

ORIGINAL ARTICLE

**Does Exogenous Application of Kinetin and Spermine Mitigate  
the Effect of Seawater on Yield Attributes and Biochemical  
Aspects of Grains?**

Heshmat S. Aldesuquy\*, Zakaria A. Baka and Bardees M. Mickky

*Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt*

\*E-Mail: [HS-Aldesuquy@hotmail.com](mailto:HS-Aldesuquy@hotmail.com)

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A pot experiment was conducted to evaluate the effect of grain presoaking in kinetin (0.1 mM), spermine (0.3 mM) and their interaction on yield components and biochemical aspects of yielded grains of wheat plants irrigated with 25% seawater. Seawater induced marked reduction in biochemical aspects of yielded grains especially carbohydrates content, nitrogenous constituents, total protein and nucleic acids contents as well as proline and organic acids (citric and keto-acids) content. Conversely, seawater stress increased phosphorus and ions (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>) content. Application of kinetin or spermine appeared to mitigate the effect of seawater stress on wheat yield and the biochemical aspects of yielded grains. The effect was more pronounced with kinetin + spermine treatment.

*Key words: Kinetin, Wheat, Seawater stress, Spermine, Yield*

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Botany Department, Faculty of Science, Mansoura University, Mansoura, Egypt

\*E-Mail: [HS-Aldesuquy@hotmail.com](mailto:HS-Aldesuquy@hotmail.com)

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*Key words:* Kinetin, Wheat, Seawater stress, Spermine, Yield

*Abbreviations:* Kinetin = K, Seawater = SW, Spermine = SPM

Yield is the ultimate outcome of all processes involved at all stages in growth and development of a crop, any one of which may limit the yield of a particular crop (Munns *et al.*, 2006). Previous reports have revealed that stressing wheat plants with different levels of saline solution resulted in significant and gradual decline in all yield components, such as number of tillers, number of spikes per plant, number of grains per plant, straw yield, grain yield, biological yield and harvest index.

In addition, the yielded grains contained less carbohydrates, nitrogen, protein, phosphorus, potassium, calcium and magnesium contents, but higher sodium content when compared with control plants (Ahmed *et al.*, 2008; Aldesuquy *et al.*, 2009).

Seed priming with cytokinins, especially kinetin, is reported to increase plant salt tolerance. It was hypothesized that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA (Iqbal *et*

al., 2006). Polyamines have been shown to be an integral part of plant stress response, as they are implicated in senescence and environmental stress (Alcázar et al., 2006). Many studies have indicated that stress tolerance of plants is correlated with their capacity to enhance the synthesis of polyamines upon encountering the stress (Kasinathan and Wingler, 2004). Which of the three polyamines plays central roles in stress responses of plants may depend on plant species and stress type (Kasukabe et al., 2004).

The present work was undertaken to assess up to what extent seed priming in kinetin, spermine or their interaction could ameliorate the deleterious effects of seawater stress on yield components and biochemical aspects of yielded grains of wheat plants.

#### **MATERIALS AND METHODS**

Plant material and growth conditions: Pure strain of *Triticum aestivum* L. var. Sakha 93 was kindly supplied by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The variety Sakha 93 is known to be more salt tolerance and commonly used by Egyptian farmers. The chemicals employed were kinetin and spermine; obtained from Sigma company.

For soaking experiment, a homogenous lot of *Triticum aestivum* L. var. Sakha 93 grains were selected. The grains were surface sterilized by soaking in 0.01 M HgCl<sub>2</sub> solution for three minutes, then washed thoroughly with distilled water. The sterilized grains were divided into four sets. Grains of the 1<sup>st</sup> set were soaked in distilled water to serve as control, while those of the 2<sup>nd</sup>, 3<sup>rd</sup>, or 4<sup>th</sup> sets were soaked in 0.1 mM kinetin (kinetin solved in 1N NaOH), 0.3 mM spermine or 0.1 mM kinetin + 0.3 mM spermine; respectively, each for about 12

hours. The choice of the above mentioned doses of the used chemicals (i.e. kinetin and/or spermine) was based on trial experiments for studying the effect of the provided chemicals on the growth of seawater-treated wheat plants. After soaking, thoroughly washed grains were drilled on 15<sup>th</sup> November of two successive seasons (2008 & 2009) in plastic pots (20 cm in diameter) filled with 5.5 kg soil (clay: sand 2/1, v/v). Fifteen grains were sown in each pot. The pots were then kept in a greenhouse at Botany Department, Faculty of Science, Mansoura University, Egypt. The plants were subjected to natural day/night conditions (minimum/maximum air temperature and relative humidity were 15/25°C and 35/45%; respectively) at mid-day during the experimental period. The plants in all sets were irrigated to field capacity by tap water.

