Effect of K- Foliar Fertilization on Biochemical Components in Wheat Plant Under Salt Stress

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Abstract: A pot experiment was conducted during the winter season of 2005/06 to define biochemical behaviors resulting from inducing salinity tolerant in wheat plant by potassium foliar application at rates of 0, 3, 6 and 9 g soluble $k_2SO_4 l^{-1}$, under irrigation with different saline waters (0.4, 2.5, 5 and 10 dSm^{-1}).Wheat *(Triticum aestivum* L.) varieties viz. Sakha 93, Gimiza 9, Sids 1 and Giza168, were grown to maturity in a sandy soil. Proline content, protein content and protein pattern were measured. Irrigation with saline water proved to increase the proline and protein contents of wheat plant and the effect was generally significant. The proline and protein contents mostly followed the order: Sakha 93 >Gimiza 9> Sids 1> Giza 68. This was particularly clear with the highest level of salinity (10 dSm^{-1}). Comparing SDS -PAGE analysis was carried out for the leaf water soluble – protein fractions of four varieties. The results revealed that's the percentage of Polymorphisms between variety and other three varieties was 74%;46.2%,79.5%,48.7% for Sakha 93 ; Gemiza 9 ;Sids 1 ;Giza 168 respectively. The changing in protein pattern due to salt stress was discussed.

Keywords: wheat varieties, salt stress, K-foliar fertilization, biochemical studies, Protein electrophoresis, proline

INTRODUCTION

Salinity stress is known to be one of the major problems causing decreases of crop production in arid and semiarid regions. More than 50 enzymes are activated by K in the metabolism of flowering plants, and Na cannot substitute in this role (Bhandal and Malik, 1988). Therefore, the development of salt tolerant plants depends on biochemical basis which may be provided more understanding the term of tolerance.

Salt damage leading to necrotic lesions and even death is attributed to disturbances of plant metabolism, including proteolysis, the accumulation of some toxic amino acids, and diamines, and the oxidation of sulfhydryl group. Phosphorus and nucleic acid metabolism appear to be affected by salinity only at the final stage of translation of genetic information into protein as evidenced by the reduced numbers of polysomes. Electron micrographs indicate mitochondria to be relatively resistant to salinity, whereas nuclei were affected somewhat and chloroplasts were severely affected (Kursanov and Genke, 1973). Proline and soluble sugars of wheat grass lines were found to be contributed to osmotic adjustment at high salinities (0.4, 0.8, 1.2 and 1.6 M Pa) but sensitive and tolerant lines did not differ in proline content (Michael, 1978). Increased amount of proline is considered to be an indication of tolerance to salt stress because proline is thought to function either as an osmoregulator and / or a protector of certain enzymes, application of k fertilization could be useful to overcome the adverse effects of salinity on whet growth (Aspinal and Paleg, 1981).A proline accumulation in sugar beet leaves under salinity stress was observed by several others(Shehata ,1989; Petrovic *et al.*, 1991; El-Noemani, 1996; Gzik, 1996). With regard to the water stress tolerance in plant, an adaptive biochemical function of osmoprotectants is the scavenging of reactive oxygen species that are by-product of hyperosmotic and ionic stresses and cause membrane disfunction and cell death (Bohnart and Jensen, 1996). Membrane stability, nitrate reductase activity and increased accumulation of proline were affected by water stress in the hexaploid wheat. Drought tolerance is a complex character and can be associated with thickness of cuticle, opening and closing of stomata, root depth and extension, hormone composition, osmotic adjustment and anti oxidant production this method, mentioned that (Szegletes *et al.*, 2000). The proline concentration in leaves tissue increased significantly with increasing NaCl concentration in the growth media, but a high prolein concentration was recorded in cv. Datt cultivar compared to the cv. Alphabet (EL-Baz *et al.*, 2003).

