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ORIGINAL ARTICLE

Varietal differences in growth vigor, water relations, protein and nucleic acids content of two wheat varieties grown under seawater stress

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Key words: Wheat / Seawater / growth vigor / Water relations / Protein

List of abbreviations: Control = Cont, days post-anthesis = d, Least significant difference = LSD, Resistant = R, Relative water content = RWC, Sensitive = S, Seawater = SW, Saturation water deficit = SWD

Plant growth under salt stress conditions is a complex mechanism and the way it is affected by the stress is not fully understood because the response of plants to excessive salinity is multifaceted and involves changes in plant's morphology, physiology and metabolism (Hilal *et al*., 1998), ultimately diminishing growth and yield (Ashraf and Harris, 2004). Morphologically the most typical symptom of saline injury to plant is reduction of growth (Azooz *et a*l., 2004; Jaleel *et* *al*., 2008), which is a consequence of several physiological responses including modification of ion balance, water status, mineral nutrition, photosynthetic efficiency, carbon allocation and utilization (Sultana *et al*., 2002; Ismail, 2003; Taylor *et al*., 2004; Yildirim *et al*., 2006).

Glycophytes, like wheat are negatively influenced by increased soil salinity resulting in significant reduction in their productivity (Flowers, 2004; Sairam and Tyagi, 2004). Since the plant life cycle of wheat is an orderly sequence of developmental stages, salinity may have a significant effect on the developmental processes that occur at particular time (El-Hendawy, 2004). In this connection, salinity not only reduces the growth rate of the entire wheat plant and its specific organs, but it also affects its development (Läuchli and Grattan, 2007). Hence, plant responses to this stress are complex but grouped into three general categories; homeostasis, detoxification and growth control (Zhu, 2001).

Usually, under saline conditions, plant growth was reduced by reducing the rate of leaf elongation, enlargement and cell division (Saqib *et al*., 2004; El-Mesiry *et al*., 2007). Therefore, increasing salinity level is detrimental to plant growth and may result in marked alteration in its morphological features including significantly reduction in shoot and root lengths, leaf area and total biomass production (Khattab, 2007; Hassanein *et al*., 2009). Many authors have observed that when wheat plants were exposed to salt stress, many growth parameters, including plant height, fresh and dry weights of shoot and root, the relative growth rate as well as leaf area tended to decrease (Aldesuquy *et al*., 2009; Shafi *et al*., 2009; Yang *et al*., 2009). Similarly and according to Cha-um *et al*. (2009), the fresh and dry weights of shoot and root, the shoot height as well as leaf area in salt-tolerant and salt-sensitive rice cultivars grown under NaCl stress were significantly decreased. Also, Widodo *et a*l. (2009) cleared that in two wheat cultivars, the stress imposed by 100 mM NaCl substantially reduced the fresh and dry weights of shoot and root, the shoot height and leaf area. Moreover, Mohammed (2007) cleared that mungbean plants subjected to 100, 200 and 300 mM NaCl induced drastic reduction in all growth characteristics through decreasing the shoot and root lengths, number of lateral roots and number of leaves, total area of leaves as well as fresh and dry weights of shoot and root.

Specific leaf area, the ratio of projected leaf area to leaf dry mass, is a critical parameter in many physiological studies. It describes the efficiency with which the leaf captures light relative to the biomass invested in the leaf (Marshall and Monserud, 2003). Moreover, specific leaf area is positively related to relative growth rates, leaf turnover rates, foliar nutrient concentrations and photosynthetic capacities (Cramer *et al*., 2000; Wright and Westoby, 2002). As a result, specific leaf area has been argued to be an important component of plant life history strategies (Grubb, 2002).

Leaves with high specific leaf area (lower leaf thickness and/or density) produce less photosynthetic machinery per unit area and therefore utilize low amounts of radiation (Sefton *et al*., 2002). These leaves also have greater relative growth rates in the absence of moisture and nutrient stress (Shipley and Vu, 2002). Conversely, leaves with low specific leaf area (greater leaf thickness and/or density) utilize high irradiances more efficiently and more tolerant to water and nutrient deficiency due to their thicker cuticle layers and slower turnover rates (Wright *et al*., 2002). In connection, Jampeetong and Brix (2009) reported that salt stress decreased the specific leaf area *in Salvinia natans* plants.

Salinity acts to inhibit plant access to soil water by increasing the osmotic strength of the soil solution (Sheldon *et al*., 2004). The inhibition of plant growth under water stress conditions is associated with altered water relations (Dichio *et al*., 2002). In this connection, Munns *et al*. (2006) reported that the presence of excessive salts in soil solution causes an osmotic stress, rapidly decreasing water uptake ultimately. Hence, less absorbed water means less water content indicated by relative water content (RWC), saturation water deficit (SWD), degree of succulence and degree of sclerophylly. In this respect, Welch and Rieseberg (2002 a&b) stated that, NaCl treatment decreased leaf succulence of three varieties of sunflower plants. In addition, Kholová *et al*. (2009) reported that in two maize genotypes, the salinity stress decreased RWC of the two varieties compared to control. In connection, Kabir *et al*. (2004) stated that in mungbean, salinity decreased RWC and water retention capacity but increased SWD and water uptake capacity. In this connection, Musyimi *et al.* (2007) cleared that leaf water content in 8 month old avocado seedlings decreased in response to increasing salt concentration in the growth medium. Also, Kaydan and Yagmur (2008) reported that NaCl significantly reduced RWC of shoot in a recently developed triticale cultivar compared to control.

