IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 5, Issue 4 (Jul. – Aug. 2019), PP 42-48
www.iosrjournals.org

Effect of Ascorbic acid in combination with NaCl for mitigating the salinity stress in sugarcane (*Saccharum officinarum* L.) variety Co 0118 and Co 0238 under *in vitro* condition

Ranjana Singh* and R.S. Sengar
College of Agriculture, Dept. of Agri. Biotechnology, Sardar Vallabh Bhai Patel University of Agriculture & Technology, Meerut-250110.

Abstract: *The ameliorating effect of exogenous application of ascorbic acid (AA) in combination with NaCl under in vitro culture on growth and associated biochemical parameters in Saccharum genotypes Co 0118 and Co 0238 were studied in the presence and absence of ascorbic acid (1mM) in combination with NaCl [0 (control), 20, 40, 80, 160 and 200 mM]. Plant growth measurements like shoot length, fresh weight, number of shoot per explant and antioxidant enzyme activities peroxidase and catalase were recorded and found significant after 4 weeks. NaCl containing medium shows the reduced growth and antioxidants activity in all the combination as compare to ascorbic acid containing medium. The AA application not only mitigated the inhibitory effects of salt stress but also induced a stimulatory effect on all the studied growth parameters. Hence, the application of AA under in vitro culture significantly alleviated the adverse effects of salinity on growth and biochemical parameters of sugarcane plantlets.*

Keywords: *Ascorbic acid, NaCl, in vitro, antioxidant enzyme, salinity*

Date of Submission: 13-08-2019 Date of acceptance: 29-08-2019

I. Introduction

Sugarcane (*Saccharum* sp.) is a phenomenal plant species growing in tropical and subtropical regions[1,2] . It is an important sugar crop of India and ranks among the top ten food crops and accounts for around 60% of the world's sugar production. The importance of cane has also increased in recent years for its wide use in industries as a raw material for sugar, alcohol, acetic acid, butanol, paper, and animal feed[3,4]. The sugarcane being a glycophyte (salt susceptible), exhibits toxic symptoms including low spout emergence, nutritional imbalance and growth reduction, leading to low productivity, especially sugar content when cultivated in the salt-affected soils[5,6]. So, the development of stress-tolerant crops will be greatly advantageous for modern agriculture in areas that are prone to such stresses. The identification of salt tolerant parents and their cross combinations that could yielded higher proportion of tolerant genotypes is most necessary phenomenon. Ion homeostasis, osmoregulation, antioxidant and hormonal systems are some of the defence mechanisms that helps halophytic species to cope up the saline conditions[7,8]. On the other hand, glycophytic species are susceptible to salt stress, because they show reduced leaf expansion, chlorophyll contents and represent the toxic damags, i.e., wilting, chlorosis, necrosis, leaf burn and senescence[9,10]. About 5% of land under sugarcane cultivation (20 million hectares) is saline[11], as per FAO 2008, about 6 % of the arable land and 20 % of the irrigated areas in the world are affected by salt stress[12]. Variety of salts present in soil that are quickly dissolved in water to produce toxic ions, especially sodium ion (Na^+). Sodium (Na^+) is frequently absorbed by root tissues and transported to the above-ground plant part through the xylem vascular tissues. Sodium ion (Na^+) is well known for negatively affecting the plant growth and development prior to plant cell death[13,14]. Salinity, a serious environmental issue affects growth and development of most of the crop plants throughout the globe[15, 16, 17,18]. High salt levels in soil causes several adverse effect viz. hyper-osmolarity, ion disequilibrium, nutrient imbalance and production of reactive oxygen species (ROS). All these, retards the plant growth through molecular damage as well as hamper many cellular processes such as photosynthesis, respiration and the plasmamembrane function[19, 20]. NaCl is responsible for both hyperionic and hyperosmotic stress in plants, and its increased accumulation in soil alters its basic structure and results in decreasing soil porosity consequently, reducing soil aeration and conductance of water[19]. Ascorbic acid (AA) is one of the most extensively studied antioxidants that have been detected in the majority of plant cell types, organelles and apoplast[21]. A fundamental role of