On the other hand, greatest value of protein content was recorded when plants were grown under 6000 $mgkg^{-1}$ salinity level then decreased gradually by increasing the level of soil salinity (Rabie *et al.*, 1985).Salinity increased the protein content in wheat grains (Francois *et al.*, 1986).Six polypeptide bands were either new or of enhance intensity were observed after SDS-PAGE of the culture medium from suspension cultures of NaCl adapted winged bean cell. (Esaka and Hanyakawa, 1995). seawater treatments Seawater treatment at 10 % and 25 % concentrations increased the protein content of the developing grains of wheat (Aldesuquy and Ibrahim, 2001). Plant adaptation to environmental stress, such as soil salinity, is expected to have a strong influence on proteins. One approach to study the molecular mechanisms of plant responses to salinity is to use 2D polyacrylamide gel electrophoresis. Furthermore, the identification of differentially regulated proteins can lead to the identification of proteins and their corresponding genes which are involved in the physiology of salt resistance. The high resolution achieved by 2D gels and computer –assisted analysis of the differentially proteins were used to examine those proteins whose synthesis was modulated by salt treatment and to quantify these changes (Munns, 2002; Apse and Blumwald, 2002).An increase in the concentration of fourteen proteins of maize (*Zea mays* L.) was recorded to be due to salt stress was reported by (Christian Zörb *et al.*, 2004).

In the present study, the main objective was to define the biochemical behaviors leading to induce salinity tolerant in wheat plant by potassium foliar fertilization under irrigation with saline water. Four wheat varieties viz. Sakha 93, Gimiza 9, Sids1 and Giza168, were investigated by measuring biochemical characters.The study was extended to use the electrophoresis analysis of protein to distinguish between the varieties under saline and non-saline conditions. The other objective of the present study was to determine proline accumulation in leaves and roots as well as the protein patterns as affected by saline water irrigation .These objectives would help to understand some of mechanisms of salt tolerance in wheat plant.

MATERIALS AND METHODS

pot experiments was carried out in the Experimental Farm of the Faculty of Agriculture, Suez Canal University, Ismailia Governorate, Egypt. During the tow winter season of 2005/06. Experiment was performed on some wheat cultivars irrigated with different levels of saline water and affected by K-foliar fertilization at different rates.

The soil used was uniformally packed in plastic bags (pots) each of 40 cm depth and 35 cm diameter at a rate of 25 Kg soil pot^{-1} and used as experimental units. The soil in each pot was thoroughly mixed with farm yard manure (FYM) at a rate 150 g .Wheat seeds were sown on 15th of November (2004 and 05) at a rate of 5 seeds pot^{-1}. N – fertilizer was added at a rate of 10 g pot^{-1}as ammonium nitrate (33.5 N), in three equal split dressings, after 15,45 and 75 days from sowing. This was based upon the results of Rabie *et al.* (1993). All pots received superphosphate (15.5% P_2O_5) at a rate 5 g pot^{-1} in two equal split dressing, before sowing and after 15 days from sowing. Potassium sulfate (48% K_2O) was applied at a rate of 5 g pot^{-1} in three equal split dressings after sowing and after 30 and 60 days from sowing. .The micronutrients were added as a mixed fertilizer (Fe 3%, Zn 3% ,Mn 3.5%) at a rate of 1 gl^{-1} of foliar spraying solution. The plants were sprayed two times, after 30 days from sowing and at preheading stage (65 days from sowing). The plants were sprayed till runoff, and the volumes of spraying solutions were ca 100 and 200 ml pot^{-1} for the first and second applications, respectively. one after 25-30 days of sowing and the second before heading. The pot experimental design was a split split with three replications. The treatments were the same as those in the field experiment. (Ebtesam- El-Hosini, 2009)

Experimental Treatments:

Four wheat cultivars were used viz, Sakha 93, Gimiza 9, Sids 1 and Giza 168. The origin of those four varieties is Egypt. The wheat plants were irrigated with saline water having four levels of salinity namely 0.4 (control), 2.5, 5 and 10 dSm^{-1}.

Foliar application of potassium was attained at the rates of 0, 3 , 6 and 9 g fertilizer l^{-1} as soluble potassium sulfate (50% K_2O), every irrigated by saline water.

The main plots were devoted to varieties while irrigation water salinity levels were located in sub plots and potassium foliar rates were presented in sub sub plots

Biochemical parameters

Proline content:

The free proline concentration in plant material (shoots and roots) was extracted and determined spectrophotometrically using the rapid method given by Bates *et al.* (1973).

The total Protein: determined according to Braford (1976).

Protein electrophoresis: The proteins of flag leaf samples were extracted and fractionated electrophoretically according to (Laemmli, 1970).

RESULTS AND DISCUSSION

Figure (1) show that irrigation with saline water proved to increase the proline and protein contents of wheat plant and the effect was generally significant with all salinity levels except for the highest level (S3) at which the same was not always true. Otherwise, the higher the salinity level the higher was the content of proline as well as the protein.

