INT. J. BIOL. BIOTECH., 9 (4): 353-358, 2012.

BIO-CHEMICAL RESPONSE BY ELEVATED SALINITY STRESS IN TWO BARLEY LINES

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ABSTRACT

A Lysimeter scale experiment was conducted to determine the effect of elevated salinity on growth parameters and changes in biochemical attributes in 15 days old barley lines PK-30108 and Haider-93 in gravel using NaCl concentrations {EC: 5 (Control), 7.5 and 15 dS m^{-1}) in 25% Hoagland nutrients solution. Under increased salinity stress both the barley lines underwent significant decrease in shoot and root dry biomass, chlorophyll (a, b and total) and total sugars whereas a significant increase in total amino acids and NIRA was recorded. The reduction was significantly more pronounced at EC 15 dS m^{-1} than EC 7.5 dS m^{-1} and the control. The line PK-30108 proved significantly more salt tolerant than the cultivar Haider-93 in respect of shoot and root biomass up to EC 7.5 dS m^{-1} while both remained similar at EC 15 dS m^{-1}. The NRA was found to be unaffected in line Haider 93 but was reduced significantly in line PK-30108 with elevating salinity levels. The parameters could be used as biochemical markers for investigating the elevated salinity effects on barley varieties.

Key words: *Gravel culture Lysimeter, Barley lines, salinity stress tolerance, biological growth markers, NRA, NIRA*

INTRODUCTION

Salinity is one of the major causes of decrease in agricultural acreage. About one third of irrigated land on the earth is affected by salinity (Taiz and Zeiger, 1991). Abiotic stresses such as salinity, high pH and drought reduce growth of plants and agricultural productivity is more important than other factors (Jaleel *et al*., 2008, Karakas *et al*., 1997). Higher salinity levels cause significant reduction in growth parameters like leaf area, leaf length, root and shoot dry weight (Ashrafuzzaman *et al*., 2002), decreases carotenoids and induce reduction in chlorophyll and photosynthetic activity (Hamada and Al-Hakimi, 2001). High pH, accumulation of salts and exchangeable sodium cause serious reduction in crop yields. Sodium chloride is predominant salt in the salt affected lands (Ahmad, 1987; Asghar *et al.*, 1962; Rehman *et al.*, 1977; Shazia *et al*., 1998). The deleterious effects of salinity on plant growth are associated with

(1) Low osmotic potential of soil solution (water stress)
(2) Nutrition imbalance
(3) Specific ion effect (salt stress)
(4) A combination of these factors

All these causes indicate adverse apheliotropic effects on plant growth and development at physiological, biochemical and molecular level. The biochemical studies of plant under saline condition provide the basis for understanding the mechanism of salt tolerance through which crop improvement is possible.

Barley, a green grass, has high contents of chlorophyll, flavonoids, beta carotene, enzymes, all the essential amino acids, calcium, iron, magnesium, phosphorus, copper, manganese, zinc, vitamins B_1, B_2, B_6, B_{12}, C, E, folic acid, and pantothenoic acid (Abadia *et al*., 1999). Saline conditions decrease the activity of ribulose 1,5-biphosphate (Rubisco), carboxylase and reduction in rubisco activity appears as drop of carbohydrates formation (El-Shihaby *et al*., 2002, Jenkins *et al*., 1998). The assimilation of nitrogen has marked effects on plant productivity, biomass, crop yield, and nitrogen deficiency leads to a decrease in structural components. It has been suggested that some methylated amino compounds and possibly some amino compounds accumulating under stress serve as osmotica for osmoregulation (Henson and Nelson, 1978). Nitrate reductase (NR) activity is main limiting step in nitrogen assimilation in plants (Srivastava, 1990; Lea, 1997). Under salinity stress, NR is inactivated and nitrogen metabolism is hampered in plants (Khan *et al*., 1990; Botella *et al*., 1993; Lea, 1997). Belkhodja *et al*. (1999) reported adverse effects of salinity on chlorophyll fluorescence and photosynthesis of barley (*Hordeum vulgare* L.). Choonwoo *et al*. (1999) reported influence of light, osmotic potential, pH and rice straw on growth of barley (*Hordeum vulgare* L.) and water foxtail (*Alopecurus aequalis var. amurensis* - Kom.).

If metabolic activities under these environments were fully elaborated and some biochemical markers identified; their incorporation in breeding program would be done through genetic engineering or through traditional

breeding. Keeping in view these factors, a study was conducted with the main objectives to identify the biochemical markers conferring the tolerance to salinity in barley.

