methionine. These deficiencies arise from the amino acid composition of the grain storage proteins, called prolamins (kafirins), which account for up to 80% of the total grain proteins. In sorghum these deficiencies are also exacerbated by cooking, which reduces sorghum protein digestibility. Similarly, binding of tannins to proteins can also reduce digestibility in high tannin lines. Improving the nutritional quality of sorghum grain protein by classical plant breeding is limited by the low level of variation in the gene pool available (Forsyth *et al* 2008). However, improved lines of the high-lysine mutant of grain sorghum have been shown to have greater digestibility of protein than normal cultivars (Hamaker *et al* 1987).

Mutations are the ultimate source of variability in organisms. Variability caused by induced mutations is not essentially different from that caused by spontaneous mutations. The direct use of mutation is a valuable supplementary approach to plant breeding, particularly when it is desired to improve one or a few characters in an otherwise well-adapted variety/hybrid. Mutation-assisted breeding can play an important role in crop improvement either directly or by supplementing the conventional breeding (Khawale *et al* 2007).

Some mutations as (G191S) in bmr6 of maize had been proved as a frame-shift mutation resulting in the truncation of the last 27 amino acids (Sattler *et al* 2010). The high-lysine mutants of grain sorghum may be

produced by frame-shift mutations that changes polypeptide amino acid constitutions and chemical characterizations and behavior. The intercalating agents are DNA modifiers that induce frame-shift mutations in microorganisms. These agents belong to a group of compounds include proflavin, acridine orange and ethidium bromides. A frameshift mutation (also called a framing error or a reading frame shift) is a genetic mutation caused by insertions or deletions of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (the grouping of the codons), resulting in a completely different translation from the original. Frameshift mutations can also be beneficial. For example, a frameshift mutation was responsible for the creation of nylonase (Lewis 2005). A frameshift mutation is not the same as a single-nucleotide polymorphism in which a nucleotide is replaced, rather than inserted or deleted. (Wikipedia 2010).

This investigation was carried out aiming to find out an answer for the question, can intercalating agents produce frame-shift mutations in the higher plants? The second objective was to induce mutations in storage protein of the cultivated grain sorghum variety Dorado aiming to improve its protein quality.

MATERIALS AND METHODS

The cultivated grain sorghum Dorado variety was used in the present investigation, it is open pollinated variety. Dorado seeds were soaked in ethidium bromide ($C_{21}H_{20}BrN_3$) solution. Ethidium bromide is commonly used as a non-radioactive marker for identifying and visualizing nucleic acid bands in electrophoresis. It fluoresces readily with a reddish-brown color when exposed to ultraviolet light, intensifying almost 20-fold after binding to DNA beside its character as intercalating in DNA causing frame-shift mutations. Ethidium bromide was added at a concentration of 0, 0.4, 2 and 8 mg/ml DW (distilled water) that contains 100g sorghum seeds and the seeds were incubated at room temperature for approximately 24 h, then the seeds were rinsed three times with DW and soaked in DW for three hours before planting.

The treated seeds of the three concentrations were planted in three plots adjacent to untreated seeds as a control at Giza Research Stations of ARC in 2007 season, where seedlings were monitored and emergence percentages were recorded. The resulted seeds of the survived plants (M_2 seed) were grown in green house in the off season and resulted panicles' seeds were used for 2008 growing season (M_3). Thirty-five heads were randomly chosen for evaluation at Giza Research Station in 2009 season (M_4). The 35 M_4 lines and the cultivated variety Dorado as check cultivar

were evaluated in field trials grown in a randomized complete block design with three replicates. Each experimental plot included one ridge, 4 meter long and spaced 0.7 m apart (2.8 m^2). Sowing was carried out in hills 20 cm apart along the ridge followed by thinning to 2 plants per hill before 1st irrigation. Recommended agricultural practices were followed at the proper time and plant protection practices were applied as needed. Data were recorded as mean performance of 10 random plants for days to 50% heading, plant height (cm), stem diameter, panicle length (cm), maximum panicle circumference (cm), 1000-kernel weight (g) and grain yield plant^{-1} (g). Data were subjected to a statistical analysis of variance of a randomized complete blocks design according to Gomez and Gomez 1984.

