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Accumulation of Osmolytes and Stress Proteins, Alteration of Levels of Gene Expressions and DNA Profiles in Two Wheat Cultivars as a Response to Amelioration of pH Stress by IAA

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Abstract: Grains of two wheat (*Triticum vulgare* L.) cultivars (Gemmiza 7 and Giza 164) previously presoaked in phosphate buffer at different pHs, as well as an optimum concentration of IAA, were sown and grown at the green house to the M2 grains in order to study the possible adaptive mechanisms to ameliorate the pH stress on osmolytes (proline and betaine as chemical chaperones), stress proteins (molecular chaperones), alteration of levels of gene expression (mRNA and proteins) and DNA profiles. The results revealed that plants under acidic pH have an obvious effect on total proteins, RNA and DNA macromolecules. Cultivar Giza 164 plants accumulated higher levels of proline than Gemmiza 7 under pH stress. In both cultivars betaine and the level of mRNA increased with pH stress and the expression was coincided with the observed betaine accumulation. The yields of poly(A)+ RNA were in the range of 0.5-1.5%±1 in Cv. Giza 164 as compared to that of Gemmiza 7 (0.3-1.1%±0.7). Exogenous application of IAA can stimulate resistance against the unfavourable environmental pH value and improve plant under stress by developing various mechanisms, which include increase in both the soluble protein, betaine and proline that acts as a free radical scavenger and limits the cytoplasmic acidification, quantitative and qualitative increase in mRNA and low molecular weight proteins (9-29 kDa) that play an important role in the readjustment of plant cell's osmotic potential and protecting cytoplasmic enzymes. RAPD analysis was effective in detecting informative qualitative and quantitative changes in both wheat cultivars as a response to pH stress and hormonal application. The thirty used different primers have different performances in detection of genetic changes. The number of amplified fragments generated ranges between 10 and 22 with molecular size ranges between 50 and 2130 bp. Quantitative changes by one common band were shown in stressed Giza 164 samples using thirteen primers as compared to nine primers in Gemmiza 7 stressed samples. Qualitative changes were recorded using all primers (thirty) in Gemmiza 7 and in Giza 164 using sixteen primers only. These changes were found to be reproducible when repeated at different times under the same amplification conditions. The expression of many proteins is known to be regulated by biotic and abiotic stresses, suggesting the occurrence of complex mechanisms that control gene expression in response to environmental stresses. The disappearance of some protein bands may be attributed to the alteration of their structural genes and RNA transcripts. The results of this study suggest that an adaptive mechanism has been developed by *Triticum vulgare* L. cultivars (Gemmiza 7 and Giza 164) in response to exogenous hormonal application under acidic pH stress. Also, the biochemical and molecular criteria which may affect on crop yield productivity and quality were improved. The synthesis of chemical and molecular chaperones to maintain osmotic adjustment is the main strategy that has evolved to maintain growth. The difference in the ability to maintain osmotic potential under pH conditions between the two cultivars reflects the differences in their genetic background.

Key words: Wheat cultivars, Gemmiza 7, Giza 164, pH stress, IAA, molecular chaperones, chemical osmolytes, RNA, DNA profiles, gene expression

INTRODUCTION

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50%[1]. The ability of plants to survive and maintain growth under saline conditions is known as salt tolerance. This is a

variable trait that is dependent on many factors including the plant species. There is a continuous spectrum of plant tolerance to saline conditions ranging from glycophytes that are sensitive to salt to halophytes that survive under very high salt concentration[2,3]. Halophytic plants are tolerant to salinity because they are able to take up water by maintaining a high osmotic potential through the accumulation of inorganic ions[4]. The ability of plants to survive under high salt conditions is important for the ecological distribution of plant species and agriculture in semi arid, arid, and salinized regions.

