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Effect of Salinity and Osmotic Stresses on Some Economic Plants

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Abstract: The effect of water stress induced by NaCl or drought stress was studied on the leaves of wheat, barley, kidney bean and mung bean. After 25 days old, iso-osmotic stresses (200 mM) NaCl and PEG 6000 (180 g 1^{-1}) were applied for 3 weeks old plants. Decreasing leaf dry weight was similar in barley, kidney bean and mung bean under both stresses, while leaf dry weight of wheat decreased more under PEG stress than under NaCl stress. Water contents decreased in all plants under osmotic stress. Ions K^+ and NO_3^- contents decreased in relation to control under salt or osmotic stresses, whereas Na^+ and Cl^- markedly increased in both wheat, barley and kidney bean. Amino acid increase was three times higher in leaves of kidney bean than in the leaves of all plants. However, the organic solutes and soluble sugar decreased in all plant leaves under both NaCl or drought stresses. Free proline increased under both stresses and its content was highest in wheat and in mung bean respectively under NaCl and PEG stresses. Malic acid accumulation was in wheat than in all plants under the PEG stress. The ability of plants to regulate their metabolic and physiological function could also play an important role under these harmful conditions.

Key words: Amino acid, proline, inorganic solutes, organic acids, mineral contents, sugars, salt stress, drought stress, wheat, barley, kidney bean, mung bean.

INTRODUCTION

Excessive salinity is an adopic factor that limits the distribution of plants in certain natural habitats, and it constitutes an increasingly severe agriculture problem in many areas of the world[40]. Salinity retarded seed germination and growth of higher plants[45,36,6,39,44]. Among the most importance factors responsible of retarded growth, under the influence of salinity, is the decline in photosynthetic performance. Thus, salinity usually causes a reduction in the leaf area which generally leads to a drastic reduction in net CO_2 assimilation[15,5]. The increased thickness of the leaf under salinity stress may also reduce the photosynthetic performance via decrease of CO_2 diffusion in the mesophyll cells[25,27,9].

The response to salinity is evaluated by using plant growth, ion balance and osmotic adjustment, both by inorganic ions and by compatible organic solutes, as selection criteria; however, the water relations of the plant are rarely used. The osmotic effect resulting from soil salinity may cause disturbances in the water balance of the plant, including a reduction of turgor and on inhibition of growth, as well as stomatal closure and reduction of photosynthesis[38]. The effect of high ambient NaCl concentrations on the internal concentrations of macronutrient elements have been extensively studied[7,31,34,43]. Na, K and Cl have been measured, most not ably those of Ca, N and P because these nutrients are of interest and required in large quantities to sustain adequate plant growth and their concentrations in the soil are often limiting to plant growth. Regarding, PEG stress effects on plant cells and tissues were investigated by many investigators; retardation of growth[24,49], acquisition of the ability to adapt to stressful[47]. PEG is an inert, non ionic, long chain polymers but highly soluble in water and available in a wide range of molecular weights[28]. PEG of high molecular weight of more than 4000 has been widely used as an osmaticum to impose water stress in plants by decreasing the water potential of nutrient solutions without being taken up and with no evidence of toxicity[23].

Thus, the present was intended to study the water content in leaves of the wheat, Barley, kidney bean and mung bean cultivated under saline and osmotic stresses. The contribution of the inorganic (Cl^-, Na^+, K^+ and NO^-_3) and organic solutes (amino acid, organic acid and soluble sugar) and proline contents.

MATERIALS AND METHODS

The grains of wheat (*Triticum aestivum*. Cv. Giza96), barley (*Hordeum vulgare* L.), kidney beans (*Phaseolus vulgaris* cv. Giza 3) and mung bean (*Vigna sativum* L.) were obtained from the Ministry of

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Agriculture, Giza, A.R. Egypt. Plants sowing was carried out in small pots (20 cm diameter) filled with constant weights of soil (mixture from clay and sandy). The three groups of pots {control and two sets representing salinized (200mM/L) and polyethylene glycol 6000 (180g 1^{-1})} treatment were placed under natural conditions in the garden of Zagazig University. When plants were 25 days old, treatments were carried out by spraying with (200 mM NaCl). Salt treatment and (180 g PEG 60001^{-1}) drought treatments. The salt and drought treatments were applied for 3 weeks. After 40 days old, the samples collected, leaf fresh weight, leaf dry weight and leaf water content (measured as < fresh weight – dry weight/fresh weight) was determined. Analysis of mineral inorganic in leaf samples, Cl^- was analysed by potentiometric titration with $AgNo_3$ of the aqueous extract as described by Johnson *et al.,*[21]. Na^+ and K^+ were estimated by atomic absorption spectrophotometry and No_3^+ content was astimated by spectrophotometrically using sulfanilic acid and α-naphthylamine Lambert and Dubois[26]. Free proline contents were detemined according to the method of Bates *et al.,*[4]. Extraction of soluble sugar were determined colorimetrically[33] using glucose as the standard. Analyses of organic acid were carried out by Timpa *et al.,*[50]. Free amino acids were measured by Russel[41].

