Mechanisms of Salt Tolerance of Wheat Cultivars

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Abstract This work was carried out to study the effect of various salinization levels (0, 20, 50, 150 and 300mM NaCl) through the whole life cycle of four wheat cultivars (Sakha94, Gimiza11, Gimiza10, and Giza 168). Accordingly the salt tolerance of four wheat cultivars during vegetative and crop yieldstages ranked according to dry matter and chemical constituents as the following: cv. Sakha 94>c.v. Gimiza 11>c.v. Gimiza 10 > cv. Giza 168. The carbohydrate and protein contents varied between the four wheat cultivars and their different plant organs, generally the soluble carbohydrate content remained more or less unchanged in cv. Sakha94 and to some extent in cv. Gimiza11 and troubled in cv. Gimiza10 and cv. Giza168. The amino acids were interesting because, they increased considerably in cv. Sakha 94 and cv. Gimiza11 accompanied with a great equilibration in protein content in the two sensitive cultivars Gimiza 10 and Giza 168. Proline content varied consequently among the four wheat cultivars and their plant organs. The results also revealed that, 23 protein bands were detected in cv. Sakha 94, 18 protein bands in cv. Gimiza 11, 16 protein bands in cv. Gimiza 10 and 18 protein bands in cv. Giza 168 in protein analysis by electrophoreses. The four cultivars possessed 17 common protein bands while they different from each other in 6 protein bands. The 14.1 KDa is specific marker for both cutivars Sakha94 and Giza 168. However, the 33.2 KDa is specific marker for cv. Sakha94 and cv. Gimiza 11. The results revealed that three bands at molecular weight 52.1 kDa is induced under salinity stress in four tested cultivars Sakha 94, Gimiza 11, Gimiza10 and Giza 168, as compared to the control treatment. It was induced at 50 mM, 150 mM in both cultivars Gimiza 11, Gimiza 10 and Giza 168 while, induced at 50 mM, 150 mM and 300 mM NaCl levels in cv. Sakha 94 as compared to control treatment. These results revealed that the 52.1 kDa protein band was commonly induced as a result of salinity treatment in the four cultivars. All the previous parameters supported the differentiation of salt tolerance between the four cultivars and open the chance for crop selection to be cultivated in saline soil.

Keywords Mechanisms; Salt Tolerance; Wheat

Introduction Several environmental factors adversely affect plant growth and development and final yield performance of a crop (Ahmad et al., 2008, 2013; Hayat et al., 2012). Plants are frequently exposed to two main types of environmental stresses while grown in nature: a- Biotic stress (Kumar et al., 2009) while caused by infection and/or competition by other organism. b- Abiotic stress may be caused by numerous factors such as drought (Simova-Stoi-lova et al., 2009; Shaddad et al., 2011 a, b), cold (Van Kumar et al., 2009), high temperature (Reynolds-Henne et al., 2010), salinity (Wang et al., 2012), heavy metals (Abd El-Samad, 2014),alkalinity (Breusgem et al., 2001), air pollution, pesticides (Hong-Bo et al., 2008), ultraviolet radiation (Gao and Zhang, 2008) and is also affected with the fertility status of soil (Sogbedi et al., 2006). Moreover, daily sudden changes in the temperature and the presence of heavy metals, toxins, and oxidants due to human activities could result in extra stresses on plant (Vierling, 1991). Soil salinity, one of the most severe abiotic stresses, limits the production of about 6% of the world’s total land and 20% of irrigated land (17% of total cultivated areas) and negatively affects crop production worldwide. On the other hand, increased salinity of agricultural land is expected to have destructive global effects, resulting in up to 50% land loss by the next couple of decades. The adverse effects of salinity have been ascribed mainly to an increase in sodium (Na+) and chloride (Cl-) ions and hence these ions produce the critical conditions for plant survival by intercepting different plant mechanisms (Hasanuzzaman et al., 2013). In saline environments, however, the difference in the water potential between soil and root cells is reduced or even inverted, leading to a reduction in water
uptake or loss of water (Boursiac et al., 2005). Growth inhibition, and ultimately, serious tissue damages, are the consequences. According to the incapacity to grow on high salt medium, plants have been classified as glycophytes or halophytes. Most plants are glycophytes and cannot tolerate salt stress (Sairam and Tyagi, 2004). There are naturally occurring salt-tolerant trees (mangroves), shrubs, grasses and herbs. However, virtually none of our crop plants is able to tolerate even a quarter of seawater without loss of yield (Flowers and Flowers, 2005; Abd El-Samad and Shaddad, 2010, 2013 and, 2014).

Thus the aim of the present work was to describing salt-induced changes on the phenology of the four wheat cultivars (Sakha94, Gimiza11, Gimiza10, and Giza 168. The correlation between growth kinetics, dry matter production, carbohydrates, proteins amino acids and proline in serving these tolerance.

1 Results
1.1 Plant growth parameters
Dry matter yield
The data in table 1 exhibited that salinity stress up to the level of 150mMNaCl, stimulated the production of dry matter of stems and leaves of wheat cultivar Sakha94. The percent of increase in dry matter yield approached 20% and 1.5- folds at the level of 150 mMNaCl in stems and leaves respectively. Dry matter of roots remained unchanged up to 150 mM NaCl then a highly significant reduction was recorded which was about 38.1% at the level of 300 mMNaCl. In spikes, there is a marked and progressive enhancement but not irregular in the production of spike in wheat cultivar Sakha94. The highest dry matter yield of spike was at the level of 150 mMNaCl more than 2 folds and the lowest accumulation of spike dry matter was at the highest salinity level used (about 16% over the control values). The data in table 1 showed that the salinity stress stimulated the dry matter yield of stems up to 150 mMNaCl of wheat cultivar Gimiza 11. At this level the percent increase in dry matter of stem was about 29.6% in relation to the control. Then a highly significant reduction was obtained (about 25% below the control). In leaves, the NaCl salinity induced insignificant changes in dry matter of leaves up to 150 mM NaCl, there after a marked and progressive reduction was obtained only at the level of 300 mMNaCl which was about (35% in relation to control). The dry matter of roots stimulated by salinity stress up to 50 mMNaCl by about 50% over those of control values, there after the dry matter of roots reduced sharply and suddenly, while the dry matter of roots stimulated by 50% at 50 mM NaCl, it on the other hand and surprisingly dropped by 22.3% at 150 mMNaCl and then continue to be reduced highly significantly up to the highest salinity level used 300 mMNaCl. At this level 300 mMNaCl the percent of reduction in root approached 80% in comparison to control. There is some irregular stimulation in the production of spikes in cv. Gimiza 11 up to the level of 150 mMNaCl. This stimulation fluctuated between 6% to 18% in relation to the control sample. However some inhibition was recorded only at 300 mMNaCl (about 19% in relation to control).

