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Effect of Chemical, Organic and Biological Fertilizers on Protein Concentration and Protein Electrophoretic Profiles of Wheat Plants Irrigated with Seawater

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ABSTRACT: *Wheat plants grown under 0%, 20% and 40% of seawater, 0, 100, 250 and 500 kg/ha of urea as chemical fertilizer; Rhizobium and Azotobacter as biofertilizer; and 0, 5, 10 and 20 kg/ha of humic acid as organic fertilizer. Soluble, insoluble and total proteins as well as RAPD-PCR were evaluated. To obtain reliable molecular markers for response to salinity in such genotype, RAPD banding patterns by using two primers. It was found that low concentration (20%) of seawater caused an observed increase in soluble protein. While, high concentration (40%) of seawater caused a significant decrease in soluble and insoluble proteins as well as total proteins. Biological and organic fertilizer treatments increased total proteins even at 40% seawater treatment as compared with unfertilized plants at the same level of seawater. The results of RAPD analysis showed that the two primers (OPUPC-75 and OPA18 could efficiently align genomic DNA of wheat. Approximately 88 bands (AF) were amplified under different treatments using the two primers. Monomorphic and polymorphic bands ware present in all individuals, and the mean percentage of polymorphic bands for all treatments was 76.2%, with molecular sizes ranging from 350 to 1900 pb. It was observed also that eight bands of the 88 commonly detected in all the samples, so it could be the specific genus bands of Triticum aestivum species. It seems that the extensive polymorphism detected among seawater and fertilizertreatments elevated the degree of change occurring in DNA sequences.*

Keywords: Chlorophyll, electrophoretic patterns, soluble proteins, salinity, sorghum.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a glycopyte species and is important crop grown in the semi-arid areas of Saudi Arabia on soils prone to salinity. Increasing wheat production is a national target to fill the gap between production and consumption. Saudi Arabia needs sustained agricultural development to cope with the social and economic obligations that are the normal consequences of the continued high rates of population growth. This urgent need requires continuous scientifically based implementation of effective agricultural practices. Problems related to the agricultural systems, deficiencies of agricultural research plans, traditional agriculture, lack of agricultural knowledge and information among most World farmers have caused irreparable damage to plant productivity in economies of these countries. Besides, salinity of soils and waters caused serious problems for crop production. Appropriate management of organic and biofertilizers reduces the potential disadvantages in comparison to the mineral fertilizers (Ahmed *et al.* 2011). Therefore, in the development and implementation of sustainable agriculture techniques, organic fertilization and biofertilization has great importance in alleviating environmental pollution and deterioration of nature (Jalilian *et al.*, 2012; Mehran *et al.*, 2011).

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Classical methods of screening for salt tolerance were based on the plant yield and are very costly and time consuming. Environmental salinity resistance in plants is recognizable through some parameters. For example, measurements of major physiological and biochemical traits, including proline content and protein concentration, can be used to monitor plant responses to salt stress (Bavei et al., 2011a). Crops in saline soils are faced with reductions in water absorption, insufficient nutrient availability, accumulation of toxic ions, or K+ and Ca++ depletion in plant tissues (Taiz and Zeiger, 2010), disturbances in metabolic activity such as respiration, photosynthesis (Ouzounidou et al., 2014), altered enzyme activity and imbalance in absorbed elements (Abdul Qados, 2015). Plant species reveal many differences in sensitivity and reaction to water potential reduction that result from drought or salinity. Molecular breeding of salt-tolerant plants using either molecular markers or genetic engineering enhances their resistance to both hyper-osmotic stress and ion toxicity (Kurepin et al., 2015).

Genetic analysis of wheat irrigated with seawater and fertilized with different kinds of fertilizers revealed some genes that exert a major effect on Na+ exclusion (Munns et al., 2010). Preferential deposition of Na+ in the leaf base has been described for rice (*Oryza sativa*) and sorghum (de Lacerda et al., 2005). One significant effect of salinity on plant growth occurs through changes induced in the osmotic strength of the growth medium. Plants have several mechanisms for balancing osmotic pressure changes in the root medium. Crops under saline conditions decrease the cellular osmotic potential by increasing the concentrations of free amino acids, inorganic cations and insoluble particles, accumulation of which helps to maintain the osmotic balance (Roy and Chakraborty, 2014). Genes that are up-regulated by salt stress mainly belong to several groups, based on their possible functionality. These genes encode proteins, enzymes involved in the biosynthesis of osmolytes, hormones, detoxification and general metabolism and regulatory molecules such as transcription factors, protein kinase and phosphatases.

The identification of specific characteristics related to salt resistance such as pralines, soluble and non-soluble proteins as well as total proteins will provide potential biological markers useful in the identification and genetic manipulation of salt-resistant plants and plant cells (Li et al., 2014). The aim of this study is to use protein electrophoretic profiles and other physiochemical traits to compare between wheat plants grown under normal water irrigation or grown under seawater irrigation in the presence or absence of chemical, bio and organic fertilization.

MATERIALS AND METHODS

Pot experiments were carried out at the King Abdulaziz University, Saudi Arabia, during winter season of 2011/2012. The average daily maximum and minimum temperature were 27/19 C^{o}, respectively (according to the Metrological Station in Jeddah). The aim of the study was to determine the effect of chemical fertilizer, bio fertilizer and organic fertilizer on proline content, protein concentration and electrophoretic patterns of leaf soluble proteins of bread wheat (*Triticum aestivum*) plants irrigated with different ratios of seawater.