After two weeks from sowing, thinning was started so that five uniform seedlings were left in each pot for the subsequent studies. The plants of each set were sub-divided into two groups. The 1<sup>st</sup> group in each set was still irrigated with normal tap water serving as control, whereas the 2<sup>nd</sup> one was irrigated with 25% seawater. Irrigation with seawater was applied after 30 days from sowing with a periodical soil washing (each two weeks) with tap water. After thinning and at heading, the plants received 36 kg N ha<sup>-1</sup> as urea and 25 kg P ha<sup>-1</sup> as super-phosphate.

The chemical analyses of the employed seawater, collected from the Mediterranean Sea, revealed that it contains Cl<sup>-</sup>, 21.6 Kg m<sup>-3</sup>; Na<sup>+</sup>, 11.1 Kg m<sup>-3</sup>; SO<sub>4</sub><sup>-2</sup>, 2.85 Kg m<sup>-3</sup>; K<sup>+</sup>, 0.49 Kg m<sup>-3</sup> and P<sup>+3</sup>, 16.6 µg dm<sup>-3</sup>. Its salinity was found to be 38.5 g kg<sup>-1</sup>; pH, 8.1 and EC, 47 mmhos cm<sup>-1</sup>.

**Estimation of yield and yield attributes:** Among the different evaluated yield attributes, the

following relations were used:

Mobilization index = Crop yield / Straw yield  
(Ray and Choudhuri, 1980)

Crop index = Economic yield / Biological yield  
(Beadle, 1993)

Harvest index = Economic yield / Straw yield  
(Beadle, 1993)

Relative grain yield = (Yield of treatment / Yield of control) X 100  
(Beadle, 1993)

Evapotranspiration efficiency = Water use efficiency / Harvest index  
(Ehdaie and Waines, 1993)

Water use efficiency = Grain yield (ton) / Total water used (gallon)  
(Stanhill, 1987)

**Estimation of carbohydrates.** Sugars were extracted by overnight submersion of dry tissue of the yielded grains in 80% (v/v) ethanol at 25°C with periodic shaking.

**Estimation of glucose.** Glucose content was estimated using O-toluidine procedure of Feteris (1965). One ml aliquot of the alcoholic extract was heated with 5 ml O-toluidine reagent (60 ml O-toluidine and 2 g thiourea made up to 100 ml with glacial acetic acid) and incubated for 15 minutes at 97°C. Optical density of the developed color was measured at 625 nm using spectrophotometer. Glucose content was calculated by the use of a calibration curve obtained using standard pure glucose solutions.

**Estimation of sucrose.** Sucrose was determined using the modification of Handel (1968). Three ml of freshly prepared anthrone reagent (150 mg anthrone + 100 of 72% H<sub>2</sub>SO<sub>4</sub>) was then added to the cooled reaction product, and the mixture was heated at 97°C for 5 minutes, cooled, and the developed color was read at 620 nm. The amounts of sucrose in plant extract were determined from calibration curve of standard pure sucrose solutions.

**Estimation of total soluble sugars.** Total soluble sugars were analyzed according to the modification of Yemm and Willis (1954) and reading the cooled samples at 625 nm using spectrophotometer.

**Estimation of polysaccharides.** To remove sugars, the plant tissue is treated with 80% ethanol then starch is extracted with perchloric acid. In hot acidic reaction, starch is hydrolysed into glucose and dehydrated to hydroxymethyl furfural. This compound forms a green colored product with anthrone reagent. The method used for estimation of polysaccharides was that of Thayermanavan and Sadasivam (1984).

**Estimation of nitrogenous constituents.** The method used for the extraction of nitrogenous constituents was essentially that adopted by Yemm and Willis (1956).

**Estimation of ammonia-N.** Ammonia-N was estimated by the method adopted by Delory (1949) using Nessler's reagent as modified by Naguib (1964).

