

Effect of Saline Stress on Antioxidative Defense and Yield of Sugar Beet

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Abstract The pot experiment was performed to study the effect of salinity stress on some antioxidants and physiological characteristics of two sugar beet varieties, PAC 60008 and SZ 35. Sugar beet seeds were collected from SES Vander Have Company (Belgium). Irrigation with 6 different salinity levels was applied after germination and analysis was done with factorial completely randomized design. Different biochemical and physiological parameters were assessed, namely antioxidant enzymes activity (POD, SOD and total antioxidant activity) and reducing sugar, fresh weight and dry weight were recorded from harvested beet root. The highest level of salinity ($EC=10dS\ m^{-1}$) treatment showed increased peroxidase (POD) activity was in PAC 60008 and decreased in SZ 35 where as in case of superoxide dismutase (SOD) activity SZ 35 showed better effect than PAC 6008 as the salinity increased. Total antioxidant activity, fresh and dry weight were decreased due to high levels of salinity in both varieties. Reducing sugar content reached at its highest level in $4\ ds\ m^{-1}$ salinity level. Considering the above biochemical parameters and yield SZ 35 is

better than PAC 6008 variety for cultivation under saline condition as well as coastal track.

Keywords Sugar beet, Saline stress, Antioxidants, Reducing sugar.

Introduction

Beta vulgaris L. is normally a biennial species, however under certain conditions it can act as an annual [1]. The sugar beet plant develops a large succulent taproot in the first year and a seed stalk the second year. Sugar beet roots are processed into white sugar, pulp and molasses for food, feed or industrial applications. A typical sugar beet root consists of 75.9% water, 2.6% non--sugars, 18.0% sugar and 5.5% pulp. Worldwide about 33% of the irrigated lands are affected by salinity. Saline soils are abundant particularly in semiarid and arid regions, where the amount of rainfall is insufficient for substantial leaching. Salinity can severely limit crop yield, especially in the most productive areas of the world.

A saline soil is the soil having a high concentration of soluble salts, high enough to affect plant growth. Salt concentration in a soil is measured in terms of its electrical conductivity. The USDA salinity laboratory defines a saline soil as having an EC_e of

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4 dS m⁻¹ or more. EC_e is the electrical conductivity of the saturated paste extract, that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. However, many crops are affected by soil with an EC_e less than 4 dS m⁻¹ [2]. Salt tolerance in plants has generally been emphasized in regulation of ionic homeostasis and osmotic adjustment [3]. Some authors have shown that, like other abiotic stresses, salinity also induces oxidative stress in plants. In pea leaves sodium chloride toxicity is associated with higher generation of hydrogen peroxide. One of the primary effects of Reactive Oxygen Species (ROS) and their products in cells is the peroxidation of membranes, which leads to leakage of low-molecular-weight solutes, particularly K⁺ [4].

Plants have evolved various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced deterioration. The enzymatic antioxidant system, which is one of the protective mechanisms, including. Superoxide Dismutase (SOD), is located in various cell compartments and catalyzes the disproportion of two O₂⁻ radicals to H₂O₂ and O₂. H₂O₂ is eliminated by various antioxidant enzymes such as catalase (CAT) and peroxidases (POD), which convert H₂O₂ into water [5].

Plant species differ greatly in their growth response to salinity. Although sugar beet is sensitive to elevated salinity at the germination and early seedling phases of development, established plants show a high osmotic adjustment and accumulation of proline, glycine betaine and inorganic ions under salt stress [6]. In terms of response to NaCl, plants were classified into four major groups, including plant species with growth stimulation at low salinity due to the presence of Na as a beneficial element. A few crop species are classified in this group, one important example being sugar beet. Salinity can occur through natural or human-induced processes that result in the accumulation of dissolved salts in the soil water to an extent that inhibits plant growth. Saline stress induces decrease in dry weights of root and shoot and leaf area, but it had no effect on leaf water contents. Plant growth is ultimately reduced by salinity stress, although plant species differ in their tolerance to salinity. Reduction of plant growth under saline condi-

tions is a common phenomenon but such reduction occurs in various ways in different plant organ [7].

