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Full Length Research Paper

Effects of gibberellic acid on sugarcane plants exposed to salinity under a hydroponic system

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A hydroponical experiment was conducted to evaluate the possible interaction of salinity (EC 0 and 9 dSm^{-1}) and occurrence of exogenous gibberellic acid (GA_3) (100 ppm) on imbalance and partitioning of nutrients in a popular sugarcane genotype (var. CP48-103) and its somaclonal tolerant variant. The results revealed that the uptake and partitioning of N, K^+, Na^+ and Cl^- content were affected as a result of salinity, so salt stress induced the accumulation of toxic elements, namely: Na^+ and Cl^-, and particularly in CP48-103. The tolerant variant performed better by maintaining higher N and K^+. Some important parameters, namely shoot/root dry matter ratio and chlorophyll content decreased, while soluble sugars and protein content increased due to salt stress. Irrespective of the genotypes, supplementing GA_3 (100 ppm) as foliar application play important role on imparting salt tolerance in terms of enhancing nutrient uptake, as well as the morphological and physiological aspects. The result of this study showed that inhibition of the growth of sugarcane plantlets by salt stress was removed by GA_3.

Key words: Sugarcane, salt stress, somaclonal variant, gibberellic acid.

INTRODUCTION

Salinization is one of the most devastating forms of land degradation threatening food production worldwide, especially in arid and semi-arid countries such as Iran. In Iran, due to a number of factors such as climate change and irrigation practices, an increasing number of landscapes became saline, causing a decreased yield of crops. However, climate change predictions indicated less rainfall and higher temperatures in the near future in most of the agricultural regions in Iran, especially in Khuzistan province, and so, experts worry that the changes will lead to even more saline lands and predict that salinity will increase from 4 to 9 dSm^{-1} in the future. Progress in developing salt tolerant varieties has been very slow because of less knowledge on the mechanism of salt damage and complex nature of salt tolerance. Thus, understanding the adaptive mechanisms of each crop becomes necessary to improve or produce the salt resistant genotypes. Salinity may cause damage to the plants through osmotic stress, nutrient imbalance and specific ion toxicity (Munns et al., 1986). As such, ionic imbalance is increasingly important among the others. The availability and uptake of essential elements are affected under salinity conditions, and they lead to accumulation of some elements in toxic concentrations (Munns, 1985; Marchaner, 1986). Palaniswamy and Moshi (1973) observed in sugarcane that high proportion of Na^+ in the exchangeable complex of soil increased the Na^+ content of the plant and decreased the yield. Syed and El-Swaif (1972) reported that as the concentrations of Na^+ and Cl^- increased, N and K^+ decreased and P did not changed in leaf tissue of salt tolerant sugarcane genotype under saline water irrigation. According to Kwon et al. (1999), salinity tolerance was associated with Na^+ exclusion, while the selectivity uptake of K^+ over Na^+ was maintained at higher K^+/Na^+ ratio in the leaf and stem of canola.

Reports are available that the external application of GA_3 can alleviate deleterious effects of salinity. Ashraf et al. (2002) showed that GA_3 application increased the nutrient uptake, dry weights, plant height, leaf area and yield of wheat under saline conditions. There is also evidence that GA_3 can significantly relieve NaCl-induced growth inhibition in rice (Wen et al., 2010). Starck and values of treatments were made by the least significant difference

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Kozinska (1980) reported that the GA_3 caused more absorption of P and Ca^{2+} and less absorption of Na^{+}, while it partially adjusted the ion ratios in bean. Bejaoui (1985) concluded that the effects of exogenously applied GA_3 in alleviation of salt stress may be caused by activation of special enzymes which participate in RNA and protein synthesis. Aloni and Pressman (1980) discussed possible interaction between salinity and the GA_3 effect on petiole elongation, cellular breakdown and bolting in celery. The response to the exogenous GA_3 treatments depends on the elapsed time from the beginning of salinization (Amzallag and Lerner, 1992). Many studies have shown positive effects of GA_3 upon photosynthesis, sucrose biosynthesis and utilization of stored sucrose in stress conditions particularly when used in a range of 100 ppm in sugarcane (Alexander, 1973; Buren et al., 1979; Moore and Ginoza, 1980). However, it can increase the sugarcane stalk length from 200 to 400% in normal conditions (Bull, 1964).