Sorghum protein isolation

Sorghum protein isolate was prepared by acid treatment (Iwabuchi and Yamauchi, 1987). A 100g sample of seeds of Mu1 (line 12 in M$_3$ generation) and Mu2 (line 12 in M$_4$ generation) was extracted once with 1.0 liter of 0.03 M Tris-HCl buffer (pH 8) containing 10 mM of 2-mercaptoethanol (2-ME) at 20°C. After centrifugation, the supernatant was acidified to pH 4.8 with 2N HCl and then centrifuged at 8000 rpm for 20 minutes. The precipitated protein was dissolved in water at 4°C and the pH adjusted to 8. After centrifugation (8000 rpm) for 10 minutes, the clear supernatant was dialyzed against distilled water for 24 h at 24°C and then freeze-dried.

SDS-polyacrylamide gel electophoresis

Protein fingerprinting was achieved using water soluble protein fraction. SDS polycarylamide gel electrophoresis (SDS-PAGE) was performed on a discontinuous buffered system according to the method of Laemmli (1970) using 15% separating gel and 3% stacking gel containing 0.1% SDS. Protein isolate samples (20 µl, 0.2%) were prepared in a Tris-glycine buffer at pH 8.8 containing 1% SDS. Electrophoresis was done at a current of 10 mA for 5 hours. After electrophoresis, the gel sheets were stained with 0.2% Coomassie brilliant blue-R250 and then distained with 10% acetic acid containing 20% methanol and de-stained with 10% methanol in 7.5% acetic acid. Gel were photographed and scored using gel documentation system manufactured by Alpha Ease FC (Alphimager 2200), U.S.A. The similarity matrices and genetic relationships between the Line 12 mutant in M$_3$ and M$_4$ (Mu1 and Mu2, respectively) and its control (Dorado variety) as revealed by dendrogram was performed using SPSS windows (Version 10) program.

Amino acids analysis

The amino acid analysis was carried out for Dorado and MU2 (in M$_4$ generation) at the Regional Center for Food and Feed (RCFF), Agricultural

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Research Center, according to AOAC (2006), and data were statistically analyzed in the same Center.

RESULTS AND DISCUSSION

Field Experiments

The germination percentages of treated seeds in M_1 generation were very low compared with untreated ones (control) so that only 120, 6, 2 and 0 plants were survived, reached to maturity and harvested for the four ethidium bromide concentrations: 0, 0.4, 2 and 8 mg/ml EtBr, respectively. These results revealed that the 2 and 8 mg/ml EtBr treatments were completely toxic for sorghum. In addition, the low germination percentage of M_2 plants grown in green house off season that didn't surpass 40%. The germination rates were improved in the followed generations. Tehreema *et al* (2010) reported that the concentration of EtBr used was 500µg mL^{-1} for 180 min which produced 96.56 % killing and 3.44 % survival rate.

Table (1) presents some agronomic traits of 35 lines in M_4 generation and the Dorado variety. Since all these lines are originally derivatives of the cultivated variety Dorado, these lines referred to one population and their means were expected to behave in a normal distribution and every line represents effective mutation have significantly diverges from the population mean. Therefore, the extreme mean for every line and every studied trait was standardized. Each line has an absolute standardized mean that exceed 2.6 was considered as does not belong to the variety Dorado and therefore is expected to be a mutation. Accordingly, three mutants were identified i.e., line 3 that had a standardized plant height of 4.6 (205.9 cm), line 16 that headed at a standardized time of -3.3 (63.5 day) and line 35 that displayed wider panicle with a standardized circumference of 4.7 (32.2 cm). Fig. (1-A) displays line 16 at heading time when other lines at early booting stage. Fig (1-B) displays line 3 that their stems surpass other lines with 30 to 50 cm.