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Experiment

Eighty one (81) pots 40 cm diameter, each was filled with fifteen (15) kilograms sandy soils mixed with perllit and peat moss in the ratio of 2:1:1 irrigated with tap water for one week to remove weeds. The pots were divided into three groups, each group was treated with different kind of fertilizers as followes:

a- Chemical fertilizer "Urea" was used in the rates of 100, 250 and 500 kg/ha. Urea is a complete water-soluble fertilizer.

b- Biofertilizer was used in the form of *Rhizobium* or *Azotobacter* treatments. Inoculation of the grains with the bio-fertilizer containing N-free living bacteria *Rhizobium* or *Azotobacter* was done just before sowing, using Arabic gum (4%) as adhesive material.

c- Organic fertilizer was used in the form of Humic acid (HA) and was applied in the rate of 5, 10 and 20 kg/ha, 7days before sowing and incorporated through soil preparation.

Ech fertilizer treatment was berformed in three replicates with the recommended dose and was irrigated with three levels (0%, 20% or 40%) of seawater designed as (low, medium or high) ratio of seawater.

Determination of proline

Plant Proline (amino acid) was determined using Bates (1973) method, by adding 10 ml aqueous sulfosalysilic acid 3% to 5 gm of the fresh plant sample. Then this was crushed and filtered, and 2 ml of the filtrate was placed in a test tube, then added 2 ml of Ninhydrin acid and 2 ml of glacial acetic acid were added. The tube was incubated in a water path (100^oC) for 1 hour, and placed on ice for 5 minutes, and 4 ml of toluene was added and then stirred using vortex for 15-20 seconds. The upper red colored part of the solution was then taken out for determination of the light intensity ay a light wave length of 520 nanometer using spectrophotometer.

Determation of proteins

Soluble and total proteins were estimated in plant leaves colorimetrically using colorimetrically using Coomassie Brilliant Blue G-250, according to the method of Bradforod (1976). The absorption was measured at 595 nm and standard curve of absorbance was prepared according to Al-Zahrani and Al-Robai (2008).

Electrophoresis study

1. DNA isolation

Total genomic DNA was isolated according to Azcárate-Peril and Raya (2001) with some modification, where 200 µl of TES buffer and 20 µl of lysozyme (10 mg/ml) were mixed with 1 mg or suitable amount of bacterial pellets of overnight culture. The mixture was incubated at 37^o C for 20 min in a water bath. 20 µl of proteinase K (10 mg/ml) was added to each sample then incubated at 37^oC for 20 min in a water bath. The mixture was transferred to ice for 5 min then 250 µl of 4M sodium acetate was added. 250 µl of chloroform: isoamyl (24:1) was added then the mixture was inverted between fingers to mix it then centrifuged at 13000 rpm for 2 min. The upper zone was transferred with care to a new clean eppendorff and 3/4 or 1 v/v of isopropanol was added then incubated at -20 overnight. Next day, the mixture was centrifuged at 13000 rpm/2 min then the supernatent was totally discarded and DNA was dried at room temperature then resuspended with 50 µl of distilled water. 10 µl of isolated

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DNA was loaded in 0.5% agarose gel in 1x of TBE buffer run at 100 V for 60-90 min and stained with ethidium bromide (Sambrook et al., 1989).

2. RAPD-PCR analysis

Polymerase chain reaction (PCR) based molecular markers has developed into controllable tools to analyze genetic relationships and genetic diversity using random amplified polymorphic DNA-RAPD. Two primers used, PCR reactions were conducted according to (Williams *et al*., 1990). Each RAPD-PCR marker was named by the primer used and DNA fragment size in base pairs (bp). RAPD patterns were scored for each treatment and genetic distances were calculated using RAPD distance. The nucleotide sequences and GC ratios of 5 primers used in RAPD-PCR were represented in (Table 1).

3. SDS-PAGE electrophoresis

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (1970) was used to study the genetic background at the vegetative stage for the studied strains by their total protein fingerprints.

Table 1: List of primers and their nucleotide sequence

Primer	**Sequence**
Prime1	AGGAGTCGGA-**3`**-5'
Prime2	AGACCCAGAG-**3`**-5'

Statistical analysis:

All data were subjected to analysis of variance and significant difference among means were determined according to (Snedecor and Cochran, 1980) with the aid of SPSS softwear. Significant difference among mean were distinguished according to the Duncans, multiple test range (Duncan, 1955). Differences between means were compared at LSD 5%.

RESULTS AND DISCUSSION

Proline concentration

Proline concentration increased in wheat shoots in response to the increase of seawater concentration. The 20% and 40% concentrations of seawater caused substantial increases (50% - 200%) in ptoline compared to control treatment. A positive correlation between sea water concentration and proline accumulation was found to be (R^2 = 92%). Data in the same figure showed a gradual increase in proline contend with increasing dose of chemical fertilizer. In this respect, the most increase in proline was recoded at Chem.3 treatment to be 18% as compared with unfertilized control plants. Moreover, chemical fertilizers resulted in an increase in proline concentration under seawater treatments compared to unsalted control plants. At 40% seawater, proline concentration increased by about 6.1% when plants were treated with Chem.3 as compared with unfertilized plants grown under same level of seawater. Biofertilizer, Bio.1 and Bio.2 treatments, caused about 12% and 13%,

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respectively, in proline concentration in plants grown under normal irrigation compared with biofertilizer-untreated control plants. While, neither biofertilizers nor organic fertilizers caused significant changes in proline content of plants grown under 20% or 40% of seawater irrigation as compared to unfertilized plants grown under same levels of seawater.

Plants grow under environmental stresses, like drought and salt stress, accumulate organic compatible substances such as proline (Cha-Um and Kirdmanee, 2009). These solutes help to maintain high water content necessary for growth and cellular function. The present study showed that seawater permitted the increase of proline in wheat plants, and the accumulation of proline was more pronounced in fertilized plants than unfertilized ones particularly at 40% seawater (Fig. 1). Physiologically, proline accumulation seemed to be a primary defense response to maintain the osmotic pressure in plant tissues (Koca *et al.*, 2007). Moreover, the role of proline in osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Kavi Kishor *et al.*, 2005). Many plants including wheat accumulate proline as an adaptive trait concerned with stress tolerance, and it is generally assumed that proline accumulation is caused by both the activation of its biosynthesis and inactivation of its degradation (Çiçek and Çakirlar, 2008).

