Characterization of Protein Patterns of Some Wheat Varieties as Affected by Some Bio-Regulators

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Abstract: Fractionation and characterization of grain storage proteins of three wheat varieties Sakha-93, Gemiza-7 and Gemiza-9, cultivated in newly cultivated land (Nubaria region) as affected by some bio-regulator treatments was carried out by Sodium Dodecyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Wheat grains of the three varieties were collected from different bio-regulator treatments. The dendrogram profile data showed the variation in the number and position of bands from one variety to another as affected by bio-regulator treatments, while some bands are considered common.

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Key words: SDS-PAGE, wheat grains, bio-regulators.

1. Introduction

Wheat is most widely cultivated cereal and important source of food and protein worldwide contain most of ingredients required for calories and health. Wheat is the basic raw material for preparation of chapatti and bread **(Haridas Rao *et al.*, 1986)**. According to solubility, wheat proteins are classified into four classes: albumin, globulin, prolamin and glutens, gluten comprising 78-85% of total protein and the large complex mainly polymeric and monomeric protein are known as glutenin and gliadins **(MacRitchie, 1994)**. Wheat contains two subunits, one is Low Molecular Weight (LMW-GS) (10,000-70,000 Da) and the High Molecular Weight (HMW-GS) glutenin subunits (80,000-130,000 Da) **(Payne *et al.*, 1980)**.

Wheat provides approximately one fifth of the calories in the human diet and is an important source of vegetable protein and nutrients for a large proportion of the world's population. (**Cakmak** *et al.,* 2000)

Mohamed and Aly (2005) studied some biochemical changes and molecular aspects of iron deficiency stress in four bread wheat (*Triticum aestivum*) genotypes (Sakha 61, Sakha 69, Sakha 93 and Gemaza 7) and found the genotypic difference in growth rate measurement can be related to iron stress and confirmed that cultivar differences in protein polymorphism could be revealed by electrophoretic patterns.

The top two major food crops in the world, wheat and rice, have been most severely affected by drought conditions. For example, wheat production in the drought-hit area of the Middle East and Central Asia has decreased by ~22% in 2009 **(De-Carbonnel, 2009).**

Plant bio-regulators (PGRs) are actively involved in a multitude of metabolic processes and play essential roles in plant growth and development under both stress and nonstress conditions. They also act as chemical messengers to modulate various processes or genes involved in plant growth and development **(Morgan, 1990)**. Plant bio-regulators also play important roles in plant adaptation to stressful environments, including drought stress (Huang et al., 2008).

Under both wild and cultivated conditions, plants often experience a multitude of environmental stresses such as drought, salinity, waterlogging, extremes of temperature, and mineral toxicities and deficiencies. Environmental stresses are undoubtedly a major cause of food insecurity in many countries around the world, in particular, in developing countries where there is a major challenge to produce sufficient food. **(Revenga *et al.*, 2000)**.

2. Materials and methods

Plant materials:

Pure lines and uniform grains of three local cultivars of wheat plant (*Triticum aestivum* L.)

- **Sakha-93**
- **Gemiza-7**
- **Gemiza-9**

These cultivars were obtained from Wheat Research Department, Agricultural Research Center, Ministry of Agriculture, Egypt.

Chemical materials:

Three types of chemical materials were used,

- Tryptophan (C6H4NH.CH:C) at 50 and 100 mgl^{-1} and Cysteine (C3H7NO2S) at 100 and 150 mgl^{-1} concentrations, as Amino Acids.

- Ascorbic Acid (Vitamin C) ($C_6H_8O_6$) at 50 and 100 mgl^{-1} and Thiamine (Vitamin B1) at 50 and 100 mgl^{-1} concentrations, as vitamins.
- Baking Yeast (*Saccharomyces cerevisiae*) extract at 1000 and 2000 mgl^{-1} concentrations .

Methods:

Field experiment was carried out at the experimental farm of National Research Center at Nubaria region to study the effect of five bio-regulators on fractionation and characterization of grain storage proteins of three local cultivars of wheat Sakha-93, Gemiza-7 and Gemiza-9. The experiment was carried out under sandy soil conditions. Grains of the three wheat varieties were collected from different bio-regulator treatments. The molecular weight for homogeneous grains were determined by Sodium dedocyl sulphate - Polyacrylamide gel electrophoresis **(Laemmli, 1970)** using Bio-Rad Mini Protein apparatus. Electrophoregrams for each variety were scored and the presence (1) or absence (0) of each band noted.