**Estimation of amide-N.** The method used with amide-N was that recommended by Naguib (1964).

**Estimation of amino-N.** The method used in the present study was designed by Muting and Kaiser (1963).

**Estimation of nitrite-N.** The method described by Snell and Snell (1939) was used to estimate nitrite-N.

**Estimation of total soluble-N.** The total soluble nitrogen was determined by the conventional semimicro-modification of Kjeldahl method of Pirie (1955).

**Estimation of total-N.** The total nitrogen was determined by the conventional semimicro-modification of Kjeldahl method of Chinbal *et al.* (1943).

**Estimation of protein-N.** Subtracting the total soluble-N from total-N gave the value of protein-N.

**Estimation of protein.** Protein content was determined according to the method adopted by Bradford (1976).

**Estimation of nucleic acids.** DNA and RNA contents were estimated according to the method of Sadasivam and Manickam (1996).

**Estimation of phosphorus.** The procedures adopted for extraction of different phosphorus compounds were essentially those described by Barker and Mapson (1964). The method of Kuttner and Lichtenstein (1932) as described by Humphries (1956) was adopted to estimate both inorganic and total phosphorus, and the difference between them was equivalent to organic phosphorus.

**Estimation of proline.** The method adopted for proline was essentially that of Snell and Snell (1954).

**Estimation of organic acids.** The method used for determining citric acid was that described by Snell and Snell (1949). To estimate keto - acids, the method of Friedman and Haugen (1943) was used.

**Estimation of elements.** Flame spectrophotometry was included for determining Na<sup>+</sup> and K<sup>+</sup> contents according to the method designed by Chapman and Pratt (1978). According to Hansen and Munns (1988), Cl<sup>-</sup> levels were determined.

**Statistical analysis.** Using SPSS program, a test for significant differences between means at P ≤ 0.05 was performed using LSD test (Snedecor and Cochran, 1976).

**Table 1:** Effect of grain presoaking in kinetin, spermine or their interaction on yield and yield attributes of wheat plants irrigated with seawater.

| Parameters<br>Treat-<br>ments | Shoot<br>length<br>(cm) | Main<br>spike<br>length<br>(cm) | Plant<br>height<br>(cm) | Main<br>spike<br>weight<br>(g) | Number<br>of tillers<br>/ plant | Number<br>of spikes<br>/ plant | Number<br>of spikelets<br>/ main<br>spike | Number<br>of spikelets<br>/ plant | Number<br>of grains<br>/ main<br>spike | Number<br>of grains<br>/ plant | Grain<br>yield<br>/<br>main<br>spike<br>(g) | Grain<br>yield /<br>plant<br>(g) |
|-------------------------------|-------------------------|---------------------------------|-------------------------|--------------------------------|---------------------------------|--------------------------------|---|-----------------------------------|--|--------------------------------|---|----------------------------------|
| Cont                          | 50.83                   | 13.80                           | 64.63                   | 3.68                           | 2.33                            | 2.00                           | 15.33                                     | 28.00                             | 41.67                                  | 75.67                          | 2.47  | 3.70                             |
| SW                            | 46.50                   | 11.07                           | 57.57                   | 3.19                           | 0.67                            | 0.33                           | 13.33                                     | 14.67                             | 30.33                                  | 31.00                          | 1.59  | 0.93                             |
| K                             | 54.00                   | 15.70                           | 69.70                   | 4.09                           | 4.67                            | 4.00                           | 19.33                                     | 79.00                             | 52.67                                  | 102.67                         | 3.48  | 4.89                             |
| SW +<br>K                     | 51.33                   | 14.07                           | 65.40                   | 3.46                           | 2.67                            | 2.33                           | 15.67                                     | 38.00                             | 42.67                                  | 72.33                          | 2.52  | 3.60                             |
| Spm                           | 52.83                   | 14.83                           | 67.67                   | 3.94                           | 4.00                            | 3.33                           | 17.33                                     | 65.33                             | 45.33                                  | 93.00                          | 2.86  | 4.26                             |
| SW +<br>Spm                   | 48.50                   | 12.60                           | 61.10                   | 3.28                           | 2.00                            | 1.67                           | 13.67                                     | 23.67                             | 36.67                                  | 60.67                          | 2.16  | 2.45                             |
| K +<br>Spm                    | 55.83                   | 17.17                           | 73.00                   | 4.28                           | 6.67                            | 5.33                           | 23.00                                     | 87.67                             | 62.33                                  | 105.00                         | 3.83  | 4.91                             |
| SW +K<br>+Spm                 | 51.83                   | 15.13                           | 66.97                   | 3.60                           | 3.00                            | 2.67                           | 15.67                                     | 37.67                             | 42.33                                  | 83.00                          | 2.45  | 4.03                             |
| LSD at<br>P ≤<br>0.05         | 1.18                    | 0.46                            | 1.27                    | 0.11                           | 0.69                            | 0.76                           | 0.98                                      | 5.81                              | 3.70                                   | 14.45                          | 0.21  | 0.28                             |