The data in table 1 revealed that, there is a highly significant reduction in the dry matter of stems in cv. Gimiza 10 at the level of 20 mMNaCl. This reduction seemed to be more or less constant up to the level of 150 mMNaCl (about 31.1%) and the highest reduction was obtained at the highest doses of NaCl, it was (about 44%) at the level 300 mM as compared with those of control. The dry matter of leaves remained mostly unchanged up to 150 mMNaCl and a highly significant reduction was recorded beyond this level (about 30%). The dry matter of roots reduced smoothly up to 150 mMNaCl, above which a sharp reduction was recorded at 300 mMNaCl. At the level of 300 mM NaCl the dry matter of roots reduced by more than 50% compared to the control values. The production of spikes dropped sharply even at the level of 20 mMNaCl (by about 40%), and remained more or less constant up to 150 mMNaCl and further excessive reduction was reported at the level 300 mMNaCl which was more than 50%. The data in table 1 revealed that there is a marked and progressive reduction in dry matter of stems even at the lowest salinity levels of NaCl in wheat cultivar Giza 168. It’s worthy to mention that the dry matter of stem dropped (by about 50%) at the level of 20 mMNaCl salinity and (by about 60%) at the level of 300mM NaCl salinity. In leaves this accelerated reduction was observed also for leaves but less than in stems and registered also at the lowest salinity levels used. Also this drastic effect of salinity was about 30% at the most salinity levels used. In roots
there is a marginal reduction in dry matter yield up to 20 mM (by about 20%) then a sharp and sudden reduction was observed beyond this level which was about 60% up to the level of 150 mM NaCl salinity and about 70% at the level 300 mM NaCl salinity. Interestingly this was the case in spikes where the production of spike reduced by more than 50% at the level of 20 mM NaCl salinity. This reduction remained more or less constant even at the highest salinity level used.

Table 1 Effect of various concentrations of NaCl on dry matter of roots, stems, leaves and spikes of cv. Sakha 94, cv. Gimiza11, cv. Gimiza10 and cv. Giza 168 wheat cultivar.

| NaCl Treat. | Root % | Stem % | Leaf % | Spike % |
|-------------|--------|--------|--------|---------|
| Cont        | 0.210a | 0.692a | 0.170a | 0.250a  |
| 20 mM       | 0.190  | 0.79   | 0.24   | 0.29    |
| 50 mM       | 0.25   | 0.89   | 0.28   | 0.41    |
| 150 mM      | 0.19   | 0.83   | 0.3    | 0.56    |
| 300 mM      | 0.13   | 0.61   | 0.16   | 0.29    |
| L. S. D. 5% | 0.64   | 0.43   | 0.69   | 0.15    |

| NaCl Treat. | Root % | Stem % | Leaf % | Spike % |
|-------------|--------|--------|--------|---------|
| Cont        | 0.185a | 0.645a | 0.230a | 0.352   |
| 20 mM       | 0.21   | 0.78   | 0.2    | 0.35    |
| 50 mM       | 0.27   | 0.98   | 0.2    | 0.38    |
| 150 mM      | 0.14   | 0.38   | 0.2    | 0.34    |
| 300 mM      | 0.04   | 0.75   | 0.15   | 0.26    |
| L. S. D. 5% | 0.46   | 0.20   | 0.46   | 0.12    |

Means not labeled with letter (a) are significantly different from control level mean.

*significant at < 0.05

1.2 Chemical constituents
1.2.1 Soluble sugar content
In roots of wheat cultivar Sakha 94, the soluble carbohydrates remained more or less unchanged even at the higher salinity (table 2). The soluble carbohydrates in stems remained more or less unchanged up to 150 mM and then a marginal increase was exhibited at 300 mMNaCl. In leaves some promotion in the soluble carbohydrate has been recorded up to 50 mM, then a highly significant increase was recorded. In spikes, the soluble carbohydrates mostly remained more or less unchanged whatever the salinity level used. In roots of Gimiza 11 salinity stress induced a slight effect if any, in the soluble carbohydrates of roots, however there is a marginal reduction at higher salinity levels which not exceeded than 15.5% (table 2). In stems the soluble carbohydrates increased progressively as the
salinity increase in the soil up to 50 mM NaCl, then it increased smoothly at 150 mM NaCl, then some reduction was exhibited which seemed to be more or less constant at the levels 300 mM NaCl, this reduction was about 27% below the control value. In leaves the soluble carbohydrates enhanced markedly and irregularly by the salinity stress, the highest increase was recorded in plants exposed to 150 mM, and the lowest increase was recorded in plants irrigated with the lowest concentrations of NaCl. In spikes the soluble carbohydrates activated progressively and irregularly by the salinity stress. The highest accumulation was found to be at 50 mM, and the lowest were at the lowest concentrations of NaCl. In roots of cv. Gimiza 10 the soluble carbohydrates decreased by salinity stress, interestingly, this reduction seemed to be more or less constant at the levels from 150 mM to 300 mM NaCl, this reduction was about 30% in relation to control (table 2).