3. Results and discussions

SDS-PAGE analysis for Sakha-93 variety:

It is well known that gene(s) acts through production of catalytic protein(s). The expression of gene(s) affected greatly by environment either biotic and/or abiotic effectors. The response of plants to treatments with bio-regulators can be qualitatively detected by the level of gene expression of certain enzymes and can be used successfully in this respect. In this investigation, total proteins were electrophoratically separated from all treated and control plants. Polyacrylamide gel electrophoresis was used for total proteins. The electrophorogram showing proteins banding pattern of different wheat varieties are given in Figures (1, 2 and 3), and total proteins were electrophoratically separated based on molecular weight in (Table 1, 2 and 3).

Different responses appeared as different banding patterns, either by appearance or disappearance of certain bands. The maximum band numbers were eleven, which was not necessarily present in all individual treated plants. The M.S of the electrophoretic products ranged from 10 to 100KDa. All bands were considered as common or monomorphic bands.

Figure (1) and Table (1) show the SDS-PAGE electrophoretic protein patterns of wheat grains cultivar Sakha-93.

The results obtained indicated the occurrence of 14 protein bands, having relative molecular weights ranging from 10 to 100 kDa. seven of these bands represented common proteins (Mws: 16, 18, 29, 40, 50, 60, 100 kDa), occurring in the control as well as with different treatments. The minimum number (8 bands) was recorded in treatment with cysteine at concentration of 100 mgl^{-1} and 50 mgl^{-1} of thiamine. However, except treatment with 50 mgl^{-1} of ascorbic acid which recorded 11 bands, 9 protein bands were assessed in the remainder seeds treated.

The application of ascorbic acid and yeast treatments with two concentrations led to the induction of only one protein having a molecular weight 98KDa. The inducible effect of the different concentrations of the cysteine 79kDa proteins. On the other hand, two proteins (Mws: 54 and 48 kDa) occurred in response to low concentrations used of ascorbic acid (50 mgl^{-1}), whereas, protein band 23KDa was specific for high concentration (100 mgl^{-1}) of thiamine treatment.

The protein band 14KDa induced only in control and treatment with tryptophan at 50 and 100 mgl^{-1}. Whereas, the protein band 10KDa repressed in plant's grains which was treated by cysteine at low concentration (100 mgl^{-1}).

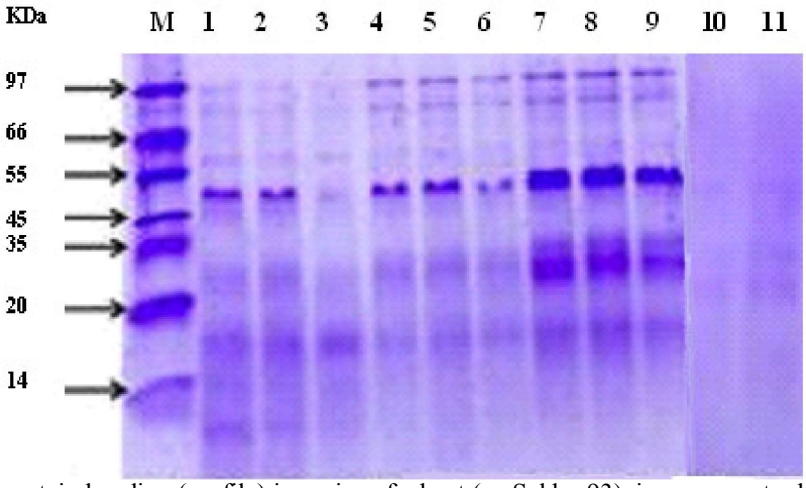

Fig. (1): Changes in protein banding (profile) in grains of wheat (cv Sakha-93), in response to different treatments of various plant bio-regulators.

Lane M refers to low molecular weight standard protein marker Lane 1 refers to control plants sprayed with (distilled water)
Lane 2 refers to plants treated with tryptophan (50 mgl^{-1}) Lane 3 refers to plants treated with tryptophan (100 mgl^{-1})
Lane 4 refers to plants treated with cysteine (100 mgl^{-1}) Lane 5 refers to plants treated with cysteine (150 mgl^{-1})
Lane 6 refers to plants treated with thiamine (50 mgl^{-1}) Lane 7 refers to plants treated with thiamine (100 mgl^{-1})
Lane 8 refers to plants treated with ascorbic acid (50 mgl^{-1}) Lane 9 refers to plants treated with ascorbic acid (100 mgl^{-1})
Lane 10 refers to plants treated with yeast extract (1000 mgl^{-1}) Lane 11 refers to plants treated with yeast extract (2000 mgl^{-1})