The adaptation to saline stress is accompanied by alterations in the levels of numerous metabolites, proteins and mRNA[5]. Concomitant to induced stress tolerance, the protein metabolism of the cells undergoes pronounced changes in terms of acquiring specific stress proteins which are either not detected, or else present in low amounts, in un-induced cells. Excellent progress has been made towards understanding the structure, function and regulation of stress proteins which are expressed in response to stresses. Stress-induced changes in gene expression have been reported for different biological systems. The levels of betaine and other osmoprotectants typically rise during exposure to stresses such as salinity, water deficit, and low temperature because the biosynthetic enzymes are stress induced[6]. Osmoprotectants are largely confined to the cytoplasm (including the organelles) and are almost absent in the vacuole[7]. Various genes which expression is activated in response to stress factors have been identified[8,9]. While some of these genes encode for protective proteins such as the osmotin, Late Embrogenesis Adundant (LEA) proteins and ion transporters[10], others code for enzymes that participate in metabolic processes specifically triggered by stress[11]. Stress is accompanied by changes in endogenous hormones which in turn perform changes in the related physiological process. However, plants can become acclimated to various stress factors by developing tolerance against the stress factor that induced the change through the activation of many genes[12,13]. The expression of most these genes is regulated by abscisic acid (ABA), a hormone produced in response to stresse factors[14]. Exogenous application of hormones can stimulate resistance against unfavourable environmental factors and improve plant growth and yield under stress[15].

The development of PCR technique is a milestone in genome analysis[16]. PCR was originally conceived as a technique for detection base changes in the genome, as a tool for DNA diagnosis of genetic diseases. In the last few years, several assays to reveal DNA polymorphism at multialellic loci have been developed in the field of PCR. Randomly amplified polymorphic DNAs (RAPDs) is essentially the most common technique used by molecular biologists. RAPD technique requires only the presence of a single 'randomly chosen' oligonucleotide. Individual RAPD primers are able to hybridize to several hundred sites within the target DNA, however, not all of these hybridizations lead to the production of PCR fragments. The ability of RAPDs to produce multiple bands using a single primer means that a relatively small number of primers can be used to generate a very large number of fragments. These fragments are usually generated from different regions of the genome and hence multiple loci may be examined very quickly[17]. The power of RAPD is that it is a fast technique, easy to perform and comparatively cheap. It is immediately applicable to the analysis of most organisms because universal sets of primers are used without any need for prior sequence information[18]. This marker system was used in many different applications involving the detection of DNA sequence polymorphisms, mapping in different types of populations[19], isolation of markers linked to various traits or specific targeted intervals[20,21] and applications such as variety identification and analysis of parentage[22]. The RAPD technology, however, has some limitations. RAPD markers are in general dominant, thereby they have a lower information content than codominant markers in the linkage analysis of F2 populations[23]. Penner *et al.*[24] reported on difficulties in obtaining identical band patterns from the same set of primers and materials among different laboratories.

Wheat is one of the most important economic crops for human nutrition in Egypt and all over the world. Using two wheat cultivars plants as models, this study examined the biochemical and molecular differences in the pH tolerance mechanisms with the aim of improving the pH tolerance, yield productivity and quality by exogenous application of the hormone (IAA).

MATERIALS AND METHODS

Plant material: Grains of two wheat (*Triticum vulgare* L.) cultivars (Gemmiza 7 and Giza 164) were brought from the Agricultural Research Center (ARC), Giza, Egypt. The grains were surface sterilized with 1% sodium hypochlorite for 20 min, then rinsed with distilled water several times. A range of pH (2-12) and IAA (0.5-3 μM) treatment were used as a preliminary experiment, the highly acidic and slightly alkaline showed lethal effects were neglected. Then three pHs were chosen (5, 7 and 9). Equal groups of grains (Gemmiza 7 and Giza 164) were soaked in phosphate buffer alone at different pHs or in combination with the optimum concentration of IAA

(1 μM) for 24 h. The grains were sown in pots containing garden soil having the same pH, then irrigated at 80% water saturation capacity, then left to grow at the natural day conditions. Samples were left until the yield maturation. Grains were collected and planted to give the M2 grains for analysis.

Measurement of proline and betaine: Ten randomly chosen dry M2 grains of each cultivar were milled separately to fine powder under liquid nitrogen. 0.5 g of mixed powder was transferred to vials and subjected to methanol extraction, and stored in the dark at 4°C. The methanol extracts were passed through Dowex-1-H and Dowex-50-H^+ columns. Betaine was eluted with 6 M NH_4OH and then analyzed by HPLC (Gulliver System, Jasco Co., Tokyo, Japan) with a Shodex NH_2 P-50-4E column. Proline was eluted with 2 N HCl and analyzed by the ninhydrin method[25].