RESULTS AND DISCUSSIONS

In the present investigation, (Fig. 1) showed that leaf dry weight and plant biomass of wheat plant decreased more under PEG than under NaCl stress. Decreasing leaf dry biomasses were practically observed under salt and osmotic stresses, in mung bean, kidney bean and barley plants than under PEG. These results could be supported by those of Perez-Alfocea *et al.,*[37] and Sanchez-Blanco *et al.,*[42]. On the other hand, osmotic stress induced reduction to the same extent, the water content of all plants compared with the control and salt treatment. Under salt-stress, reduced water content was found in barley. In this regard, barley, being highly resistant to drought and salinity[31], is the only crop agronomically feasible; in the Ebro river basin, rainfed barley covers more than 10000 km^2. Barley grows in these salt affected areas in the presence of high concentration of not only sodium and chloride, but also calcium, in the soil water solution. The osomatic effect resulting from soil salinity may cause disturbances in the water balance of the plant, including a reduction of turgor and an inhibition of growth, as well as stomatal closure and reduction of photosynthesis[38]. In This respect, Shalhevet *et al.,*[46] found that salinity generally reduces shoot and root growth of maize and soybean plants

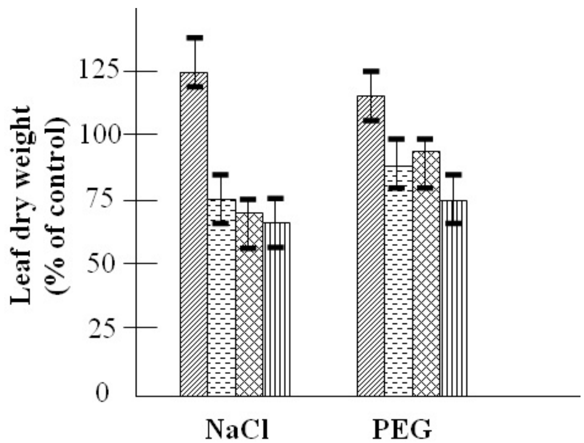

Fig. 1: Effects of NaCl (200 mM) and PEG 6000 (180 g l-1) iso-osmatic treatment on the leaf dry weight of:

wheat , barley, kidney bean and mung bean . Vertical bars represent SE values.

based on dry weight and length measurements. While, Abo-Hamed *et al.,*[1] revealed that the reproductive stage of sorghum reduced the relative water content, leaf conductance and transpiration rate with the increase of stress period. Also, El-Darier[10] revealed that root and shoot dry weights of Zeamays seedlings were decreased in response to salinity. In agreement with the result obtained in the present investigation, Badawi,[3] showed that, the dry weight and water contents of both roots and shoots of Hyoscyamus muticus plants were significantly reduced following treatment with salinity and PEG as compared with control plants.

Inorganic Solutes: The results obtained in Table (1) showed that total ion content in leaves of Na and Cl^- was high in NaCl solution and drought stress PEG treatment for all plants. However, $K^+$and NO_3^- ions in all plant leaves decreased under saline stress or PEG. In this respect studied, Gadallah,[16] reported that salinity decreased the contents of dry mass and enhanced content of Na^+, Ca^{+2} and Cl^-. In saline treatment the contribution of inorganic solutes was high in mung bean. Na^+ and Cl, whose contents were two-fold higher than in wheat. Among the wheat, the contribution of inorganic solutes was highest in kidney bean, however, the specific contribution of Na^+ and Cl^- was almost the same as that of wheat and barley.

Salt-stress caused a more pronounced nutritional imbalance than drought stress. Whatever all the plants were; however, drought stress reduced the No^-_3 level

Table 1: Effect of NaCl and PEG on the water content and inorganic solutes' mmol (g D wt)$^{-1}$of wheat , barley, kidney bean and mung bean leaves.