Table 2 Effect of various concentrations of NaCl on carbohydrate content (soluble fraction) as mg/gm dry weight in roots, stems, leaves and spikes of wheat cultivar cv. Sakha 94 , cv. Gimiza11, cv. Gimiza10 and cv. Giza 168.

| NaCl Treat. | Root % | Stem % | leaf % | Spike % |
|-------------|--------|--------|--------|---------|
| Cont        | 72.6a  | 100    | 84.9   | 40.6    | 20.4    | 100 |
| 20 mM       | 68.6   | 94.4   | 84     | 98.9    | 33.6    | 82.7 | 21.4 | 104.9 |
| 50 mM       | 63.0   | 86.7   | 63.0   | 74.2    | 46.4    | 114.2 | 21.2 | 103.9 |
| 150 mM      | 69.0   | 95.0   | 73.7   | 86.7    | 49.3    | 121.3 | 19.4 | 95.0  |
| 300 mM      | 62.9   | 86.5   | 100.9  | 118.9   | 46      | 113.3 | 25.6 | 125.4 |
| L. S. D. 5% | 0.64   | 0.24   | 0.22   | 0.22    |         |       |      |       |

| cv. Sakha 94                           |          |        |        |         |          |       |
|----------------------------------------|----------|--------|--------|---------|----------|-------|
| Cont                                   | 79.8a    | 100    | 89.4a  | 100     | 92.7a    | 100   |
| 20 mM                                  | 85.8     | 107.5  | 90.6   | 101.3   | 93.9     | 101.2 | 40.9 | 101.8 |
| 50 mM                                  | 75.2     | 94.1   | 153.2  | 171.3   | 107.4    | 115.8 | 54.9 | 136.5 |
| 150 mM                                 | 100.2    | 125.5  | 105.6  | 118.1   | 109.2    | 117.7 | 45.2 | 112.4 |
| 300 mM                                 | 67.5     | 84.5   | 67.8   | 75.8    | 98.7     | 106.4 | 52.9 | 131.7 |
| L. S. D. 5%                            | 0.12     | 0.16   | 0.72   | 0.37    |          |       |      |       |

| cv. Gimiza 11                           |          |        |        |         |          |       |
|----------------------------------------|----------|--------|--------|---------|----------|-------|
| Cont                                   | 68.9a    | 100    | 60.5   | 100     | 77.6     | 100   |
| 20 mM                                  | 64.5     | 93.6   | 79.5   | 131.5   | 73.8     | 95.1  | 93.9 | 58   |
| 50 mM                                  | 51.6     | 74.9   | 83.9   | 138.7   | 73.6     | 94.8  | 50.0 | 72.6 |
| 150 mM                                 | 44.9     | 65.1   | 36.2   | 59.9    | 71.4     | 92.0  | 50.0 | 72.6 |
| 300 mM                                 | 46.9     | 68.1   | 35.3   | 58.3    | 61.2     | 78.8  | 44.7 | 64.9 |
| L. S. D. 5%                            | 0.85     | 0.54   | 0.21   | 0.40    |          |       |      |       |

| cv. Gimiza 10                           |          |        |        |         |          |       |
|----------------------------------------|----------|--------|--------|---------|----------|-------|
| Cont                                   | 88.7a    | 100    | 73.6a  | 100     | 158.8a   | 100   |
| 20 mM                                  | 93.4     | 105.2  | 67.2   | 91.3    | 138.6    | 87.2  | 44.9 | 140.3 |
| 50 mM                                  | 98.4     | 110.9  | 67.6   | 91.8    | 138.8    | 87.4  | 42.5 | 132.8 |
| 150 mM                                 | 86.2     | 97.1   | 59.4   | 73.6    | 1146.5   | 92.2  | 33.3 | 104.2 |
| 300 mM                                 | 45.6     | 54.4   | 54.4   | 73.9    | 120.6    | 75.9  | 43.9 | 1.37 |
| L. S. D. 5%                            | 0.85     | 0.16   | 0.41   | 0.23    |          |       |      |       |

Means not labeled with letter (a) are significantly different from control level mean.

*Significant at < 0.05

In stems the soluble carbohydrates stimulated markedly and highly significantly up to 50 mM NaCl, then it reduced quickly and surprisingly. In leaves the soluble carbohydrates remained more or less unchanged at the most salinization levels, with a minor reduction only at the higher salinity level used. In spikes, the soluble carbohydrates dropped highly significantly and quickly, whatever the salinity levels used. The soluble carbohydrates reduced by about 35% at 300 mM NaCl. In roots of cv. Giza 168, the
soluble carbohydrates remained more or less unchanged up to 150 mM NaCl, and then it reduced quickly and highly significantly at 300 mM NaCl (table 2). In stems the soluble fraction remained more or less unchanged up to 50 mM and reduced highly significantly beyond this level, which seemed to be more or less similar this reduction was about 25%. In leaves the soluble carbohydrates were reduced marginally up to 150 mM and highly significantly at 300 mM NaCl. In spikes the soluble carbohydrate in spike enhanced markedly at the most salinity level used. The magnitude of this effect was higher at the moderate doses of the salt.

1.2.2 Soluble protein
In roots the soluble protein of wheat cultivar Sakha 94 enhanced markedly by salinity stress, this enhancement was more pronounced up to 150 mM NaCl (table 3). In stems, there is a marked increasing trend in the accumulation of soluble proteins as the salinity increase in the culture media. In leaves, the soluble protein content remained more or less unchanged up to 150 mM NaCl, then some activation was exhibited which was much more pronounced and 300 mM NaCl (about 21.7% over the control). In spikes salinity induced an accumulation in soluble protein reach a maximum value at 150 mM NaCl levels.

Table 3 Effect of various concentrations of NaCl on protein content as mg/gm dry weight in roots, stems, leaves and spikes of wheat cultivar cv. Sakha 94, cv. Gimiza11, cv. Gimiza10 and cv. Giza 168.

| NaCl Treat. | Root | % | Stem | % | leaf | % | Spike | % |
|-------------|------|---|------|---|------|---|-------|---|
| Cont        | 62.6a| 100| 50.4a| 100| 90.9a| 100| 38.6  | 100|
| 20 mM       | 99.3 | 158.7| 58.8 | 116.6| 84.6 | 93.0| 39.9  | 103.5|
| 50 mM       | 96.9 | 154.9| 59.6 | 118.1| 72.9 | 80.1| 60.9  | 157.9|
| 150 mM      | 99.9 | 159.7| 58.9 | 116.9| 84.0 | 92.4| 50.4  | 130.7|
| 300 mM      | 75.6 | 120.8| 84.9 | 168.4| 109.5| 120.4| 60.2  | 156.0|
| L. S. D. 5% | 0.21 | 0.11| 0.19 | 0.13|      |     |       |     |