RNA analysis: Grains of each M2 samples were germinated in the greenhouse to mid-tillering stage (20 cm long). Leaves of ten plants were randomly chosen from each cultivar, and were harvested. Total RNA was isolated from the leaves of pH stressed plants using the methods described by Chrigwin *et al.*[26]. Promega's PolyATtract® System was used to quickly purify large amounts of undegraded mRNA from total RNA using streptavidin coupled paramagnetic particles (SA-PMPs) to bind biotinylated oligo (dT):mRNA hybrids[27]. Samples (three replicates per treatment were used for analysis) of 20 μg each (to ensure equal loading) were electrophoretically separated using non-denaturing agarose gel analysis (1.4% agarose) in TBE buffer (pH 8.0) containing 0.5 μg mL^{-1} ethidium bromide and analysed.

Protein analysis: Ten randomly chosen dry M2 grains of each cultivar were milled separately to fine powder under liquid nitrogen. 0.5 g of mixed powder was reconstituted in 1 mL of PSA, pH 7.2, containing 0.1 mM PMSF and cleared by centrifugation at 9000 x g for 5 min at 4°C. Protein content in the extracts was determined using a Protein Assay Kit (Bio-Rad, USA). Extracts were stored at -20°C. Samples (10 μg) of each extract were separated by 12.5% SDS-PAGE[28] in minigels, stained , destained and documented by photography. Data were analyzed and identified by Gel Documentation System (GDS) which comparing polypeptide maps on the basis of band intensity, molecular weight and the rate of mobility of each polypeptide with standard markers using Gel Proanlyzer Version 3 Media Cyberene Tice Imaging Experts Software. Scoring of protein bands was made for each cultivar and treatment yielded a clear pattern as present (1) or absent (0).

Table 1: The codes, sequences and GC % of the thirty used reproducible primers, total number of amplified fragments and polymorphic bands generated in M2 seedlings of two wheat (*Triticum vulgare* L.) cultivars (Gemmiza 7 and Giza 164) whose parents previously exposed to various treatments of phosphate buffer at different pHs and IAA

Primer				Total amplified	Polymorphic
No.	Code	Sequence 5`→3`	GC%	fragments	fragments
1	OPD-01	ACCGCGAAGG	70	12	6
2	OPD-02	GGACCCAACC	70	14	6
3	OPD-05	TGAGCGGACA	60	20	8
4	OPD-09	CTCTGGAGAC	60	16	6
5	OPD-12	CACCGTATCC	60	18	12
6	OPE-03	CCAGATGCAC	60	17	9
7	OPE-05	TCAGGGAGGT	60	14	11
8	OPE-08	TCACCACGGT	60	20	10
9	OPE-11	GAGTCTCAGG	60	10	8
10	OPE-15	ACGCACAACC	60	12	5
11	OPE-20	AACGGTGACC	60	15	11
12	OPJ-01	CCCGGCATAA	60	22	18
13	OPJ-03	TCTCCGCTTG	60	12	9
14	OPJ-05	CTCCATGGGG	70	14	11
15	OPJ-08	CATACCGTGG	60	20	9
16	OPJ-12	GTCCCGTGGT	70	14	11
17	OPJ-15	TGTAGCAGGG	60	20	13
18	OPJ-17	ACGCCAGTTC	60	14	8
19	OPJ-18	TGGTCGCAGA	60	22	11
20	OPJ-19	GGACACCACT	60	20	10
21	OPJ-20	AAGCGGCCTC	70	12	9
22	OPK-01	CATTCGAGCC	60	18	6
23	OPK-05	TCTGTCGAGG	60	10	6
24	OPK-06	CACCTTTCCC	60	14	12
25	OPK-07	AGCGAGCAAG	60	15	11
26	OPK-08	GAACACTGGG	60	19	12
27	OPK-09	CCCTACCGAC	70	16	12
28	OPK-10	GTGCAACGTG	60	12	10
29	OPK-11	AATGCCCCAG	60	14	8
30	OPK-12	TGGCCCTCAC	70	16	12
		Total		472	290

DNA analysis: Grains of each M2 samples were germinated in the greenhouse to mid-tillering stage (20 cm long). Leaves of ten plants were randomly chosen from each cultivar and were harvested. Total genomic DNA was extracted from young leaves of fresh seedlings using the standard method of Sambrook *et al.*[29]. DNA was quantitated and qualitated by spectrophotometry and agarose gel electrophoresis (0.5%).