The line 12 had been identified at M_3 because its panicle displayed aberrant kernels with opaque appearance (Fig.1-C), therefore grain protein percentage was estimated for this line along with a sample of the variety Dorado. The chemical analysis revealed that protein percentage for the line 12 and the cultivar Dorado was about 12.5 and 11.9%, respectively, with insignificant increment about 0.6% (P>0.15). Nevertheless, this result encouraged us to perform more investigations.

Table 1. Means of some agronomic traits of 35 lines in the M₄ generation and the Dorado variety.

Line	Morphological traits			Panicle length		Yield components		Panicle weight	Grain Yield
	PLH	Stem D	HD	PL	PC	KW500	KN		
1	135.0	2.2	80.5	27.7	16.1	11.7	2479.5	83.4	58.0
2	132.5	2.4	72.0	24.7	11.3	16.1	1832.0	79.9	56.5
3	205.9	2.6	75.5	30.9	14.3	14.1	2780.0	107.5	78.0
4	139.2	2.5	72.5	22.3	12.7	10.9	2429.0	76.1	52.8
5	135.0	2.4	80.0	28.8	13.3	14.5	1532.5	75.5	44.5
6	123.3	2.2	70.0	27.9	15.5	14.1	2492.0	98.0	70.5
7	148.4	2.1	76.0	31.2	13.8	14.2	1766.5	110.5	50.5
8	146.7	2.3	73.5	27.0	15.8	13.1	2155.0	79.5	58.0
9	139.2	2.7	74.5	25.1	13.5	13.7	1412.0	56.6	38.5
10	135.0	2.9	76.0	24.9	13.3	14.5	1735.5	69.0	50.5
11	146.7	2.8	74.5	23.4	12.1	12.9	1438.5	57.0	37.5
12	135.0	2.6	75.5	24.2	13.0	14.0	1586.0	60.5	44.0
13	133.4	2.7	79.0	22.3	10.9	14.5	1819.5	73.5	53.0
14	151.7	1.8	73.0	25.7	11.9	13.3	1916.5	74.3	51.0
15	135.0	2.6	73.5	28.3	13.3	13.9	2272.5	75.0	63.0
16	151.7	1.8	63.5	23.1	11.0	13.0	1729.0	64.9	45.0
17	146.7	2.6	77.5	31.0	14.4	12.7	1459.5	62.0	36.5
18	143.4	2.2	76.5	29.1	11.6	13.7	2236.5	87.5	60.0
19	133.3	2.5	70.5	29.3	18.3	16.6	2496.5	89.0	82.9
20	126.7	2.8	71.5	30.1	13.6	14.8	1988.0	89.0	59.0
21	151.7	2.3	72.0	24.3	13.0	14.4	1415.5	58.8	41.0
22	130.0	2.6	75.0	25.8	14.8	14.9	2317.0	91.0	70.0
23	136.7	2.7	78.5	23.5	13.8	14.5	1685.5	73.0	48.5
24	130.0	2.5	78.5	25.9	12.0	15.8	1434.5	64.8	45.0
25	135.0	2.8	73.0	22.9	12.3	14.4	1625.0	56.5	46.5
26	143.4	2.8	74.5	27.9	14.8	15.0	1967.0	81.5	58.5
27	150.0	2.4	73.5	27.5	14.3	14.2	2162.5	87.5	62.0
28	127.5	2.8	74.0	26.3	14.8	14.7	2087.0	87.9	61.0
29	140.0	2.7	74.0	27.6	13.9	16.0	2012.0	87.0	63.0
30	145.0	2.0	69.0	30.2	13.2	14.4	1745.5	77.0	50.0
31	141.7	2.6	72.5	28.3	16.0	14.2	2544.5	87.5	72.0
32	153.4	2.2	77.0	27.8	14.6	15.4	2077.0	86.5	64.0
33	125.0	1.9	77.5	27.8	10.4	14.2	1289.5	44.5	36.0
34	153.4	2.5	76.0	27.8	23.2	14.4	2627.0	111.5	72.0
35	140.8	2.4	75.0	23.0	32.2	10.5	2512.5	75.3	52.5
Dorado	141.7	2.7	75.0	24.2	14.3	13.6	2182.0	78.5	59.0
LSD 5%	10.9	19.3	4.7	10.1	14.1	12.3	24.3	22.0	24.0
MIN	123.3	1.8	63.5	22.3	10.4	10.5	1289.5	44.5	36.0
X`	141.3	2.5	76.6	14.4	78.3	14.1	1978.9	55.3	55.3
MAX	205.9	2.9	80.5	31.2	32.2	16.6	2780.0	111.5	82.9

