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Index of Root Carbohydrates Contents for Salt Tolerance in Alfalfa

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Abstract. A study was conducted to identify some salt tolerant alfalfa (*Medicago sativa*) ecotypes from its centre of origin in Iran based on the biochemical responses of five Iranian genotypes of alfalfa to salt stress at mature plant growth satge. Among the responses measured were carbohydrate components (root soluble sugars) including fructose, glucose, and sucrose using high performance liquid chromatography (HPLC). The results indicated that the levels of sugars in root increased when the plant was exposed to salt stress but the increase in monosaccharide was greater than disaccharides. Based on results of physiological and biochemical responses of alfalfa ecotypes under salt stress it was found that the ecotypes *Gharghologh* and *Nik-Shahri* were the most tolerant among the 20 ecotypes.

Keywords: Salt stress, Alfalfa, carbohydrates signaling

1. Introduction

When exposed to salt stress plants need to maintain internal water potential through an increase in its osmotic potential, either by uptake of soil solutes or by synthesis of metabolic (compatible) solutes [1]. Low molecular weight organic solutes or inorganic ions can accumulate in cell and play major roles to adjust osmotic potential in plant under salt stress. However their relative contribution varies among species, among cultivars and even between different compartments within the same plant under salinity condition [2]. The main compatible osmolytes include low molecular weight sugars, organic acids, polyols, and nitrogen containing compounds such as amino acids, amides, imino acids, ectoine (1,4,5,6-tetrahydro-2-methyl-4-carboxylpyrimidine), proteins and quaternary ammonium compounds [2]. Some evidence have shown that among the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes exposed to salt stress [3]. The pattern of sugar accumulation in plants under stress varies with genotype where reports indicated that in *Vitis vinifera* there was a decrease in sucrose, and an increase in reducing sugars [4], while in *Cenchrus pennisetiformis* there was a decrease in sucrose but no increase in glucose and fructose [5]. In *Hordeum vulgare* sucrose concentration increased while reducing sugars did not [6]. In *Glycine max* salinity resulted in an increase in sucrose [7] and in *Lepidium crassifolium* all soluble sugars increased in concentration [3]. There are some reports that indicated the content of soluble sugar in tolerant cultivars was higher than sensitive cultivars to salt stress in sunflower [8]-[10]. Since alfalfa is perennial and frequently cut the concentration of soluble sugars in roots has a major role for regrowth and survival of plants under stress.

2. Materials and Methods

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Five ecotypes respectively 1- *Rehnani*, 2- *Ghargholough*, 3- *Shorkat*, 4- *Bami* and 5- *Nik-shahri* selected from three climatic regions of Iran according to classification by Dinpashoh [11]. Seeds were planted in jiffy pot, with 4 seeds per pot. After one week the jiffy pots were transferred to polybags (30 x15 cm) filled with sand and then thinned to 2 plants and all polybags were inserted in the long hydroponic tray. All the polybags were subjected to complete nutrient solution according to Utah State University formula [12]. After 2 month from planting, salt was added to nutrient solution to bring the EC to 3 dS/m. Salt was increased gradually where every 15 days the NaCl increased by 3 dS/m, and the final EC was 18 dS/m. The plants underwent three stages of salt increase bringing up the EC to 6, 12, and 18 dS/m. The roots harvested in three stages of salt concentration, and the soluble sugar including sucrose, glucose, and fructose extracted. For extraction, three gram of root dry matter was heated with 100 ml of methanol on a steam bath for 30 min. After cooling the obtained mixture was filtered through Whatman No. 1 filter paper into a flask, and the residue was re-extracted twice in 75 ml portions of methanol, and filtered. The obtained filtrate was evaporated up to below 10 ml under vacuum at 50 ℃ in a rotary evaporator, and then volume was made up to 10ml in a volumetric flask. Then the solution was filtered through a cellulose nitrate membrane filter (0.2 μm, nylon type) using a syringe. To determine the root soluble sugar including sucrose, glucose, and fructose the high performance liquid chromatography (HPLC) was used. Fructose, glucose, and sucrose were collected in the nonretained fraction during this HPLC purification step. For the HPLC mobile phase the acetonitrile and water (80/20, v/v) were used as eluent, and the sugar standards used were fructose, glucose, and sucrose in the concentration range 1 to 5% (w/v). A calibration curve was obtained for each of the four sugars. For quantification of the sugars sucrose, glucose, and fructose standard were used.

3. Results and Discussions

The concentration of the three soluble sugars in root, fructose, glucose, and sucrose changed with increasing levels of salt stress. There were no significant differences between ecotypes in terms of all sugar concentrations. The interaction of salinity and ecotypes for all soluble sugar was significant indicating differences in the responses of ecotypes to level of salt stress. The two way analysis of variance indicated that when alfalfa was exposed to salt stress all sugars were affected. The influence of salt stress on fructose in root was positive (Figure 1) where with increasing salt the content of fructose in roots of alfalfa increased. Fructose levels increased slowly at low levels of stress but the increase became more accentuated at levels of stress above 12 dS/m.