Plant acclimation to stress is associated with profound changes in proteome composition. Since proteins are directly involved in plant stress response, proteomics studies can significantly contribute to unravel the possible relationships between protein abundance and plant stress acclimation [8].

Increased activity of the antioxidant enzymes SOD, POD, CAT, DPPH and MAD not only contributes to the protection of tolerant cultivars from severe salt stress, but also can explain the beneficial effect of mild salinity in some cultivars. Therefore, this study has been undertaken with the objective to estimate the effect of different soil salinity level on biochemical parameters of sugar beet varieties as well as study effect of salinity stress on growth and yield attributes of sugar beet.

Materials and Methods

Seed germination and treatments

Two varieties of sugar beet, *B. vulgaris* cv ans PAC 60008 and SZ 35 were taken from SESV ander Have Company Belgium. Seeds were sown into the pots (20-30 cm²) filled with black soil and grown under poly house condition (light/dark regime of 16/8 h at 25/35°C, relative humidity of 60-70%). After germination, seedlings were watered regularly with different levels of saline water prepared by dilution of sea water. Salt treatment started as 0 (control), EC 2 ds m⁻¹, EC 4 dS m⁻¹, EC 6 ds m⁻¹, EC 8 dS m⁻¹ and EC 10 dS m⁻¹ at the stage of about four true leave. All biochemical and physiological analysis were done at harvesting stage from the mature beet root.

Antioxidant enzymes and total antioxidant activity

Analysis of peroxidase (POD) activity was checked by the oxidation of O- dianisidine method and recording the absorbance for 1 minute was recorded at 430 nm enzyme activity and was expressed as ΔA/g/min. The super oxide dismutase (SOD) assay was based on the capacity of extracts to inhibit photochemical

reduction of Nitro Blue Tetrazolium (NBT) in the ribo-flavin-light-NBT system and one unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. The total antioxidant activity was determined using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. Percent scavenging of the DPPH free radical were plotted against respective concentrations from which IC_{50} was calculated and expressed as $\mu\text{g/ml}$ sample.

Reducing sugar content

The reducing sugar was measured by the reduced copper reacting with arsenomolybdic acid and recording the absorbance of molybdenum blue at nm using dextrose as standard.

Dry and fresh root weight

At full maturity all plants were root harvested and then washed with water to remove excess soil from beet root and weight was taken in grams for each sample. Then root samples were kept in hot air oven at $65 \pm 5^\circ\text{C}$ overnight and then kept for sun drying. After drying again root weight of all samples were measured.

Statistical analysis

The experiment was setups as a factorial completely randomized design with two factors and six salinity levels. All data were examined by one-way ANOVA. Values are given as mean $\pm$ standard error of mean (SEM) at ($p = 0.05$) significance level.

Results and Discussion

POD activity has increased in PAC 60008 while it has decreased in SZ 35 with the increasing salinity level (Fig 1). Some previous studies, as parallel with our results, mentioned the increased POD activity under salinity stress conditions in *Beta vulgaris* L. cv *ansa* and wild beet *Beta maritima* L. From the earlier stud-