PLH= plant height, Stem D= stem diameter, HD= days to 50% heading,
PL= Panicle length, PC= Panicle circumference, K500= 500 kernel weight and
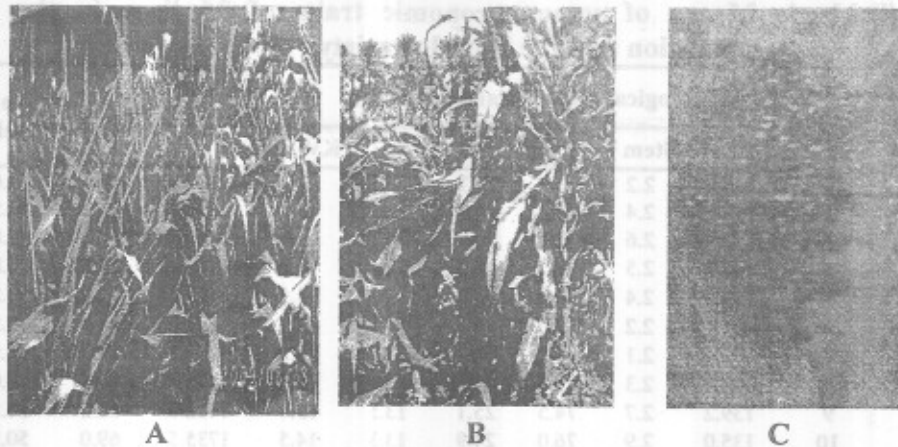KN = Kernel Number

Fig. 1. Some morphological changes due to treatment with EtBr, (A)
Line 16 at heading time, (B) Line 3 (tall plants), (C) Line 12
of panicle opaque appearance.

Protein electrophoresis

Protein markers, including seed storage protein was among the first
group of molecular markers exploited for genetic diversity assessment. Seed
storage protein (water-soluble fraction) was used in this study to assess
polymorphism between Dorado and the mutant in M_3 and M_4 generations.
Electrophoretic separation of water soluble extracted protein in the studied
Dorado and its mutants is shown in Figure (2) and their densitometric
analyses are illustrated in Table (2). A positive sign in the tables represents
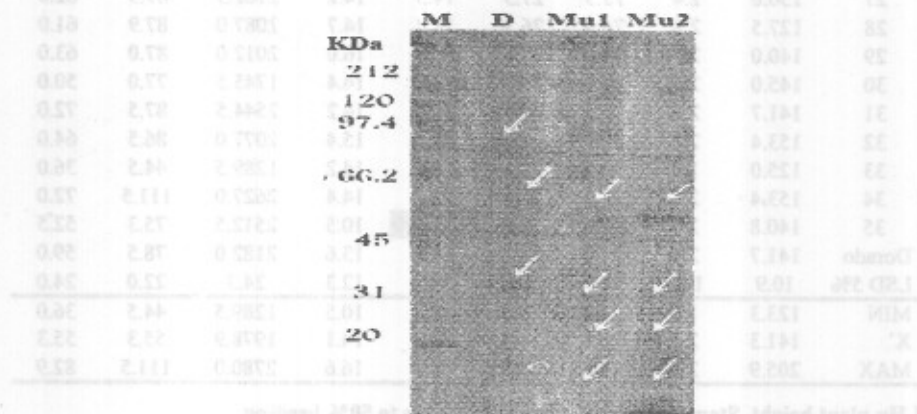the presence of each corresponding band.