| NaCl Treat. | Root | % | Stem | % | leaf | % | Spike | % |
|-------------|------|---|------|---|------|---|-------|---|
| Cont        | 26.9a| 100| 32.2a| 100| 94.2a| 100| 24.5  | 100|
| 20 mM       | 44.6 | 165.7| 36.4 | 113.0| 85.8 | 91.0| 36.4  | 148.5|
| 50 mM       | 42.8 | 159.1| 49.4 | 153.4| 83.1 | 88.2| 21.2  | 86.5|
| 150 mM      | 53.6 | 199.2| 30.2 | 93.7 | 114.9| 121.9| 21.9  | 89.3|
| 300 mM      | 33.0 | 122.6| 29.4 | 91.3 | 135.9| 144.2| 35.6  | 145.3|
| L. S. D. 5% | 0.17 | 0.14| 0.4  | 0.10|      |     |       |     |

| NaCl Treat. | Root | % | Stem | % | leaf | % | Spike | % |
|-------------|------|---|------|---|------|---|-------|---|
| Cont        | 58.4a| 100| 101.2a| 100| 74.8a| 100| 88.4a | 100|
| 20 mM       | 73.2 | 125.3| 124.0| 122.5| 80.8 | 108.0| 72.4  | 81.9|
| 50 mM       | 61.2 | 104.7| 104.4| 103.1| 83.0 | 110.9| 75.2  | 85.0|
| 150 mM      | 71.2 | 121.9| 104.2| 102.9| 48.6 | 64.9 | 66.4  | 75.1|
| 300 mM      | 57.6 | 98.6| 55.855.1| 97.2| 129.9| 63.6| 71.9  |     |
| L. S. D. 5% | 0.47 | 0.23| 0.22 | 0.33|      |     |       |     |

| NaCl Treat. | Root | % | Stem | % | leaf | % | Spike | % |
|-------------|------|---|------|---|------|---|-------|---|
| Cont        | 80.4a| 100| 93.2a| 100| 92.4a| 100| 65.6a | 100|
| 20 mM       | 84.8 | 105.4| 83.4 | 89.4 | 88.8 | 96.1 | 67.4  | 102.7|
| 50 mM       | 86.0 | 106.9| 87.2 | 93.5 | 81.6 | 88.3 | 65.0  | 99.0|
| 150 mM      | 80.4 | 100 | 75.2 | 80.6 | 77.2 | 83.5 | 60.2  | 91.7|
| 300 mM      | 87.6 | 108.9| 67.2 | 72.1 | 72.0 | 77.9 | 52.6  | 80.1|
| L. S. D. 5% | 0.21 | 0.51| 0.27 | 0.15|      |     |       |     |

Means not labeled with letter (a) are significantly different from control level mean.

*Significant at < 0.05

In roots of cv. Gimiza 11, the soluble protein content accumulated hugely and unexpectedly up to 150 mM NaCl approached 2-folds and the lowest accumulation of protein was reported in plants
irrigated with 300 mM NaCl (about 22.6% over the control) (table 3). In stems, the soluble protein content accumulated markedly up to 50 mM NaCl, and remained more or less unchanged at the other salinization levels. The soluble protein content increased by about 53.3% at 50mM NaCl. In leaves, the soluble protein content remained more or less unchanged up to 50 mM NaCl, there after some inconsistent accumulation has been recorded. In spikes, the soluble contents increased at most salinization levels, which was much more prominent at higher doses of salinization levels (300 mM NaCl level). In roots of cv. Gimiza 10, the soluble protein content enhanced slightly up to 150 mM NaCl and remained more or less unchanged beyond this level (table 3). In stems, the soluble protein remained more or less unchanged up to 150 mM NaCl then, it slow down greatly, the soluble protein reduced by about 45% at 300 mM NaCl. In leaves, three faces occurred in the effect of various salinization levels of soluble proteins in cv. Gimiza 10, a- Some promotion occurred up to 50 mM NaCl. b- A marked and progressive reduction was recorded at 150 mM NaCl. c- A significant enhancement was obtained at 300 mM NaCl. In spikes the soluble protein content reduced marginally at the most salinization levels; the highest reduction about 30% was recorded at the highest concentrations of NaCl. In stems, the soluble protein remained more or less unchanged up to 50 mM NaCl, above which a gradual reduction was exhibited. This reduction approached 30% at 300 mM NaCl. In leaves the soluble protein remained more or less unchanged up to 150 mM NaCl, there after some reduction in soluble protein content was obtained. In spikes, while salinity stress induced a slight reduction, if any in the soluble protein.

Table 4 Protein pattern of cv. Sakha 94, cv. Gimiza 11, cv. Gimiza 10 and Giza 168 in response of salinity stress (50 mM, 150 mM and 300 mM NaCl levels) as compared with control plants.

| MW   | G-10 0.0mM | G-11 0.0mM | G-168 0.0mM | SHK-94 0.0mM |
|------|------------|------------|-------------|-------------|
| 63.5 KD | +          | +          | +           | +           |
| 61.6 KD | +          | +          | +           | +           |
| 57.1 KD | +          | +          | +           | +           |
| 52.1 KD | +          | +          | +           | +           |
| 47.2 KD | +          | +          | +           | +           |
| 44.1 KD | +          | +          | +           | +           |
| 42.2 KD | +          | +          | +           | +           |
| 38.2 KD | +          | +          | +           | +           |
| 37.0 KD | +          | +          | +           | +           |
| 35.1 KD | +          | +          | +           | +           |
| 33.2 KD | +          | +          | +           | +           |
| 32.3 KD | +          | +          | +           | +           |
| 28.2 KD | +          | +          | +           | +           |
| 26.4 KD | +          | +          | +           | +           |
| 24.1 KD | +          | +          | +           | +           |
| 20.2 KD | +          | +          | +           | +           |
| 18.8 KD | +          | +          | +           | +           |
| 17.1 KD | +          | +          | +           | +           |
| 16.3 KD | +          | +          | +           | +           |
| 15.6 KD | +          | +          | +           | +           |
| 14.8 KD | +          | +          | +           | +           |
| 12.8 KD | +          | +          | +           | +           |
| 10.1 KD | +          | +          | +           | +           |
In roots cv. Giza 168 the soluble protein in root remained more or less unchanged up to 150 mM NaCl, and then some activation was recorded about 14.3% at levels from 150 mM to 300 mM NaCl (table 3). In stems the soluble protein remained more or less unchanged up to 50 mM NaCl, above which a gradual reduction was exhibited. This reduction approached 30% at 300 mM NaCl. In leaves the soluble protein remained more or less unchanged up to 50 mM NaCl, there after some reduction in soluble protein content was obtained. In spikes while salinity stress induced a slight reduction, if any in the soluble protein.