ies it is clear that catalase and peroxidase enzymes plays a significant role in quenching H_2O_2 and complementary to each other as peroxide detoxification takes place by a fine tuning of up regulation and down regulation of both of the enzymes [9]. SOD activity has decreased in PAC60008 while it has increased in SZ35 as the salinity level increased (Fig 2). It has been demonstrated that salinity leads to a decrease in SOD activity in salt sensitive plants of *Sesamum indicum* than salt tolerant ones [10]. There is a possibility that high amount of Na^+ and Cl^- ions in leaves directly inhibit chloroplast SOD catalysis *in vivo*, because in plant most of the SOD is present in chloroplasts. The increase in SOD activity under NaCl-salinity stress suggests better oxidative stress tolerance and according in this fact that SOD processing is known to be substrate inducible [5], an increase in the SOD activity may be attributed to the increased production of active oxygen species as substrate that lead to increased expression of genes encoding SOD [11]. DPPH radical scavenging activity was also significantly increased in roots of both sugar beet varieties (Fig. 3) as more amount of sample is needed to obtain the IC_{50} value for DPPH radical scavenging and similar results were reported in seedlings of chickpea genotypes [3]. In this study moderate levels of salt stress increased the root reducing sugar content in both of the sugar beet varieties as compared to the control (Fig. 4) and similar results were recorded previously in sugar beet. A reason for this might be a storage root of many small cells would be more efficient at accumulating photosynthate than composed of large cells. The accumulation of sucrose under salt stress supported the well-established role of this sugar as an osmoprotectant that stabilizes cellular membranes and maintains turgor [12]. It was observed that salt stress increases the level of reducing sugars (sucrose and fructans) within the cell in a number of plants belonging in different species [13].

Sugar beet is considered to be relatively salt tolerant although our work showed sugar beet root fresh weight (Fig. 5); dry weight (Fig. 6) were reduced as the salinity levels were increased as well as the dry matter accumulation and deformity had been decreased with the increasing of salinity level (Fig. 7 and Fig. 8). Similar results were found in *Beta vulgaris* and *Brassica oleracea* var *capitata* [7].

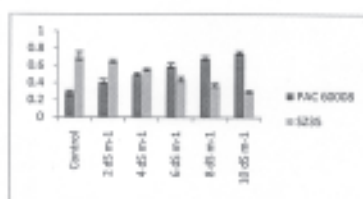


Fig 1: Effect of different salinity level on peroxidase activity in root of sugar beet.
C.D.@5%=0.0110, CV%=1.48

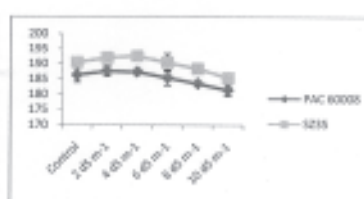


Fig 4: Effect of different salinity level on reducing sugar content of beet root.
C.D.@5%=0.5597, CV%=0.20

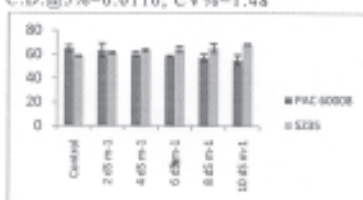


Fig 2: Effect of different salinity levels on super oxide dismutase activity in root of sugar beet.
C.D.@5%=0.4168, CV%=0.47

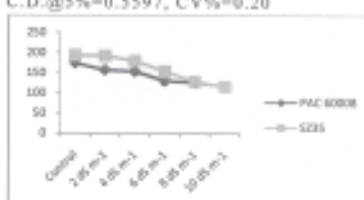


Fig 5: Effect of different salinity level on fresh weight of sugar beet root.

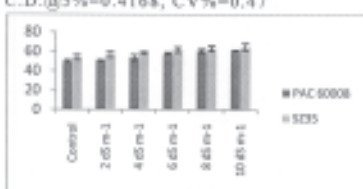


Fig 3: Effect of different salinity level on total antioxidant activity of sugar beet root.
C.D.@5%=0.4523, CV%=0.33

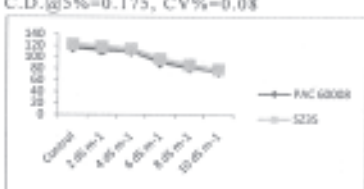


Fig 6: Effect of different salinity level on dry weight of sugar beet root.
C.D.@5%=0.208, CV%=0.14

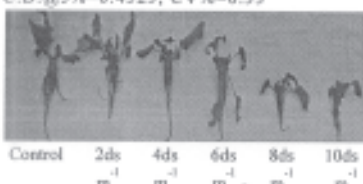


Fig 7: Effect of different level of salinity on size of SZ35 variety beet root.