With respect to the cultivars effect, the proline and protein contents mostly followed the desending order: Sakha 93 >Gimiza 9> Sids 1> Giza 68. This was particularly clear with the highest level of salinity (10 dSm^{-1}).

Regarding the effect of potassium application, no clear trends are observed concerning the proline and protein contents under the different experimental treatments. However, Rabie (1990) concluded that potassium deficiency resulted in a considerable increase in the proline content in wheat shoots (19.9 micromole g^{-1} dry matter) as compared with the control (13.7 micro mole g^{-1}dry matters).

Presented data also reveal that the proline content was considerably higher in plant shoots than roots, and this was particularly pronounced under the highest salinity level (10 dSm^{-1}) with Sakha 93 and Gimiza 9 cultivars.

Ahmad *et al.* (1981) found that salinity- resistant ecotypes of *Agrostis stolonifera* accumulated more proline and several other amino acids than susceptible ecotypes under salinity stress. Voetberg and Stewart (1984) found that proline accumulated in barley leaves in response to salinity and its maximum concentration was linearly related to Na concentration. Pandey and Ganapathy (1985) found that NaCl – tolerant cell lines of *Cicer arietinum* accumulate more proline than NaCl-susceptible lines, when grown on NaCl-containing medium. The significance of proline accumulation has been attributed to the ability of proline to act as a protective agent for cytoplasmic enzymes Samaras *et al.* (1995) reported that proline accumulated in the cytoplasm to balance the osmotic potential of vacule where toxic solutes such as Na and Cl were found. Ashraf (1994) found that proline can effectively regulate the accumulation of essential N and it is

