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Role of Proline, Na and Chlorophyll Content in Salt Tolerance of Corn (*Zea mays* L.)

[1]D. Molazem, [2]E.M. Qurbanov and [3]S.A. Dunyamaliyev

[1]Islamic Azad University, Ardabil Branch, Ardabil, Iran
[2]Baku State University
[3]Aqrarian Science Center Institute of Crops Husbandry

Abstract: Considering Iran and Azerbaijan as origin countries in Astara region and in order to study the effects of salt stress (NACL) on proline, Na and Chlorophyll Contents of 8 maize cultivars were experimented in three replications on the basis of randomized complete block design. Cultivars included K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2 and Zaqatala and they were cultivated in two pieces of land in Astara: one with normal soil and the other with salty soil. During the experiment, proline, leaf relative water content (LRWC), Chlorophyll a and Chlorophyll b contents were measured. Results showed that, regarding proline, Chlorophyll a, Chlorophyll b, total chlorophyll, Na content, plant biomass and yield, there were significant differences between cultivars in combined analysis under normal and salinity conditions, but leaf relative water content (LRWC), there weren't any significant differences between cultivars. Leaf chemical analysis of different maize cultivars indicated that sodium (Na) content was increased under saline condition. Maize cultivar S.C704 and zaqatala showed higher accumulation of proline than others but was not observed significant difference between them. The least proline content was observed in B73 that didn't have any significant difference with S.C302, K3615/1and K3545.6. The greatest biomass was recorded in Zaqatala, B73 and S.C704 that was never noticed significant difference between them.

Key words: Salinity · Proline · Chlorophyll content · Maize

INTRODUCTION

High soil salinity is one of the important environmental factors that limit distribution and productivity of major crops [1, 2]. Agricultural productivity in arid and semiarid regions of the world is very low due to accumulation of salts in soils [3, 4]. All around the world, about 100 million ha, or 5% of arable land has already been adversely affected by high salt concentrations which reduce crop growth and yield [5]. Saline medium causes many adverse effects on plant growth, which is due to low osmotic potential of soil solution (osmotic stress) specific ion effects (salt stress), nutritional imbalance or a combination of these factors [6, 7]. All these factors cause adverse effects on plant growth and development at physiological and biochemical activities [3, 8]. Maize (*Zea mays L.*) is considered as one of the most important cereal crops used in human consumption, animal feeding and starch industry and oil production [9]. It is the most important cereal crop in the world after rice and wheat. As regard cultivated area, it ranks third position after wheat and rice in world statistics. It is also year-round crop for its wider range of climatic adaptability. According to Mass and Hoffman [10], maize is generally regarded as a highly salt sensitive species.

Tolerances to environmental stresses as salinity of plants can be determined by using different parameters. Plants need to have special mechanisms for adjusting internal osmotic conditions and changing of osmotic pressure in the root environment. In salt stressed plants osmotic potential of vacuole decreased by proline accumulation [11]. It was thought that accumulated proline under environmental stress do not inhibit biochemical reactions and plays a role as an osmoprotectant during osmotic stress [11]. In addition, several possible roles have been attributed to supraoptimal levels of proline;

Corresponding Authers: Davar Molazem, Islamic Azad University, Ardabil Branch, Ardabil, Iran.
Tel. +989141525496.

osmoregulation under drought and salinity conditions, stabilization of proteins, prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period [12]. It is suggested that the low osmotic potential may cause proline accumulation in tissues [13, 14].

Plants differ genetically in their response to salt stress [15]. Different mechanisms of salt tolerance by plants have been suggested by Flowers and Hajibagheri [16] and Schachtman and Munns [17]. Keeping in view the importance of maize and salinity, present study has been planned to examine the role of proline, Na and chlorophyll content in salt tolerance of maize (*Zea mays* L.).

MATERIALS AND METHODS

Considering Iran and Azerbaijan as origin countries in Astara region and in order to study the effects of salt stress (NACL) on proline, leaf relative water content (LRWC), Chlorophyll a and Chlorophyll b content of 8 maize cultivars were experimented in three replications on the basis of randomized complete block design. Cultivars included K3615/1, S.C704, B73, S.C302, Waxy, K3546/6, K3653/2 and Zaqatala and they were cultivated in two pieces of land in Astara: one with normal soil and the other with salty soil. During the experiment, before dealing amount of proline, leaf relative water content (LRWC), chlorophyll a and Chlorophyll b Content were measured in the laboratory. Photosynthetic pigments (chlorophyll a and b) were measured in fresh leaf samples, a week before the harvest. One plant per replicate was used for chlorophyll determination. Prior to extraction, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Leaf samples (0.5 g) were homogenized with acetone (80% v/v), filtered and make up to a final volume of 5 mL. Then the solution for 10 minutes away in 3000 (rpm) centrifuged. Pigment concentrations were calculated from the absorbance of extract at 663 and 645 nm using the formula [18, 19] given below:

$$\text{Chlorophyll a (mg/g FW)} = [12.7\times (A663)\ \_\ 2.69\times (A645)]\times 0.5$$

$$\text{Chlorophyll b (mg/g FW)} = [22.9\times (A645)\ \_\ 4.69\times (A663)]\times 0.5$$

$$\text{Chlorophyll a+b (mg/g FW)} = [20.2\times (A645)\ \_\ 8.02\times (A663)]\times 0.5$$

Sodium concentrations were determined by using Eppendorf Elex 6361 model flame photometry described by Miller [20].