Plants	Treatment	Water content (%w/w)	Na^+	Cl^-	K^+	No_3^-	Sum inorganic solutes
Wheat	Control	89.0	0.8	2.4	32.4	36.6	72.2
	NaCl	75.5	28.2	17.8	13.0	20.6	79.6
	PEG	52.0	4.8	13.4	23.8	23.0	65.0
LSD	5%	1.27	2.16	1.92	3.09	4.12	2.99
	1%	1.86	3.26	2.89	4.66	6.26	4.54
Barley	Control	89.6	0.6	3.6	31.9	38.0	74.1
	NaCl	79.4	18.5	20.3	23.6	13.4	75.8
	PEG	61.0	4.8	3.4	22.6	18.0	48.8
LSD	5%	2.07	2.19	1.22	1.38	3.72	2.13
	1%	3.12	3.24	1.86	2.09	5.65	3.27
kidney bean	Control	90.0	1.1	3.8	42.7	37.4	85.0
	NaCl	81.0	22.8	17.0	16.4	29.5	85.7
	PEG	55.7	1.7	3.2	23.7	27.0	55.6
LSD	5%	2.24	2.22	1.01	3.19	1.83	2.37
	1%	3.27	3.26	1.53	4.81	2.72	3.55
Mung bean	Control	91.7	1.5	2.7	26.5	57.6	88.3
	NaCl	75.0	37.3	26.2	8.1	21.0	92.6
	PEG	54.0	7.0	3.4	26.1	36.1	72.6
LSD	5%	1.85	0.21	1.18	1.27	2.07	2.42
	1%	1.97	0.31	1.75	1.89	3.10	3.65

more significantly than saline stress. In this connection, a number of low molecular weight organic solutes and inorganic ions known to be associated with osmotic adjustment under water stress[18,19]. Similar results were recorded by Cramer *et al.,*[7] studies that the concentration of Na, Ca, Mg, K, S, P, Fe and Cu elements increased with time in the barley plant under salt-stressed with either NaCl or KCl salinity mostly depressed the growth and nitrate uptake and accumulation as well as nitrate reductase activity in two-week-old maize seedlings[22]. In this respect, Nesiem and Ghallab,[35] showed that total N, K^+, Ca^{+2}, Mg^{+2} and Na^+ of Triticum aestivum decreased by increasing saliniyy level. Also, Botella *et al.,*[6] found that the inhibtory effect of salinity on K^+ translocation from root to shoot of maize phants.

Organic Solutes: Data presented in Table (2) demonstrated that, the amino acid contents decreased in wheat and barley plant under salt and drought stress. However, they increased in kidney bean and mung bean under both salt and osmotic stress. Also, Table (2) shows that, upon NaCl or PEG stresses, organic acids in leaves were always lower in all plants. However, the sugar contents of all plants increased after 45 days under polyethylene glycol or salt stress. Compared with controls, organic acids decreased in all planted under saline stress; conversely they were constant in wheat and barley or increased in kidney bean and mung bean under osmotic stress. Zayed and Zeid[53] found that, the activity of alpha-amylase and sugars decreased in the germinating seeds of mung bean during a 3-day stress period under different concentration of polyethylene glycol (PEG) or salt solution. However, after 10 days, the contents of organic solutes and the activity of hydrolytic enzymes increased. The seedlings under water stress induced by PEG were affected much more than under salinity. This may be due to the maintenance of higher succulence under salt stress than under PEG-induced water stress. Also, Gadallah[16] reported that, salinity enhanced contents of total free amino acids, Na^+ Ca^{++} and Cl^- of *Vicia faba* plants. Fernandez *et al.*[13] reported that, kidney bean plant under NaCl or PEG stresses, malate and most amino acid reached a maximum 3-12 hours after the initiation of the osmotic shock, and were then restored to values observed for control plants.

In this respect many authers reported that salt stress is a key factor influencing the accumulation of organic solutes and acids which may reflect an adaptational trait of maize plant in response to external salinity treatments[12]. However, improving crop tolerance requires an understanding of what resource is most limiting and the traits (leaf area index, plant height, dry matter accumulation and partitioning and metabolite synthesis and accumulation) that improve the acquisition and preemption of these resources[29]. The decline in water contents of shoots tissues attributed to

Table 2: Effect of NaCl and PEG on the organic solutes of wheat leaves, barley, kidney bean leaves and mung bean leaves (mmol/g Dwt.)$^{-1}$.