The present study focuses on the following objectives:

(a) To understand the effect of salt on nutrient imbalance and variability in partitioning of nutrients in different organs, and (b) to test the efficiency of GA_3 on the impart of salt tolerance in terms of better nutrient uptake under salt stress situation.

MATERIALS AND METHODS

A hydroponic culture with the use of ¼ strength Hoagland's solution (Hoagland and Arnon, 1950) was conducted by using two salinity treatments (NaCl), which include EC = 0 and 9 dSm^{-1} mixed with and without 100 ppm GA_3 as foliar spray, for the 60 days old plantlet. The experiment was carried out in the Department of Agronomy and Plant Breeding, Ahvaz University, Iran, in 2010 using a popular sugarcane (*Saccharum officinarum* L.) genotype, namely CP48-103, and its salt-tolerant variant was derived from a tissue culture process in this department. The experimental trays were arranged in a factorial complete randomized design with 4 replicates.

The observations on physiological parameters and nutrient analysis were recorded when the plants were 150 days old, after which the plants were harvested, dried in an oven at 70°C, weighed and grounded to constant mass for further analysis. Dry matter partitioning to shoots and roots was estimated by calculating shoot:root ratios in dry weight basis. Nitrogen was estimated following micro Kjeldal's method described by Humphries (1956) and then the total nitrogen concentration was determined according to the method of Lindner (1944). The potassium and sodium content was estimated by the flame photometer (Jenway PFP 7, ELE Instrument Co. Ud.) method. Calcium was estimated by versene titration method as described by Jackson (1973). Chloride content was estimated by volumetric method with $AgNO_3$ solution using potassium chromate as an indicator after extraction of leaf powder with 0.05 N potassium sulphate solutions as described by Saffiqua et al. (1977). Chlorophyll content was determined using the spectrophotometric method of Hille et al. (1986). Soluble sugars were determined by the anthrone-sulphuric acid method (Irigoyen et al., 1992), while soluble proteins were determined according to the method of Bradford (1976). All measurements were conducted on three plants per each treatment. The data were subjected to analysis of variance (ANOVA), and comparisons between the mean (LSD) tests using the statistical software SAS (SAS Institute. 1992).

RESULTS

Uptake and partitioning of nutrients

Results indicated that irrespective of genotypes and plant parts, reduction in N content was observed under salinity condition; however, the reduction was higher in shoot as compared to root. An overall reduction in N content in different plant parts was also associated with more accumulation of toxic ions in different plants (Table 1). Although, Nasir et al. (2010) mentioned that nitrate reductase is the enzyme that catalyses the N assimilation, which appears to suppress its activities by salinity, GA_3 can overcome its effect. In this study, GA_3 improved non-significantly the N content in two types of sugarcane plants even under NaCl-stress.

The plants supplemented with NaCl exhibited a significant increase in Na^{+} and Cl^{-} content of shoot and root as compared to the plants grown under non-saline condition. GA_3 diminished this increase clearly in the tolerant variant grown with or without NaCl. In the tolerant variant, the accumulation of these harmful ions (that is, Cl^{-} and Na^{+}) was lower than the parent as there was limited transfer. Tolerant variant seems to have a genetically ability to absorb Cl^{-} in low rate and a mechanism to transfer small amounts of that toward shoot. However, GA_3 decreased markedly the accumulation of Cl^{-} in both shoot and root, particularly in tolerant variant. While concentration of K^{+} decreased markedly in shoots and roots of source variety due to salt stress, no significant change was observed in shoots of tolerant variant, except in its roots. After application of hormone, K^{+} accumulation in the shoots of all sugarcane plants increased under non-saline and saline stress (Table 1). In contrast, Ca^{2+} contents of shoot and root showed a little increase in both sugarcane type plants under NaCl stress, but the effect of hormone application was observed adversely even without the presence of NaCl.