In plants, salt stress retards all major growth processes such as cell division and enlargement, production of nucleic acids and proteins as well as energy metabolism (Yagi and Al-Abdulkareem, 2006). Plant growth, being an integral of cell elongation, is well documented to be negatively affected by salinity through reducing the cellular content of nucleic acids required for various metabolic processes (Zeid, 2009b). In this respect, El-Sawy (2009) reported that irrigation of wheat plants with seawater (10, 25 or 50%) resulted in marked reduction in total protein content. Moreover, Siddiqui (2006) cleared that salinity markedly decreased RNA and protein contents of *Arthrocnemum indicum* plants. In this connection, Younis *et al*. (2009) indicated that, increasing NaCl concentrations in the culture medium induced significant decrease in both DNA and RNA contents of *Vicia faba* plants. Also, Zeid (2009a&b) found that in both *Zea mays* and *Vicia faba* plants, both DNA and RNA contents were reduced under salt stress. Similarly, Jaleel *et al*. (2008) reported that in roots, stems and leaves of *Catharanthus roseus* plants under water stress, there was marked decrease in both DNA and RNA contents. Also, salt stress by NaCl induced significant decrease in both DNA and RNA contents of *Vigna radiata* plants (Mohammed, 2007).

The present study was undertaken to clarify the impact of seawater stress salinity on growth vigor and water relations variability, which in turn affect protein and nucleic acids (DNA and RNA) content in flag leaves of two wheat cultivars.

MATERIALS AND METHODS

Plant material and growth condition

Pure strains of *Triticum aestivum* L. Gemmieza-9 (salt sensitive cultivar) and Sids-1 (salt resistant cultivar) were kindly supported by the Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. For soaking experiment, a homogenous lot of *Triticum aestivum* L. (either sensitive or resistant cultivar) grains were selected. The grains were separately surface sterilized by soaking in 0.01 M $HgCl_2$ solution for three minutes, then washed thoroughly with distilled water. The sterilized grains from each cultivar were drilled in plastic pots (25 cm in diameter) filled with 7 kg soil (clay: sand 2/1, v/v), where 15 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 (min./max. air temperature and relative humidity were 15/25^{o}C and 35/45%, respectively) at mid-day during the experimental period. The plants 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 from each cultivar were divided into three sets. The 1st set was still irrigated with normal tap water serving as control, whereas the 2nd or 3rd ones were irrigated with 10% and 25% seawater receptively. 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^{-1} as urea and 25 kg P ha^{-1} as superphosphate. The chemical analyses of the employed seawater, collected from the Mediterranean Sea, revealed that it contains Cl^-, 21.6 Kg m^{-3}; Na^+,11.1 Kg m^{-3}; SO_4^{-2}, 2.85 Kg m^{-3}; K^+, 0.49 Kg m^{-3} and P^{+3} 16.6 μg dm^{-3}. Its salinity was found to be 38.5 g kg^{-1}; pH, 8.1 and EC, 47 mmhos cm^{-1}. To estimate the growth parameters during grain-filling (14 & 21 days post-anthesis) (i.e. 99 & 106 days after sowing), ten samples were taken from each treatment. Moreover, only triplicate samples were taken from each treatment for the biochemical analyses. Data were obtained and the mean values (per plant) were computed for each treatment.

Growth parameters

Leaf area = Length X Breadth X 0.75 (Quarrie and Jones, 1979)

Specific leaf area = Leaf area / Dry mass (Beadle, 1993)

Water amount = Fresh Mass - Dry mass

Degree of succulence = Water amount / Leaf area (Delf, 1912)

Degree of sclerophylly = Dry mass / Leaf area (Witkoswski and Lamont, 1991)

Shoot or root distribution = Fresh Mass / Length (Arduini *et al.*, 1994)

Shoot or root density = Dry Mass / Length (Arduini *et al.*, 1994)

Determination of relative water content (RWC)

For measuring relative water content, the method of **Weatherly (1950)** and its modification by Weatherly and Barrs (1962) was adopted.

Determination of saturation water deficit (SWD)

Saturation water deficit was calculated according to Weatherly and Barrs (1962) from the following equation: SWD (%) = 100 - RWC (%).

Estimation of protein

The method of protein extraction was adopted by Scarponi and Perucci (1986). Protein content was determined spectrophotometrically according to the method adopted by Bradford (1976).

Estimation of DNA and RNA

Nucleic acids (DNA and RNA) content were determined according to the method adopted by Sadasivam and Manickam (1996) as described by Devi (2000).

Statistical analysis

It should be mentioned that the sample numbers which were taken for investigation were as follows: ten for growth parameters and three for all chemical analyses and only the mean values were represented in the respective figures. A test for significant differences between means at $P \leq 0.05$ was preformed using least significant difference (LSD) test (Snedecor and Cochran, 1976). The correlation coefficients were estimated according to SPSS programme.

RESULTS

CHANGES IN GROWTH CRETERIA

Changes in growth vigor of root

The pattern of results showed that, there is a noticeable increase in all root growth criteria in control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. In relation to wheat cultivar, the resistant one had higher root criteria values than the sensitive ones. As compared to the control values, all concentrations of seawater salinity led to marked decrease ($P \leq 0.05$) in root biomass (fresh and dry masses), root length also number of adventitious as well as root/shoot ratio, root density and distribution of both wheat cultivars during anthesis stages. Magnitude of reduction was greater in salt sensitive cultivar than salt tolerant one (Fig 1).

Changes in growth vigor of shoot

The data presented revealed that, there is a tendency among control and seawater-stressed plants to a progressive increase in the growth vigor of shoot from 14 to 21 days post-anthesis in both wheat cultivars, except number of tillers. In relation to control values, all the used levels of salinity stress caused a noticeable decrease ($P \leq 0.05$) in all shoot characteristics (i.e. shoot length, plant height, shoot biomass (fresh and dry masses), number of tillers as well as shoot density and distribution of both wheat cultivars during anthesis stages. Salinity stress affected the salt sensitive cultivar more as compared to salt tolerant one (Fig 2).

Changes in growth vigor of flag leaf

Perusal of the data cleared that, there is a marked increase in flag leaf growth vigor in control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars, except degree of succulence in sensitive cultivar and degree of sclerophylly in both cultivars. In comparison with control values, all the applied doses of seawater resulted in general significant reduction ($P \leq 0.05$) in growth vigor of flag leaf, especially its biomass (fresh and dry masses), degree of succulence as well as leaf area and specific leaf area of both wheat cultivars during anthesis stages. On the other hand, seawater stress induced an increase in the degree of leaf sclerophylly. Meanwhile, 10% seawater caused a non-significant effect on all previous parameters in case of resistant cultivar in both stages. Among the cultivars, Sids-1 (salt tolerant) showed a better performance and produced more biomass under salt stress when compared with Gemmieza-9 (Fig 3).