**Continued table 1:** Effect of grain presoaking in kinetin, spermine or their interaction on yield and yield attributes of wheat plants irrigated with seawater.

| Parameters      | Grain biomass (mg) | 100 kernel weight (g) | Biological yield / plant (g) | Economic yield / plant (g) | Straw yield / plant (g) | Crop yield / plant (g) | Mobilization index | Crop index | Harvest index | Relative grain yield (%) | Evapo-transpiration efficiency |      |
|-----------------|--------------------|-----------------------|------------------------------|----------------------------|-------------------------|------------------------|--------------------|------------|---------------|--------------------------|--------------------------------|------|
| Treatments      | Fresh mass         | Dry mass              |                              |                            |                         |                        |                    |            |               |                          |                                |      |
| Cont            | 59.67              | 51.67                 | 5.13                         | 7.82                       | 3.70                    | 4.12                   | 6.26               | 1.517      | 0.473         | 0.897                    | 100.00                         | 5.69 |
| SW              | 54.67              | 46.67                 | 4.72                         | 3.28                       | 0.93                    | 2.35                   | 3.19               | 1.357      | 0.283         | 0.395                    | 25.16                          | 5.64 |
| K               | 65.00              | 57.67                 | 5.66                         | 9.86                       | 4.89                    | 4.97                   | 7.59               | 1.526      | 0.496         | 0.983                    | 132.28                         | 6.87 |
| SW + K          | 60.33              | 53.67                 | 5.25                         | 7.50                       | 3.60                    | 3.90                   | 6.39               | 1.638      | 0.480         | 0.923                    | 97.39                          | 5.69 |
| Spm             | 63.33              | 56.00                 | 5.48                         | 8.79                       | 4.26                    | 4.53                   | 7.07               | 1.561      | 0.485         | 0.940                    | 115.24                         | 5.85 |
| SW + Spm        | 57.00              | 49.00                 | 4.88                         | 5.13                       | 2.45                    | 2.68                   | 5.62               | 2.096      | 0.478         | 0.915                    | 66.37                          | 5.63 |
| K + Spm         | 68.00              | 60.33                 | 5.91                         | 9.84                       | 4.91                    | 4.93                   | 8.30               | 1.682      | 0.499         | 0.995                    | 132.82                         | 6.88 |
| SW+K+Spm        | 60.67              | 53.67                 | 5.24                         | 8.13                       | 4.03                    | 4.10                   | 6.69               | 1.633      | 0.496         | 0.985                    | 109.11                         | 5.94 |
| LSD at P ≤ 0.05 | 1.75               | 1.52                  | 0.15                         | 0.31                       | 0.28                    | 0.15                   | 0.19               | 0.07       | 0.024         | 0.08                     | 17.39                          | 0.03 |

**Table 2:** Effect of grain presoaking in kinetin, spermine or their interaction on carbohydrates content and nitrogenous constituents (mg g<sup>-1</sup> d wt) in yielded grains of wheat plants irrigated with seawater.