Protein analysis by electrophoresis
The results in table 4 and figure 1 revealed that 23 protein bands were detected in cv. Sakha 94, 20 protein bands in cv. Gimiza 11, 18 protein bands in cv. Gimiza 10 and 21 protein bands in cv. Giza 168. The four cultivars possessed 17 common protein bands with molecular weight 63.5 KDa, 61.6 KDa, 57.1 KDa, 47.2 KDa, 42.2 KDa, 38.2 KDa, 37.0 KDa, 35.1 KDa, 28.2 KDa, 26.4 KDa, 24.1 KDa, 20.2 KDa, 17.1 KDa, 16.3 KDa, 15.6 KDa, 12.8 KDa and 10.1 KDa. While they different from each other in 6 protein bands with molecular weight 52.1 KDa, 44.1 KDa, 33.2 KDa, 32.3 KDa, 18.8 KDa and 14.1 KDa. The 44.1 kDa is specific marker for both cultivars Sakha 94 and Giza 168. However, the 33.2 KDa is specific marker for cv. Sakha 94, cv. Gimiza 11 and Giza 168. The 32.3 KDa is specific marker for cv. Sakha 94 and cv. Gimiza 11. Studying the pattern of protein synthesis under salt stress may help to identify a proteins associated with stress. In the present study, salinity induced the synthesis of newly proteins and simultaneously reduced other protein sets (Table 1). The results revealed that three bands at molecular weight 52.1 kDa is induced under salinity stress in four tested cultivars Sakha 94, Gimiza 11, Gimiza 10 and Giza 168, as compared to the control treatment. It was induced at 50 mM, 150 mM in both cultivars Gimiza 11, Gimiza 10 and Giza 168 while, induced at 50 mM, 150 mM and 300 mM NaCl levels in cv. Sakha 94 as compared to control treatment. This was detected in cv. Sakha 94 with molecular weight 14.8 kDa at 50 mM and 150 mM NaCl levels but it disappeared at 300 mM NaCl. This band was absent in the cultivars Gimiza 11, Gimiza 10 and Giza 168. This result suggested that this band was specific and responsible for halo tolerant character features of cv. Sakha 94 and supported the growth parameters (crop yield), metabolic constituents and minerals. In addition to these newly synthesized proteins, salinity stress also reduced the production of 14.8 in cv. Sakha 94, 52.1 KDa, 44.1 kDa, 18.8 kDa, 14.8 kDa in cv. Gimiza 11, 52.1 kDa, 44.1 kDa, 33.2 kDa, 32.3, 18.8, 14.8 in cv. Gimiza 10 and 52.1 kDa, 32.3 kDa, 14.8 kDa protein in cv. Giza 168 kDa, as compared with the control treatment.

![Polyacrylamide gel electrophoresis of soluble protein profiles separated from wheat cultivars Sakha 94 (SHK-94), Gimiza 11 (G11), Gimiza 10 (G10) and Giza 168 (G168) treated with 0.0, 50 mM, 150 mM, 300 mM NaCl levels](https://example.com/polyacrylamide-gel-electrophoresis)

**Figure 1** Polyacrylamide gel electrophoresis of soluble protein profiles separated from wheat cultivars Sakha 94 (SHK-94), Gimiza 11 (G11), Gimiza 10 (G10) and Giza 168 (G168) treated with 0.0, 50 mM, 150 mM, 300 mM NaCl levels

1.2.3 Total free amino acids content
The data in figure 2 illustrated that while salinity stress induced insignificant changes in the amount of amino acids in roots wheat cultivar Sakha 94. It on the other hand, induced a considerable activation in the
Contents of amino acids in the other three plant organs (stems, leaves and spikes).

Figure 2 Effect of various concentrations of NaCl on amino acids as mg/ gm dry weight in roots, stems, leaves and spikes of wheat cultivar cv. Sakha 94, cv. Gimiza 11, cv. Gimiza 10 and cv. Giza 168.

In stems the highest accumulation of amino acids was obtained in plants irrigated with 20 mM NaCl (about 65.6% over the control value), in leaves and spikes the highest accumulation of amino acids was recorded in plants subjected to 300 mM NaCl (45.8% over the control value), in leaves and spikes respectively. In roots wheat cultivar Gimiza 11 amino acids content remained more or less unchanged up to 150 mM NaCl, then they decreased highly significantly (figure 2). At the level of 300 mM NaCl the amount of amino acids reduced by 31.4% below the control value. In stems, the accumulation of amino acids enhanced highly significantly, whatever the concentration of NaCl might be. The highest concentration of amino acids was recorded at the level of 300 mM NaCl (about 54.8% over the control value). In leaves there is a marginal increase in the total amino acids content at the most salinization level used. In spikes, the amount of amino acids content is maintained mainly around the control value. The data in figure 2 showed that while roots kept the amino acids mainly around the control, they reduced marginally in stems of wheat cultivar Gimiza 10. In leaves, the salinity stress up to 150 mM NaCl induced a marked accumulation in the content of amino acids. Above which, they remained more or less unchanged even at the highest doses of the salt. In spikes the amount of amino acids still around those of control plants up to 50 mM NaCl, and then there is a gradual increase in these contents up to 150 mM NaCl. There after the concentration of 300 mM NaCl retained the amount of amino acids around those of control plants. It is worthy to mention that the percent of increase in the total amino acids in spikes subjected to 300 mM NaCl was 5% respectively that indicated the surprising differences between the different salinity levels in the
accumulation of amino acids in spike of wheat cultivar Gimiza 10. In roots of cv. Giza 168 amino acids content reduced slightly by salinity stress which seemed to be pronounced at the higher doses of the salt. In stems there is some promotion in the accumulation of amino acids at the most salinization levels. However this trend in the accumulation of amino acids differed greatly from salinization level to another. In leaves the salinity stress up to 150 mMNaCl induced insignificant changes in the amount of amino acids, and then there is a marked and progressive increasing trend in the amount of amino acids by increasing the salinity stress in the soil. The magnitude of this promotion was the highest at the highest salinization level (300 mMNaCl).