Polymerase chain reaction amplifications were performed using a PJ2000 Thermal Cycler (Perkin Elmer, Norwalk, Connecticuts, USA) with standard optimized control. Each PCR reaction was repeated three times to ensure repeatability of the amplification patterns. DNA was amplified according to Williams *et al.*[22] using thirty arbitrary primers (Operon Tech., Kits D, E, J and K, USA) (Table 1). RAPD-PCR conditions were optimized for annealing temperatures (33-36°C), numbers of thermal cycles (30-45), DNA template concentration (1-50 ηg), and magnesium chloride concentrations (1.5-6 mM). The optimal conditions for RAPD-PCR were as follows: PCR was carried out in a volume of 25 μL containing 1x PCR

buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.0, 0.1% gelatin), 20 ηg DNA, 40 pmol primer, 100 μM dNTPs mix, 2 mM $MgCl_2$ and 2 U Taq polymerase. The thermal cycling conditions were as follows: 10 min at 94°C (one cycle, hot start), 40 cycles (30 sec at 94°C, 45 sec at 36°C and 45 sec at 72°C) and a final one cycle post-extension of 7 min at 72°C.

The amplified PCR products were electrophoresed on an agarose (2 %) (DNA grade, Sigma) in 1x TAE buffer. Electrophoresis was performed for 1 h at 80 V in submarine electrophoresis cell (Pharmacia). Gels were stained with ethidium bromide for visualization of bands, examined by Gel Doc 2000™ Gel Documentation System (Bio Rad) and video printing of gel image. The RAPD-gels were scanned and analyzed using Gel Pro-Analyzer as in protein analysis. The size of the amplified products was estimated from the gel by comparison to a standard DNA molecular size marker. Scoring of bands was made for each cultivar, treatment and primer that yielded a clear pattern as present (1) or absent (0).

RESULTS AND DISCUSSION

Osmolytes or compatible solutes (glycine, betaine, glycerol, proline and trehalose), are a group of low-molecular-weight organic compounds that accumulate in organisms in response to osmotic stress. From Table 2, it is clear that Cv.Giza 164 plants accumulate higher levels of proline under pH stress conditions than Cv. Gemmiza 7 plants. However, Cv. Gemmiza 7 plants exhibited higher betaine content than Cv.Giza 164. The accumulation of these osmolytes represents an important adaptive response to such stress[5]. In higher plants, the pathway of betaine synthesis is short and straightforward: choline monooxygenase (CMO) converts choline to betaine aldehyde and betaine aldehyde dehydrogenase converts this product to betaine. Accumulation of BADH transcripts was a common response to changes in osmotic stress regardless of the cause[30,31]. In the present study, the level of mRNA under different pH conditions increased due to pH stress and the expression was coincided with the observed betaine accumulation. As with betaine accumulation under pH conditions, the activity of betaine enzymes was regulated by pH stress. These results are consistent with the findings reported by several investigators using other plants[32,33]. It is well known that osmotic adjustment involves the net accumulation of solutes in a cell in response to stress. Consequently, the osmotic potential decreases, which in turn attracts water into the cell and enables turgor to be maintained. The observed difference in øs and osmotic adjustment seemed to be related to the accumulation of betaine and proline.