| Parameters      | Carbohydrates content (mg g <sup>-1</sup> d wt) |         |                      |                 |                     | Nitrogenous constituents (mg g <sup>-1</sup> d wt) |                |                |                  |                        |                |                  |
|-----------------|---|---------|----------------------|-----------------|---------------------|--|----------------|----------------|------------------|------------------------|----------------|------------------|
|                 | Glucose   | Sucrose | Total soluble sugars | Polysaccharides | Total carbohydrates | Ammonia nitrogen                                   | Amino nitrogen | Amide nitrogen | Nitrite nitrogen | Total soluble nitrogen | Total nitrogen | Protein nitrogen |
| Cont            | 0.53  | 13.03   | 15.56                | 704.69          | 721.50              | 2.96   | 3.22           | 2.72           | 0.041            | 14.60                  | 20.81          | 6.21             |
| SW              | 0.40  | 11.97   | 14.31                | 677.19          | 691.50              | 2.27   | 2.84           | 2.42           | 0.045            | 13.66                  | 19.45          | 5.79             |
| K               | 0.86  | 17.69   | 18.03                | 732.50          | 750.53              | 3.19   | 3.56           | 2.96           | 0.046            | 16.34                  | 22.94          | 6.60             |
| SW + K          | 0.55  | 13.58   | 15.97                | 704.84          | 720.81              | 2.99   | 3.27           | 2.60           | 0.049            | 15.22                  | 21.55          | 6.33             |
| Spm             | 0.73  | 16.92   | 17.84                | 721.09          | 738.94              | 3.07   | 3.42           | 2.90           | 0.044            | 15.45                  | 21.73          | 6.28             |
| SW + Spm        | 0.41  | 12.61   | 15.69                | 694.22          | 709.91              | 2.84   | 3.05           | 2.54           | 0.046            | 14.42                  | 20.67          | 6.25             |
| K + Spm         | 0.89  | 17.89   | 19.38                | 739.53          | 758.91              | 3.31   | 3.68           | 3.10           | 0.047            | 16.61                  | 23.24          | 6.63             |
| SW+K+Spm        | 0.54  | 13.81   | 16.47                | 714.69          | 731.16              | 3.04   | 3.32           | 2.75           | 0.048            | 15.72                  | 22.09          | 6.37             |
| LSD at P ≤ 0.05 | 0.03  | 0.25    | 0.52                 | 0.69            | 6.96                | 0.13   | 0.09           | 0.06           | 0.003            | 0.62                   | 0.13           | 0.62             |

**Table 3:** Effect of grain presoaking in kinetin, spermine or their interaction on total protein, nucleic acids, proline, organic acids, phosphorus contents ( $\text{mg g}^{-1}$  d wt) and ionic content ( $\text{mmole g}^{-1}$  d wt) in yielded grains of wheat plants irrigated with seawater.

| Parameters      | Total protein ( $\text{mg g}^{-1}$ d wt) | Nucleic acids ( $\text{mg g}^{-1}$ d wt) |       | Proline ( $\text{mg g}^{-1}$ d wt) | Organic acids ( $\text{mg g}^{-1}$ d wt) |            | Phosphorus content ( $\text{mg g}^{-1}$ d wt) |         |       | Ionic content ( $\text{mmole g}^{-1}$ d wt) |                |                 |
|-----------------|--|--|-------|------------------------------------|--|------------|---|---------|-------|---|----------------|-----------------|
|                 |  | DNA                                      | RNA   |                                    | Citric acid                              | Keto acids | Inorganic                                     | Organic | Total | Na <sup>+</sup>                             | K <sup>+</sup> | Cl <sup>-</sup> |
| Cont            | 86.67                                    | 0.176                                    | 0.074 | 0.792                              | 0.289                                    | 0.167      | 0.108   | 0.509   | 0.617 | 1.53  | 2.03           | 0.056           |
| SW              | 81.46                                    | 0.160                                    | 0.068 | 0.714                              | 0.258                                    | 0.140      | 0.164   | 0.530   | 0.695 | 1.69  | 2.59           | 0.085           |
| K               | 89.84                                    | 0.184                                    | 0.081 | 0.896                              | 0.336                                    | 0.180      | 0.159   | 0.533   | 0.693 | 1.62  | 2.54           | 0.075           |
| SW + K          | 87.40                                    | 0.177                                    | 0.076 | 0.818                              | 0.305                                    | 0.170      | 0.207   | 0.554   | 0.761 | 1.78  | 2.91           | 0.096           |
| Spm             | 87.33                                    | 0.181                                    | 0.076 | 0.831                              | 0.313                                    | 0.173      | 0.132   | 0.552   | 0.684 | 1.59  | 2.38           | 0.066           |
| SW + Spm        | 86.98                                    | 0.164                                    | 0.070 | 0.779                              | 0.281                                    | 0.160      | 0.190   | 0.540   | 0.730 | 1.73  | 2.76           | 0.094           |
| K + Spm         | 89.97                                    | 0.185                                    | 0.082 | 0.948                              | 0.328                                    | 0.187      | 0.150   | 0.541   | 0.692 | 1.65  | 2.67           | 0.080           |
| SW+K +Spm       | 87.58                                    | 0.179                                    | 0.078 | 0.831                              | 0.297                                    | 0.173      | 0.192   | 0.556   | 0.747 | 1.80  | 2.93           | 0.113           |
| LSD at P ≤ 0.05 | 0.50                                     | 0.003                                    | 0.003 | 0.028                              | 0.018                                    | 0.014      | 0.022   | 0.028   | 0.032 | 0.08  | 0.42           | 0.010           |