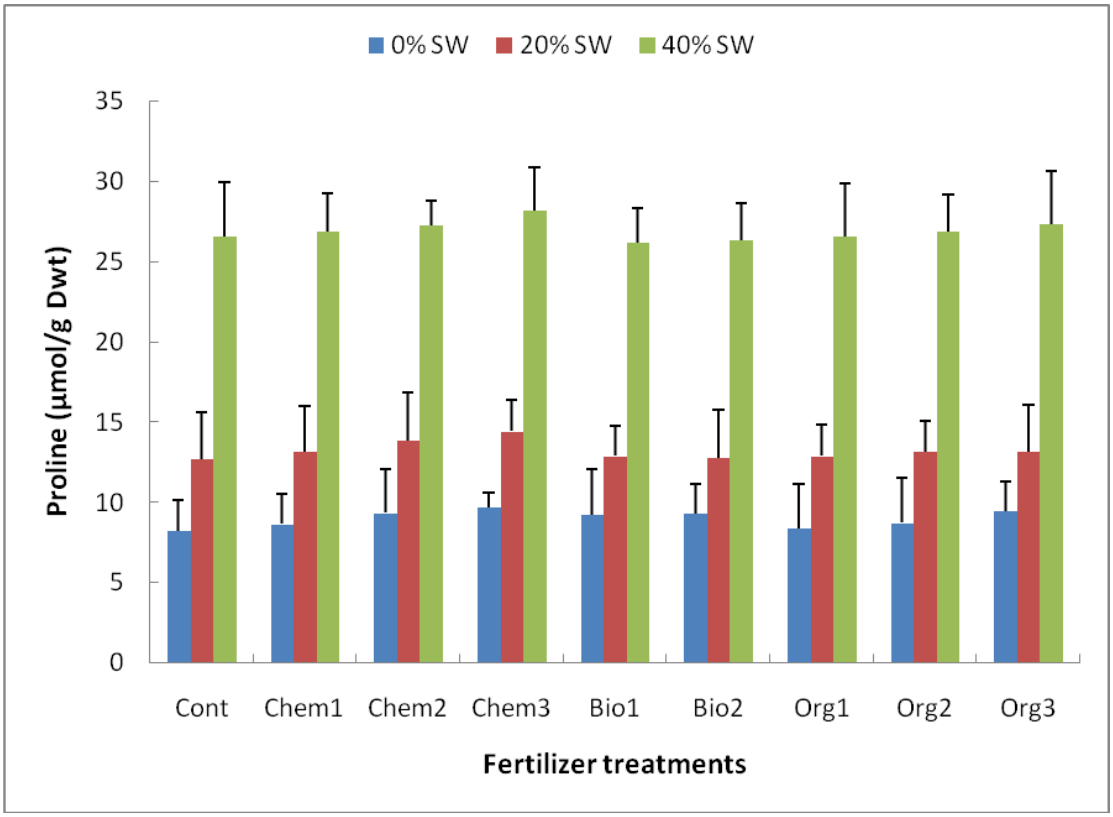

Fig. (1): Effect of Chemical fertilizer (Chem1-Chem3), Biofertilizer (Bio1-Bio2) and Organic fertilizer (Org1-Org3) on proline concentration of wheat plants grown under different concentrations of seawater (SW). (Cont = Control ; vertical lines indicate SD values).

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Soluble and insoluble proteins

The 20% concentration of seawater treatments caused an observed increase in soluble and to a relatively little extent in the insoluble proteins as compared with 0% control treatment (Table 2). On the other side, the 40% of seawater caused a significant decrease in soluble and insoluble proteins as compared with control treatment. At 20% concentration of seawater, Chem.1 treatment improved the soluble proteins by about 16.3% and insoluble proteins by about 31.8% as compared with 0% level of sea water. Under biofertilizer treatments, only soluble proteins were increased with the 20% concentration of seawater, at which an increase in soluble proteins by Bio.1 and Bio.2 treated plants was about 14.5% and 12.2%, respectively. Organic fertilizer showed gradual increases in soluble proteins and gradual decreases in insoluble proteins. In this regard, soluble proteins increased by about 5%, 14.8% and 22.6% at Org.1, Org.2 and Org.3 treatment, respectively, as compared with 0% sea water treatment. The comparable decreases in the insoluble proteins were about 3%, 4.9% and 11.2%, respectively. Both, soluble and insoluble proteins were drastically decreased at 40% concentration of seawater eather in fertilizer-treated or untreated plants.

Table (2): Effect of different fertilizer treatments on soluble and insoluble proteins (mg/g Fwt) of wheat (*Triticum aestivum*) shoots grown under different concentrations of seawater.