At this level the percent of increase in the amino acids content was 79.1% over the control values. In spikes the amount of amino acids remained more or less unchanged up to 50 mMNaCl, and then they reduced quickly and suddenly by increasing the salinity stress in the soil. The highest reduction was observed at the higher doses of salt (300 mMNaCl), which was about 36% below the control values.

Figure 3 Effect of various concentrations of NaCl on proline as mg/ gm dry weight in roots, stems, leaves and spikes of wheat cultivar cv. Sakha 94, cv. Gimiza11. cv. Gimiza10 and cv. Giza 168.

1.2.4 Proline content
In wheat cultivar Sakha 94, the data in figure 3 showed that the salinity stress dropped greatly the amount of proline in roots, but more so at the higher salinity. Proline content is reduced by about 43.5% at 300 mM NaCl. In stems, proline content enhanced greatly and irregularly at the most salinization levels, so the amount of proline differed greatly among the different salinization levels. At the lower salinization levels while proline content increased by 83% at 50 mMNaCl, it increased by only 8.3% at 20 mMNaCl. At the highest salinization levels (300 mM NaCl), the percent of increase in proline at 300 mM NaCl was 50%.Moderate salinization level (150 mMNaCl), the percent of increase in proline content of stem was 50%.In leaves, proline content remained more or less
unchanged up to 150 mMNaCl, there after a highly significant accumulation was recorded at 300mMNaCl. Also the percent of increase at 300 mMNaCl was 29%. In spikes proline content enhanced slightly up to 50 mMNaCl, and then it accumulated progressively. The highest accumulation was reported at 300 mMNaCl which approached 47% than that in control plants.

The data in figure 3 illustrated a marked variation in proline content in the four plant organs of wheat cultivar Gimiza 11. In roots, there is a great reduction in proline content up to 150 mM NaCl, the magnitude of this reduction was the highest at the lowest concentration of NaCl, then it remained unchanged up to 150 mM NaCl, then a quick and sharp accumulation was reported at 300 mM NaCl. At this level the percent of increase in the proline content was about 130.7% in relation to the control values. In stems, the amount of proline reduced marginally up to 150 mM NaCl then some promotion was exhibited at 300 mM NaCl (about 21.4% over control values). In leaves tended in most cases to kept proline content around the control values whatever the salinity levels used. In spikes, there is a great and fast accumulation in proline content at all salinity levels. The highest accumulation was recorded at 300 mM NaCl (about 121.4% over the control values).

In roots and stems of cv. Gimizia 10 proline content dropped progressively whatever the salinity level used. Interestingly the highest reduction was recorded in plants irrigated with 150 mM NaCl in both plant organs. At this level the percent of reduction of proline in roots and stems was 51.5% and 68.5% respectively, which considered unexpected results in the accumulation of proline under stress condition especially when we take into consideration, that this cultivar was categorized as a salt sensitive cultivar. In leaves proline content remained more or less unchanged up to 50 mMNaCl, and then some reduction was exhibited. In Spikes proline content dropped quickly, it dropped by about 57% at 300 mMNaCl respectively.

The data in figure 3 illustrated the variation in proline content in the four plant organs of cv. Giza168. In roots, proline content remained more or less unchanged up to 50 mMNaCl, there after it reduced marginally beyond this level. In stems, proline content slightly accumulated up to 50 mMNaCl, and then gives the value of control up to 150 mMNaCl levels. At the highest salinization levels 300 mMNaCl, it enhanced greatly. In leaves and spikes, there is no significant change in proline content, whatever the salinity levels used.

2 Discussion

From previous results it can be said that cv. Sakha 94 was the most salt tolerant cultivar moreover this cultivar has halophytic character because stimulation rather than inhibition was recorded even at the highest doses of NaCl which could be linked with the success of breeders to select parents which can produce this surprising tolerant cultivar followed by cv. Gimiza11 which tolerated NaCl salinity mainly up to the level of 150 mMNaCl salinity but a marked reduction was observed at the level of 300 mM (about 20%), surprisingly the production of spike of cv. Giza168 and cv. Gimiza 10 slow down earlier and markedly, that is while, the production reduced by about 40% at the level of 20 mM in cv. Gimiza 10, it reduced by more than 50% at the same salinization level in wheat cultivar Giza 168 which indicated the great susceptibility of these cultivars to salinity, these cultivars cannot be cultivated even at the lowest saline soil and indicated the unsuccessfulness of the breeding program of these susceptible cultivars. Accordingly, the salt tolerance of the four wheat cultivars, during vegetative growth and crop yield stages ranked as the following:

cv. Sakha 94> cv. Gimiza 11 >cv. Gimiza 10 > cv. Giza 168

Interestingly, the differences in the production of spike among the four wheat cultivars under salinity conditions were found to be greatly concomitant with the data of shoot system. cv. Sakha 94 was the superior in the production of dry matter yield of shoot system followed by cv. Gimiza 11 and cv. Gimiza 10, so cv. Giza 168 was the salt susceptible dry matter producer at the vegetative growth. Thus the differences in the phenological characters among the studied cultivars, during vegetative growth draw the future of crop yield. This was also draw by the direction of various metabolic processes as well as the transportation between carbon and nitrogen. It is worthy to mention that the difference in the salt
tolerance of glycophytes differed greatly not only among species and cultivars, but also among the different organ of the same cultivar, for example:

In cv. Sakha 94 while, stems, leaves and spikes enhanced by lower and moderate salinization levels. Roots reduced by about 38% beyond 150 mM NaCl at 300 mM. In cv. Gimiza 11, while roots reduced by 50% and stem reduced by 25%, leaves reduced about 30% and only 20% in spike at 300mM NaCl. However, in cv. Gimiza 10 and cv. Giza 168, the reduction in the four plant organs (stem, leaf, root, and spike) seemly to be more or less comparable and appeared earlier (at the lower salinization level). It is worthy to note that lower concentrations of NaCl stimulated the growth of wheat shoot and broad bean root and cotton shoot and root plants (Hamdia and Shaddad, 2014). Abdul Qados (2011) investigated the effect of sodium chloride (NaCl) concentrations on growth, of (Vicia faba L.) seedlings. NaCl caused an increase in plant height with low and medium concentrations and a decrease with the highest concentration, in both measurement periods. No significant effect was observed in the number of leaves or leaf area with low concentration, while a decrease was noticed for each, with two higher concentrations and in both measurement periods. The inhibitory effect of salinity of growth as in cv. Gimiza 10 and cv. Giza 168 may be attributed to the effects of salinity on several facets of plant activities such as enzyme activity (Seckin et al., 2009), DNA, RNA, protein synthesis (Anuradha and Rao, 2001) and mitosis (Tabur and Demir, 2010). However, plant species differ in their sensitivity or tolerance to salt stress (Ashraf and Harris, 2004; Roy et al., 2014), osmotic adjustment Hamdia and El-Komy (1998), hormonal balance (Jackson, 1997; Debezet et al., 2001; Iqbal and Ashraf (2013) and photosynthesis (Amuthavalli and Sivasankaramoorthy 2012). The great differences in the accumulation of carbohydrates among of the four wheat cultivars and even in their plant organs could use as a suitable trait for the differences in the gene expression (Hamdia and Shaddad 1996, Hamdia and Azooz, 2002 and Balibrea et al., 1997). They stated that the sugar accumulation and its distribution in different parts of plants could be a valid trait to discriminate cultivars of different tolerant to saline and osmotic stress. In some cases the soluble fraction increased highly significantly which could share in osmotic pressure, for example leaves of cv. Sakha 94 and to some extent cv. Gimiza 11 accumulated a suitable amount of soluble sugars in increasing the osmotic pressure of leaves which could play a pivotal roles which could achieved water flow from the down ward into upward, this strategy considered important in the field of the salt tolerance in the glycophytes. Plants accumulated the soluble component in leaf to pooling water from the down ward into upward. On the other hand and interestingly the most sensitive cultivars cv. Giza168 and cv. Gimiza 10 failed to accumulate the soluble sugars in their leaves, this component dropped markedly beyond 150 mM NaCl, which might a suitable sign for the great susceptibility (please again see the observable reduction in the crop yield of this cultivar). Reduction in plant biomass is sometimes observed under sever salt stress, and this is possibly because of the decrease in carbohydrate accumulation caused by reduction in carbon assimilation (Pattanagul and Thitisaksakul, 2008).

Protein contents were also varied among the four wheat cultivars and their plant organs. It is worthy to mention that wheat cultivar Sakha 94 maintained, there is a huge accumulation in the soluble protein in roots, stems, leaves and spikes whatever the salinity level used (as in the case of soluble carbohydrates). In cv. Gimiza 11 the soluble protein induced highly significantly in leaves, roots and in stems up to 50 mM NaCl, while in spikes soluble protein contents remained more or less unchanged up to 150 mM NaCl and accumulated highly significantly and progressively at 300 mM NaCl, which indicated also that the four plant organs responded differently to the soluble fraction under the salt stress conditions. In Gimiza 10 also this cultivar failed to accumulate the soluble protein especially under moderate and higher salinization levels.

In cv. Giza 168 the most sensitive cultivar also in most cases failed to accumulate a suitable amount of soluble protein especially in the principle plant organ (roots, stems, and leaves). Shaddad et al. (2006) and Chen et al., (2007) stated that the accumulation of soluble proteins helps in triggering and transition of cells from a state of active growth to a state of salinity tolerance. The accumulation of compatible solutes is often regarded as a basic strategy for the production and survival of plants under salinity stress and
oxidative stress. Accordingly, protein concentrations showed different results with increasing of salinity level which depended on plant species and genotypes (Rahdar et al., 2012). Shaddad et al., (2012) suggest a greater participation of soluble sugars and proteins than free amino acids in maintaining water relations in both roots and shoots.

The results in table 3 and figure 1 revealed that 23 protein bands were detected in cv. Sakha 94, 18 protein bands in cv. Gimiza 11, 16 protein bands in cv. Gimiza 10 and 18 protein bands in cv. Giza 168. The four cultivars possessed 17 common protein bands while they different from each other in 6 protein bands.

Studying the pattern of protein synthesis under salt stress may help to identify a protein(s) associated with stress. In the present study, salinity induced the synthesis of newly proteins and simultaneously reduced other protein sets. The results revealed that three bands at molecular weight 52.1 kDa is induced under salinity stress in four tested cultivars Sakha 94, Gimiza 11, Gimiza10 and Giza 168, as compared to the control treatment. It was induced at 50 mM, 150 mM in both cultivars Gimiza 11, Gimiza 10 and Giza 168 while, induced at 50 mM, 150 mM and 300 mMNaCl levels in cv. Sakha 94 as compared to control treatment. These results revealed that the 52.1 kDa protein band was commonly induced as a result of salinity treatment in the four cultivars. Another important point is the induction of protein band with molecular weight 14.8 in cv. Sakha 94 at 50 mM and 150 mm NaCl levels but it disappeared at 300 mMNaCl. This band was absent in the cultivars Gimiza11, Gimiza 10 and Giza 168. This result suggested that this band was specific and responsible for halotolerant characteristic features of cv. Sakha 94 and supports the growth parameter (crop yield), metabolic constituents and minerals. Tammam (2003) found that salt treatment of broad bean seedling resulted in the disappearance of five polypeptides, while the peptides with molecular mass 26, 18, 14and 12kDa increased in their intensity and two peptides 99 and 102 kDa appeared on the gel. Amini and Ehsnapour (2005) stated that accumulation of proteins in plant grown under saline condition may provide a storage form of nitrogen that is re-utilized when stress is over and might be due to osmotin like protein in a particular synthesis of those proteins which are involved in modification of cell wall. Witzel et al., (2014) study the root proteome was analyzed based on two-dimensional gel electrophoresis. A number of cultivar-specific and salinity stress-responsive proteins were identified. Mass spectrometry-based identification was successful for 74 proteins, and a hierarchical clustering analysis grouped these into five clusters based on similarity of expression profile.