The total protein, RNA and DNA contents of control wheat grains as well as those subjected to various treatments are represented in Table 2. The contents in Cv. Giza 164 are greater than those of Gemmiza 7 which indicates that Giza 164 cultivar is more tolerant than Gemmiza 7. Moreover, addition of IAA at the optimal level (1 μM) has a stimulator effect on such parameters which indicates that the inhibitory action of pH stress could be ameliorated by hormonal application. The present results showed a similar tendency to that by Gao *et al.*[34], who found that the protein content in tobacco and soybean decreased by 2.6±15.8% after being treated with pH 2.0. The low and high pH could inhibit the protein synthesis in plants because some metals might be binded with DNA and the nitrogen metabolism could be obstructed. When plants subjected to various form of stresses, their endogenous hormonal level are undergone various and rapid changes[35]. Steward [36] reported that auxins, gibberellins and cytokinins stimulate the synthesis of nucleic acids and inhibit the activities of RNase, DNase and 3-nucleotidase. Botia *et al.*[37] reported that elongation of etiolated hypocotyl segments and contents of nucleic acids (5 mm long) of lupinus (*Lupinus albus* Cv. Multolupa) were stimulated and increased by acid pH (4.5 vs 6.5) and by IAA for periods of upto 4 h. After this time, the segments were unable to grow further. In the presence of an optimal IAA concentration (10 μM), acid pH increased the growth rate but had no effect on final growth. With sub-optimal IAA (0.1 μM), however, acid pH increased growth and total nucleic acids in a more than additive way, suggesting a synergistic action between the two factors. This synergism could be explained by the increased IAA uptake and decarboxylation seen at an acid pH. Vanadate inhibited growth and IAA uptake and decarboxylation suggesting that this inhibitor probably inhibits growth not only by decreasing ATPase-mediated acidification but also by decreasing H^+-dependent IAA uptake from the apoplast. Also, the dependence of IAA uptake on ATPase may be mediated by apoplastic acidification. The amount of IAA decarboxylated increased when the assay conditions favoured the growth of segments indicating that IAA could be destroyed by decarboxylation during the auxin-induced growth.

The level of mRNA under different pH conditions increased due to pH stress and the expression was coincided with the observed betaine accumulation. The yields of poly(A)+ RNA were in the range of 0.5-1.5%±1 in Cv. Giza 164 as compared to that of Gemmiza 7 (0.3-1.1%±0.7). Fine regulation of gene expression is

Table 2: Analysis of total protein, DNA, RNA, proline and betaine accumulation in M2 grains of two wheat (*Triticum vulgare* L.) cultivars (Gemmiza 7 and Giza 164) under pH stress and IAA

Cultivar	Treatment		Total protein (μg g^{-1} wt.)	Total DNA (mg/100 g wt.)	Total RNA (mg/100 g wt.)	Proline (mg/g wt.)	Betaine (μg g^{-1} wt.)
Gemmiza 7	H_2O		5.33	210	550	0.66	1.21
	pH	5	8.12**	280**	630**	0.71	2.16*
		7	7.23**	310**	680**	0.74	3.01**
		9	9.51**	335**	710**	0.83**	3.86**
	IAA		6.88	240*	696**	0.74	1.62
	pH +IAA	5	10.11**	311**	730**	0.87**	3.17**
		7	9.54**	342**	720**	0.89**	3.88**
		9	12.22**	365**	686**	0.93**	4.50**
Giza 164	H_2O		5.10	218	506	0.52	1.44
	pH	5	6.11	260*	611*	0.76*	2.18*
		7	6.88	316**	632**	0.82**	3.22**
		9	7.11*	372**	661**	0.73*	4.15**
	IAA		7.20*	397**	655**	0.80**	3.56*
	pH+IAA	5	8.14**	365**	681**	0.93**	4.12**
		7	8.33**	374**	692**	0.89**	4.88**
		9	10.56**	391**	698**	1.11**	4.18**

*Significant, **Highly significant

Table 3: Comparative analysis of relative concentrations (band %), molecular weight (M.wt.) and mobility rate (R_m) of the different types of protein bands in M2 grains of wheat (*Triticum vulgare* L.) cultivar (Gemmiza 7) whose parents previously exposed to various treatments of phosphate buffer at different pH and IAA