Table (1): Protein banding (profile) in grains of wheat (cv. Sakha-93), as affected by different treatments of various plant bio-regulators. (M: protein marker; **1:** control; **2:** 50 mgl^{-1} of tryptophan; **3:** 50 mgl^{-1} of tryptophan; **4:** 100 mgl^{-1} of cysteine; **5:** 150 mgl^{-1} of cysteine; 6: 50 mgl^{-1} of thiamine; **7:** 100 mgl^{-1} of thiamine; **8:** 50 mgl^{-1} of ascorbic acid; **9:** 100 mgl^{-1} of ascorbic acid; **10:** 1000 mgl^{-1} of yeast extract; 11: 2000 mgl^{-1} of yeast extract).

	MW	1	2	3	4	5	6	7	8	9	10	11
1	**100**	+	+	+	+	+	+	+	+	+	+	+
2	**98**	-	-	-	-	-	-	-	+	+	+	+
3	**79**	-	-	-	+	+	-	-	-	-	-	-
4	**60**	+	+	+	+	+	+	+	+	+	+	+
5	**54**	-	-	-	-	-	-	-	+	-	-	-
6	**50**	+	+	+	+	+	+	+	+	+	+	+
7	**48**	-	-	-	-	-	-	-	+	-	-	-
8	**40**	+	+	+	+	+	+	+	+	+	+	+
9	**29**	+	+	+	+	+	+	+	+	+	+	+
10	**23**	-	-	-	-	-	-	+	-	-	-	-
11	**18**	+	+	+	+	+	+	+	+	+	+	+
12	**16**	+	+	+	+	+	+	+	+	+	+	+
13	**14**	+	+	+	-	-	-	-	-	-	-	-
14	**10**	+	+	+	-	+	+	+	+	+	+	+

SDS-PAGE analysis for Gemiza-7 variety:

Figure (2) and Table (2) show the SDS-PAGE electrophoretic protein patterns of wheat grains cultivar Gemiza-7.

The SDS-PAGE electrophoretic protein profiles indicated the occurrence of 11 polymorphic bands, having relative molecular weights ranging from 16 to 100kDa. five of these bands represented common proteins (Mws: 18, 40, 57, 60, 90kDa), that occurred in the control's grains and those with different treatments. The minimum number of bands (7) was shown in the grains collected from control and thiamine treatments with high concentration (100 mgl^{-1}). The maximum number of bands (10) occurred in response to treatments with two concentrations of yeast. The results also indicate the absent of protein band 35KDa in the grains of control only.

The results showed that, except plants treated with high concentration of ascorbic acid (100 mgl^{-1}) and both concentrations of yeast extract (1000, 2000 mgl^{-1}), the protein band 100 KDa was disappeared from all others bio-regulator treatments.

Data also indicated that except of control and tryptophan with their concentrations and low concentration of cysteine, the new protein band having 98KDa was presented in the others treatments. In the contrary, protein band having 16 KDa M.W. was absent from all treatments except control and tryptophan with their two concentrations and low concentration of cysteine. Protein band 54 KDa molecular weight was absented only in plants treated with 100 mgl^{-1} of thiamine. In the contrary, except the low concentration of cysteine (100 mgl^{-1}) and both concentrations of yeast (1000 and 2000 mgl^{-1}) the protein band of 28KDa was disappeared from all treatments in addition the control plants.

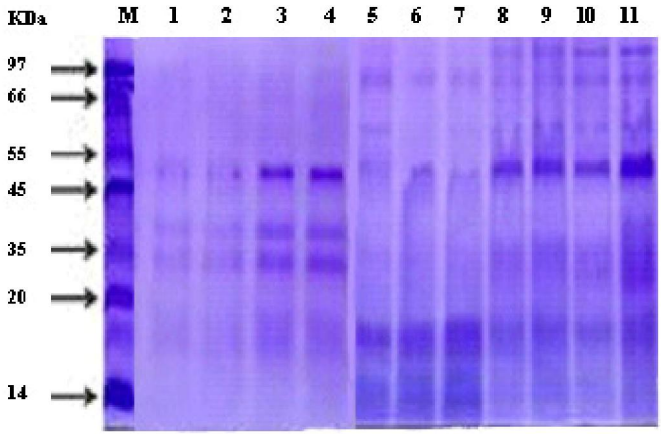

Fig. (2): Changes in protein banding (profile) in grains of wheat (cv Gemiza-7), in response to different treatments of various plant bio-regulators.