Changes in relative water content (RWC %)

The data cleared that, there is a progressive decrease in turgidity in both control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. As compared to control plants, all the used concentrations of seawater caused additional reduction ($P \leq 0.05$) in RWC % of wheat flag leaf during anthesis stages in both cultivars with more reduction in salt sensitive cultivar in comparing to salt resistant one (Fig 4).

Changes in saturation water deficit (SWD %):

The data in figure 4 showed that, SWD % appeared to increase in flag leaf of control and seawater-stressed plants from 14 to 21 days post-anthesis in both wheat cultivars. In general, all the applied doses of seawater increased significantly ($P \leq 0.05$) SWD % in flag leaf of both cultivars during anthesis stages. Comparing both the cultivars higher SWD% was observed in salt sensitive cultivar than salt resistant one under saline conditions.

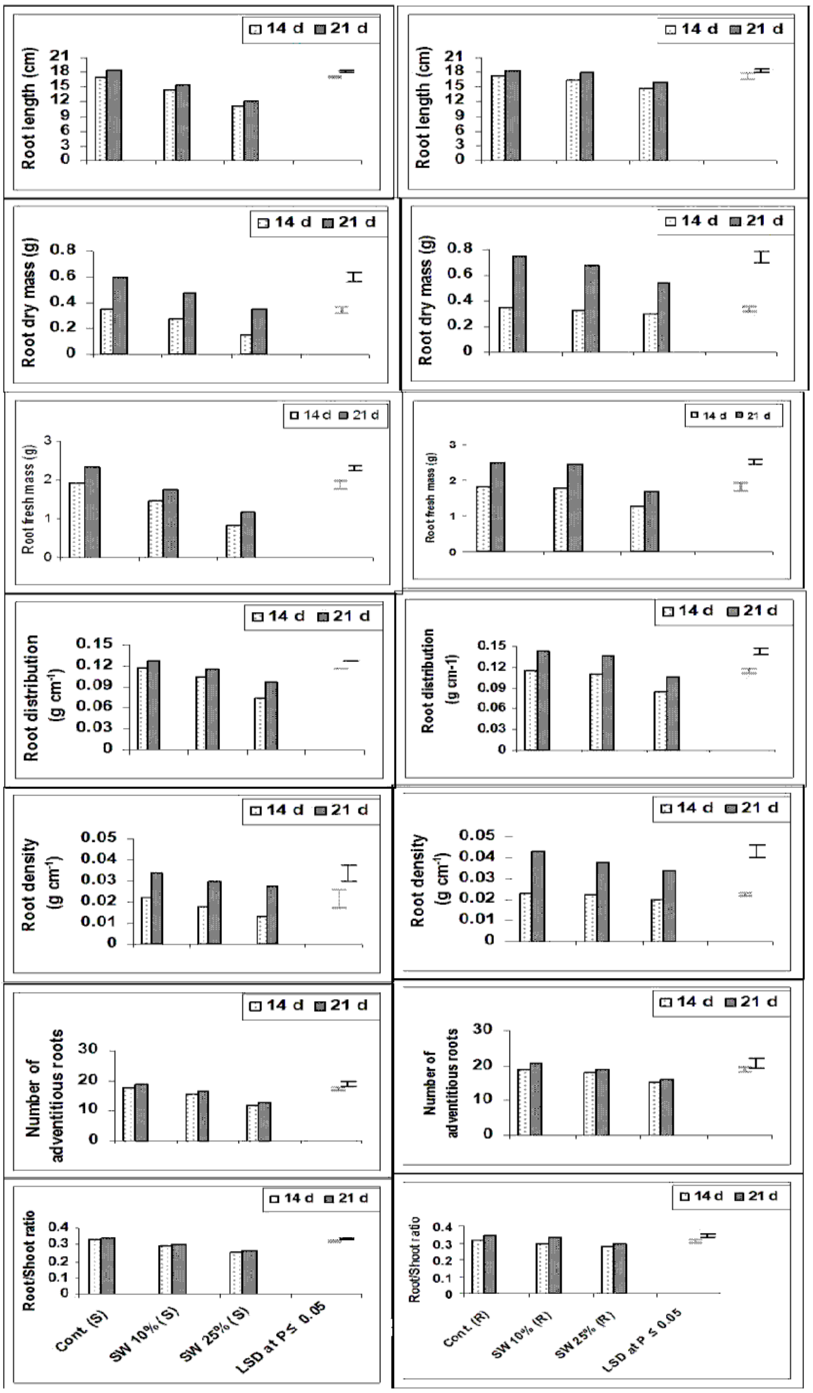

Fig. 1. Effect of different concentrations of seawater on root fresh mass (g), root dry mass (g), length (cm), root/shoot ratio, number of adventitious roots, root density (g cm^{-1}) and root distribution (g cm^{-1}) of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at $P \leq 0.05$.