Plants	Treatment	Amino acid	Organic acids	Sugars	Sum organic solutes
Wheat	Control	3.0	18.3	6.4	27.8
	NaCl	2.9	10.2	8.5	21.6
	PEG	2.3	17.0	13.2	33.5
LSD	5%	0.17	2.03	3.09	4.62
	1%	0.25	3.10	4.60	7.02
Barley	Control	3.5	14.5	8.4	26.4
	NaCl	3.5	9.3	9.1	21.9
	PEG	3.3	14.0	14.7	32.5
LSD	5%	0.09	1.02	1.11	1.35
	1%	0.13	1.56	1.68	2.05
Kidney bean	Control	1.0	2.2	5.7	15.0
	NaCl	3.0	5.6	4.2	12.8
	PEG	3.2	15.2	10.4	28.8
LSD	5%	1.02	1.31	1.21	1.29
	1%	1.54	1.97	1.86	1.94
Mung bean	Control	2.5	6.0	3.1	11.7
	NaCl	2.7	3.1	1.9	6.7
	PEG	3.96	8.9	8.2	21.06
LSD	5%	0.39	1.98	2.28	2.38
	1%	0.58	2.98	3.39	3.64

reduction in water uptake. Mehta *et al.,*[32] found that both chloride and sulphate at various concentrations induced an increase in carbohydrate contents that was followed by a decrease which was greater with chloride than with sulphate are in disagreement with the results obtained here with wheat,barley, kidney bean and mung bean leaves where the treated plants attained mostly high values of sugars as compared with their respective controls. In agreement with, Badawi[3] revealed that Na_2So_4 and PEG induced variable increase in carbohydrate functions of Hyoscymus muticus plant. Also, Inal *et al.,*[20] noticed that fresh and dry weigths of tomato plants decreased with increasing salinity, plant Na^+, Cl^- and proline while the chlorophyll contents increase and K^+ and total-N decreased.. In respect to Table (2) total free amino acid, including proline decreased in all treated plants under stress conditions. Free amino acid contents were higher in mung bean than wheat under saline conditions, while under osmotic stress mung bean leaves accumulated more amino acids than wheat plants. Table (3) shows that, under salt stress, wheat-treatment plant accumulated more proline than mung bean, free proline contents in salt and PEG-treated wheat plants represented 35-40% of the total amino acids. Conversely, high levels of proline were found in PEG-treated mung bean compared with salt-treated plants. So, that proline represented, respectively, 50% and 30% of total amino acids under PEG-and salt-stresses. These results agreed in part with those of Gulati and Jaiwal[17] who found that higher internal ions and proline contents impart dual resistance to drought and salt stress. Lyer and Caplan[30] showed that, many plants accumulate high levels of free proline in response to osmotic stress. This amino acid is widely believed to function as a protector or stabilizer of enzymes or membrane structures that are sensitive to dehydration or ionically induced damage. Intermediates in proline

Table 3: Effect of NaCl and PEG on the proline content of wheat, barley, kidney bean and mung bean leaves (mmol/gD.wt.)$^{-1}$.

Plants	Treatment	Proline contents
Wheat	Control	0.1
	NaCl	1.8
	PEG	2.5
LSD	5%	0.27
	1%	0.39
Barley	Control	0.1
	NaCl	2.3
	PEG	1.7
LSD	5%	0.24
	1%	0.35
Kidney bean	Control	0.1
	NaCl	1.5
	PEG	2.6
LSD	5%	0.15
	1%	0.22
Mung bean	Control	0.1
	NaCl	0.7
	PEG	4.4
LSD	5%	1.02
	1%	1.49

Table 4: Effect of NaCl and PEG on the organic acids contents of wheat, barley, kidney bean and mung bean leaves (mg/g dry wt.) $^{-1}$.