Lane M refers to low molecular weight standard protein marker Lane 1 refers to control plants sprayed with (distilled water)

Lane 2 refers to plants treated with tryptophan (50 mgl^{-1}) Lane 3 refers to plants treated with tryptophan (100 mgl^{-1})

Lane 4 refers to plants treated with cysteine (100 mgl^{-1}) Lane 5 refers to plants treated with cysteine (150 mgl^{-1})

Lane 6 refers to plants treated with thiamine (50 mgl^{-1}) Lane 7 refers to plants treated with thiamine (100 mgl^{-1})

Lane 8 refers to plants treated with ascorbic acid (50 mgl^{-1}) Lane 9 refers to plants treated with ascorbic acid (100 mgl^{-1})

Lane 10 refers to plants treated with yeast extract (1000 mgl^{-1}) Lane 11 refers to plants treated with yeast extract (2000 mgl^{-1})

Table (2): Protein banding (profile) in grains of wheat (cv. Gemiza-7), as affected by different treatments of various plant bio-regulators. **(**M: protein marker; **1:** control; **2:** 50 mgl^{-1} of tryptophan; **3:** 50 mgl^{-1} of tryptophan; **4:**100 mgl^{-1} of cysteine; **5:**150 mgl^{-1} of cysteine; **6:** 50 mgl^{-1} of thiamine; **7:** 100 mgl^{-1} of thiamine; **8:** 50 mgl^{-1} of ascorbic acid; **9:** 100 mgl^{-1} of ascorbic acid; **10:** 1000 mgl^{-1} of yeast extract; **11**: 2000 mgl^{-1} of yeast extract).

	MW	1	2	3	4	5	6	7	8	9	10	11
1	**100**	-	-	-	-	-	-	-	-	+	+	+
2	**98**	-	-	-	-	+	+	+	+	+	+	+
3	**90**	+	+	+	+	+	+	+	+	+	+	+
4	**60**	+	+	+	+	+	+	+	+	+	+	+
5	**57**	+	+	+	+	+	+	+	+	+	+	+
6	**54**	+	+	+	+	+	+	-	+	+	+	+
7	**40**	+	+	+	+	+	+	+	+	+	+	+
8	**35**	-	+	+	+	+	+	+	+	+	+	+
9	**28**	-	-	-	+	-	-	-	-	-	+	+
10	**18**	+	+	+	+	+	+	+	+	+	+	+
11	**16**	+	+	+	+	-	-	-	-	-	-	-

SDS-PAGE analysis for Gemiza-9 variety:

The SDS-PAGE electrophoretic protein profiles for cultivar three (Gemiza-9) indicated the occurrence of 12 polymorphic bands, having relative molecular weights ranging from 10 to 90 kDa. seven of these bands represented common proteins (Mws: 15, 18, 20, 35, 54, 57, 60 kDa), that occurred in the control's grains and those with different treatments. The minimum number of bands (9) was shown in the grains collected from plants treated with 100 mgl^{-1} of tryptophan, cysteine and ascorbic acid. The maximum number of bands (12) occurred in response to treatments with low concentration of tryptophan (50 mgl^{-1}). The results also indicate the absence of protein band 50KDa in the control plants only.

The results showed that, the protein band 90 KDa was disappeared from all others bio-regulator treatments except low concentration of tryptophan in addition the control.

Data also indicated that, the protein band having M.W. of 45KDa was disappeared in the grains treated with high concentration of tryptophan (100 mgl^{-1}) and both concentrations of cysteine (100 and 150 mgl^{-1}). Also the protein band having M.W. of 28 KDa was absent only from ascorbic acid treatment at concentrations 100 mgl^{-1}. On the other hand, protein band having 10KDa was absent in grains collected from plants treated with 100 mgl^{-1} of tryptophan, 100 mgl^{-1} of cysteine, 100 mgl^{-1} of thiamine and both concentrations of ascorbic acid (50 and 100 mgl^{-1}).

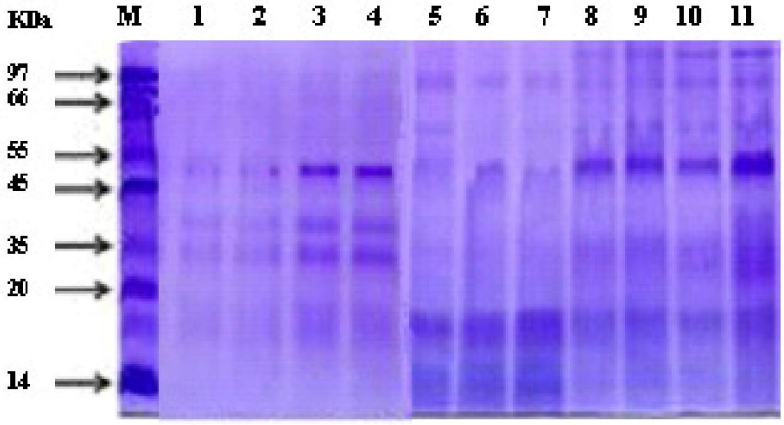

Fig. (3): Changes in protein banding (profile) in grains of wheat (cv Gemiza-9), in response to different treatments of various plant bio-regulators.