Fertilizer treatments	Seawater concentration					
	0%		20%		40%	
	soluble	insoluble	soluble	insoluble	soluble	insoluble
Cont	1.22±0.32	3.26±0.42	1.35±0.16	4.14±0.71	1.15±0.16	2.26±0.40
Chem1	1.84±0.41	3.58±0.46	2.14±0.22	4.72±0.75	1.43±0.11	3.11±0.67
Chem2	2.55±0.36	4.11±0.72	2.75±0.25	4.88±0.82	2.06±0.24	3.34±0.71
Chem3	2.17±0.29	3.85±0.66	2.16±0.31	3.88±0.74	1.48±0.24	3.09±0.72
Bio1	1.45±0.25	4.12±0.88	1.66±0.26	3.55±0.92	1.22±0.12	3.13±0.69
Bio2	1.56±0.23	4.23±0.82	1.75±0.25	3.26±0.88	1.16±0.23	3.02±0.72
Org1	1.19±0.25	3.66±0.82	1.25±0.26	3.55±0.93	1.05±0.18	3.22±0.85
Org2	1.88±0.18	3.85±0.74	2.16±0.22	3.67±0.87	1.34±0.12	3.42±0.74
Org3	2.16±0.23	4.35±0.79	2.65±0.30	3.91±0.90	1.66±0.17	3.65±0.62
LSD5%	0.26	0.56	0.34	0.48	0.20	0.35

Total protein content

All forms of fertilizers caused an increase in total proteins of wheat plants under normal irrigation (Fig. 2). At 0% seawater, the highest increase in total proteins was observed at Chem.2, Bio.2 and Org.3 treatments at which the increase was 48%, 29.2% and 45.35, respectively, as compared with unfertilized control plants. It was also clear that total proteins were negatively affected by 40% of seawater, at which total protein was reduced by about 31.3%, as compared with control plants under 0% level of seawater.

It seemed that fertilizer treated plants could withstand the harm effect of salt stress and total protein was increased even at high concentrations of seawater as compared

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with non-fertilized plants. In this regard, Chem.2 treatment showed the highest level of total protein among other chemical fertilizer treatments (58.8% at 40% seawater); Biofertilizers positively affected total protein (27.5% at Bio.1 and 22.5% at Bio.2 treatments under 40% seawater) and Organic fertilization also improved total proteins (55.7% at Bio.3 treatment under 40% of seawater).

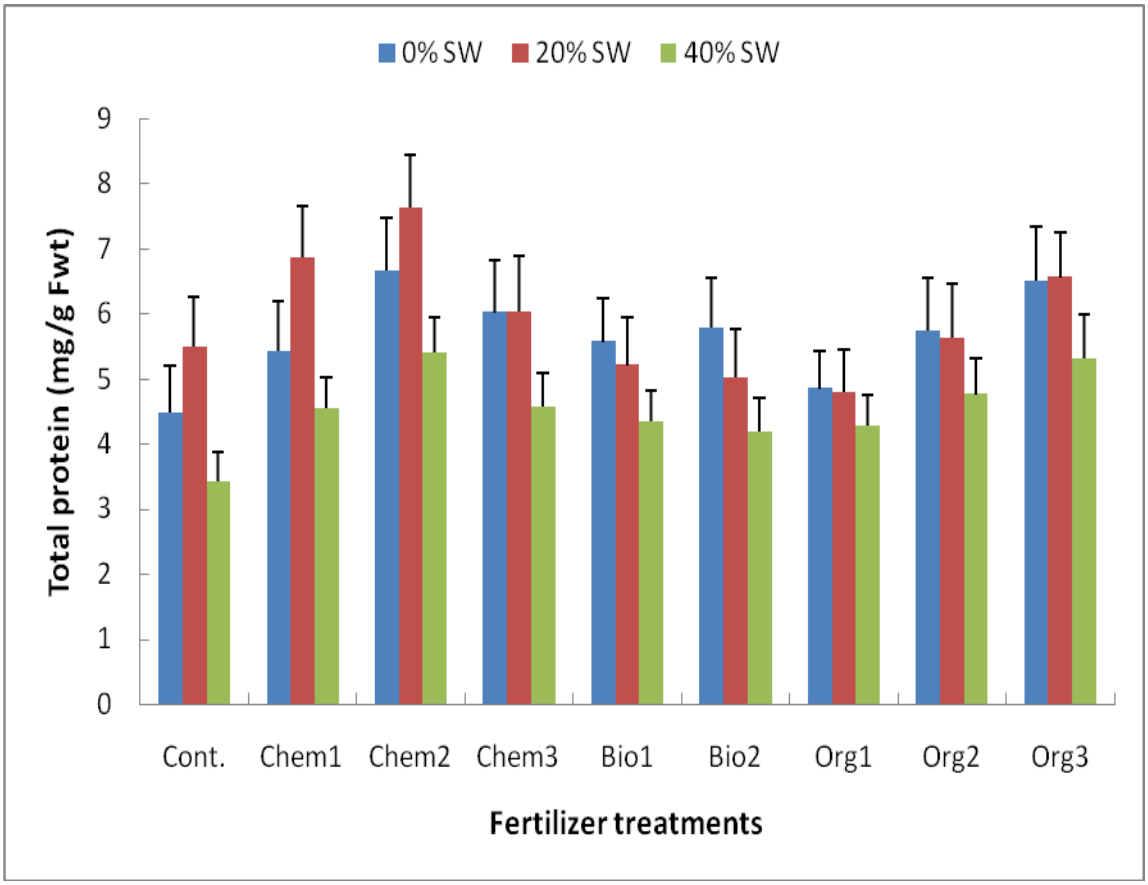

Fig (2): Effect of Chemical fertilizer (Chem1-Chem3), Biofertilizer (Bio1-Bio2) and Organic fertilizer (Org1-Org3) on total protein concentration of wheat plants grown under different concentrations of seawater (SW). (Cont = Control ; vertical lines indicate SD values).

The inhibiting effects of saline water on plant growth and biochemical composition have previously been reported by many researchers (Abdalla, 2011; Hefny, 2011). It is well known that high salinity stress conditions cause a multitude of molecular, biochemical and physiological changes, thereby affecting plant growth and development (Boutraa, 2010). The depressive effect of high salt stress on growth parameters may also be attributed to a reduction in the assimilation of nitrogen compounds (Reddy *et al.*, 2003), affecting the rate of protein formation (Yazdanpanah *et al.*, 2011) and cause oxidative damage to DNA, lipid and proteins. On the other side, accumulation of protein under low concentration of seawater has been correlated with stress avoidance (Goudarzi and Pakniyate, 2009).