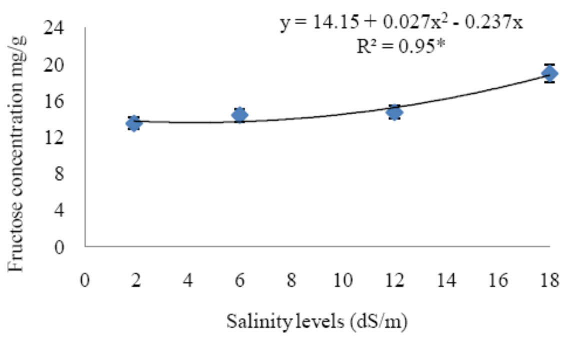

Fig. 1: Effects of salinity levels on fructose concentration

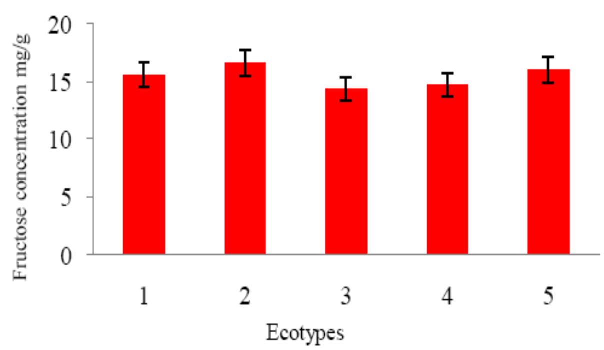

Fig. 2: Variation of fructose concentration among the 5 ecotypes under saline condition

There was no significant difference among the ecotype in fructose concentration. Ecotypes number 2 and 5 had apparently greater fructose content than other ecotypes (Figure 2). Previous work show that the reducing sugar like fructose increased in *Vitis vinifera* [4], did not change in *Cenchrus pennisetiformis* [5], and increased in *Lepidium crassifolium* [3] under salt stress. The soluble sugar content can be an index for salt tolerance [8], [9], [10] so that our results indicate that the tolerance of ecotypes number 3 and 4 to salt stress may be lower than other ecotypes although there was no significant difference between them. The pattern of changes in glucose concentration in root (Figure 3) was different from that of fructose (Figure 1) although both of them are monosaccharide and reducing sugar. With increasing salt stress the concentration of glucose increased up to a peak at12 dS/m and then it declined but for fructose, the concentration was increasing even at 18 dS/m. dS/m.

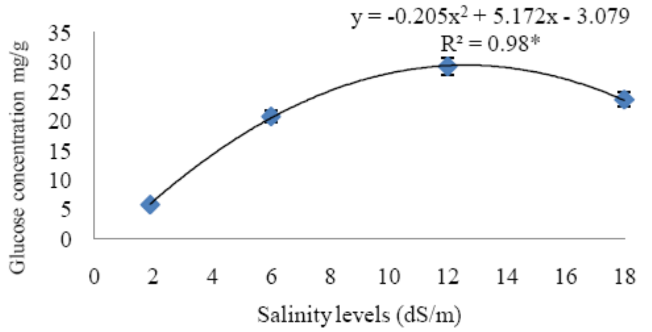

Fig. 3: Effects of salinity levels on glucose concentration

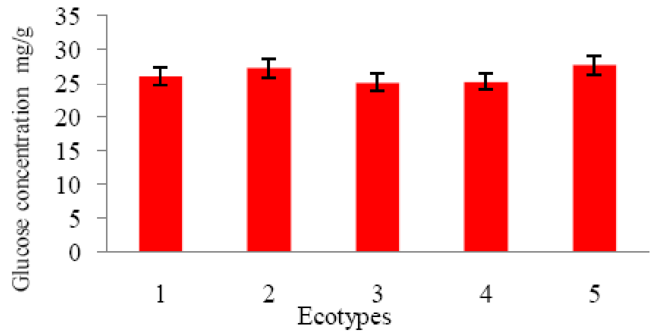

Fig. 4: Variation of glucose concentration among the 5 ecotypes under saline condition

There was no significant difference between ecotypes in terms of concentration of glucose under salt stress but the root concentration glucose for ecotype numbers 5 and 2 appear greater than other ecotypes (Figure 4).

Ashraf and Harris [2] stated that genotypes that can accumulate more sugar are considered as tolerant genotypes, so that ecotypes numbers 5, and 2 are more tolerant to salt stress than ecotypes numbers 1, 3 and 4. For most plants, when they are subjected to salt stress the concentration of sugars such as sucrose, fructose and glucose increased so that it can be considered as one of the most important mechanisms to overcome salt stress [2], [13].

Unlike reducing sugars and monosaccharides, the concentration of sucrose declined with increasing salinity (Figure 5). The breakup of disaccharides to monosaccharides is one of the mechanisms for re-growth and survival in alfalfa under salt stress. The change in sucrose concentration of plants under salt stress reported in literature has been varied, for example, in *Vitis vinifera*, and *Cenchrus pennisetiformis* sucrose concentration decreased under stress while that in *Hordeum vulgare*, and *Glycine max* sucrose increased under salt stress [3], [4], [7], [14]. Our results indicate sucrose concentration of roots under salt was not significantly different among ecotypes (Figure 6). The role of soluble sugars in adaptation of plants to salt stress is verifiable and soluble sugar accumulation can be an indicator for salt tolerance in breeding programs for some species [2].