## RESULTS AND DISCUSSION

The reduction in yield of stressed wheat plants (Table 1) can be attributed to the decrease in photosynthetic pigments, carbohydrates accumulation (polysaccharides) and nitrogenous compounds (total nitrogen and protein) as well as the reduction in the rate of translocation of photoassimilates from source (flag leaf) toward the sink (developing grain) across the conductive canals (phloem). The decrease in yield and yield components in different crops under similar conditions has also been reported by Aldesuquy *et al.* (2009).

Irrigation of wheat plants with 25% seawater caused marked reduction ( $P \leq 0.05$ ) in shoot length, main spike length and plant height (Table1). This was in good agreement with the results of Islam *et al.* (2007) who observed great reduction in these

parameters of rice plants stressed with different levels of salinity. The recorded reduction in the estimated lengths (shoot length, main spike length as well as plant height) in response to seawater stress (Table1) could be explained by the fact that salinity may exert negative effect on plant elongation via its reverse effect on cell division, expansion and enlargement during the early growth stages. Salinity reduces growth of the plant shoot through osmotic effects that reduce the ability of plants to take up water and this causes reduction in growth. If excessive amount of salt enters the plant, the concentration of salts eventually rises to a toxic level in older transpiring leaves causing premature senescence and reduction in the photosynthetic leaf area to a level that the plant cannot sustain growth and produce reasonable yield (Munns, 2002).

The number of spikes per plant as well as the number of tillers per plant significantly decreased ( $P \leq 0.05$ ) by the irrigation of wheat plants with 25% seawater (Table1). This may probably due to the number of spikes per plant depends on tillering ability of the plant which is negatively affected by salinity (Hasamuzzaman *et al.*, 2009). These results were in accord with the findings of Zeng and Shannon( 2000). Seawater stress induced marked decline in the number of spikelets and grains per spike and also per plant (Table1). In addition, the number of grains was observed to be less than the number of spikelets in the same spike. This difference was more obvious with salt treatment rather than the control and this may be attributed to the reduced spikelet fertility (the failure of grain set in its spikelet) in response to salt stress which may be caused by the lack or reduced pollen viability (Abdullah *et al.*, 2001). In addition, seawater irrigation induced drastic reduction in grain biomass and 100-kernel weight. Similar observations were recorded by Hasamuzzaman *et al.* (2009) who found that 1000-grain weight of rice plants decreased with increasing salinity levels.

Grain yield of wheat plants is the ultimate product of almost all yield components which are inversely influenced by salinity levels (Table1). Under seawater stress, the loss of grain yield may result from a combination of reductions in plant stand, spikelet number per spike and fertility (Hasamuzzaman *et al.*, 2009). In addition, spike length and spike number are two important affected characters that contribute to grain yield. The reason for reduced grain yield in wheat plants irrigated with 25% may result from increased  $\text{Na}^+$  uptake through root before flowering and its subsequent distribution in different vegetative and floral parts; especially in leaves where it causes leaf

mortality thereby reducing transportation of total assimilates to the growing region (Muuns, 2002). Furthermore, Seawater stress also caused massive reduction ( $P \leq 0.05$ ) in straw yield of wheat plants. This result was in accordance with that obtained by Naeem and Muhammad (2006). The decreased in harvest index with salinity may be due to the severe inhibitory effects of salts on fertility. This is in accord with Abdullah *et al.* (2001).