The results obtained here are consistent with those of Ashraf et al. (2002) in wheat (*Triticum aestivum* L.), Kaya et al. (2006) in maize (*Zea mays* L.), Rodriguez et al. (2006) in rice (*Oriza sativa* L.) and Nasir et al. (2010) in linseed (*Linum usitatissimum* L.); however, they were in contrast with Maggio et al. (2010) findings in tomato (*Lycopersicon esculentum* L.). As a result, application of GA_3 to the NaCl-exposed plants was found to be more effective in the reversal of the adverse effect of NaCl stress, which caused significant responses.

Dry matter accumulations and physiological responses to salt stress

The results for dry matter accumulation of shoots and roots

Table 1. Changes in sugarcane nutrient content in response to salinity stress and exogenous GA_3.

Salinity (dSm^{-1})	GA_3 (ppm)		Tolerant variant					Source variety				
			N	Na^+	K^+	Ca^{2+}	Cl^-	N	Na^+	K^+	Ca^{2+}	Cl^-
0	0	Shoot	1.45	0.20	1.43	0.14	0.19	1.32	0.31	1.46	0.10	0.26
		Root	1.04	0.31	1.48	0.09	0.16	1.03	0.62	1.39	0.04	0.24
	100	Shoot	1.50	0.20	1.78	0.12	0.18	1.48	0.24	2.00	0.09	0.20
		Root	1.07	0.24	1.20	0.08	0.12	1.07	0.32	1.15	0.04	0.15
9	0	Shoot	1.35	0.27	1.44	0.15	0.39	1.12	0.49	1.17	0.11	0.43
		Root	0.96	0.55	1.19	0.11	0.48	0.94	0.95	1.14	0.06	0.87
	100	Shoot	1.39	0.24	1.69	0.15	0.26	1.20	0.39	1.35	0.11	0.35
		Root	0.99	0.41	0.95	0.11	0.29	0.98	0.95	1.31	0.05	0.49
L.S.D. (5%)			0.08	0.06	0.12	0.01	0.05	0.08	0.12	0.12	0.02	0.11

Table 2. Changes in sugarcane dry matter accumulation in response to salinity stress and exogenous GA_3.

Salinity (dSm^{-1})	GA_3 (ppm)	Tolerant variant			Source variety		
		Dry matter ($gplant^{-1}$)	Shoot mass ($gplant^{-1}$)	Root mass ($gplant^{-1}$)	Dry matter ($gplant^{-1}$)	Shoot mass ($gplant^{-1}$)	Root maas ($gplant^{-1}$)
0	0	1.41	0.94	0.47	1.53	1.02	0.51
	100	1.43	0.96	0.47	1.61	1.15	0.46
9	0	1.29	0.81	0.48	0.98	0.59	0.39
	100	1.37	0.95	0.42	1.21	0.76	0.45
L.S.D. (5%)		0.05	0.06	0.04	0.21	0.18	0.04

showed that NaCl had a significant adverse effect on them (Table 2). Both the two plant types mirrored this response with approximately 36 and 8.5% reduction for parent variety and its tolerant variant, respectively. Application of GA_3 also had a significant positive effect on the biomass production of both sugarcane types. From the mean data of dry weights of shoots and roots, it was evident that although dry matter accumulation declined consistently with increase in NaCl level, application of GA_3 was found to have alleviated the effect of salt stress on both sugarcane types.

In the absence of salinity, the parent variety was affected by GA_3 as compared to its tolerant variant. So, it had a superior distribution of dry matter benefit to shoot; although, this condition was completely reversed when NaCl was added. Table 2 clearly depicts that the tolerant variant had an