Free proline accumulation was determined using the method of Bates *et al*. [21]. 0.04 gram dry weight of leaves was homogenized with 3% sulfosalicylic acid and after 72h that proline was released; the homogenate was centrifuged at 3000 g for 20 min. The supernatant was treated with acetic and acid ninhydrin, boiled for 1 hour and then absorbance at 520 nm was determined by Uv-visible spectrophotometer.

Leaf relative water content (LRWC) was calculated on the basis of Yamasaki and Dillenburg method [22]. Two leafs were randomly chosen from middle parts of the plants in each repetition. At first, leafs were separated from the stems and their fresh masses (FM) were calculated. In order to measure the saturation mass (TM), they were placed into the distilled water in closed containers for 24 hours under the air condition of 22° C, for the purpose of being reached to their greatest amount of saturation mass and then, they were weighed. Then leafs were placed inside the electrical oven for 48 hours under the air condition of 80° C and the dry mass of the leaves (DM) were obtained (DM). All of the measurements were done by scales with 0.001g accuracy and were placed into the following formula and into the following formula:

$$\text{LRWC (\%)} = [(\text{FM-DM})(\text{TM} - \text{DM})] \times 100$$

Statistical analysis of the data was done on the basis of randomized complete block design. The average of attendances was calculated on the basis of Duncan method at 5% probability level.

RESULTS AND DISCUSSION

Results from the experiment showed that, regarding proline, Chlorophyll a, Chlorophyll b, Chlorophyll a+b, total chlorophyll, Na content, plant biomass and yield, there were significant differences between cultivars in combined analysis in normal and salinity conditions, but leaf relative water content (LRWC), there weren't any significant differences between cultivars. Leaf chemical analysis of different maize cultivars indicated that sodium (Na) contents increased under saline condition (Table 1). In the normal condition, was not observed significant difference between cultivars, but under salt condition significant difference between cultivars was noticed. The greatest sodium content was recorded with S.C704 and zaqatala under salinity condition that was noticed significant difference between them. In studies where salinity is developed with NaCl, a focus has been

Table 1: Comparing the average of understudy characteristics in eight cultivars of the maize in combined analysis

condition	cultivars	Proline µmol/g FW	Na mg g^{-1} DW	Chlorophyll a mg/g FW	Chlorophyll b mg/g FW	Total Chlorophyll mg/g FW
Normal	1-Zaqatala	1.033^{fgh}	0.33^{g}	1.113^{bcd}	1.107^{cd}	2.217^{cd}
Normal	2-S.C302	1.010^{gh}	0.3010^{g}	1.483^{abc}	1.523^{bcd}	3.010^{abcd}
Normal	3-K3653.2	1.323^{ef}	0.2890^{g}	1.197^{abcd}	2.037^{ab}	3.233^{abc}
Normal	4-B73	1.30^{efg}	0.2957^{g}	1.627^{ab}	2.510^{a}	4.140^{a}
Normal	5-S.C704	1.150^{efgh}	0.3497^{g}	1.847^{a}	1.850^{abc}	3.697^{ab}
Normal	6-Waxy	1.430^{e}	0.2730^{g}	1.113^{bcd}	1.280^{bcd}	2.393^{cd}
Normal	7-K3615.1	0.953^{h}	0.3320^{g}	1.050^{bcd}	1.513^{bcd}	2.563^{bcd}
Normal	8-K3545.6	0.987^{h}	0.3163^{g}	1.060^{bcd}	1.033^{d}	2.097^{d}
salty	1-Zaqatala	4.847^{ab}	4.094^{b}	0.95^{cd}	1.113^{cd}	2.063^{e}
salty	2-S.C302	4.660^{bc}	3.277^{d}	0.9067^{cd}	1.037^{d}	1.943^{e}
salty	3-K3653.2	3.91^{d}	2.840^{e}	1.037^{bcd}	1.2^{cd}	2.24^{e}
salty	4-B73	4.443^{c}	2.753^{e}	0.98^{cd}	1.13^{cd}	2.10^{e}
salty	5-S.C704	5.067^{a}	4.215^{a}	0.9967^{bcd}	1.16^{cd}	2.153^{e}
salty	6-Waxy	3.743^{d}	2.611^{f}	0.7167^{d}	0.8633^{d}	1.573^{e}
salty	7-K3615.1	4.663^{bc}	3.769^{c}	1.013^{bcd}	1.28^{bcd}	2.293^{e}
salty	8-K3545.6	4.710^{bc}	3.314^{d}	0.8867^{cd}	1.017^{d}	1.903^{e}