Irrigation of wheat plants with 25% seawater significantly reduced ( $P \leq 0.05$ ) evapotranspiration efficiency. Salts in the soil water solution can reduce evapotranspiration by making soil water less available for plant root extraction (Ouda, 2006). Shalhevet (1994) reported that the effects of salinity and water stress are generally additive in their impacts on crop evapotranspiration. However, under saline conditions, many plants are able to partially compensate for low osmotic potential of the soil water by building up higher internal solute contents (Ouda, 2006), and this may explain the improvement of evapotranspiration efficiency with the application of kinetin, spermine or their interaction, where it was found that these chemicals caused marked increase in osmotic pressure and different osmotically active metabolites in water extract of flag leaves of stressed wheat plants (Unpublished data).

The reduction in wheat yield and yield components in response to seawater stress might be attributed to the inhibiting effects of salinity on plant growth due to the suppression of many metabolic processes including protein, nucleic acids and polyamine synthesis (Reggiani *et al.*, 1994), activity of mitochondria and chloroplasts (Singh and Dubey, 1995), decreasing transpiration, stomatal conductance and photosynthesis (Sharma, 1995), restriction in the absorption of water by plant roots



and water use efficiency (Mansour, 1994), the toxic effects of certain ions present in soil solution and/or imbalance in phytohormone levels through its effects on either the biosynthesis or the destruction of the plant hormones (Nesiem and Ghallab, 1999).

The results in table1 indicated that application of kinetin, either alone or in combination with spermine, enhanced all yield components of stressed or unstressed wheat plants. In accordance with these findings, it was earlier noticed that presoaking of wheat grains in phytohormones before subjection to salinity stress increased the plant yield (Farida *et al.*, 2003). It is possible that under salt stress, the amount of naturally occurring cytokinins are suppressed; probably through the inhibition of their *de novo* synthesis, conversion from active to inactive or bound form, or the acceleration of their degradation (Iqbal and Ashraf, 2006). In addition, it was previously postulated that salinity stress could reduce cytokinins export from the plant root, where they are synthesized, into the shoot of most plants (Kuiper *et al.*, 1990). These two reasons may explain why the exogenous application of kinetin could alleviate the inhibitory effect of seawater stress on the yield of wheat plants.

Gadallah (2004) reported that in salt-stressed wheat plants, increased soil salinity resulted in appreciable inhibition of grain yield. However, kinetin application ameliorated the deleterious effects of salinity and enhanced grain yield. Furthermore, Angrish *et al.* (2001) noticed that increasing levels of salinity decreased straw and grain yield of wheat (*Triticum aestivum* L.) plants, but presowing seed soaking in kinetin alleviated the harmful effects of salinity.

Seed priming with cytokinins has been reported

to have beneficial effects on wheat under seawater stress. These findings indicate that relief of the damage and restoration of normal conditions was maintained either partially or completely by application of kinetin. This recovery may be a consequence of several roles played by such hormones, which can cause triggering of the internal cellular metabolism and also induce alterations in the ratios of growth regulators (Younis *et al.*, 2003). Furthermore, Ray *et al.* (1983) found that the number of spikelets per panicle, number of panicles, percentage of filled grains, panicle weight and grain yield per plant and the mobilization and harvest indices of salt-stressed rice plants were significantly increased by kinetin treatment. The possibility of increased grain-filling and thus yield due to delayed foliar senescence by kinetin treatment was possible.

The results in table1 clearly indicated that application of spermine was significant in alleviating the adverse effects of seawater on yield and yield components of wheat plants irrigated with seawater at 25%. The increase in yield production may be due to increase in longevity of leaves by spermine application which perhaps contributed to grain filling by enhancing the duration of photosynthate supply to grains (Kaur-Sawhney *et al.*, 1982).

The increment in yield components due to spermine treatment, either alone or along with kinetin, may be attributed to the increase in growth rate (Unpublished data). In this respect, Davies (1995) reported that polyamines play a critical role in different biological processes, including cell division, growth, somatic embryogenesis, floral initiation, development of flowers and fruits. This could be explained by the fact that polyamines, and spermine with special concern, have the ability to

increase the efficiency of solar energy conversion into different photosynthetic outputs which maximized the growth rate of wheat plant and consequently increased its productivity and yield components. In addition, spermine may exert a stimulatory effect on stressed or unstressed wheat plants through their role in increasing the endogenous phytohormones (in particular cytokinins) which in turn increase the yield components through breaking the apical dominance of wheat plants leading to the increase in flowering tillers and consequently the number of spikes and their weight and/or through increasing the assimilates and their translocations from leaves to spikes as the spike weight increased (El-Bassiouny *et al.*, 2008).