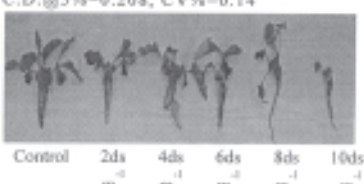


Fig 8: Effect of different level of salinity on size of variety of PAC 6008 beet root.

Fig. 1. Effect of different salinity level on peroxidase activity in root of sugar beet. **Fig. 2.** Effect of different salinity levels on super oxide dismutase activity in root of sugar beet. **Fig. 3.** Effect of different salinity level on total antioxidant activity of sugar beet root. **Fig. 4.** Effect of different salinity level on reducing sugar content of beet root. **Fig. 5.** Effect of different salinity level on fresh weight of sugar beet root. **Fig. 6.** Effect of different salinity level on dry weight of sugar beet root. **Fig. 7.** Effect of different level of salinity on size of SZ35 variety beet root. **Fig. 8.** Effect of different level of salinity on size of variety of PAC 6008 beet root.

Conclusion

According to the experimental results, different antioxidant activities could be suggested for differences between responses of two contrasting sugar beet cultivars to mild and severe salinity. Protection against

various reactive oxygen radicals, were considerable different between the two cultivars as well as mechanisms for growth stimulation and tolerance to mild and severe salinity showed difference in both of the varieties. It seems likely that the activity of antioxidants are important for the expression of growth im-

provement under mild salinity and on the basis of that SZ 35 has performed well under salinity stress as compare to PAC 60008.

References

1. Abbas F, Al-Jbawi E, Ibrahim M (2013) Growth and chlorophyll fluorescence under salinity stress in sugar beet (*Beta vulgaris* L.). Int J Environ 3 : 2091—2854.
2. Munns R (2015) The impact of salinity stress [Online]. CSIRO Division of Plant Industry Canberra ACT, Australia. http://www.plantstress.com/articles/salinity_i/salinity_i.htm [2 Feb. 2015].
3. Kaur N, Kumar A, Kaur K, Gupta AK, Singh I (2015) DPPH radical scavenging activity and contents of H₂O₂, malondialdehyde and proline in determining salinity tolerance in chickpea seedlings. Ind J Biochem and Biophysics 51 : 407—415.
4. Wu GQ, Liang N, Feng RJ, Zhang JJ (2013) Evaluation of salinity tolerance in seedlings of sugar beet (*Beta vulgaris* L.) cultivars using proline, soluble sugars and cation accumulation criteria. Acta Physiol Pl 35 : 2665—2674.
5. Abili J, Zare S (2014) Evaluation of antioxidant enzymes activity in canola under salt stress. Int J Farming and Allied Sci 3 : 767—771.
6. Szabados L, Savoure A (2011) Proline : A multifunctional amino acid. Trends in Pl Sci 15 : 89—97.
7. Jamil M, Rehman S, Rha ES (2007) Salinity effect on plant growth, PSII photochemistry and chlorophyll content in Sugar beet (*Beta vulgaris* L.) and cabbage (*Brassica oleracea* var capitata L.). J Bot 39 : 753—760.
8. Kosova K, Vitamvas P, Prasil IT, Renaut J (2011) Plant proteome changes under abiotic stress-contribution of proteomics studies to understanding plant stress response. J Proteomics 74 : 1301—1322.
9. Abogadallah GM (2010) Antioxidative defense under salt stress. Landes Biosci 5 : 369—374.
10. Koca M, Bor M, Ozdemir F, Turkan I (2007) The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ and Experim Bol 60 : 344—351.
11. Abedi T, Pakniyat H (2010) Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.). Czech J Genet and Pl Breed 46 : 27—34.
12. Radi AA, Farghaly FA, Hamada MA (2013) Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. J Biol Earth Sci 3 : 72—88.
13. Gupta B, Huang B (2014) Mechanism of salinity tolerance in plants: Physiological, biochemical and molecular characterization. Int J Genomics 2014 : 1—18.