*Within each column, same letter indicates no significant difference between treatments (p<0.05)

Table 2: Comparing the average of understudy characteristics in eight cultivars of the maize in combined analysis

condition	cultivars	LRWC(%)	Grain yield ton/ha	Biomassg/plant
Normal	1-Zaqatala	61.02^{1}	4.49^{1b}	833.5^{1}
Normal	2-S.C302	57.88^{1}	3.70^{1bc}	297d
Normal	3-K3653.2	63.78^{1}	3.43^{bcd}	580.8^{bc}
Normal	4-B73	67.15^{1}	3.58^{bc}	738^{1}
Normal	5-S.C704	62.57^{1}	5.87^{1}	695^{1b}
Normal	6-Waxy	61.89^{1}	3.07^{bcde}	550.5^{bc}
Normal	7-K3615.1	61.07^{1}	4.77^{1b}	540^{bc}
Normal	8-K3545.6	58.43^{1}	4.34^{1b}	522.3^{c}
salty	1-Zaqatala	61.47^{1}	2.16^{cdef}	550^{bc}
salty	2-S.C302	57.27^{1}	2.40^{cdef}	280.8^{d}
salty	3-K3653.2	61.70^{1}	1.53^{f}	270d
salty	4-B73	61.68^{1}	1.73^{ef}	423.8^{cd}
salty	5-S.C704	62.36^{1}	2.60^{cdef}	432.8^{cd}
salty	6-Waxy	61.60^{1}	1.84^{def}	301.8^{d}
salty	7-K3615.1	58.44^{1}	1.24^{f}	332.2^{d}
salty	8-K3545.6	63.62^{1}	1.31^{f}	294.3^{d}

*Within each column, same letter indicates no significant difference between treatments (p<0.05)

the transport systems that are involved in the utilization of Na as an osmotic solute [23].Literature indicated that intracellular Na homeostasis and salt tolerance are modulated by calcium and high Na concentrations negatively affect K acquisition [4]. Biochemical analysis of leaves of different maize cultivar for proline accumulation and chlorophyll contents indicated that proline accumulation increased and chlorophyll contents decreased under saline condition (Table1 2). Maize cultivar S.C704 and zaqatala showed higher accumulation of proline than others but was not noticed significant difference between them. The least proline content was observed with B73 that didn't have any significant difference with S.C302, K3615/1and K3545.6. Accumulation of solutes especially proline, glycine-betaine and sugars is a common observation under stress condition [18, 24]. Ashraf *et al.* [25] reported that proline is an important osmolyte to adjust the plant under drought/saline conditions. There is however reasons to believe that proline accumulation may play a role in the salinity tolerance. Firstly it is an osmolyte accumulated under stress in almost all the plant species. Secondly a high proline concentration has been described in organs which naturally have low water contents such as seed and inflorescence. The results of present study showed that there is a positive relationship between praline accumulation and performance of maize cultivar in terms of grain yield under salinity stress.