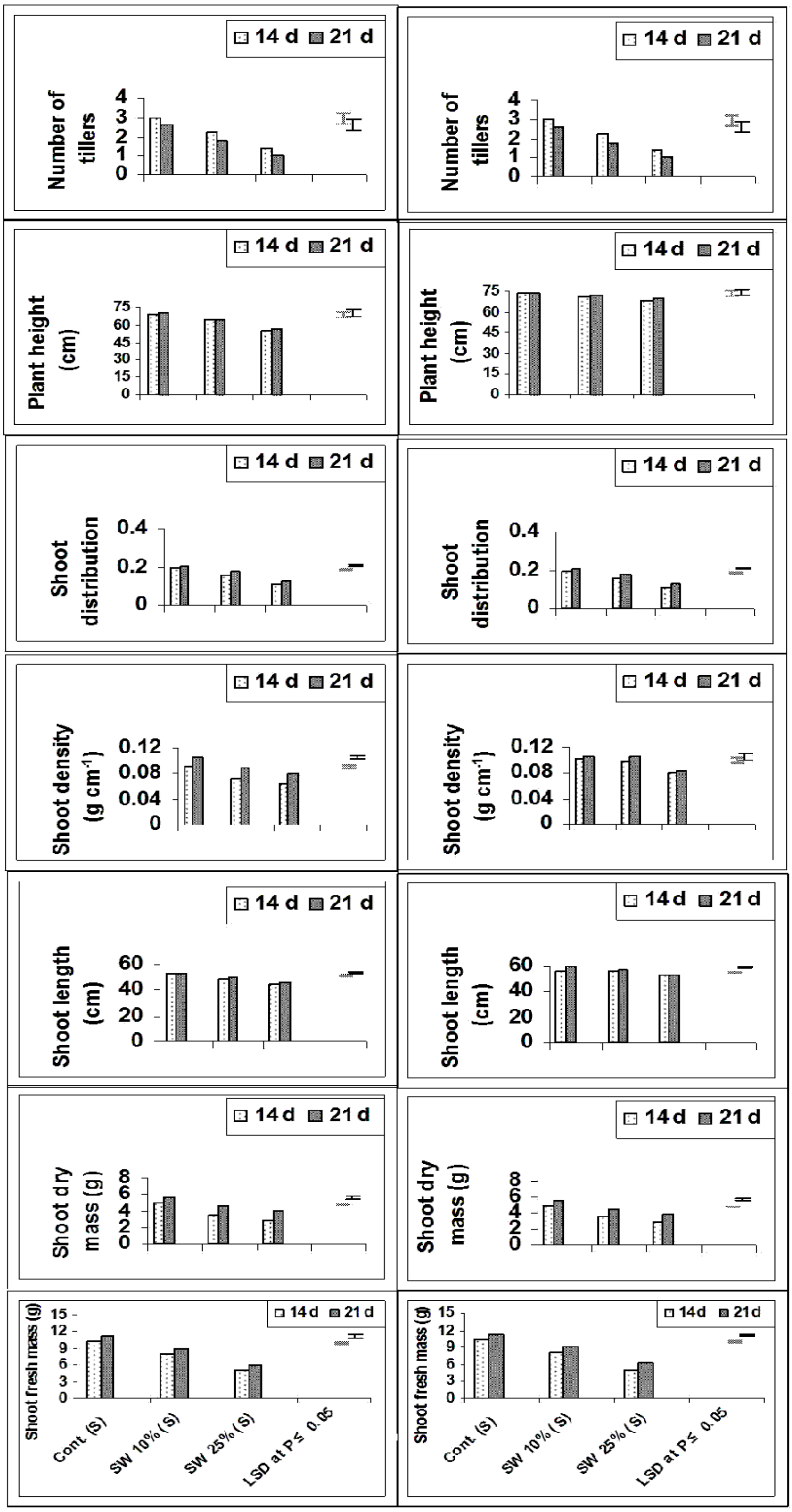

Fig. 2. Effect of different concentrations of seawater on shoot fresh mass (g), dry mass (g), length (cm), shoot density (g cm^{-1}), shoot distribution (g cm^{-1}), plant height (cm) and the number of tillers of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at $P \leq 0.05$.