Plants	Treatment	Citric	Pyruvic	Malic	Succinic	Fumaric	Total
Wheat	Control	29.5	0.16	41.3	2.0	0.19	73.15
	NaCl	19.6	0.13	34.2	3.1	0.06	57.1
	PEG	11.3	0.50	53.3	1.3	0.07	66.47
LSD	5%	2.08	0.09	2.93	0.17	0.03	4.08
	1%	3.00	0.13	4.21	0.24	0.04	5.95
Barley	Control	12.3	0.19	37.1	2.3	0.09	52.0
	NaCl	10.8	0.21	28.8	2.0	0.08	42.0
	PEG	7.0	0.53	42.2	1.3	0.07	51.2
LSD	5%	1.17	0.13	2.76	0.14	0.02	2.07
	1%	1.79	0.20	3.86	0.21	0.03	3.12
Kidney bean	Control	21.6	0.26	28.0	1.6	0.13	51.6
	NaCl	14.2	0.94	36.9	3.4	0.14	65.6
	PEG	11.0	0.5	53.1	1.8	0.09	66.5
LSD	5%	2.78	0.24	3.68	0.09	0.02	3.65
	1%	4.05	0.35	6.59	0.13	0.03	5.40
Mung bean	Control	11.0	0.13	9.6	8.0	0.09	28.8
	NaCl	7.6	0.14	9.4	8.4	0.06	23.6
	PEG	12.9	0.13	22.3	1.6	0.10	37.0
LSD	5%	1.01	0.05	2.16	0.07	0.02	1.82
	1%	1.48	0.07	3.17	0.10	0.03	2.63

biosynthesis and catabolism such as glutamine and DELTA1-pyroline-5-carboxylic acid (P5C) could increase the expression of several osmotically regulated genes in rice. Osmotically stressed rice induced increases in the concentration of one or more intermediates in proline metabolism could be influencing some of the characteristic responses to osmotic stress. Tipirdamz and Cakirlar[51] reported that stem and proline contents increased more in wheat stem (Bez-Ostaya) than in wheat (Gerek 79) under NaCl with or without drought induced by iso-osmotic polyethylene glycol. Wyn Jones and Storey[52] noticed that stress conditions induced by PEG and NaCl caused an accumulation of proline and glyinebetaine in the shoot of two barley culltivars. A gradual increase in the stress led to higher glyinbetaine than proline levels whereas salt and osmotic shock led to proline levels far in excess of glyinbetaine levels. Wyn Jones and Storey[52] also suggested that, proline may be used as an internal assay for water stress levels. Comparison of the physiological respones of the barley cultivars to NaCl and PEG stress suggested that the suppession of the growth by salt treatment was not due directly to the low external water potential but across either from the ions, Na^+ and Cl^-,interfering with metabolic process or from a number of possible but unsubstantiated interactions leading to a reduction in turgor potential and thus in the rate of cell expansion. Also, Al-Hakimi[2] showed that the soluble carbohydrate, soluble protiens, proline and other free amino acids of Vicia faba increased with the rise of NaCl concentration. Rapid accumulation of proline, is one of the most remarkable metabolic consequenes of salt stress in higher plants[8]. Also, Stewart[48] proposed that accumulation of sucrose prevents oxidation of proline and increased the conversion of proline to protiens. Moreover, it was suggested that free proline accumulation in salt-stressed seedlings may be related to decrease in proline oxidase activity or to an impaired incorporation of proline into protien. On the other hand, Ford and Wilson[14], proposed that proline plays an indirect role in osmoregulation by increasing the water-binding capacity of plant cell walls to maintain the hydration of protoplasm and to increase membrane permeability. Meanwhile, Hanada *et al*.,[18] stated that proline affects the solubility of various protiens and could protect them against denaturation caused by water stress, thus the interaction between proline and hydrophobic residues on the protien surface increase the stability of protiens. In addition, Eberhardt and Wegmann[11], mentioned that proline may act as non-toxic osmotic solute, preferentially located in the cytoplasm and as enzyme protectant, stabilizing the structures of macromolecules and organelles. It is apparent from Table (4) that total organic acid contents were 2-3 times greater in wheat than in mung bean irrespective of the treatment. Compared with controles, organic acids decreased in all plants under saline stress, conversely they were practically constant in wheat and barley or increased in kidney bean and mung bean under osmotic stress. High levels of malate (over 50%) we found in the organic acid pools of wheat, irrespective of treatment (table 4). Malate contents decreased only in wheat plant under salt stress, but increased malate contents were found in PEG-treated plants, mainly at the expense of citrate, as in wheat plant. Thus, malate content was two-fold higher in PEG treated wheat than in mung bean. Pyruvate increased under PEG stress in leaves of all plants, and only in leaves of kidney bean under salt stress. Fernandez-Ballesteretal.,[13] revealed that treatment of kidney bean by NaCl or PEG stresses, malate and most amino acids reached a maximum 3-12 hrs. after the initiation of the osmotic shock and were then restored to values observed for control plants .

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