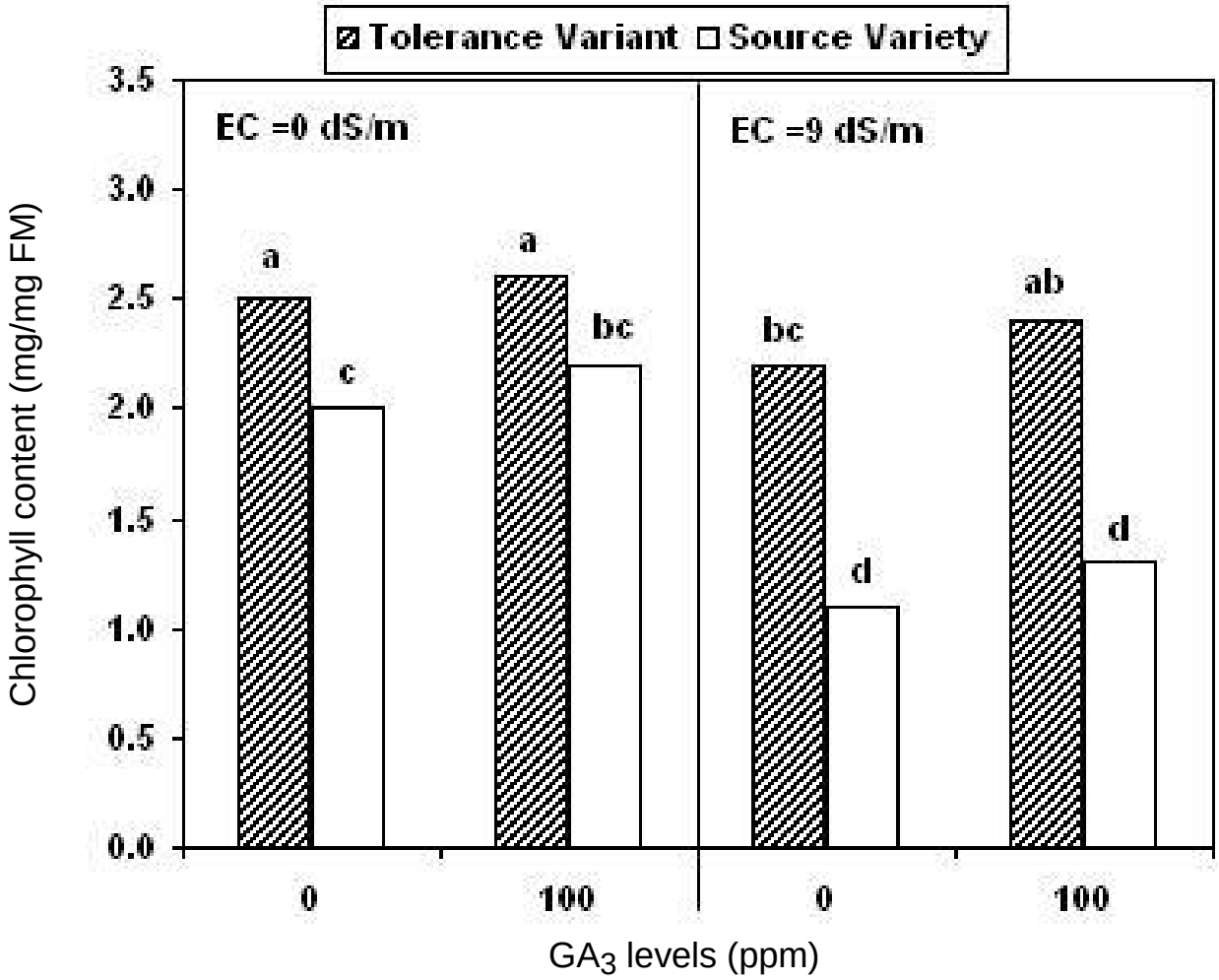


Figure 1. Effects of salinity and GA3 on protein content of sugarcane plant tissue (mg.g-1 fresh matter).

aimed response to GA_3 application under salinity.

Salinity and GA_3 treatments significantly affected protein content both in the source variety and in its tolerant variant (Figure 1). The significant interaction between GA_3 and salinity treatments revealed a positive effect of GA_3 on the protein content of plants, as the highest level of protein content was found in salt stressed plants, which were subjected to GA_3.

The presence of NaCl caused a significant decrease in chlorophyll content as compared to the control (NaCl-free stress). The highest pigment content was found in the tolerant variant which was subjected to GA_3. However, under salt stress, the lowest chlorophyll content was recorded in the source variety plants, supplied with or without GA_3 treatments (Figure 2). Application of GA_3 could not bring about a significant change in chlorophyll content especially in tolerant variant.