	Band %									
Band No.	1	2	3	4	5	6	7	8	R_m	M. wt. (kDa)
1	1.18	2.17	3.11	2.77	3.16	6.88	-	1.12	0.08	202.00
2	7.11	-	6.06	4.61	2.16	-	8.17	-	0.10	199.11
3	-	2.11	7.40	-	-	5.60	-	5.50	0.12	188.16
4	5.52	3.50	6.40	8.11	4.16	7.11	8.12	9.11	0.13	170.55
5	-	4.50	5.60	6.70	-	-	1.10	2.10	0.15	165.16
6	2.10	1.40	-	-	-	-	-	-	0.17	159.11
7	-	-	-	-	-	-	-	6.14	0.18	141.50
8	-	2.65	-	-	-	-	-	4.88	0.22	116.11
9	4.16	1.11	2.50	-	5.10	3.14	-	-	0.25	110.16
10	2.18	12.50	2.16	6.11	7.14	-	3.21	-	0.28	92.11
11	-	2.44	1.70	-	-	1.77	-	1.66	0.30	88.52
12	-	3.61	1.46	5.11	-	1.76	-	2.76	0.31	80.10
13	1.17	4.15	-	-	14.50	-	3.18	2.11	0.32	73.15
14	2.11	-	-	4.87	-	2.89	7.17	8.16	0.36	71.10
15	1.17	-	4.50	-	6.70	-	2.11	-	0.38	66.12
16	-	1.15	-	2.17	-	3.16	3.74	1.12	0.40	59.10
17	-	2.13	-	-	8.13	-	-	-	0.42	57.50
18	2.50	1.70	-	-	-	-	3.88	-	0.45	55.10
19	6.10	4.60	2.11	8.10	6.11	7.10	8.11	6.32	0.48	50.11
20	3.44	2.63	-	2.14	-	-	-	14.50	0.49	47.11
21	-	1.26	-	3.68	4.68	2.13	2.36	3.77	0.50	45.91
22	2.15	2.39	-	-	-	1.24	4.15	-	0.55	39.11
23	-	2.15	-	5.11	2.50	-	3.22	-	0.58	34.16
24	8.11	2.71	7.14	-	-	-	5.11	2.42	0.61	29.56
25	3.16	-	5.20	1.10	2.70	11.06	10.50	2.68	0.63	29.10
26	-	8.60	-	2.13	-	7.20	6.33	2.35	0.65	26.15
27	-	1.98	3.11	2.16	-	4.65	3.26	-	0.68	22.16
28	-	2.87	-	1.48	-	-	-	3.07	0.70	18.11
29	-	3.58	1.66	-	2.16	-	1.50	-	0.72	16.55
30	2.33	-	1.54	2.08	-	1.87	-	3.11	0.74	15.66
31	-	-	-	5.50	2.17	2.68	-	-	0.76	14.11
32	-	5.10	-	1.14	2.50	-	4.88	2.59	0.78	13.50
33	-	2.65	-	-	4.16	-	-	-	0.80	12.55
34	4.08	-	2.10	1.98	1.72	2.06	1.46	2.15	0.82	11.66
35	-	3.10	-	-	2.07	-	-	-	0.83	11.10
36	6.11	5.18	5.38	-	-	4.25	-	3.12	0.84	10.12
37	-	3.25	-	-	4.12	6.23	2.13	2.50	0.86	9.50
Total	18.00	29.00	18.00	20.00	19.00	19.00	21.00	23.00		

Lane 1: control (H_2O), Lane 2: pH 5, Lane 3: pH 7, Lane 4: pH 9, Lane 5: IAA, Lane 6: pH 5 + IAA, Lane 7: pH 7 + IAA, Lane 8: pH 9 + IAA

Table 4: Comparative analysis of relative concentrations (band %), molecular weight (M.wt.) and mobility rate (R_m) of the different types of protein bands in M2 grains of wheat (*Triticum vulgare* L.) cultivar (Giza 164) whose parents previously exposed to various treatments of phosphate buffer at different pHs and IAA