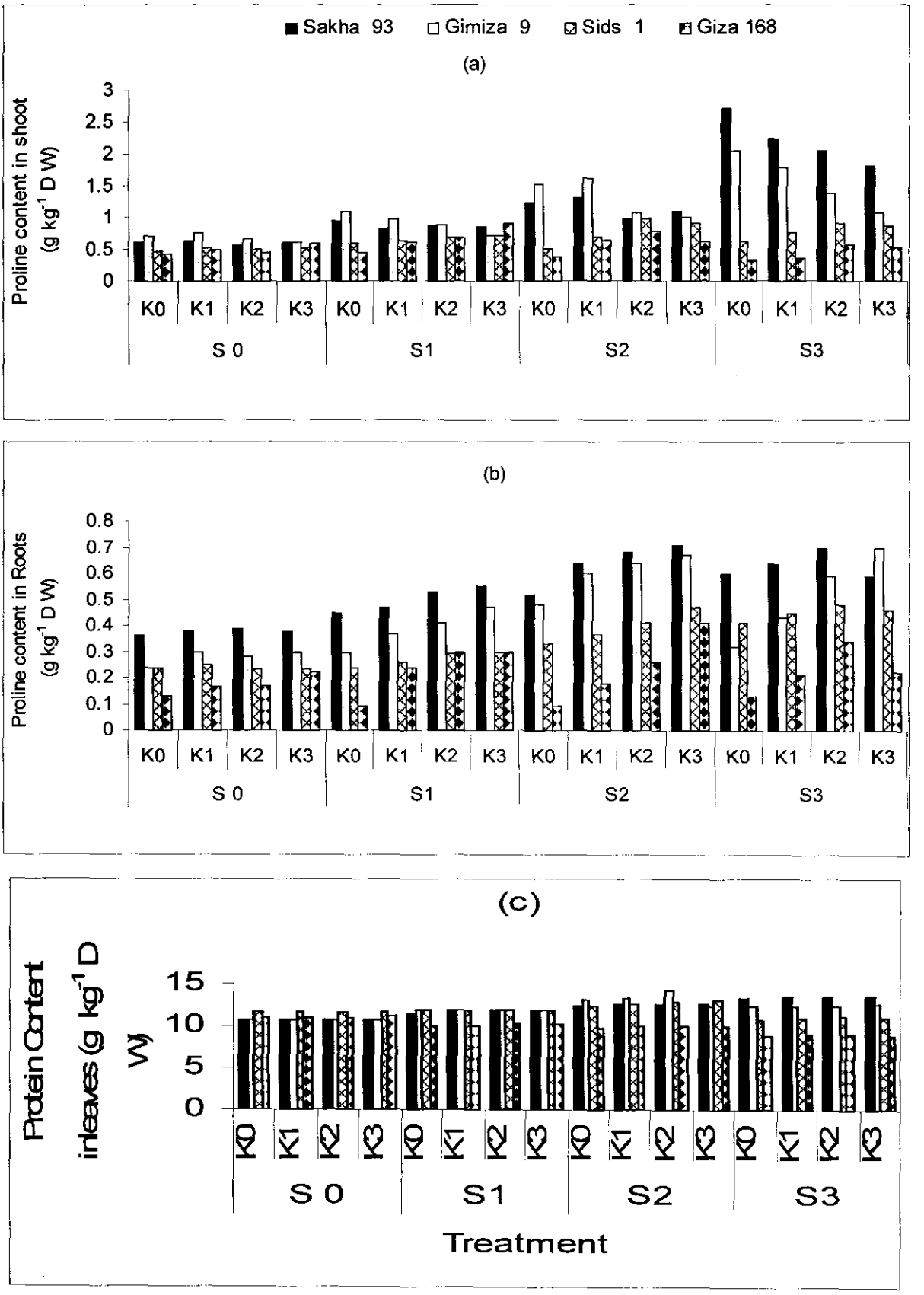

Fig. (1): Effect of Salinity and Potassium Levels on (a) Proline in shoot (b) and Proline in Roots and (c) Protein Content 2005 / 06 Season.

- S0, S1, S3 and S3 refer to irrigation water salinity at 0.4, 2.5, 5 and 10 dSm^{-1} , respectively.
- K0, K1, K2 and K3 refer to K-foliar application rates of 0, 3, 6 and 9 g $K_2SO_4^{-1}$, respectively.

osmotically very active. It also is compatible with other cytoplasmic components and can be easily converted to glutamate via transamination reaction. This conversion is very important because glutamate takes part in the synthesis of other essential amino acids. Thus, proline in a plant under salt stress could act both a nitrogen reserve and in osmoregulation.

Schobert (1977) proposed that proline protects the hydration of proteins rather than acting as an osmotic solute under salt stress. Bartels and Nelson (1994) reported that tobacco cells adapted to NaCl accumulate proline to 80 fold higher levels and this is largely accounted for increased synthesis and this accounted for almost 50% of the total osmotic adjustment. They considered that the accumulation to such level is consistent not with its role as an adjusting solute but also its role as compatible solute.

Serrano and Gaxiola (1994) suggested that proline protect plant tissues against osmotic stress because it is an osmosolute, a source of nitrogen compounds, and protectant for enzymes and cellular structures and a scavenger for hydroxyl radicals.

Because proline accumulates in plants subject to severe conditions of both drought and salt, it may be that the synthesis of proline is a non-response to low water stress (Greenway and Munns, 1980; Wyn Jones, 1981). On the other hand, Moftah and Michel (1987) found that the proline content could not be used as an indicator of salt tolerance in soybean.

Wyn Jones (1981) reported that proline may not play an adaptive role in plants in response to stresses because it accumulates at the extremes of stress. This may help plants to temporarily override highly damaging stress and thus its use as a selection criterion for salt tolerance does not seem plausible. However, Lui and Zhu (1997) concluded that proline accumulation is merely a consequence of stress and dose not lead to salt tolerance because the lack of correlation between proline level and salt tolerance in certain plant species. Hasegawa *et al.* (2000), Ismail (2003) and El-Kholy (2004) reported that proline is believed to facilitate osmotic adjustment by which the internal osmotic potential is lowered and may then contribute to tolerance. Proline as a compatible solute is typically hydrophilic, which suggest that it could replace water at the surface of protein, protein complexes, or membrane, thus acting as a nonenzymatic osmoprotectant.

The above-mentioned interpretation concerning the interrelationship between the proline content in plant and salt stress effect seem to be not contradictory but rather complementary as far as the plant diversity and plant growth conditions are concerned. The high concentration of K under salinity stress declares the importance of K in shoot to activate the enzymes which is shown to be related to salting tolerant. As for SDS – PAGE analysis of the tolerant variety (sakha 93), the results in plate(1) revealed that the maximum numbers of bands were 39 with molecular weight ranged from 234 k Da to 12 k Da. 12 bands (unique band)of 31% and 29 bands showed polymorphisms of 74% with 10 common ones. Plate 1 shows that the polymorphism between Sakha 93 and other three cultivars was 74.4%. Plate 2 shows that the polymorphism between Gemiza 9 and the other three cultivars was 46.2%.