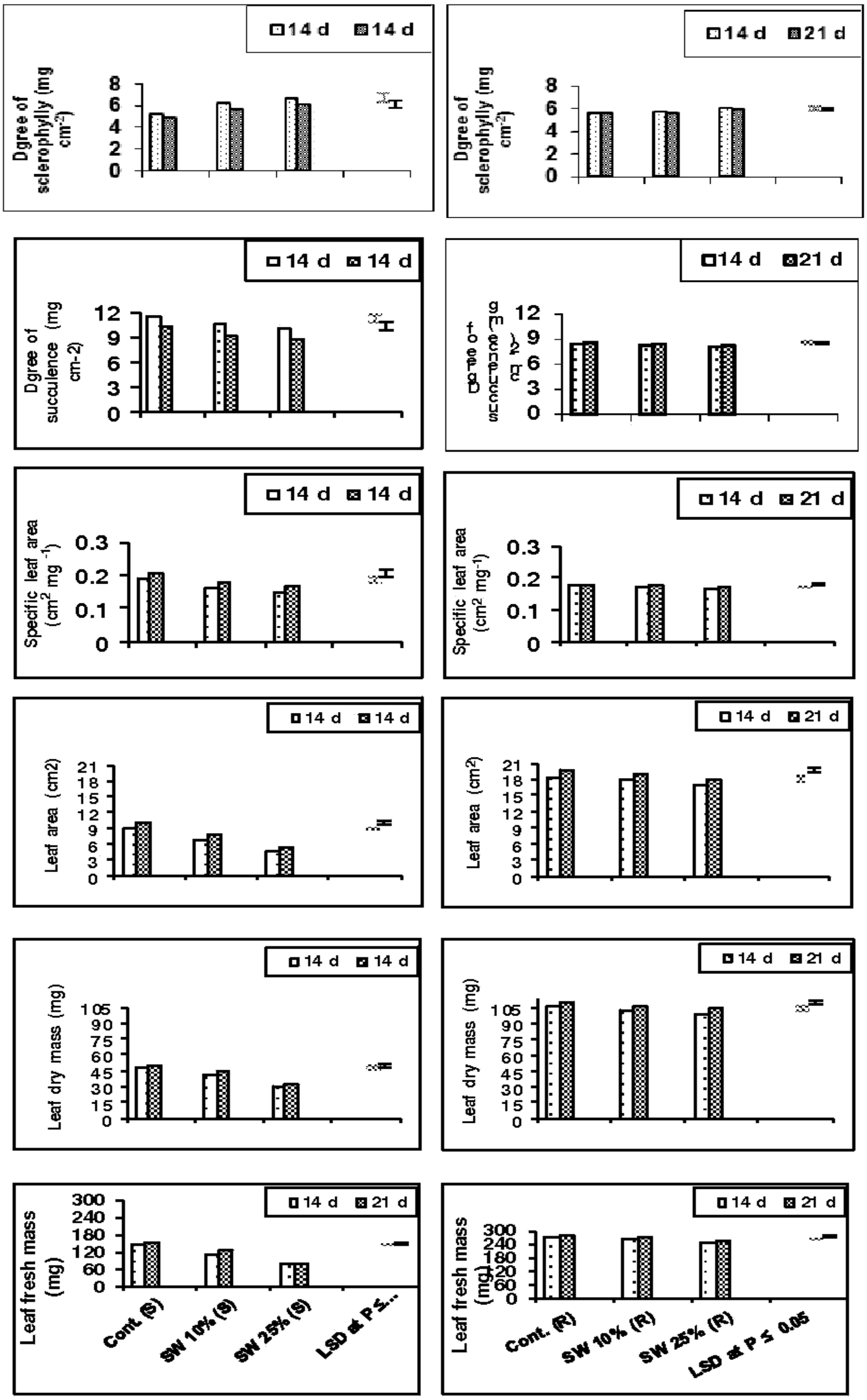

Fig. 3. Effect of different concentrations of seawater on flag leaf fresh mass (mg), dry mass (mg), area (cm^2), specific area (cm^2 mg^{-1}), degree of succulence (mg cm^{-2}), degree of sclerophylly (mg cm^{-2}) of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at $P \leq 0.05$.

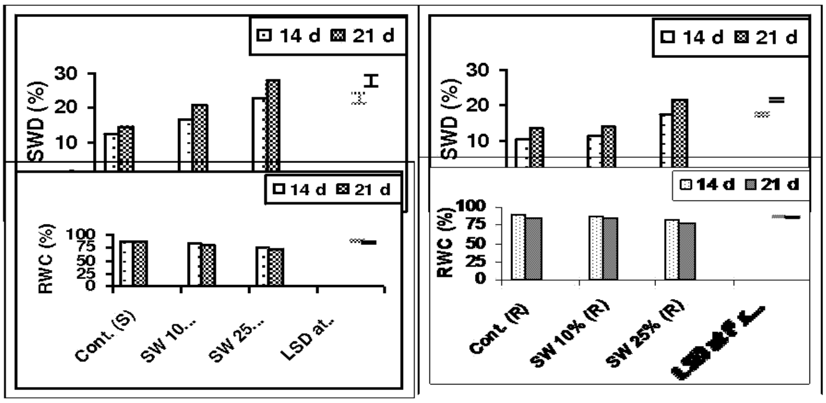

Fig. 4. Effect of different concentrations of seawater on RWC (%) and SWD (%) of flag leaf of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at $P \leq 0.05$.

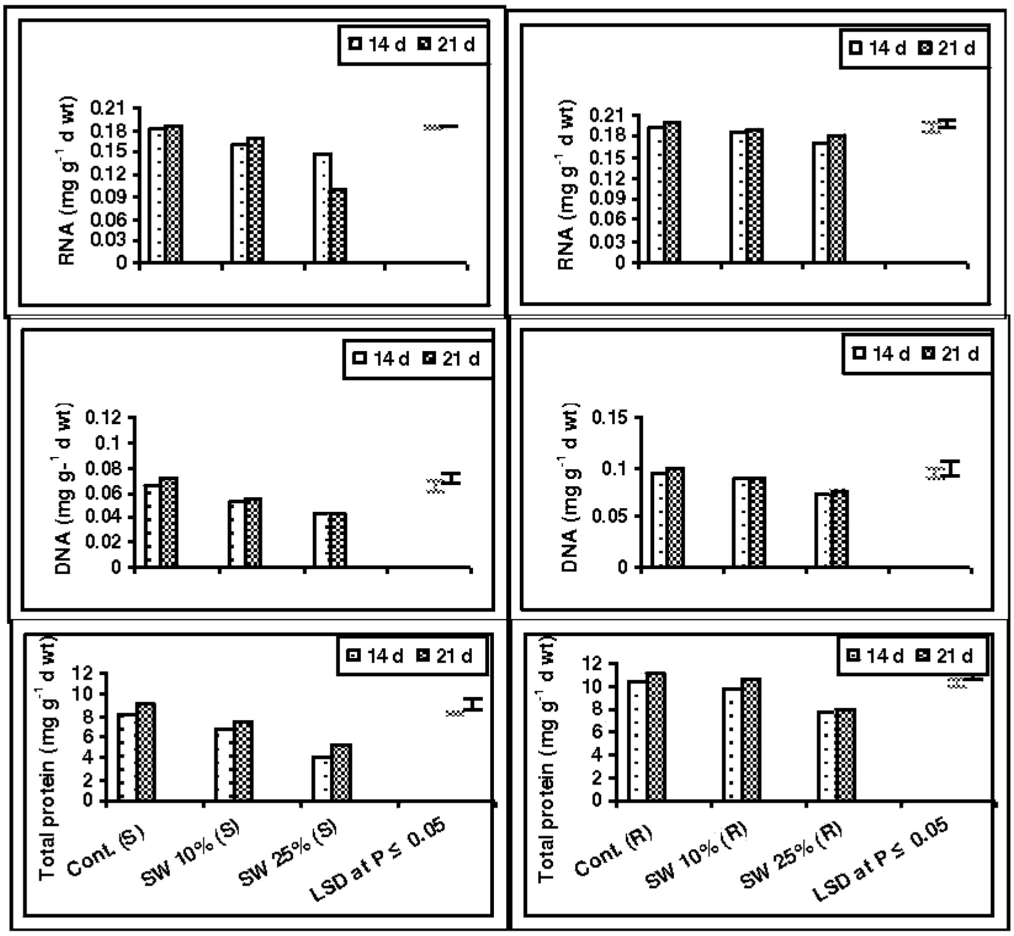

Fig. 5. Effect of different concentrations of seawater on total protein and nucleic acids (DNA & RNA) content (mg g^{-1} d wt) in flag leaf of wheat cultivars during grain-filling (14 & 21 days post-anthesis). Vertical bars represent LSD at $P \leq 0.05$.