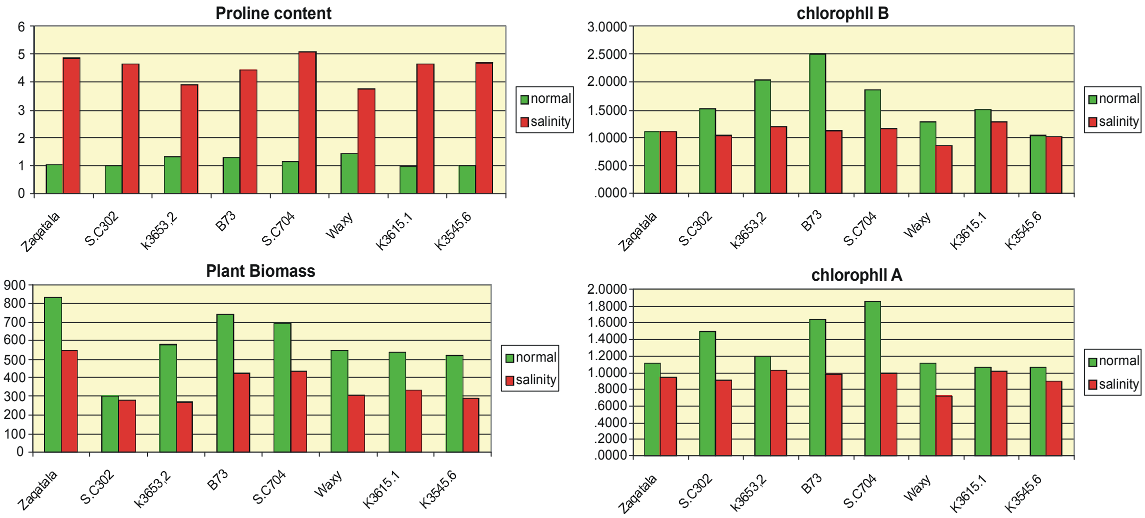

Fig. 1: Diagram of different understudy characteristics in eight cultivars of the maize under the two normal and salty conditions

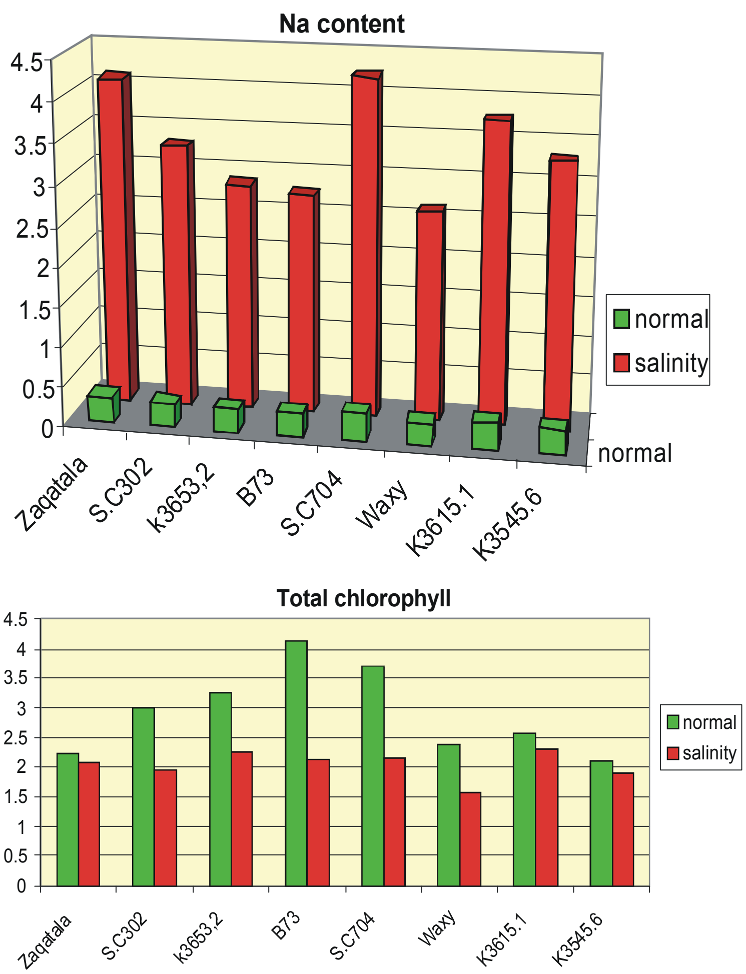

Fig. 2: Diagram of different understudy characteristics in eight cultivars of the maize under the two normal and salty conditions

The greatest Chlorophyll a was recorded by S.C704, B73, K3653/2 and S.C302 that was no significant difference between them was observed. The greatest Chlorophyll b was recorded with B73 that didn't have any significant difference with S.C302, K3615/1, S.C704 and K3653/2. The most Chlorophyll a and b was obtained by S.C704 in normal condition. The least Chlorophyll a and b seen in Waxy in salinity condition. In the two conditions, was seen significant difference between cultivars and salinity had caused reduction in their values (Table 1) there are some reports where an increase in chlorophyll contents was observed in 6 genotypes of rice [26]. However, the reduction in chlorophyll contents is to be expected under stress; being membranous bound, its stability is dependent on membrane stability, which under saline condition seldom remains intact [1]. was Our results are in agreement with those reported by Iqbal *et al.* [27] and Ashraf *et al.* [1], who reported that chlorophyll content was decreased under saline conditions. The decrease is significant in sensitive genotypes in comparison to tolerant. Moussa Helal [28] showed that salt stress significantly decreased both chlorophyll a and b content in an experiment done by Ashrafuzzaman *et al.* [29] showed with increasing salinity Chlorophyll a, b, total Chlorophyll (a+b) and Chlorophyll a/b were reduced.

The results of biomass indicated that applied NaCl inhibited the growth of maize plant and led to a decrease in biomass (Table 2). The greatest biomass was recorded with Zaqatala, B73 and S.C704, while no significant difference between them was noticed. This may be related to the effect of salt stress which resulted in the limitation of water absorption and biochemical processes [30, 31].

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