Soluble sugar content had an increasing trend in shoot and root tolerant variant and no significant change in source variety with the increase in salt concentration, whereas with application GA_3, the effects on this variable were better only in tolerant variant as was markedly reported in its shoots (Figure 3a and b). Overall, the effect of growth regulator application on the source variety was not significant with respect to this attribute.

DISCUSSION

The results reported in the present study showed that GA_3 application helped the salinity-challenged plants to a different degree in the reversal of altered growth and physio-chemical processes in sugarcane. It has been suggested that salt stress modifies the biochemical changes taking place in the cell wall during growth, thereby preventing extension (VanVolkenburgh and Boyer, 1985; Kaya et al., 2006). GA_3 has been reported to promote cell division and cell elongation in sugarcane (Alexander, 1973). Thus, the observed increase in seedling growth of salt stressed sugarcane with GA_3

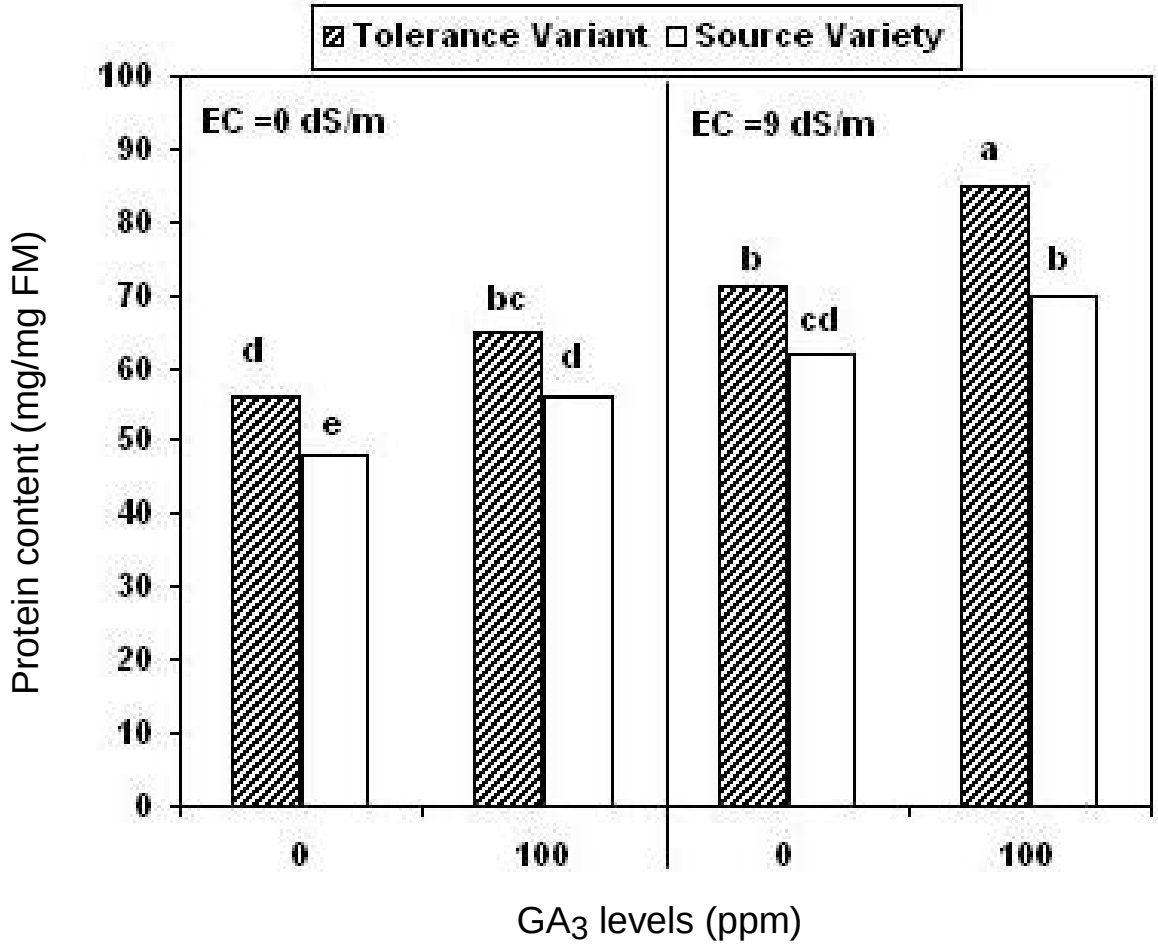
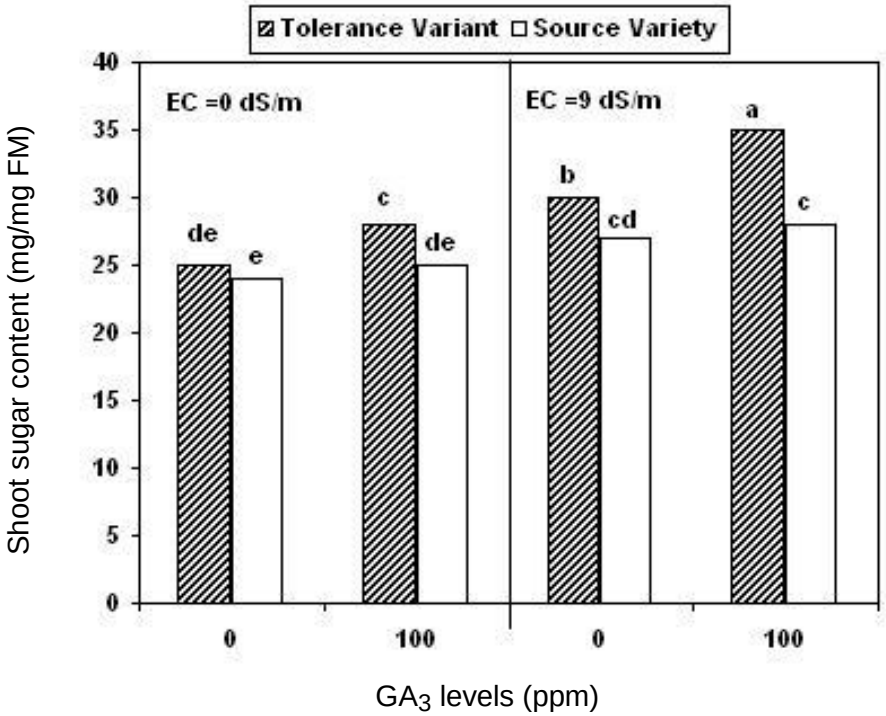