It is worthy to mention that salinity stress exerted multiple effects on the production of free Amino acids among the four wheat cultivars and their plant organs. The correlation between proteins and amino acids in the four wheat cultivars and their plant organs was interestingly because:

While the total protein remained more or less unchanged in cv. Sakha 94, amino acids enhanced markedly in (stems, leaves, and spikes) and remained unchanged in roots. Thus the two processes was not interdependent, the high accumulation of amino acids in this state is not incorporated into proteins because it was equilibrated, there is no need to this elevation in amino acids for protein content (a sufficient amount of protein was synthesized in this cultivar), the high accumulations of amino acids in this state could directed into biological functions rather than protein synthesis they used functionally rather than structurally. They used as:

a-Osmoregulation to increase the internal osmotic potential (osmotic adjustment). (Dubey and Rani, 1989; Seki et al., 2007) stated that the major osmoregulator substance such accumulation of amino acids in salt-stressed plants equalizes the osmotic potential of the cytoplasm, thus maintaining the cellular function and structure.

b-Non-enzymatic free radical scavenging components kept the cell membranes mainly around absolute control. The function of the accumulation of amino acids is often associated with osmotic adjustment by lowering the water potential to improve the water uptake against the external gradient (Gilbert et al., 1998; Abd El-Samad et al. 2013). This agree with Sato et al., (2006) who stated that the increase of some amino acids might be result of reduced water transport under saline stress and/ or active physiological reaction of decreasing the water potential to cope with
the stress. (Gilbert et al., 1998) added other possible roles played by amino acids such as serving as readily available energy source or as nitrogen source during limited growth and photosynthesis, detoxification of excess ammonia under periods of stress and stabilization of enzymes and /or membranes might be achieved.

In cv. Sakha 94 proline content dropped quickly and surprisingly in roots at all salinization levels, increases in highly significant in stems and spikes and remained unchanged in leaves up to 150 mMNaCl. Also, the growth and the crop yield in this genotype were estimated greatly under salinity condition. In cv. Gimiza 11 three faces occurred on the effect on the accumulation of proline in roots according to salinity level: a- It reduced markedly up to 150 mM NaCl. a-Accumulated highly and quickly at 300 mM NaCl. In leaves of cv. Gimiza 11 proline content remained unchanged up to 150 mM afterthat, it accumulated at 300 mM NaCl and reduced in stems. While in spikes, proline accumulated greatly in the all salinity levels. Also, the dry matter accumulation in this cultivar remained more or less unchanged whatever the salinity level used.

It is worthy to mention that proline content in cv. Gimiza 10 reduced sharply in roots, stems, leaves and spikes which were interestingly and surprisingly accompanied with the excessive reduction in dry matter yield in the four plant organs especially at higher salinity stress levels. Thus in this cultivar, the two processes (the accumulation of dry matter and proline) seemed to be revealed parallel to each other. In the most sensitive cultivar Giza 168 proline content seemly to be in the most cases remained more or less unchanged in four plant organs at most salinization level. Interestingly it was found to be accompanied with a great reduction in the dry matter yield in four plant organs and consequently in crop yield production. Accordingly the correlation between proline content and dry matter yield in the four cultivars revealed 3 scenarios. The two processes went to opposite to each other in the most cases in cv. Sakha 94 and to some extent in cv. Gimiza 11, (i.e. the great reduction in proline accompanied with a great stimulation in dry matter yield).Proline content remained more or less unchanged in Giza 168 accompanied with a great reduction in dry matter yield, they also went opposite to each other. Interestingly and surprisingly the two processes revealed parallel to each other only in wheat cultivar Gimiza 10, where the great reduction in proline content accompanied with a great reduction in dry matter yield in the four plant organs. All the three scenarios and interpretations indicated the complexity and diversity in the correlation between criteria of proline and the differences in the salt tolerance among the four studied cultivars and their organs which weakness the physiological significance of proline even to what can proline is a sign for salt tolerance or saline injury should be taken with care.

The problematical behavior of proline could accompany with the criteria in the synthesis and proline degradation during stress condition. The accumulation or reduction of proline could accompanied with the activity of the enzymes responsible for the synthesis or the degradation of proline (proline turn over).The biosynthesis and degradation of proline did not equilibrated. (I.e. troubling in the de novo synthesis).Some researchers have suggested that proline accumulation may be related to the degree of salt tolerance and/ or osmotic tolerance (Ashraf and Iram, 2005; Hassine et al., Ghanem, 2008; Rajaravindran; Natarajan, 2012). While, in contrast, others have suggested that it is a symptom of salt-stress injury rather than an indicator for resistance (Lutts et al., 1996 a, b; Ashraf and Harris, 2004; Mansour et al., 2005).

The response of crops to salinity is a complex phenomenon and involves changes in plant morphology and physiology. Such modifications in plant physiology may lead to the accumulation or depletion of certain metabolites, alteration in the behavior of many enzymes and synthesis of new sets of proteins referred as salt-tolerant proteins (Ben-Hayyimet et al., 1989).

3 Materials and Methods
Vegetative growth stage and crop yield stage (long duration experiment): A pot experiment was carried out in open air at the garden of the Faculty of Science-Minia University during winter season (from the beginning of November to the middle of March). Wheat seeds cultivars (Sakha94, Gimiza11, Gimiza10, and Giza168) which brought from three different
Wheat seeds were surface sterilized by immersion in a mixture of ethanol 96% and H₂O₂ (1:1) for 3 minutes, followed by several washings with sterile distilled water. The concentrations of NaCl were chosen after preliminary experiments in which the seeds were subjected to different concentrations of NaCl. Eight seeds were sown per pot. Each pot contained 3.8 kg of garden clay soil in three replicates. All pots were irrigated with tap water for two weeks until full germination. The seedlings were then irrigated by different concentrations of NaCl solutions (0, 20, 50, 150 and 300 mM) after two weeks from sowing. In order to maintain the osmotic potential, the soil moisture content was kept near the field capacity using tap water. Plants were left to grow in natural conditions under these conditions until crop yield production. Dry weight was determined at the end of the experimental period yields of the different organs (roots, stems, leaves and spikes). To determine the dry matter yields of the different organs they were dried in an oven at 80°C. Successive weighting was carried out until the constant dry weight of each sample was reached. Carbohydrates were determined by the anthrone-sulfuric acids method (Fales, 1951). Free amino acids, proline and a soluble protein contents were measured according to Moore and Stein (1948), Bates et al. (1973) and Lowry et al. (1951) respectively.

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