Changes in total protein and nucleic acids content

Changes in total protein content of wheat flag leaf followed the same trend of the changes in nucleic acids (DNA & RNA) content in response to salinity stress (Fig. 5). As compared to control, irrigation of wheat plants with seawater induced drastic reduction ($P \leq 0.05$) in total protein and nucleic acids (DNA & RNA) contents of the flag leaf during anthesis stage in both cultivars. Gemmieza-9 being susceptible to salinity induced more protein and nucleic acids contents lost than Sids-1.

DISCUSSION

Abiotic pressures like salt stress can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress (Joseph et al., 2010).Water is imperative for plant growth and development. The scarcity of fresh water and soil salinity are the two most important abiotic stresses facing today's agriculture (Mahajan and Tuteja, 2005). Availability of water is one of the most important factors, which determine geographical distribution and productivity of plants (Bartels, 2001). Water stress is perceived as water deficit and can occur with different severity (Ramanjulu and Bartels, 2002). Hence, water stress is a very important limiting factor at the initial phase of plant growth and establishment (Shao et al., 2008). The quantity and quality of plant growth depend on cell division, cell enlargement and cell differentiation and all of these events are affected by water stress (Borsani *et al*., 2001; Kusaka *et al*., 2005; Jaleel *et al*., 2007 a&b).

The controlled use of alternative water resources, like brackish or seawater could be a valid tool to face drought in the Mediterranean regions. The efficient application of seawater depends on the convenient dilution and use of suitable plant genotypes and growing techniques (Malorgio *et al*., 2001). Plants respond in many ways to salinity and at a number of levels (Munns and Tester, 2008). In order to define salt stress tolerance or sensitivity of both cultivars, growth parameters like lengths, dry and fresh weights of roots and shoots as well as flag leaf were tested under the effect of 10 and 25% seawater treatments during grain filling.

The current results showed that irrigation of wheat plants with 10 or 25% seawater generally caused a noticeable reduction in almost all growth criteria of root, shoot and flag leaf of both wheat cultivars Gemmieza-9 (salt sensitive) and Sids-1 (salt tolerant) at 14 and 21days post-anthesis. Extent of reduction was more obvious at higher salinity levels than at lower one particularly in Gemmieza-9 (Fig. 1-3). These results were in agreement with those of many authors (Ahmed and Jabeen, 2009; Zaki *et al*., 2009; Khalid and Teixeira da Silva, 2010) who reported that salinity causes growth reduction through its osmotic effect, which is equivalent to a decrease in water activity through specific toxic effects of ions and by disturbing the uptake of essential nutrients. Hence, the inhibition of wheat growth characters under seawater irrigation would almost certainly be due to exposure to injurious levels of drought causing a decrease of turgor which would result in a decrease of growth and development of cells.

Salt stress has a strong influence on the allocation of energy between assimilation and dissimilation. Assimilation was found to be reduced (photosynthesis slowed and root uptake decreased), while respiration was found to be increased (to synthesize organic solutes that neutralize the inorganic ions and restore damage caused by the stressor). These may explain why growth is slowed under salt stress (Guo *et al*., 2009). A common adverse effect of water stress on crop plants is the reduction in fresh and dry biomass production (Farooq *et al*., 2009). Diminished biomass due to water stress was observed in almost all genotypes of sunflower (Tahir and Mehid, 2001), common bean and green gram (Webber *et al*., 2006), *Poncirus trifoliatae* seedlings (Wu et al., 2008) and Petroselinum crispum (Petropoulos et al., 2008).

More interestingly, the morphology of roots affects the amount of salt taken into the plant (Maggio and Ramnarayan, 2001). Hence, plants must develop a vigorous root system that allows them to grow and overcome any stress conditions. However, salt-stress could lead to marked changes in the growth, morphology and physiology of roots that will in turn change water and ion uptake and as a result, the whole plant is then affected when its root is growing in unfavorable medium with high salt concentration (Hajer *et al*., 2006).

It is evident from the current studies that root biomass (fresh and dry masses), root length, number of adventitious roots, root/shoot ratio as well as density and distribution were significantly and adversely influenced by the two used concentrations of seawater in both wheat cultivars where, magnitude of reduction was greater in Gemmieza-9 than Sids-1(Fig. 1). These results were supported by Nabti *et al.* (2010) who observed that irrigation of durum wheat with 160 or 200 mM NaCl partial inhibit or total inhibit root development. Hence, seawater irrigation assumed to reduce root growth of seawater-stressed wheat plants by inhibiting root formation and branching, growth of the existing roots and inducing root decay.

Seawater stress significantly decreased root fresh and dry masses of both wheat cultivars. Earlier, Grewal (2010) stated that increasing levels of subsoil NaCl salinity had significant depressing effect on root biomass (fresh and dry masses). In this investigation, the deleterious effect of salinity on root biomass may be attributed to the inhibitory effect of abscisic acid (ABA), induced by salinity, on cell division and/or cell expansion as stated by Hassanein (2000) or reduced water absorption due to osmotic effect, specific ion toxicity and nutritional imbalances as mentioned by Tahir *et al.* (2006) and Joseph *et al.* (2011).

The present work showed that, root length and the number of adventitious roots as well as root/shoot ratio calculated on length basis adversely affected by seawater-stress in both cultivars (Fig. 1). Current results were also in harmony with those of El-Sawy (2009) who reported that massive decrease in both root length and number of adventitious roots as well as root/shoot ratio of wheat plants in response to seawater-stress. Similar findings have been reported by many authors (Kaymakanova and Stoeva, 2008; Ali, 2009). This could be evaluated as salinity reduces growth rate and tissue differentiation of the plant root (Shannon and Grieve, 1999).