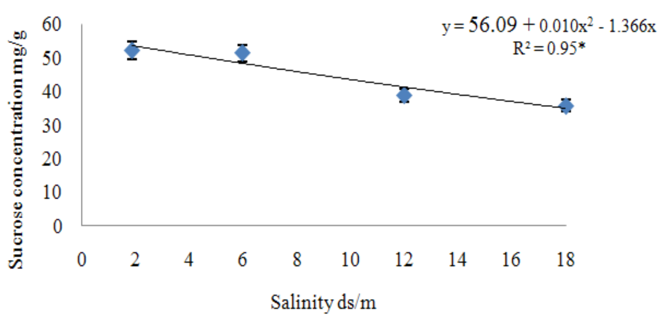

Fig. 5: Effects of salinity levels on sucrose concentration

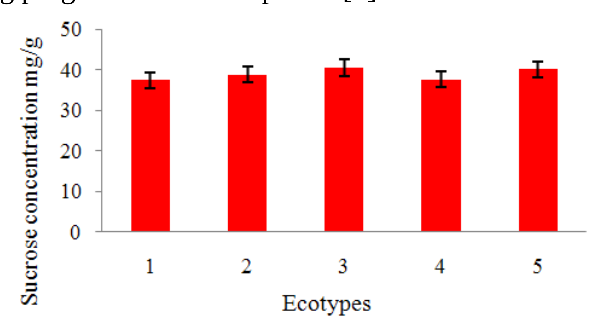

Fig. 6: Variation of sucrose concentration among the 5 ecotypes under saline condition

For total soluble sugar with increasing salinity from 6 to 12 dS/m there was a sharp increase of total soluble sugar but it decreased from 12 to 18 dS/m (Figure 7). It appears that from 3 to 12 dS/m alfalfa had a relative tolerance due to increasing total soluble sugars, whereas from 12 to 18 dS/m with reduction of tolerance in alfalfa the respiration of plants elevated and subsequently total soluble sugars declined (Figure 7). The concentration of total soluble sugar in ecotype 3 decreased with increasing salt stress and it was considered as not tolerant ecotype compared to other ecotypes.

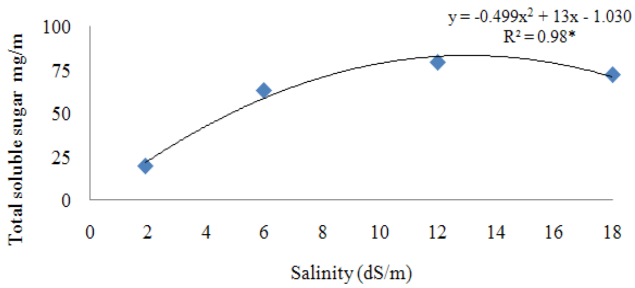

Fig. 7: Effects of salinity levels on total concentration of soluble sugar

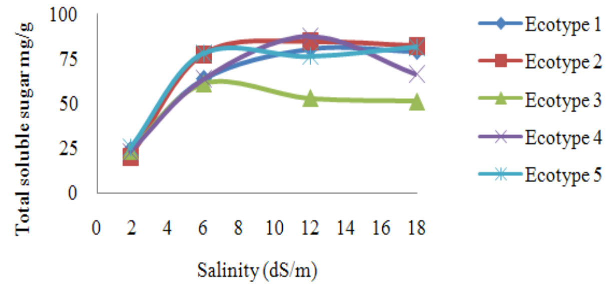

Fig. 8: Total soluble sugar of different ecotypes with increasing salinity

The ecotype 2 not only accumulated more soluble sugar in root but also maintained the concentration of soluble sugars, showing that it is a salt tolerant ecotype (Figure 8). Ashraf and Tufail [8], [10] concluded that the ecotypes that accumulated more carbohydrates under stress is considered as tolerant genotypes hence ecotypes numbers 2, 5 and 1 in this case were higher and they were more tolerant (Figure 8). Parida *et al*. [15] noted that the sugars such as glucose, fructose, and sucrose, accumulate in plants under salt stress, playing a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging.

4. Conclusion

Alfalfa is perennial forage so that the total soluble sugar in the root plays an important role for regrowth of alfalfa and the concentration of soluble sugar is one of the determining factors for salt tolerance. Our results showed that the concentration of all soluble sugars changed in response to levels of salinity and varied among ecotypes. Glucose as a monosaccharide and reducing sugar was more affected under salt stress in alfalfa than fructose and sucrose, and it demonstrated that when the mature alfalfa plants were exposed to salt stress the level of glucose increased in roots. The increase in reducing sugar content such as glucose and fructose was more than sucrose in roots of alfalfa under salt stress. The soluble sugar content can be an index for salt tolerance and based on this ecotypes *Gharghologh* and *Nik-Shahri* are regarded as more salt tolerant than other ecotypes.

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