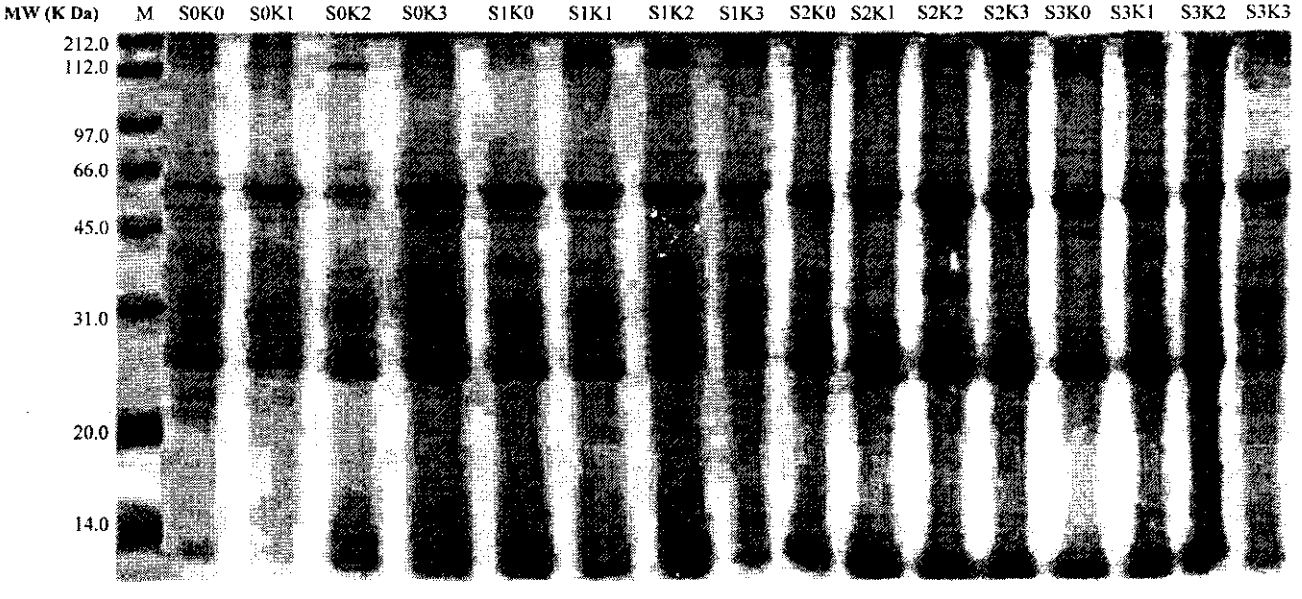

Plate (1): Effect of irrigation water salinity and potassium foliar application levels on schematic representation of SDS-PAGE analysis of water – soluble protein for Sakha 93 cultivar.

- S0, S1, S2 and S3 refer to irrigation water salinity at 0.4, 2.5, 5 and 10 dSm^{-1}, respectively.
- K0, K1, K2 and K3 refer to K-foliar application rates of 0, 3, 6 and 9 g $K_2SO_4l^{-1}$, respectively.

Plate 3 shows that the polymorphism between Sids 1 and the other three cultivars was 79.5. Plate 4 showed that the polymorphism between Giza 168 and the other three cultivars was 48.7%. The appearance of new bands and the absence of others, in the four cultivars under salt stress would indicate either enhancement or repression of gene expression in these plants. This might alter the produced proteins in response to salt stress either on the transcription or post-transcription levels of gene expression. These findings are in agreement with those of, Ramagopal (1987) in barley, Abdel- Tawab *et al* (1997 and 1998) in maize and sorghum, respectively, and Fahmy *et al* (1992) in maize.

Salt stress was shown to elicit quantitative and qualitative changes in protein (Han *et al*., 1997). Salt – induced polypeptide have been observed in many studies Arora *et al*, 1998), Riccardi *et al*, 1998) which are assumed to play a role in water stress tolerance. The results of Jiang and Huang (2002) indicated that accumulation of some polypeptides were responsive for salt stress treatment. Proteins synthesized during prolonged salt stress in plant of documented tolerance to extreme water deficits may indicate the presence of gene that control traits of adaptive value Vance *et al*, 1990).