Accumulation of proteins under moderate level of seawater (20%) is known to occur widely in plants in response to salinity to protect plant cells by balancing the osmotic strength of cytosol with the vacuole and external environment (Kavi Kishor *et al.*,

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2005). In addition, protein accumulations are particularly important for cell survival under low concentration of salt stress and causes membranes stabilization under salinity stress (Goudarzi and Pakniyate, 2009). Thus, in response to salinity, plants make new proteins that help them to grow and develop under low saline conditions (Rani and Rose, 2012). The soluble protein and free amino acids in barley and maize plants increased with NaCl increasing (El-Tayeb, 2005; Hussein *et al.*, 2012). The marked increase in soluble and insoluble protein in shoots might indicate the superiority of shoots to alleviate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells (Hefny, 2011).

Nitrogen containing fertilizer, improves protein contents within plant tissues, because nitrogen play a role in the structure of protein and nucleic acids. Therefore, additions of N fertilizers can substantially increase protein content and plants yield. However, plants protein concentration can decrease if the amount of added N is not adequate for potential yield (Hafez *et al.*, 2012). Many researchers have found that N additions as fertilizer materials were effective in attaining higher plants protein concentration (Namvar and Sharifi, 2011). However, high amount of fertilizers increases the salt concentration in the soil and has an adverse effect on growth and protein and other constituents in plant tissues (Daneshmand *et al.*, 2012)

As for Bio and Organic fertilizers, Abd El-Razek and El-Sheshtawy (2013) found that bio-fertilizer inoculation gave high nitrogen percentage and total protein percentage. In this regard, many investigators found that inoculation of wheat grains with nitrogen fixing bacteria significantly enhanced the growth and protein of wheat plants (Zaki *et al.*, 2012). Daneshmand *et al.* (2012) indicated that the use of biological and organic fertilizers lead to significant increase in yield and protein content of wheat (*Triticum aestivum*), thus such fertilizers can be used to reduce the harmful effect of chemical fertilizers and to improve soil and plant nutrition and reduce the environmental pollution.

4-12 Effects on DNA

RAPD data analysis

PCR amplification of genomic DNA was tested on 2 RAPD primers in two rounds of amplification. RAPD analysis evaluated the response of *Triticum aestivum* genomes challenged with seawater and fertilizer treatments, thereby to detect the molecular changes associated with the presence of fertilizer priming in wheat leaves. Results in Figs (3, 4 and 5) and Tables (3A, 4A and 5A) indicated that the two primers (OPUPC-75 and OPA18 could efficiently align genomic DNA of wheat. Approximately 88 bands (AF) were amplified under different treatments using the two primers (Figs 3, 4 and 5; Tables 3B, 4B and 5B). Monomorphic and polymorphic bands are present in all individuals, and the mean percentage of polymorphic bands for all treatments was 76.2%, with molecular sizes ranging from 350 to 1900 pb. It was observed also that eight bands of the 88 commonly detected in all the samples, so it could be the specific genus bands of *Triticum aestivum* species.

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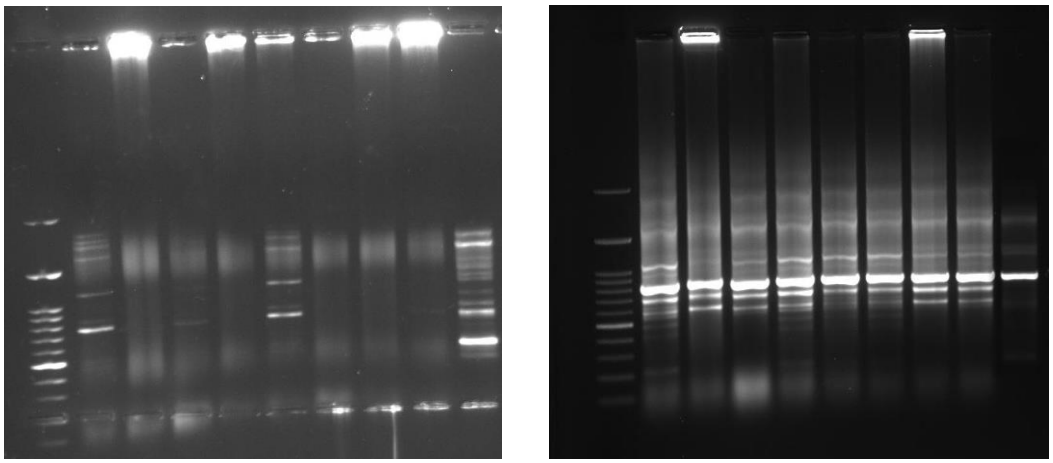
It was obvious that the two primers gave clear and reproducible banding patterns, however primer1 was more clear than primer2 in producing more bands at sea water treatment of 20% and 40%. But for control plants, where no sea water treatment, primer 2 was more active than primer 1 in producing the bands. In this regard, total bands produced by primer1 in 0% sea water treatment was 46 while those produced by primer2 were 62, to make a sum of 108 bands for both primers. The polymorphic bands for primer1 accounted for 87.5% of the total bands of this primer, while the polymorphic bands for primer2 accounted for 81.2% of the total bands produced by primer2, thus the mean polymorphism for both RAPD primers was 84.4% of the total sum of produced bands.