	Band %									
Band No.	1	2	3	4	5	6	7	8	R_m	M. wt. (kDa)
1	-	-	-	-	3.25	-	-	3.25	0.11	198.23
2	2.69	2.50	1.30	3.25	2.87	2.87	4.20	1.68	0.12	196.16
3	3.69	-	-	2.69	-	-	-	2.80	0.13	191.26
4	3.25	2.55	1.50	-	-	2.36	-	-	0.14	177.25
5	-	-	-	-	3.66	-	2.58	1.23	0.15	170.25
6	5.24	-	2.56	-	1.65	-	1.69	-	0.17	166.23
7	2.50	4.20	3.25	4.58	3.69	6.58	4.21	5.82	0.20	152.36
8	-	-	-	2.58	2.36	-	1.25	3.68	0.21	125.68
9	3.65	4.25	3.66	-	-	5.26	-	4.39	0.24	120.58
10	-	2.50	-	1.63	-	-	2.58	-	0.26	98.57
11	-	-	-	2.69	-	-	3.69	2.85	0.28	90.25
12	2.30	1.25	-	-	1.36	-	-	2.58	0.30	82.35
13	1.36	2.30	-	-	-	-	-	3.69	0.32	74.25
14	1.36	-	2.50	3.25	1.58	1.96	2.47	-	0.35	71.98
15	-	3.67	-	-	6.23	-	-	8.26	0.38	65.28
16	9.50	-	-	-	11.30	-	-	12.20	0.40	60.27
17	-	2.30	3.60	2.34	1.24	1.25	2.68	1.25	0.41	55.69
18	3.20	4.25	4.29	3.68	1.28	8.25	3.66	2.69	0.45	54.28
19	-	3.25	1.25	-	2.36	-	-	1.25	0.46	48.58
20	-	-	2.36	1.25	2.87	-	-	1.26	0.49	47.50
21	4.25	8.36	4.22	4.58	-	-	-	5.32	0.51	42.52
22	2.31	1.22	-	-	-	5.36	-	3.55	0.53	38.87
23	6.35	3.25	3.58	2.87	3.96	1.25	1.87	-	0.56	33.58
24	-	-	-	-	5.25	-	-	-	0.60	28.58
25	-	-	-	-	4.25	-	-	3.65	0.63	27.96
26	4.81	3.25	-	-	5.36	-	-	-	0.64	24.74
27	-	2.20	3.25	3.58	1.20	4.25	8.25	2.87	0.68	21.55
28	5.20	-	-	-	-	-	-	-	0.70	19.50
29	1.25	3.69	2.35	-	1.27	-	-	3.66	0.73	15.20
30	-	3.22	4.22	3.46	4.58	7.24	6.35	2.25	0.74	14.28
31	-	1.20	1.58	1.87	1.99	2.50	1.87	1.10	0.75	13.25
32	6.20	-	-	-	3.58	-	-	2.50	0.78	12.52
33	2.50	-	-	-	-	-	-	-	0.80	10.24
34	-	3.33	2.85	5.28	6.11	3.12	4.00	2.80	0.81	9.50
35	2.87	2.55	-	4.22	-	-	-	3.66	0.83	8.20
Total	20.00	22.00	17.00	17.00	24.00	13.00	15.00	26.00		

Lane 1: control (H_2O), Lane 2: pH 5, Lane 3: pH 7, Lane 4: pH 9, Lane 5: IAA, Lane 6: pH 5 + IAA, Lane 7: pH 7 + IAA, Lane 8: pH 9 + IAA

required for normal growth and development, as well as for stress adaptation. Although research has focused on regulation that is exerted at the transcriptional level, post-transcriptional control of gene expression is now emerging as a pivotal check point. mRNA stability is one of the major post-transcriptional regulation mechanisms. Changes in gene expression at the transcriptional and post-transcriptional levels were initially demonstrated by analysis of protein profiles elicited in plants using stress factors. These studies revealed both qualitative and quantitative changes in the pattern of polypeptides synthesized following stress[38]. Urao *et al.*[39] reported that, some of the stress-responsive genes are those that encode proteins involved in the regulation of other stress-responsive genes and that these regulatory genes are mostly transacting factors. Concerning the protein profile in this study, 35 and 37 bands were separated in Cv. Giza 164 and Gemmiza 7, respectively, of molecular weights ranged between 8.20 and 202.00 kDa. The most pronounced increment was at the lower molecular weight (11-29 kDa) in all treatments as being compared with the control (Tables 3 and 4). However, the changes seem to be quantitative especially at 50 and 170 kDa. Exogenous application of IAA induced quantitative and qualitative increase in low molecular weight proteins (9-29 kDa) that play an important role in the readjustment of plant cell's osmotic potential and protecting cytoplasmic enzymes. These results are confirmed by those obtained by Claes *et al.*[40], who found that, at least nine proteins has been recorded in roots of rice plants exposed to stress factors and one of these proteins (salt protein, 14.5 kDa) has been isolated and characterized. Wang *et al.*[41] reported that, abiotic stresses usually cause protein dysfunction. Maintaining proteins in their functional conformations and preventing the aggregation of non-native proteins are particularly important for cell survival under stress. Additional studies have reported the induction of the stress protein in response to abiotic