Lane M refers to low molecular weight standard protein marker Lane 1 refers to control plants sprayed with (distilled water)

Lane 2 refers to plants treated with tryptophan (50 mgl^{-1}) Lane 3 refers to plants treated with tryptophan (100 mgl^{-1})

Lane 4 refers to plants treated with cysteine (100 mgl^{-1}) Lane 5 refers to plants treated with cysteine (150 mgl^{-1})

Lane 6 refers to plants treated with thiamine (50 mgl^{-1}) Lane 7 refers to plants treated with thiamine (100 mgl^{-1})

Lane 8 refers to plants treated with ascorbic acid (50 mgl^{-1}) Lane 9 refers to plants treated with ascorbic acid (100 mgl^{-1})

Lane 10 refers to plants treated with yeast extract (1000 mgl^{-1}) Lane 11 refers to plants treated with yeast extract (2000 mgl^{-1})

Table (3): Protein banding (profile) in grains of wheat (cv. Gemiza-9), as affected by different treatments of various plant bio-regulators. **(**M: protein marker; **1:** control; **2:** 50 mgl^{-1} of tryptophan; **3:** 50 mgl^{-1} of tryptophan; **4:** 100 mgl^{-1} of cysteine; **5:** 150 mgl^{-1} of cysteine; **6:** 50 mgl^{-1} of thiamine; **7:** 100 mgl^{-1} of thiamine; **8:** 50 mgl^{-1} of ascorbic acid; **9:** 100 mgl^{-1} of ascorbic acid; **10:** 1000 mgl^{-1} of yeast extract; **11**: 2000 mgl^{-1} of yeast extract).

	MW	1	2	3	4	5	6	7	8	9	10	11
1	**90**	+	+	-	-	-	-	-	-	-	-	-
2	**60**	+	+	+	+	+	+	+	+	+	+	+
3	**57**	+	+	+	+	+	+	+	+	+	+	+
4	**54**	+	+	+	+	+	+	+	+	+	+	+
5	**50**	-	+	+	+	+	+	+	+	+	+	+
6	**45**	+	+	-	-	-	+	+	+	+	+	+
7	**35**	+	+	+	+	+	+	+	+	+	+	+
8	**28**	+	+	+	+	+	+	+	+	-	+	+
9	**20**	+	+	+	+	+	+	+	+	+	+	+
10	**18**	+	+	+	+	+	+	+	+	+	+	+
11	**15**	+	+	+	+	+	+	+	+	+	+	+
12	**10**	+	+	-	-	+	+	-	-	-	+	+

In this respect **Ma *et al.*, (1994)** reported that, plant growth regulators (PGR) have potential to increase grain yield and may also alter grain protein levels of cereal crops. On the other hand, **Shuaib *et al.*, (2007)** reported that, seed storage protein profiles could be useful markers in the studies of genetic diversity and classification of adapted cultivars, thereby improving the efficiency of wheat breeding programs in cultivar development especially in a developing country. Also **Abedi *et al.*, (2010)** demonstrated that, The SDS-PAGE was performed to investigate differences between proteins banding pattern in different growth stages of wheat under different levels of N and compost.

Kamel *et al.*, (2011) assessed the genetic diversity among five Egyptian bread wheat genotypes (Misr1 and Sids 13 cultivars and the promising lines No.1, No.2 and No.11) using SDS-PAGE and RAPD markers and found that, the total number of SDS-bands was seven. Six SDS- bands were monomorphic while the other was polymorphic. Line-1 was characterized by the presence of band -3 with a molecular weight of 41.56 kDa. RAPD analysis showed that the number of polymorphic amplicons was 66 out of a total of 93 amplicons, thus revealing a level of 70.97 % polymorphism. The highest genetic similarity revealed by RAPD analysis (93.1%) was between Misr1 and Line 2 genotype. While, the lowest similarity (85.2 %) was between Line 1 and Line 2. The dendrogram separated Line1 from all the other genotypes. The four genotypes constituted a subcluster divided into two groups, one group composed of Misr 1 and Line 2, while the second group comprised Sids 13 and Line 11.

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