As for seawater treatments, it is clear that at 20% seawater, PCR amplification with RAPD primer1 gave a total number of 84 RAPD fragments of different molecular weight, out of which 90.4% were polymorphic, while Prime2 produced a total of 48 RAPD fragments, out of which 66.7% were polymorphic. The mean value of the polymorphism for both RAPD primers was 78.5% of the total bands.
At 40% treatment of sea water, it is obvious that RAPD primer1 yield 79 RAPD fragment, 73.6% of which were polymorphic. While RAPD primer2 produced 50 RAPD fragments, of which 57.1% were polymorphic. Thus, the mean value of polymorphism for Primer1 and Primer 2 was 65.4% of total bands.

0% seawater



Prim1 prim2

Fig (3): Effect of fertilizers on RAPD-PCR polymorphism of DNA using OPUPC-75 (Prim1) and OPA18 (Prim2) primers on *Triticum aestivum* plants grown under 0% seawater.

Table (3A): Effect of the interaction of 0% seawater and fertilization treatments on RAPD analysis from the DNAs of *Triticum aestivum* using 2 random primers.

Primer name	Total # bands	Total AF	Monomorphic bands	Polymorphic bands	% of Polymorphism
Prim.1	46	16	02	14	87.5
Prim.2	62	16	03	13	81.2

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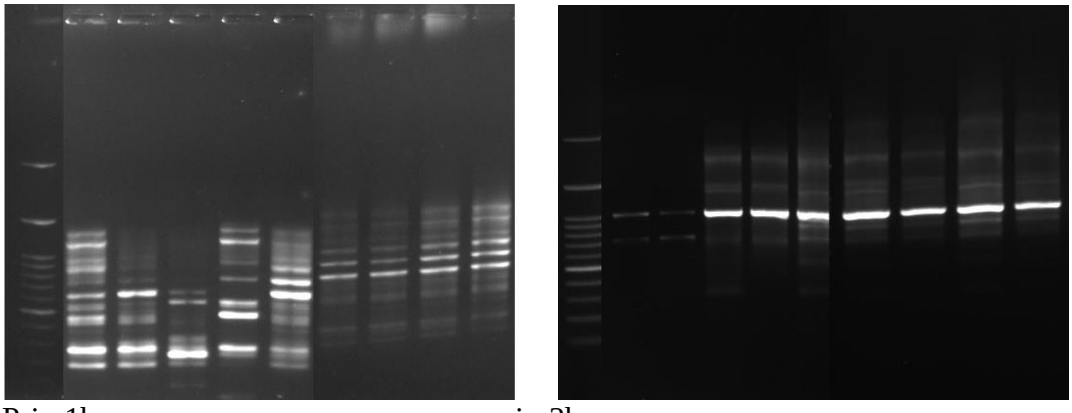
Table (3B): Molecular weight base pairs (bp) of amplified DNA fragment that produced by using RAPD analysis with two primers at 0% seawater.

DNA marker	Size (bp)	L1	L2	L3	L4	L5	L6	L7	L8	L9
Prim.1	3000	+	-	-	-	+	-	-	-	+
	2800	+		-	+	+	-	-	-	+
	2600	+	+	+	+	+	+	+	+	+
	2500	-	-	-	-	-	-	-	-	+
	1800	-	-	-	-	-	-	-	-	+
	1500	+	-	-	-	-	-	-	-	+
	1200	-	-	-	-	-	-	-	-	+
	900	+	-	+	-	+	-	-	+	-
	800	-	--	+	-	+	-	-	+	+
	700	-	-	-	-	-	-	-	-	-
	600	-	-	-	-	-	-	-	-	+
	500	+	-	-	-	-	-	-	-	-
	400	+	+	+	-	+	-	-	-	-
	300	+	-	-	-	-	-	-	-	+
	200	+	+	+	+	+	+	+	+	+
	100	-	-	-	-	-	-	-	-	+
Total		9	3	5	3	6	2	2	4	12
Prim.2	1300	+	+	+	+	+	-	-	-	+
	1250	+	-	-	-	-	-	-		
	1200	+	-	-	-	-	-	-	-	+
	1150	-	-	+	-	+	+	+	+	-
	1100	+	+	+	+	+	+	+	+	+
	1000	-	-	-	-	-	-	-	-	-
	950	+	+	+	+	+	-	-	-	+
	900	-	-	-	-	-	+	+	+	-
	450	+	+	+	+	+	+	+	+	+
	400	+	-	-	-	-	-	-	-	-
	350	+	-	-	-	+	+	+	+	+
	300	-	-	-	-	-	-	-	+	-
	250	+	+	+	+	+	+	+	+	+
	200	+	-	-	+	-	-	-	-	-
	150	-	-	-	+	-	-	-	-	-
	100	-	-	-	+	-	-	-	-	-
Total		10	5	6	8	7	6	6	7	7

20% seawater



Prim1b prim2b

Fig (4): Effect of fertilizers on RAPD-PCR polymorphism of DNA using OPUPC-75 (Prim1) and OPA18 (Prim2) primers on *Triticum aestivum* plants grown under 20% seawater**.**

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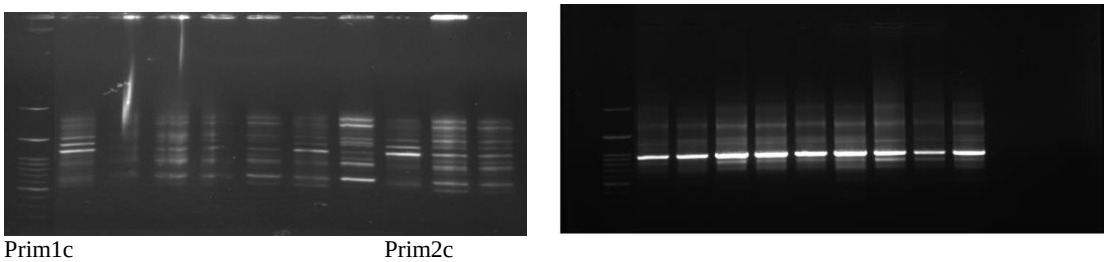
Table (4A): Effect of the interaction of 20% seawater and fertilization treatments on RAPD analysis from the DNAs of *Triticum aestivum* using 2 random primers.