Fig. 2. Water soluble protein banding patterns of Dorado and its
mutant (Line 12) in M3 (Mu1) and in M4 (Mu2).

Table 2. Banding patterns of SDS-PAGE for water soluble protein fraction of Dorado and its mutant (Line 12) in M3 (Mu1) and in M4 (Mu2).

No of band	MW KDa	Dorado	Mu1(M3)	Mu2(M4)
1	152.7	+	+	+
2	121.7	+	+	+
3	115.8	+	+	+
4	98.4	+	–	–
5	88.7	+	+	+
6	68.3	+	+	+
7	63.9	+	–	–
8	59.3	–	+	+
9	55.2	+	+	+
10	44.2	+	+	+
11	42.0	+	+	+
12	35.0	+	+	+
13	32.6	+	–	–
14	28.5	–	+	+
15	24.6	+	+	+
16	21.4	–	+	+
17	20.2	+	+	+
18	18.0	+	+	+
19	16.8	–	+	+
20	15.5	+	+	+
Total No. of bands		16	17	17

From the protein banding patterns and densitometric analysis of SDS-PAGE for water soluble protein fraction of sorghum Dorado variety and its Line 12 mutant (in both M3 and M4 generations), 20 bands were obtained with different molecular weights ranging from 152.7 to 15.5 KDa. Some bands were absent in Line 12 mutant (in M3 and M4) such as bands no. 4 (98.4 KDa), 7 (63.9 KDa) and 13(32.6 KDa). On the other hand, band no. 8 (59.3KDa), 14 (28.5 KDa), 16 (21.4 KDa) and 19 (16.8 KDa) were expressed only in the mutant (M3 and M4 generations). The result in Table (2) showed that two bands in Dorado variety disappeared in the mutant (in both M3 and M4 generations) and appeared at low molecular weight, such as the band with MW of 63.9 KDa in Dorado, which disappeared in Dorado

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and appeared with new molecular weight in the mutant in both M_3 and M_4 (59.3 KDa) and also the band with MW of 32.6 KDa that appeared in Dorado, disappeared in the mutant and appeared with low MW (28.5 KD) in Line 12 (in both M_3 and M_4), which may indicate an occurrence of frameshift mutations due to the use of EtBr.

The highest similarity index (100%) was recorded between Mu1 and Mu2 (Line 12 mutant resulted from treatment with EtBr in both M_3 and M_4 generations, respectively), as shown in Table (3) and Fig. (3). While the similarity index between the original or parental variety (Dorado) and its mutant in M_3 and M_4 generations were 71%, indicating the occurrence of mutation due to the intercalating agent used for inducing variation that may be a frameshift mutation.

Table. 3. Similarity index of Dorado and its mutant (Line 12) in M_3 (Mu1) and in M_4 (Mu2)

Genotypes	Dorado	Mu1 (M_3)
Mu1 (M_3)	71%	100%
Mu2 (M_4)	71%	100%

Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine

Genotype

Mut1(M_3)		
Mut2(M_4)	100%	71%
Dorado		

Fig.3. A dendrogram showing the genetic distance among Dorado sorghum variety and its mutant (Line 12) in M_3 and M_4 generations using SDS- protein data.

Since the frameshift mutation alters amino acid sequences and polypeptide chain length resulted from affected genes, it is conceived that most of these mutations may be lethal if these genes control enzymes or active polypeptides, but if mutated genes control storage proteins of their plants have a great potential to survive. So we performed amino acid analysis to check this expectation.