The results indicated that, seawater-stress caused noticeable decreases in root density and root distribution of both cultivars. Since root density relates dry mass production to the unit root length and root distribution represents the fresh mass accumulated per the unit root length, the reduction in both density and distribution of wheat root may reflect the effect of salinity on decreasing root biomass (fresh and dry masses). In this respect, Chopart *et al.* (2008) stated that evaluation of root density and distribution could be considered as a key factor for water and nutrient uptake by a plant in soil.

Seawater-stress induced a significant decrease in all growth parameters of the shoot i.e. shoot biomass (fresh and dry masses), shoot length, plant height, spike length and the number of tillers, as well as shoot density and shoot distribution of both wheat cultivars with more pronounced effect on Gemmieza-9 as compared to Sids-1. These results are also in conformity with those obtained by Ali (2009) who stated that seawater stress caused marked reduction in the shoot biomass, shoot length and plant height as well as the number of tillers in wheat plants. The inhibition of shoot growth of both wheat cultivars under seawater irrigation could be explained on the basis that the presence of excessive salts in soil solution reduced the ability of plants to take up water ultimately causing the slower plant growth rate. Additionally, the initial reduction in shoot growth was probably due to hormonal signals generated by the roots (Munns, 2002) and/or inhibition of the efficiency of the translocation and assimilation of photosynthetic products (Xiong and Zhu, 2002).

In wheat plants, shoot biomass (fresh and dry masses) were adversely affected by seawater irrigation (Fig. 2). These results were in harmony with those obtained by Seckin *et al.* (2010) who reported that shoot fresh and dry weights of two barley cultivars were decreased at 300 mM NaCl. This reduction in shoot dry matter yield of seawater-stressed wheat plants could be attributed to inadequate availability of nutrients present in growth medium and the decreased water entry rate into the plants and/or the decreased in photosynthetic output with suppressed supply of CO_2. Moreover, Chaparzadeh *et al.* (2004) stated that the reduction in shoot dry mass may be a consequence of turgor limitation or cell wall hardening which may be due to altered wall structure induced by salinity (Chaparzadeh *et al.*, 2004).

The pattern of shoot growth revealed that seawater stress greatly affected shoot length, the development and viability of tillers per plant as well as plant height (Fig. 2). These results were also in good harmony with those obtained by Hasamuzzaman *et al.* (2009). This reduction in plant height of seawater-stressed plants may be attributed to reduction in peduncle length and/or spike length as water stress appeared to decrease the meristematic activity as well as cells elongation and enlargement (Manivannan *et al.*, 2007). In this regard, the decrease in fresh and dry masses of the shoot system of seawater-stressed wheat plants was assumed to be due to the decrease in either the number of tillers and/or plant length, which might be due to the disturbance in metabolic activities affected by the decrease in water absorption and/or disturbance in water balance. This conclusion came also in agreement with those of Suleiman *et al.* (2002) and Sairam and Tyagi (2004).

Flag leaf plays a key and the most important role in plant life as it transport assimilates to spike and developing grains. It is clear from the results in figure 3 that, all growth parameters of wheat flag leaf (i.e. biomass, area, specific area as well as the degree of succulence and sclerophylly) were negatively affected by seawater-stress in both cultivars with more conspicuous effect in Gemmieza-9. Overall, these results were in accordance with those obtained by many authors (Jampeetong and Brix, 2009; Silva *et al.*, 2010) with different plant species.

The flag leaf biomass (fresh and dry masses) of both cultivars was greatly inhibited by seawater stress (Fig. 3). In wheat, genotypic differences in biomass production under seawater-stress were observed but all showed a reduction in this growth parameter. These results were in agreement with those obtained in other studies (Djanaguiraman *et al.*, 2006; Siddiqui *et al.*, 2008). The observed reduction in leaf biomass of seawater-stressed wheat plants might be due to induction of ionic stress by salinity, which causes premature abscission and senescence of adult leaves, causing a reduction in the available photosynthetic area. Additionally, this reduction also could be attributed to a combination of slower growth and development as a result of osmotic stress (Shani and Ben-Gal, 2005; Hatzig *et al.*, 2009), as well as the inhibition in photosynthesis as a result of direct effects of salinity on the photosynthetic apparatus or indirect effects caused by the reduction in sink capacity (Moradi and Ismail, 2007).

Development of optimal leaf area is important to photosynthesis and dry matter yield. Salt stress mostly reduced leaf growth and in turns reduced the leaf area and specific leaf area of seawater-stressed wheat plants (Fig. 3). The observed reduction in leaf area and dry mass of seawater-stressed wheat plants could be attributed to the changes in plant water relations under seawater stress, which cause a reduction in meristem activity as well as cell elongation, thereby inhibiting leaf expansion after the loss of cell turgor pressure. These results were in a good agreement with those obtained by Choluj *et al.* (2004) and Shah (2007). Moreover, plant tried to cope with the salt stress by reducing its leaf area in order to allow the conservation of energy, minimize the deleterious effects of salt and to complete their life cycle under stress conditions (i.e. avoidance and/or tolerance mechanisms).

Seawater-stress induced a significant decline in the degree of leaf succulence with a concomitant increase in its degree of sclerophylly. In accordance with these results, leaf succulence was found to decrease in three varieties of salt-stressed sunflower plants (Welch and Rieseberg, 2002 a&b). This may be explained on the basis that less absorbed water means less water content of the growing leaves, indicated by less relative water content and more saturation water deficit (Fig. 4) as well as less succulence and more sclerophylly (Fig. 3). From our results we could concluded that, greater leaf succulence and less leaf sclerophylly could be recorded as a means of increasing salt tolerance since, the resistant cultivar showed less reduction in the degree of leaf succulence and more increment in the degree of leaf sclerophylly in comparing to sensitive cultivar under salt stress.