Primer name	Total # bands	Total AF	Monomorphic Bands	Polymorphic bands	% of Polymorphism
Prim1	84	21	02	19	90.4
Prim2	48	09	03	06	66.7

Table (4B): Molecular weight base pairs (bp) of amplified DNA fragment that produced by using RAPD analysis with two primers at 20% seawater.

DNA marker	Size (bp)	L1	L2	L3	L4	L5	L6	L7	L8	L9
Prim.1	1600	-	-	-	+	-	-	-	-	-
	1450	-	-	-	+	-	-	-	-	-
	1400	-	-	-	-	-	+	+	+	+
	1350	+	-	-	-	-	-	+	+	+
	1300	+	-	-	+	-	-	+	+	+
	1250	-	-	-	-	-	-	+	+	+
	1200	+	-	-	-	-	-	+	+	+
	1150	+	+	-	+	-	-	-	-	-
	1100	+	+	+	-	-	+	-	-	-
	1000	-	+	-	-	+	+	-	-	-
	950	+	+	+	+	+	+	+	+	+
	900	+	+	+	+	+	+	-	-	-
	650	+	-	-		-	-	-	-	-
	600	+	+	+	+	+	+	+	+	+
	550	+	+	-	-	-	-	-	-	-
	500	-	-	-	-	+	+	-	-	-
	450	+	+	+	-	+	-	-	-	-
	400	-	-	-	-	+	-	+	+	+
	600	-	-	+	-	-	+	+	+	+
	260	+	+	+		+	+	-	-	-
	100	+	+	+	+	+	-	-	-	-
Total		13	10	8	8	9	9	9	9	9
Prim.2	1900	+	+	+	+	+	+	+	+	+
	1500	-	-	-	-	-	+	+	+	+
	1000	+	+	+	+	+	+	+	+	+
	750	+	+	+	+	+	+	+	+	+
	700	-	-	-	-	+	+	+	+	+
	600	-	-	-	-	+	-	-	-	-
	500	+	+	+	+	-	+	+	+	-
	350	-	-	+	+	+	-	-	-	-
	200	-	-	-	-	+	-	-	-	-
Total		4	4	5	5	7	6	6	6	5

40% seawater



Prim1c Prim2c

Fig (5): Effect of fertilizers on RAPD-PCR polymorphism of DNA using OPUPC-75 (Prim1) and OPA18 (Prim2) primers on *Triticum aestivum* plants grown under 40% seawater.

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Table (5A): Effect of the interaction of 40% seawater and fertilization treatments on RAPD analysis from the DNAs of *Triticum aestivum* using 2 random primers.

Primer name	Total # bands	Total AF	Monomorphic Bands	Polymorphic Bands	% of Polymorphism
Prim1	79	19	05	14	73.6
Prim2	50	07	03	04	57.1

Table (5B): Molecular weight base pairs (bp) of amplified DNA fragment that produced by using RAPD analysis with two primers OPUPC-75 (Prim1) and OPA18 (Prim2) at 40% seawater.

DNA marker	Size (bp)	L1	L2	L3	L4	L5	L6	L7	L8	L9
Prim.1	2000	-	-	-	-	-	-	+	-	+
	1900	-	-	-	-	-	-	-	+	-
	1800	+	+	+	+	+	+	+	+	+
	1500	-	-		-	-	-	-	-	-
	1300	+	+	+	+	+	+	-	-	-
	1100	-	-	-	-	-	-	-	+	-
	1000	+	-	-	-	-	-	-	-	-
	950	-	-	+	+	+	+	+	-	+
	900	-	-	-	-	-	-	-	+	-
	850	-	-	-	-	-	-	+	-	+
	800	+	+	+	+	+	+	+	+	+
	750	-	-	-	-	-	-	+	+	+
	700	+	+	+	+	+	+	+	+	+
	650	+	-	+	-	+	+	-	-	-
	550	-	-	-	-	-	-	+	-	+
	500	+	+	+	+	+	+	+	+	+
	450	-	-	-	-	-	-	-	+	-
	400	+	+	+	+	+	+	+	+	+
	300	+	-	-	-	+	+	-	-	+
Total		9	6	8	7	9	9	10	10	11
Prim.2	3000	-	-	+	+	+	+	+	-	-
	1500	+	+	+	+	+	+	+	+	+
	900	-	-	+	+	+	+	-	-	+
	800	+	+	+	+	+	+	+	+	+
	650	+	+	+	+	+	+	+	+	+
	600	+	-	+	+	-	+	+	+	+
	500	-	-	+	+	+	+	+	-	+
Total		4	3	7	7	6	7	6	4	6

Data in Table (6) indicated that, except for 0% seawater primer1 was more effective in producing RAPD fragments than primer2, while the reverse was true for 20% and 40% of seawater. In this regard the 20% treatment of sea water produced the highest mean of RAPD fragments number (9.9) with primer1, while 40% sea water produced 8.8 fragments with the same primer. As for primer2, it shows most activity with 0% of seawater under which it produced RAPD fragments more than that produced with 20% or 40% of seawater.Regardless of seawater treatments, data in the same table showed that the form of fertilization has its impact on the RAPD fragments produced with both primers. In this concern, organic fertilization produced RAPD fragments (7.2) more than other kind of fertilizations followed by biofertilizer treatments (6.9) while chemical fertilization produced only 6 RAPD fragments.

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Table (6). Effect of fertilizer treatments on RAPD analysis from DNA 2 random primers of *Triticum aestivum* grown under different concentrations of seawater. (values are the mean of bands number).