Because three bands have disappeared from Dorado column and four new different bands displayed in Mu1 (M_3) and Mu2 (M_4) generations of the line 12, we believed that two frame-shift mutations had occurred in two genes encoding for albumin protein which are soluble in water (Table 2). Storage protein in sorghum (kafirins represents 80%) is mainly prolamins, therefore the altered albumin protein is a part from the remainder 20%. This information with the weightier new bands illustrate the trivial increase of the protein percentage from 11.9 of Dorado to 12.5% of the line 12.

Table 4 represent the grain sorghum protein constitution of amino acids for the variety Dorado and it`s derivative line 12 (MU2) only, according to the results of Table (3) and Fig. (3), which indicated that both Mu1 and Mu2 are similar (with 100% similarity index and are located in the same cluster).

Table 4. **The amount of essential amino acids (g) in one kilogram grain of the derivative mutant line 12 and its basic precursor variety Dorado.**

Amino acid	Human requirement	MU2	Dorado	Difference
Isoleucine	essential	4.3 ± 0.17	4.2 ± 0.16	0.1
Leucine	essential	16.9 ± 0.59	13.7 ± 0.54	1.2
Tyrosine	essential	3.6 ± 0.14	3.5 ± 0.14	0.1
Phenylalanine	essential	6 ± 0.24	5.8 ± 0.23	0.2
Histidine	essential	2.4 ± 0.04	2.4 ± 0.04	0
Lysine	essential	2.3 ± 0.09	2.6 ± 0.1	-0.3*
Argnine	non	4 ± 0.16	4.2 ± 0.16	-0.2*
Aspartic acid	non	8.7 ± 0.17	8.5 ± 0.17	0.2*
Thrionine	essential	3.7 ± 0.07	3.6 ± 0.01	0.1*
Serine	non	5 ± 0.2	4.8 ± 0.019	0.2*
Glutamic acid	non	24.4 ± 0.48	22.8 ± 0.45	1.6*
Proline	non	9.1 ± 0.54	8.4 ± 0.5	0.7*
Glycine	non	3.7 ± 0.14	3.8 ± 0.15	-0.1
Alanine	non	9.2 ± 0.55	8.7 ± 0.52	0.5
Valine	essential	5.6 ± 0.22	5.6 ± 0.22	0

These results revealed that some amino acids conserved their percentages like isoleucine, leucine, tyrosine, phenylalanine, histidine, alanine and valine, while the amino acids Aspartic acid, thrionine, serine, glutamic acid and proline increased significantly. Unfortunately, two amino

acids containing lysine and argnine proportionally decreased their percentage.

Finally, we concluded that the intercalating agent ethidium bromide has a great potentiality to induce frame-shift mutations and consequently alter storage protein constitution of higher plants like grain sorghum. In the course of this work the essential amino acid lysine had been decreased but in the future may be a high protein quality with suitable lysine percentage will be captivated.

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امكانيات تحسين جودة البروتين فى الذرة الرفيعة

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يبلغ إنتاج مصر من الذرة الرفيعة حوالي ٠.٨ مليون طن تحتوي على ٨٣,٤٠٣ طن من البروتين الخام. هذا البروتين إلى جانب كونه منخفض في الحامض الأميني الأساسي ليسين، فإنه أقل من بروتينات الحبوب الأخرى فى القابلية للهضم. ولذلك ، فإن الدراسة الحالية هى محاولة أولية لاستحداث طفرات تغيير تتابع الاحماض الامينية (Frame-shift mutation) لتعديل البروتين المخزن فى الذرة الرفيعة، وذلك باستخدام عوامل اقحام النيوكليوتيدات (intercalating agents) ,والتى تؤدى لإدراج أو حذف النيوكليوتيدات في المناطق المشفرة من

الحمض النووي وبالتالي تؤدى الى تغيير البوليبيبتيدات والبروتينات الناتجة. عوملت حبوب الصنف المنزرع دورادو بثلاث جرعات من بروميد الإثيديوم والتي تمت زراعتها مع الكنترول (بدون معاملة) في موسم ٢٠٠٧. وتم رصد النباتات الحية اثناء اربعة أجيال متعاقبة، (من الجيل الطفورى الاول الى الجيل الطفورى الرابع) حيث تم تقييمها.