Figure 2. Effects of salinity and GA_3 on protein contents of sugarcane plant tissue (mg mg^{-1} fresh matter).

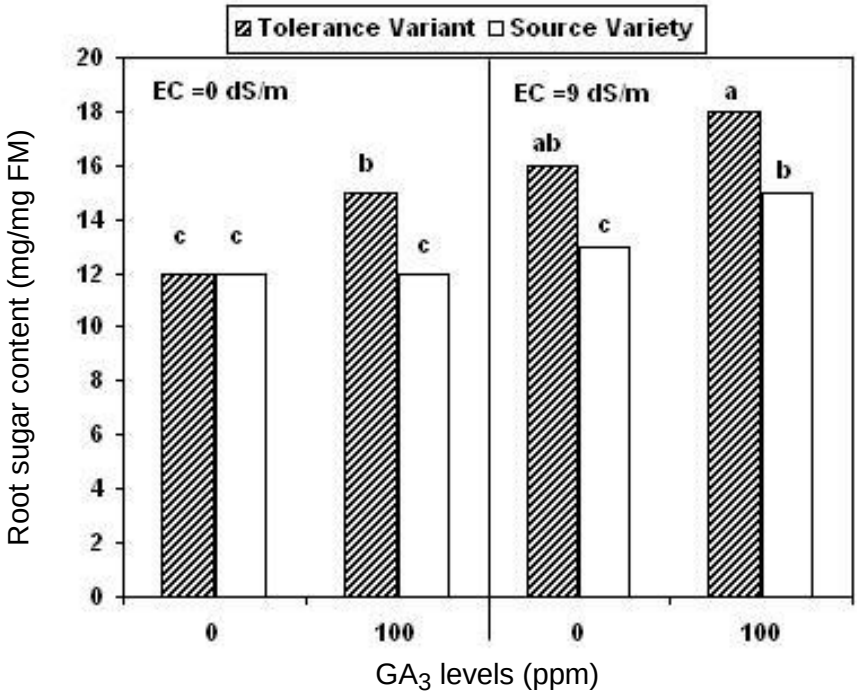
treatment could be due to the inherent attribute of GA_3 in increasing impaired cell division and cell elongation under stressed conditions. As such, salt stress was strong enough to inhibit plant growth due to reduction in gibberellin production (Kaya et al., 2006). Addition of exogenous gibberellic acid might increase seedling growth by enhancing the content of endogenous gibberellic acid as that mentioned by Rodriguez et al. (2006).