Sea water Conc.	RAPD primer	Fertilizer treatments				Mean
		Control	Chemical	Bio. Fert.	Org. Fert.	
		Total No. of bands				
0%	Prim1	9.0	3.7	4.0	6.0	5.7
	Prim2	10.0	6.3	6.5	6.7	7.3
20%	Prim1	13.0	8.7	9.0	9.0	9.9
	Prim2	4.0	4.7	6.5	5.7	5.2
40%	Prim1	9.0	7.0	9.0	10.3	8.8
	Prim2	4.0	5.6	6.5	5.3	5.4
Mean		8.2	6.0	6.9	7.2	

Data recorded in Table (7) showed that 20% of seawater treatment produced the highest number of RAPD fragments with primer1 as compared with other treatments. Moreover, despite seawater treatments, data in the same table indicated that organic fertilizer treatments produced the highest number of RAPD fragments. In this respect, Org.1, Org.2 and Org.3 treatments produced a mean number of 7.8, 7.2 and 8.0, respectively, of RAPD fragments. While, Bio.1 and Bio.2 treatments produced a mean number of 7.7 and 7.8, respectively, of fragments. On the other side, Chemical fertilizer treatments produced lower number of fragments. In this regard Chem.1, Chem.2 and Chem.3 treatments produced a mean number of 5.3, 7.2 and 6.8, respectively, of RAPD fragments.

Table (7). Effect of sea water treatments on RAPD analysis from DNA 2 random primers of *Triticum aestivum* grown under different concentrations of seawater . (values are the mean of bands number).

Sea water	Primer	Cont	Chem1	Chem2	Chem3	Bio1	Bio2	Org1	Org2	Org3	Mea
0%	Prim1	9	6	8	7	9	9	10	10	11	8.7
	Prim2	10	3	7	7	6	7	6	4	6	6.2
20%	Prim1	13	10	8	8	9	9	9	9	9	9.3
	Prim2	4	4	5	5	7	6	6	6	5	5.3
40%	Prim1	9	6	8	7	9	9	10	10	11	8.7
	Prim2	4	3	7	7	6	7	6	4	6	5.6
Mean		8.1	5.3	7.2	6.8	7.7	7.8	7.8	7.2	8.0	

It is is obvious from data analysis of DNA that RAPD assay can efficiently generate both randomly dispersed markers as well as markers linked to specific genes. In this study, the RAPD analysis with 2 random primers gave totally 108, 132 and 129 fragments of which 84.4 %, 78.6 % and 65.5 % were polymorphic at 0%, 20% and 40% of sea water, respectively. These results are close with results of other studies on corn lines show similar ratio of polymorphic RAPD fragments (Bauer *et al.*, 2005).

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The molecular mechanism of salt stress tolerance in plant could be elucidated using cDNA microarrays. However, mRNAs may not be transcribed or that changes in the protein level or enzyme activity can occur without any detectable change in transcript abundance due to translational or other levels of control. Therefore, it is necessary to study the salt stress responses at the protein level (Shunping *et al.*, 2005). In this study the major protein profile of wheat leaf under control conditions show protein bands that can categorized in high molecular weight, medium range molecular weight and low range molecular weight. The protein profile patterns emphasize the closely relationship between Biofertilizers and Organic fertilizers in their effect (7).

Under salt stress conditions, protein profile of the wheat show high homogeneity pattern among the different treatments of the same fertilization. Comparing to non-stress protein pattern, the high molecular weight bands were disappeared and the major protein bands were located at the medium molecular weight range. This could be due to the inhibitory effects of salt stress at 40% of sea water on transcriptional process (Abbas and Fayed , 2014). The fast breakdown of cellular protein during salt stress, induced by 40% sea water, might be also due to the increasing activity of acid and alkaline protease (Sohrabi *et al.*, 2011). Homeostasis and mineral deficiencies has been a consequence of salt stress condition (Abbas *et al.*, 2013).

The obtained results showed that the using of RAPD analysis to characterize each treatment with the appearance of specific markers and produce informative bands that distinguished all the treatments. The expressions of many genes induction by stress, involved directly in stress tolerance and regulation of gene expression and signal transduction (Zhou *et al.*, 2010). The expression of stress-responsive genes is important for the plants' ability to grow under different environmental stress conditions (Chinnusamy *et al.*, 2007). In this regard, Malik *et al.*, (2000) revealed that RAPD technique has a potential to find DNA-based polymorphisms between the stress resistant and stress-susceptible genotypes of the same varieties. Abdel-Bary *et al.*, (2005) recorded positive and negative RAPD markers for salinity tolerance, thus, the different primers have different performances for evaluation of genetic polymorphism. The extensive polymorphism detected among seawater and fertilizer treatments elevated the degree of change occurring in DNA sequences. The results of RAPD-PCR indicated the existence of differences in RAPD fragments. The quantitative polymorphism obtained might be due to the changes of some regions of the nucleotide sequences aligned by arbitrary primers. This promotion may be due to effects of fertilization or due to the enhancement of annealing between primers and DNA templates by activation and recognition of sequences and / or activation of Taq polymerase activity (Afiah *et al*., 2007). In addition, the action of fertilization was achieved by enhancing the activity level of free radical scavenging enzymes could reduce the incidence of DNA damage, explaining the appearance of new DNA in fertilizer treatments (Mohsen *et al.*, 2013; Bavei *et al.*, 2011b).

CONCLUSION AND RECOMMENDATION

Results of this study indicated that seawater irrigation of wheat plants caused an observed increase in proline and significant reduction in soluble, insoluble and total

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proteins, while fertilizers increased all fractions of proteins either under normal or seawater irrigation. In addition, the obtained results showed that RAPD analysis can be used to characterize each treatment with the appearance of specific markers and produce informative bands that distinguished all the treatments.

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