وقد كانت نسبة الإنبات فى البذور المعاملة في الجيل الطفورى الاول منخفضة جدا بالمقارنة مع النباتات غير المعاملة (الكنترول)، حتى أن أعلى تركيز من بروميد الإثيديوم كان بالغ السمية لبادرات الذرة الرفيعة. ولم تتجاوز نسبة الإنبات المنخفضة فى نباتات الجيل الطفورى الثانى [تحت ظروف الصوبة فى الموسم الربيعى (off season) ٢٠٠٨] ٤٠ % إنبات.

وقد تم تمييز ثلاثة طفرات طبقا لقيم صفاتها مقدرة على وحدة الاختلاف القياسى ، وهو ما لا يتجاوز ٢٫٦ كقيمة مطلقة. وأظهر السلالة رقم ٣ طول نبات قدره ٢٠٥٫٩ سم مع قيمة قياسية تبلغ ٤٫٦ ، و السلالة رقم ١٦ أظهرت طرد للقناديل في وقت قياسى بقيمة -٣٫٣ (٦٣٫٥ يوم) والسلالة رقم ٣٥ أظهرت محيط اكبر للقنديل بقيمة قياسية ٤٫٧ (٣٢٫٢ سم). وهناك الطفرة الرابعة في السلالة رقم ١٢، والتي تم تحديدها في الجيل الطفورى الثالث لحبوبها المعتمة. ومحتوى البروتين لهذا الخط قدر بـ ١٢٫٥% وبذا تفوق على الصنف الأصلي دورادو بمحتوى بروتين (١١٫٩ وباحتمالية = ٠٫١٥).

تم تقدير الاختلافات في البروتين المخزن في الحبوب بين الصنف دورادو وطفرة السلالة رقم ١٢ في الجيل الطفورى الثالث والرابع، وأظهر التفريد الكهربائى وتحليله للبروتينات الذائبة في الماء و المستخلصة من الصنف دورادو وطفرة السلالة رقم ١٢ في الجيل الطفورى الثالث والرابع، اختفاء ثلاث حزم بروتينية في الطفرة في كلا الجيلين الطفوريين، كما ظهر بها أربعة حزم بروتينية ذات اوزان جزيئية منخفضة غير موجودة بالصنف الاصلى مما يشير إلى حدوث طفرات تغيير تتابع الاحماض الامينية (Frame shift mutation) نظرا لاستخدام بروميد الإثيديوم . وأيد هذا الاقتراح تقدير الأحماض الأمينية للصنف دورادو وطفرة السلالة رقم ١٢ في الجيل الطفورى الثالث والرابع. هذه النتائج تكشف عن أن بعض الأحماض الأمينية احتفظت بنسبتها مثل الاحماض الامينية: الآيزوليوسين ، ليوسين ، التيروزين ،فينيل الانين ، هستدين، ألانين و ڤالين ، بينما زادت معنويا نسبة الأحماض الأمينية: الأسبارتيك ،ثريونين، سيرين، حمض الجلوتامين والبرولين زيادة كبيرة. لسوء الحظ ان اثنين من الأحماض الأمينية وهي الليسين والارجنين انخفضت نسبتهما نسبيا. ويستنتج من ذلك أن بروميد الإثيديوم له مقدرة عالية على استحداث طفرات تغيير تتابع الاحماض الامينية وبالتالي أحداث تغيير في مكونات البروتينات المخزنة فى الحبوب فى النباتات الراقية مثل الذرة الرفيعة.