On the contrary, Jiang *et al* (1998) found that SDS-PAGE revealed no linear relationship between drought resistance and changes in the content of particular soluble proteins in leaves.

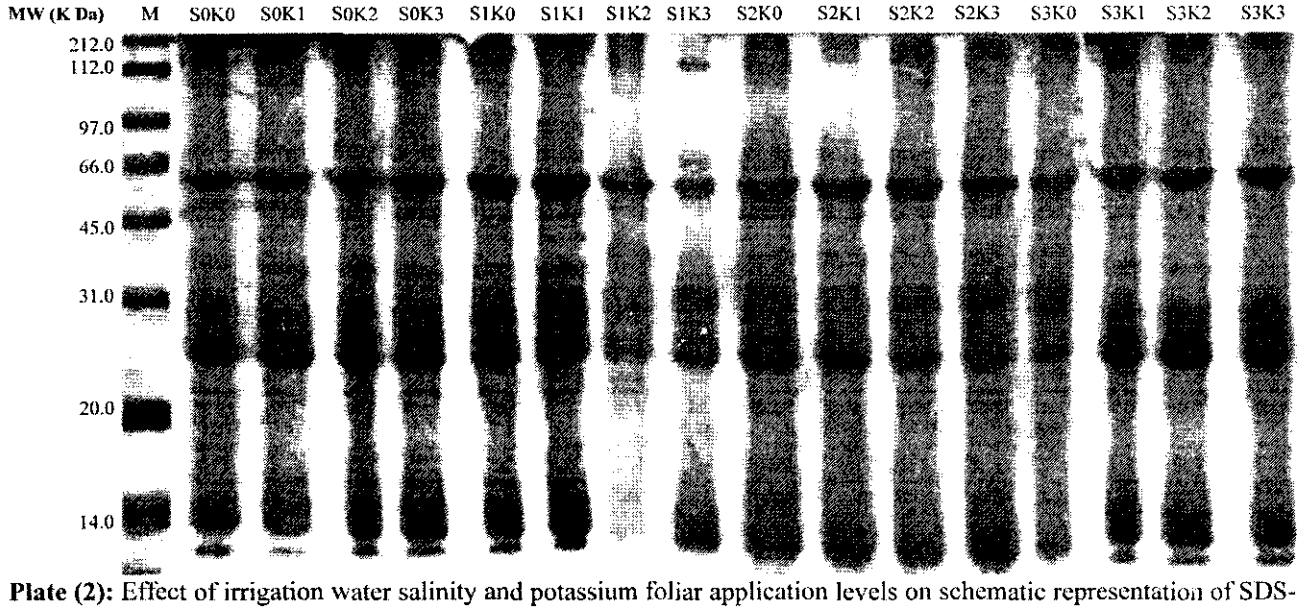

Plate (2): Effect of irrigation water salinity and potassium foliar application levels on schematic representation of SDS-PAGE analysis of water – soluble protein for Gimeza 9 cultivar.

- S0, S1, S2 and S3 refer to irrigation water salinity at 0.4, 2.5, 5 and 10 dSm^{-1}, respectively; K0, K1, K2 and K3 refer to K-foliar application rates of 0, 3, 6 and 9 g $K_2SO_4l^{-1}$, respectively.

Plate (3): Effect of irrigation water salinity and potassium foliar application levels on schematic representation of SDS-PAGE analysis of water – soluble protein for Sids 1 cultivar.

- S0, S1, S2 and S3 refer to irrigation water salinity at 0.4, 2.5, 5 and 10 dSm^{-1}, respectively.
- K0, K1, K2 and K3 refer to K-foliar application rates of 0, 3, 6 and 9 g $K_2SO_4l^{-1}$, respectively.