Salt stress is known to enhance the uptake and accumulation of toxic ions such as Na^+ and Cl^- in crop species, including sugarcane (Muniaswamy, 1998; Ashraf et al., 2002; Kaya et al., 2006; Nasir et al., 2010). However, GA_3-treated plants accumulated less Na^+ and Cl^- in both shoots and roots than the untreated plants but in significant lower range in tolerant variant. These results agree with Aldesuquy (1995) who reported that GA_3 reduced the accumulation of toxic ions in leaves of wheat under saline conditions. Although the shoot and root N content decreased consistently in both types of sugarcane plants under salt stress, GA_3 application promoted the uptake of N in both shoots and roots. It is pertinent to mention that GA_3 application might have mitigated the adverse effect of salt stress on nitrate reductase and enhanced its activity as mentioned by Nasir et al. (2010). In order to maintain K^+ transport, GA_3 was more effective on tolerant variant than on source variety in the presence of salinity; but without salt stress, the effects were higher in source variety.

This study showed that inhibition of the growth of sugarcane plantlets by salt stress was removed by GA_3. In contrast, increasing salinization (EC) of the nutrient solution caused a reduction of the leaf area, shoot dry matter percentage and total dry matter. The observed chlorophyll depletion may be considered to be a result of the inhibition of chlorophyll biosynthesis following an increase in ethylene production brought about by the elevated NaCl content (Shah, 2007). Moreover the GA_3 can promote the synthesis of ethylene (Alexander, 1973), so this study did not have a positive effect on chlorophyll content. Consequently, in this study, GA_3 did not increase the chlorophyll in sugarcane; so, these results do not agree with those of Ashraf et al. (2002) study. During salt stress, plants adapt to osmotic stress by accumulating



(a)



(b)

Figure 3. Effects of salinity and GA_3 on (a) shoot and (b) root soluble sugar contents of sugarcane plant tissue (mg mg^{-1} fresh matter).

some compatible solutes such as proteins, soluble sugars, proline, glycine betaine, polyols and trehalose (Ryoun et al., 1999; Sakamoto and Murata, 2002). These compounds play a predominant role in protecting plants from osmotic stress. Although many reports showed that the activities of some antioxidant enzymes were increased by salt stress, it was observed that it may be due to the application of GA_3 to salt-stressed plants which enhanced the activities of these enzymes and so diminished the disadvantage of salinity effects (Nasir et al., 2010). Thus, antioxidants and compatible solutes may provide a strategy to enhance salt tolerance in plants and the later studies need to determine the appearance of these agents especially in tolerant plants as quality and quantity amounts. The cumulative response of these parameters might have provided an environment to the plants to perform photosynthesis normally, which is further confirmed by the enhanced dry matter production.

On a whole-plant basis and under field conditions, there are evidences which indicate that vigorous plants may better cope with salinity (Munns et al., 2006), possibly by delaying the onset of the salinity tolerance threshold (Dalton et al., 2000). In contrast, GA_3-dependent growth reductions were reported to be critical in stress adaptation and/or survival (Achard et al., 2006). Although the independent roles of GA_3 have been well documented (Maggio et al., 2010), it remains uncertain how this hormone coordinately regulate plant growth and stress adaptation.

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