Edwards *et al.* (2000) reported that the functional significance of sclerophylly remains controversial, with three main groups of hypotheses proposed to explain its adaptive significance. These centre on sclerophylly as (1) an adaptation to water deficit, (2) an adaptation to, or consequence of, low nutrients in the growing medium, and (3) enhancement of leaf longevity by leaf protection, thereby increasing leaf carbon gain.

It is clear from the current study that seawater irrigation significantly decreased almost all growth criteria of root, shoot and flag leaf of both wheat cultivars. Among the cultivars used in the experiment, Gemmieza-9 showed more susceptibility to seawater-stress since it showed a worse performance and produced less biomass under seawater-stress when compared with Sids-1. Hence, this reduction in growth might be due to toxicity of the ions, low osmotic potential and a decrease in wall extensibility. In this respect, morphologically, the most typical symptom of saline injury in plants is growth retardation due to inhibition of cell expansion.

Water stress is one of the first and most evident effects of salinity so the determination of water relations is critical for any study of plant resistance to salinity (Netondo *et al*., 2004a). Plant water status is important not only for their growth under favorable environmental conditions but also for their ability to tolerate water deficit and high salt levels (Blumwald, 2000). Additionally, the importance of the internal water balance in plant water relations is generally accepted because of the close relationship between the balance and turgidity to the rates of physiological processes that control the quality and quantity of growth (Aldesuquy *et al*., 2009).

Leaf relative water content (RWC) has been emphasized as a better indicator of water status of a plant than water potential (Farhat et al., 2008). Thus, data in figure 4 showed that seawater-stress considerably lowered the RWC % with a concomitant increase in saturation water deficit (SWD) % of flag leaf of both wheat cultivars. Hence, Gemmieza-9 showed more reduction in RWC % and more increment in SWD% under saline conditions in comparing to Sids-1. These results were in agreement with those obtained by Aldesuquy and Ibrahim (2001) who demonstrated that irrigation of wheat plants with seawater significantly reduced RWC % and increased SWD % of the stressed plants as compared with the control ones. The suppression of leaf RWC under salt stress implies that there is a reduction in turgor and the plant suffers from restricted water availability to the cells (Munns, 2002). This decrease in turgor under salinity conceivably reduces expansive growth of cells (Munns *et al*., 2000; Netondo *et al*., 2004a). Furthermore, Garg and Singla (2009) ascribed the reduction in RWC under salt stress to decreased water uptake due to the injury to the root system.

Water molecules are critical components of the reaction mechanism, they contribute to the stability of proteins, nucleic acids (DNA and RNA) and lipids. Stress conditions, as manifested by salinity, are a detrimental factor which adversely affects the cellular contents of plant cells of different species, such as proteins and nucleic acids (Bor *et al*., 2003; JungKlang, 2005). In the present investigation, irrigation of wheat plants with 10 or 25% seawater induced a noticeable decrease in total proteins and nucleic acids (DNA and RNA) content in stressed leaves of both wheat cultivars during anthesis. Note, for resistant cultivar in order to increase its ability to overcome salt stress, it was able to keep out its protein and nucleic acids (DNA and RNA) contents under salt stress at higher levels than the sensitive one (Fig 5). In this respect, a close relation between the changes in total protein and nucleic acids contents under salt stress conditions was observed. Since the processes of DNA and RNA synthesis are related to protein synthesis, reductions in RNA synthesis ultimately reduce the enzymatic protein content (Siddiqui, 2006). In agreement with our results, Debouba *et al.* (2006) found that salt stress decreased total leaf protein and nucleic acids (DNA and RNA) contents in tomato plants.Likewise, seawater-induced depletion of proteins and nucleic acids could be ascribed to the enhancement of ROS production. ROS can affect many cellular functions by rapidly attacking all types of bio-molecules such as proteins and nucleic acids (Foyer *et al*., 1994; Luna *et al*., 1994), leading to irreparable metabolic dysfunction and cell death. Furthermore, ROS may spontaneously react with nucleophilic centers in the cell, thereby forming covalent bonds to protein and nucleic acids (Mates and Sanchez-Jimenez, 1999).

The protein content in plant cells is an important indicator of their physiological state but under salt stress, total protein contents is usually decreased (Muthukumarasamy *et al*., 2000; Parida *et al*., 2002). The reduction in protein content of various plant tissues under saline conditions may be due to the inhibition of transamination process, the increase of proteolysis as well as the decrease of protein synthesis (Baraka, 2008). The adverse effect of salt stress on protein content could be also attributed to disturbance in nitrogen metabolism, inhibition of nitrate absorption or the decrease in the availability of amino acids and denaturation of the enzymes involved in amino acid and protein synthesis (Tawfik *et al.*, 2006). Moreover, Mohammadkhani and Heidariturk (2008) found that, the decrease in total soluble proteins under water stress conditions was due to a severe decrease in photosynthesis and as a result materials for protein synthesis aren't provided. Therefore, protein synthesis dramatically reduced or even stopped.

Nucleic acids metabolism is another cellular function known to be affected by exposure to salt stress. Therefore, the observed reduction in nucleic acids (DNA and RNA) contents in flag leaves of salt-stressed wheat plants (Fig. 5) can be attributed to cations imbalance, such as the abundance of sodium cations in the cell and the reduction of phosphorus incorporation into nucleic acids as reported by Zeid (2009a). Moreover, Bassuony *et al.* (2008) stated that, levels of nucleic acids in plants growing under saline stress are affected by salt-induced alteration in the activities of their synthetic and/or hydrolytic enzymes.

It was concluded from this work that the plant growth is significantly affected by seawater stress in both wheat cultivars (Gemmieza-9 and Sids-1) in comparison to non-saline conditions. According to these results, Gemmieza-9 would be classified as a species susceptible to seawater salinity, because its growth and physiological parameters, such as dry and fresh weight at the whole plant level, leaf area, specific leaf area, shoot to root ratio on the basis of length, some water relations (i.e. relative water content, saturation water deficit, degree of leaf succulence and degree of leaf sclerophylly) and leaf protein and nucleic acids (DNA and RNA) contents were severely affected by seawater stress in comparison to Sids-1.

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