MATERIALS AND METHODS

The research study was conducted in the Enzyme Research Laboratories of Biochemistry, Department of Chemistry, University of Agriculture, Faisalabad and Plant Physiology Lab., Nuclear Institute for Agriculture & Biology, Faisalabad, Pakistan. Barley lines PK-30108 and Haider-93 were grown in pots under hydroponic (gravel) culture in Hoagland solution. Two salinity levels (EC 7.5 and 15 dS m-1) were maintained in addition to control. The salinity levels were established by addition of NaCl salt in ¼ strength of Hoagland solution with pH of 7 (Nelson, 1991). The shoots were collected after 15 days, rinsed with distilled water, dried with filter paper, chopped in very small pieces and stored in the refrigerator. Stored samples were used for estimation of nitrate reductase activity, chlorophyll-a and Chlorophyll-b (Arnon, 1949), total soluble sugars (Riaz *et al*., 1985), total amino acids (Moore and Stein, 1948) and soluble proteins (Lowery *et al*., 1951). Plant samples for shoot and root biomass were also collected and dried in air blown oven at 60^{o}C till to a constant weight and dry weights were recorded. The data was subjected to analysis of variance technique followed by Duncan's Multiple Range (DMR) test for multiple comparisons of paired means (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Barley (*Hordeum vulgare* L.) is rated as salt tolerant forage crop and highly salinity tolerant as grain crop (Maas, 1984). Seedling stage is relatively the most sensitive growth stage determining the plant stand density, which affects the final yield. Under osmotic stress, accumulation of osmotically active compounds called osmolytes takes important consideration in order to lower the osmotic potential. These are referred as compatible metabolites because they do not apparently interfere with the normal cellular metabolism. Molecules like glycerol, sucrose and proteins, etc, were discovered by empirical methods to protect macro-biological molecules against the damaging effect of salinity (Jefferies, 1980, Sairam and Tyagi, 2004). Depressive effects of salinity are thought to arise from stomatal and/ or nonstomatal limitations (i.e., stomatal closure and/ or damage to Calvin Cycle enzymes (Meloni *et al.*, 2003).

Shoot dry biomass (SDB)

The barley line PK-30108 produced significantly more shoot dry biomass (SDB) under salinity stress (T1 and T2) compared to Haider-93 (Fig.1.1). However, with line PK-30108, the increment of 58% more shoot dry biomass (SDB) yield in T1 compared to line Haider-93 gradually decreased with increasing salinity and the SDB contents were comparable in T3. Both varieties showed significant gradual reduction with increasing salinity and the SDB reduced to 18-28 % at T3. The line Haider-93 revealed 62% decrease under the salinity level of 15 dS m^{-1} (T3) when compared to control. These results are in accordance with general observation of growth depression, retardation or inhibition under increased salinity stress as reported by Hamda and Al-Hakimi (2001). The line Haider-93, however, having less SDB at control (T1) produced almost comparable SDB at 15 dS m^{-1} salinity stress treatment.

Root Dry biomass (RDB)

The line PK-30108 produced 72% more root dry biomass (RDB) compared to line Haider-93 in control (T1) but under salinity stress (Fig.1.2) the RDB of line PK-30108 reduced significantly. The RDB of line Haider-93 was 22% more than PK-30108 in T3 treatment. The salinity stress significantly reduced the RDB of PK-30108 (83%) and Haider-93 (63%) in T3 compared to T1. The reduction in RDB with increasing salinity observed by both lines is in accordance to the findings of Ashrafazzaman *et al*., (2002). At control the RDB of line PK-30108 was 72 % more than line Haider-93. The line Haider-93, being the salt sensitive line, however impaired most of its photosynthetic material towards root development enhancing root biomass (22 %) more than line PK-30108 at 15 dS m^{-1} treatments (T3). It showed that salt sensitive line translocated most of its photosynthate material towards root development/ maintenance to maintain nutrients availability under salinity stress environment, leaving the above ground biomass less than expected.

Chlorophyll-a (Chl-a)

The cholorophyl-a assimilation under salinity stress (Fig.1.3) was significantly higher in line PK-30108 at T2 but more in line Haider-93 at T3. Under elevated salinity stress both varieties; PK-30108 and Haider-93 respectively

revealed 34 and 16 % decrease in Chl-a content in T3 compared to T1 which were in line with the findings of Jaleel et al., (2008). A comparison of two barley lines showed that the Haider-93 had less content of Chl-a in control and 7.5 dS m^{-1} but showed 15 % increase over PK-30108 at 15 dS m^{-1} treatment.