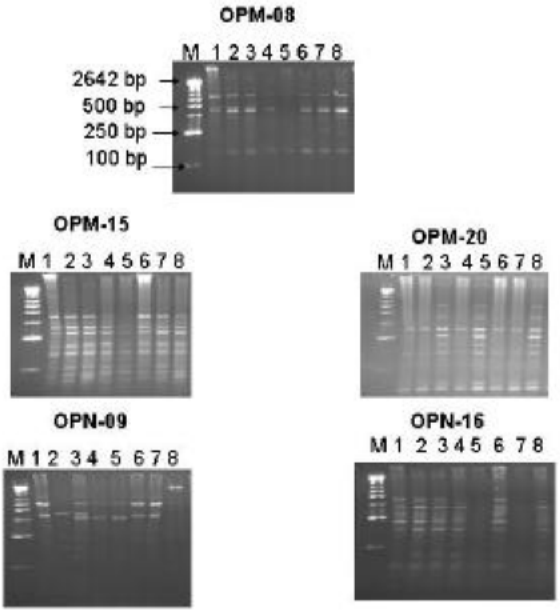

Fig. 1: RAPD fingerprints detected in M2 seedlings of two wheat (*Triticum vulgare* L.) cultivars (Gemmiza 7 and Giza 164) whose parents previously treated with phosphate buffer at different pH values and IAA concentrations using some decamer primers (OPM-08, -15 and -20 and OPN-09 and -16). Lane M: DNA molecular size marker, lane 1: control (H_2O), lane 2: pH 5, Lane 3: pH 7, lane 4: pH 9, lane 5: IAA, lane 6: pH 5 + IAA, lane 7: pH 7 + IAA and lane 8: pH 9 + IAA

factors, ABA and others hormones[42]. More recently, El-Khawas[43], who found that the addition of IAA can improve tolerance to pH stress by the accumulation of low molecular weight proteins in Sids 1 wheat cultivar treated with acidic pHs. Hirano *et al.*[44] and Zhang *et al.*[45] provided biochemical evidence demonstrating that the stress protein may exhibits mannose-binding lectin activity. An increasing number of studies suggest that the molecular chaperones (that responsible for protein folding, assembly, translocation and degradation in many normal cellular processes, stabilize proteins and membranes, and can assist in protein refolding under stress conditions) interact with other stress-response mechanisms. In *E. coli*, it was suggested that the proline and betaine osmolytes can also act as 'chemical chaperones' by increasing the stability of native proteins and assisting in the refolding of unfolded polypeptide[46].

RAPD analysis was effective in detecting informative qualitative and quantitative changes in both wheat cultivars as a response to pH stress and IAA hormonal application. The RAPD profiles of the thirty decamer primers used are shown in Fig. 1 and analyzed in Table 1. The thirty used different primers have different performances in detection of genetic changes. The numbers of amplified fragments generated ranges between 10 and 22 with molecular size ranges between 50 and 2130 bp. Quantitative changes by one common band were shown in stressed Giza 164 samples using thirteen primers as compared to nine primers in Gemmiza 7 stressed samples. Yang and Quiros[47] reported that , quantitative changes could be explained on the basis of alterations of some DNA sequences. Qualitative changes were recorded using all primers (thirty) in Gemmiza 7 but recorded in Giza 164 using sixteen primers only. The recorded changes were found to be reproducible when repeated at different times under the same amplification conditions. These changes may be due to slight changes of the nucleotide sequences recognized by primers as a result of pH stress or due to the reduction of annealing efficiency between primers and DNA templates by masking the recognition sequences or due to the inhibition of Taq polymerase activity[48]. These results are in agreement with that reported by Emam *et al.*[49], who used five arbitrary primers to detect the effect of different salt concentrations on different cultivars of rice. The disappearance of some protein bands may be attributed to the alteration of their structural genes and mRNA transcripts. The expression of many proteins is known to be regulated by biotic and abiotic stresses, suggesting the occurrence of complex mechanisms that control gene expression in response to environmental stresses. Similarly, there are many evidences that there is a crossed response by different

stimuli. Thus, various genes and proteins that respond to stress are induced by stress factors[50].

The results of this study suggest that an adaptive mechanism has been developed by *Triticum vulgare* L. cultivars (Gemmiza 7 and Giza 164) in response to exogenous hormonal application under acidic pH stress. Also, the biochemical and molecular criteria which may affect on crop yield productivity and quality were improved. The synthesis of chemical and molecular chaperones to maintain osmotic adjustment is the main strategy that has evolved to maintain growth. The difference in the ability to maintain osmotic potential under pH conditions between the two cultivars reflects the differences in their genetic background.

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