As an explanation to the ability of exogenously-applied polyamines to reverse the deleterious effects of salt stress, Liu *et al.* (2006) suggested that polyamines, including spermine, are highly protonated at a physiological pH, which favors electrostatic binding of polyamines to negatively charged components of membranes, leading to membrane stabilization through ionic interactions. In addition, polyamines may be effective in scavenging free radicals. Recently, several reports suggested that exogenous polyamines could activate the antioxidant systems, thereby controlling free radical generation and preventing membrane lipid peroxidation, which resulted in improved cell growth under stress (Verma and Mishra, 2005).

Application of spermine, whether alone or combined with kinetin, appeared to mitigate the deleterious effects of seawater stress on grain biomass of wheat plants (Table 1). The repairing effect of the two chemicals may be attributed to their effects on leaf area expansion and

consequently increase the import of dry matter towards the drying grains. Furthermore, these chemicals induce faster translocation of photoassimilates from leaves towards developing grains particularly after looking to its effect on phloem area in both flag leaf and its peduncle.

The reduction in different organic constituents of developed wheat grains in response to seawater stress (Table 2) may be attributed to the fact that salt stress could increase ABA levels in flag leaves which in turn induced stomatal closure and consequently decreased photosynthetic activity in flag leaves (the main source of photo-assimilates towards developing grains), this effect may result in a decrease in biochemical aspects of yielded grains. Also, salinity stress may stimulate the early senescence in wheat leaves which also affected the translocation of the photo-assimilates from leaves (particularly flag leaf). These results were in good accordance with those obtained by Elhakem (2008). In addition, grain priming with kinetin increased carbohydrates and protein contents in yielded grains of stressed sorghum plants (Aldesuquy *et al.*, 2005). Moreover, Zeid (2004) mentioned that putrescine application resulted in increased in the biosynthesis of nucleic acids, synthesis of macromolecules particularly protein and photosynthetic pigments.

The results in table 2 cleared that seawater induced massive decrease ( $P \leq 0.05$ ) in total protein and nucleic acids (DNA and RNA) contents in the developed wheat grains. On the other hand, application of kinetin and/or spermine caused significant increase ( $P \leq 0.05$ ) in total protein and nucleic acids contents of yielded grains of stressed wheat plants, particularly when compared with the stressed plants. The interaction of kinetin and spermine was the most effective treatment in

mitigating the deleterious impact of salinity on total protein and nucleic acids contents of wheat grains.

The decrease in protein contents in yielded grains of stressed wheat plants (Table 3) may be due to less transport of protein from source (flag leaf) to the sink (grain). In support to this finding, water stress induced remarkable decrease in soluble protein in flag leaf at heading stage (Unpublished data). Application of kinetin under seawater salinity was found to synergistically increase seed protein content, being in accordance with the results of Singh and Sharma (1996). Hormone pretreatment is known to have a secondary enhancement effect on protein content through the intensification of nitrate reductase activity (Shah, 2004).

Phosphorus, sodium, potassium and chloride levels in yielded wheat grains were increased in response to seawater stress (Table 3). These results match those obtained by Elhakem (2008)). The increase in ionic content of developed grains is a logic consequence to the irrigation using seawater which is rich in different ionic components. This increase in ions levels may result from transportation of these elements from root to shoot through the transpiration stream to the developing grains. El-Tayeb (2005) recorded that phosphorus increased in barley plants due to salinity. Application of kinetin and spermine caused additional increase in phosphorus, sodium, potassium and chlorides contents of yielded wheat grains (Table 3). As a similar trend, grain priming with kinetin increased ion contents in yielded grains of stressed sorghum plants (Aldesuquy et al., 2005). Moreover, polyamines increased water uptake by root and consequently increased the uptake and translocation of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> contents which were driven by transpiration (Alcázar et al., 2006).

## CONCLUSION

On the basis of the results obtained, we concluded that when it is necessary to cultivate wheat cultivars Sakha 93 in seawater-irrigated soil, presoaking in kinetin and spermine is required to increase the tolerance ability of wheat plants to seawater stress conditions.

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