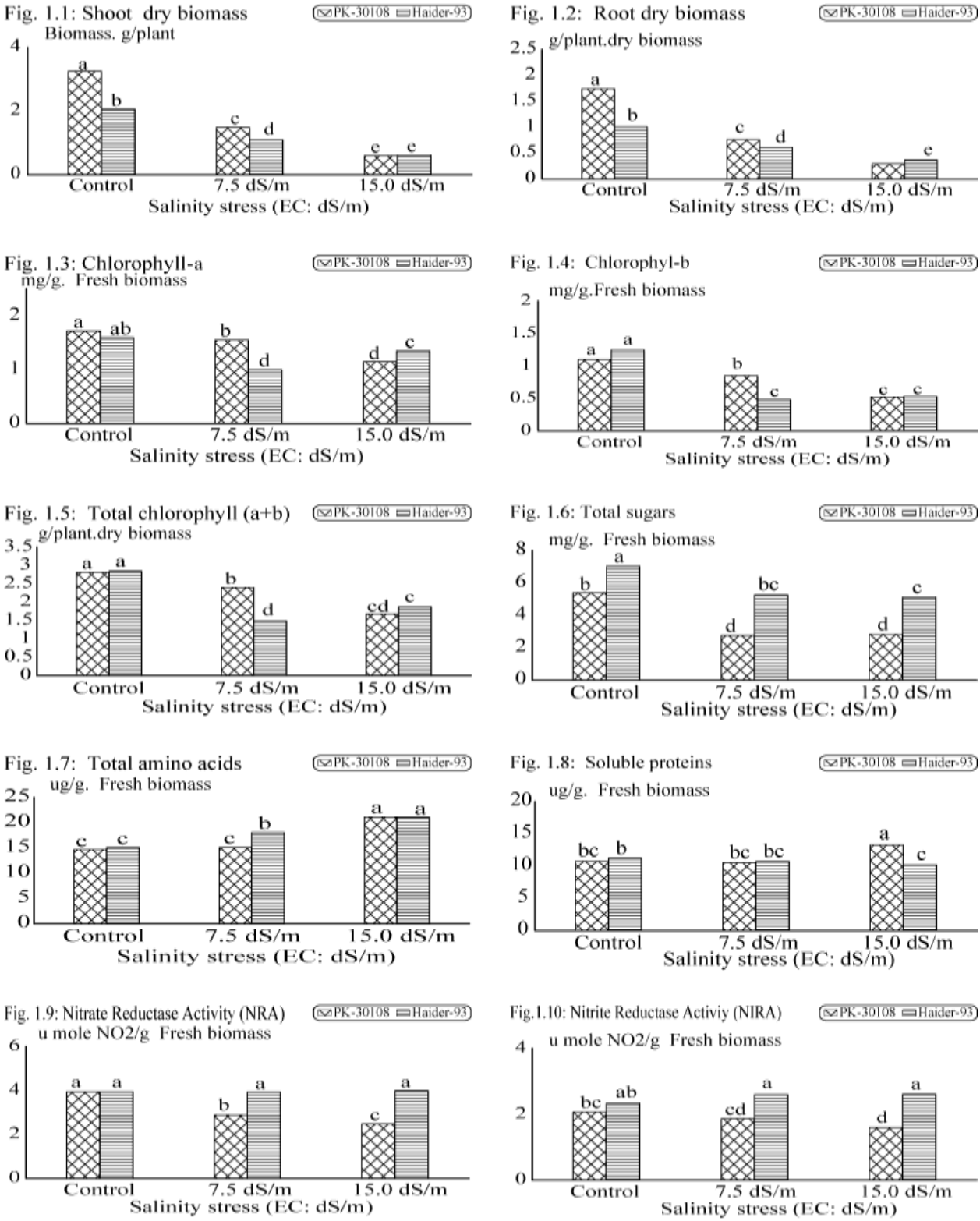

Fig.1. Effect of salinity stress on different physiological parameters of barley plants growth.