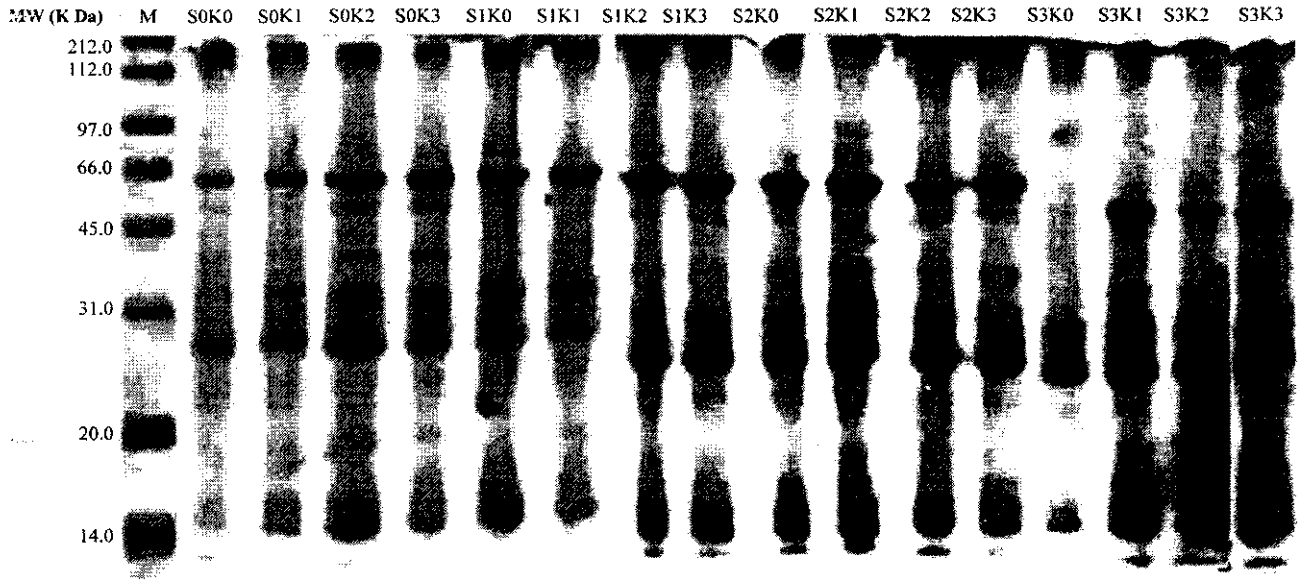

Plate (4): Effect of irrigation water salinity and potassium foliar application levels on schematic representation of SDS-PAGE analysis of water – soluble protein for Giza 168 cultivar.
- S0, S1, S2 and S3 refer to irrigation water salinity at 0.4, 2.5, 5 and 10 dSm^{-1}, respectively.
- K0, K1, K2 and K3 refer to K-foliar application rates of 0, 3, 6 and 9 g $K_2SO_4l^{-1}$, respectively.

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تأثير الرش الورقي بالبوتاسيوم على المكونات البيوكيميائية في نبات القمح

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أجريت تجربة أصص خلال موسم ٢٠٠٥-٢٠٠٦ لتقدير المكونات البيوكيميائة التي تؤدى لتحمل الملوحة في النبات وذلك من خلال الرش الورقي بمعدلات من البوتاسيوم هي المقارن و ٣ و ٦ و ٩ جرام K_2SO_4/لتر تحت مستويات مختلفة من الماء المالح وهي ٠,٤ و ٢,٥ و ٥ و ١٠ ديسي سيمينز / م وكانت الأصناف المستخدمة تحت الدراسة (سخا ٩٣ وجميزة ٩ و سدس ١ وجيزة ١٦٨) . تم إنماء هذه الأصناف حتى مرحلة النضج في تربة رملية بمزرعة كلية الزراع بالكيلو ٤,٥ بمحافظة الإسماعيلية . تم تقدير محتوى البرولين والبروتين والتفريد الكهربي للبروتين . ادى الري بالماء المالح الى زيادة معنوية فى محتوى البرولين والبروتين لنبات القمح . محتوى البرولين والبروتين تم ترتيبهما فى الأصناف كالتالى سخا ٩٣ > جميزة ٩ > سدس ١ > جيزة ١٦٨ وهذا كان واضحا مع المستويات العالية للملوحة (١٠ ديسي سيمينز م$^{-1}$) . وجد أن التحليل الكهربي بنظام SDS-PGE للأوراق وجد به نسبة أختلاف بين الأصناف حيث كانت نسبة الاختلاف بين كل من الأصناف سخا ٩٣ وجميزة ٩ و سدس ١ وجيزة ١٦٨ مع الثلاثة أصناف الأخرى ٧٤% و ٤٦% و ٧٩,٥% و ٤٨,٧% على التوالى.