Chlorophyll-b (Chl-b)

Both of the barley lines showed almost gradual decrease in Chlorophyll-b (Chl-b) synthesis under salinity stress (Fig.1.4). The line Haider-93, having more (12 %) Chl-b content in control treatment compared to the line PK-30108 that rapidly lost its Chl-b synthesis activity at moderate salinity level (7.5 dS m^{-1}) confirming the salinity sensitive nature of the line. The line PK-30108 assimilated significantly more Chl-b in T2 level.

Chlorophyll a+b (Chl_{a+b})

A comparison of sum of Chlorophyll-a and Chlorophyll-b (Total chlorophyll content) for the two barley lines was conducted as well and it was observed that difference in between studied cultivars was not assessable satistically in T1 and T3 whilst significantly more quantity of this parameter was recorded in T2 for cultivar PK-30108. The PK-30108 synthesized significantly more Chl-a+b. The two varieties synthesized statistically comparable Chl_{a+b} in control treatment, however, it increased by 12 % in line Haider-93 compared to line PK-30108 in T-3 under salinity stress (Fig.1.5). These results are in accordance with the findings of Jaleel et al. (2008). Both of the barley lines showed regular decrease in Chl_{a+b} content under increasing salinity within the studied range of NaCl salinity. Munns (2002) and Ashraffazzaman et al. (2002) also reported impairment of the photosynthate/ chlorophyll activity, which was greatly accounted for growth restrictions of non-halophytes under salinity stress.

Total Soluble sugars (TSS)

The inter-varietals comparison showed that barley line Haider-93 produced 23 and 45 % more total soluble sugar (TSS) in T1 and T3 salinity treatments respectively compared to line PK-30108. Similarly, maximum decrease in total soluble sugars under salinity stress in T3 (Fig.1.6) was observed to be 48 and 27 % for PK-30108 and Haider-93 respectively. Overall, line Haider-93 produced significantly higher TSS at all salinity levels compared to line PK-30108. The two barley lines showed decreasing trend with increased salinity stress, however, the salinity sensitive line Haider-93 undergo far less decrease compared to line PK-30108. These findings are supported by results of Sairam and Tyagi (2004) who reported that such osmolytes/ compatible metabolites protect macrobiological molecules against the damaging effect of salinity.

Total amino acids (TAA)

The TAA gradually increased with increase in salinity. However, TAA were significantly more in line Haider-93 under T2 compared to PK-30108. The inter-varietals comparison showed that both lines produced almost comparable total amino acids (TAA) in T1 and T3 levels each. The TAA increased to 143% and 139 % under salinity stress (Fig.1.7) for the barley lines PK-30108 and Haider-93, respectively in T3 compared to control. The gradual increase in total amino acids (TAA) with increasing salinity was is in accordance with the findings of Ali (1991).

Total soluble proteins (TSS)

Under salinity stress, comparable soluble protein (TSP) were produced in control conditions by the two varieties. However, the barley line PK-30108 produced 31 % more SP (Fig.1.8) content in T3 salinity treatment compared to control which was significantly higher than the line Haider-93. This finding is in line with Cusido et al. (1986) who also reported increase in total soluble proteins at higher salinity levels. At other treatments, there were insignificant differences between the two barley lines. At T3 treatment in line PK-30108, the salinity stress increased the TSP content by 22 %. The line Haider-93 showed sensitivity to change in salinity in respect of TSS.

Nitrate Reductase Activity (NRA)

The inter-varietals comparison showed that the two barley lines produced comparable NRA in control (T1) but under elevated salinity stress (Fig.1.9), the line Haider-93 exhibited 38% more NRA compared to line PK-30118 at T3. No significant change in NRA activity was observed under elevated salinity stress by line Haider-93 within the studied range of salinity. The nitrates are absorbed from soil solution and converted to nitrite through nitrate reductase activity. Under salinity stress, the NRA reduced significantly in barley line PK-30108 but there was no significant change under salinity stress in case of line Haider-93.

Nitrite reductase activity (NIRA)

The inter-varietal comparison of the barley lines showed that Haider-93 possessed 11 % more NIRA in T1 that was enhanced by 38% in T3 compared to the line PK-30118. The two lines responded differently to salinity stress (Fig.1.10); the line PK-30118 showed inhibition of NIRA by 23% while in line Haider-93 NIRA this characteristic increased by 11% in T2 & T3 compared to control. The nitrates are reduced to nitrite under nitrite reductase activity

(NiRA). The barley line PK-30118 exhibited significant reduction in NIRA under increased salinity stress compared to control. However, there was no significant change observed at T2 and T3 in both lines.

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(*Accepted for publication July 2012*)