ascorbic acid is providing defence system in plant and protecting the metabolic processes against H_2O_2 and other toxic derivatives of oxygen. AA reacts non–enzymatically with superoxide, hydrogen peroxide and singlet oxygen[22], and acts as a primary substrate in the cyclic pathway for the enzymatic detoxification of hydrogen peroxide; thus, it has a significant role in the activation of various biological mechanisms and plant defence[23]. AA is signified as one of the most efficient growth regulators against abiotic stresses and its cellular levels are correlated with the activation of complex biological defence mechanisms. It has also used to counteract the adverse effects of salt stress in many crop plants [24,25,26]. Salt-induced adverse effects could be reduced significantly with the exogenous application of AA and increase the growth and yield of plant[27]. In recent years, the involvement of AA in response to abiotic stresses has come into light. Several studies support a major role of AA in plant adaptation to the changing environment, and induce plant tolerance to various abiotic stresses including elevated NaCl.[28,29,30] It is a well observed fact that AA potentially generates a wide array of metabolic responses in plants and also affects plant water relations.[31] It controls cell division and cell expansion, acts as a cofactor of many enzymes, modulates plant sense, and is involved in photosynthesis, hormone biosynthesis, and regeneration of antioxidants.[32,33] The AA pre-treatment effect is studied earlier in sugarcane under *in vitro* condition with significant result under NaCl stress[34]. Keeping the above facts in mind present work has been designed with two sugarcane cultivar for their comparative analysis towards facing the salt stress in the presence and absence of AA under *in vitro* culture condition.

II. Materials And Methods

Plant material and media Composition

For this experiment 3 weeks old micropropagated shoots of variety Co 0238 and Co 0118 were taken for the experiment and subcultured in the MS medium[35] induced with NaCl [0 (control), 20, 40, 80, 160 and 200 mM] and NaCl+AA (0+1, 20+1, 40+1, 80+1, 160+1, 200+1 mM) separately, supplemented along with 0.5 mg/l BAP (*N6*-benzylaminopurine), 0.5 mg/l kinetin (N6-furfuryladenine), 3% sucrose, 0.8% agar and 300 mg/l PVP (polyvinylpyrrolidone) (Hi-media, India). pH of each nutrient medium was adjusted between 5.6±2 before its sterilization (autoclave at 121ºC and 15 lb/inch^2 pressure for 20 minutes) and cultures were maintained in 16/8 hours day and night light conditions with light intensity 3000 lux with culture room temperature 25ºC±2. Data were taken after 4 weeks, with each repeated five times. Data for morphological (fresh weight, shoot length, number of shoot per explants) and biochemical parameters (catalse and peroxidase) were estimated accordingly.

Enzyme assay

For enzyme extraction, a sample of fresh leaves (0.5 g) was homogenized in 1 mL of 0.1 M phosphate buffer, 0.1 g of polyvinylpolypyrollidone (PVP), and 0.5% (v/v) Triton X-100. The resultant mixture was centrifuged at 14,000 rpm at 4 °C for 30 min. The supernatant was carefully separated and used further for quantitative estimation of catalase and peroxidase. Catalase (CAT) (EC 1.11.1.6) activity was assayed calorimetrically according to the method given by Sinha[36], (1972) and expressed as (units/ ml enzyme) and Peroxidase (POD) (EC 1.11.1.7) activity was assayed by the method of Kumar and Khan [37] (1982), and expressed as (mg/ g tissue). Absorbance for both the enzyme activities were taken by UV-visible range Spectrophotometer (PerkinElmer VIS/UV, Lambda 25).

Experimental design and statistical analysis

The experiment was laid out as Completely Randomized Design with five replications (*n*=5), two shoots per replication per test-tube, in each concentration of NaCl and NaCl+AA. Analysis of variance (ANOVA) in each parameter was computed, and the mean values of morphological and biochemical parameters were compared with the significance level at $P < .05\%$ and $P < .001\%$.

III. Results and Discussion

Growth parameter

The present study shows the effect of NaCl in combination with AA to the *in vitro* grown sugarcane plants viz. variety Co 0238 and Co 0118, and simultaneously the ameliorative effect of AA on plant under saline conditions. Our results for the experiment showed that salt stress attenuated all the studied growth parameters (fresh weight, shoot length and no. of shoots per culture vessel) and enzyme activities (catalase, and peroxidase) as well. Ascorbic acid is known to protect organelles and cells from the adverse effect of ROS (reactive oxygen species), which over-accumulate due to stress-induced oxidative damages[38,39,40,41]. Efficient detoxification of ROS help plants in growth and development, thorugh increased activity of SOD and POD that scavenge $O2^-$ and H_2O_2 radicals quickly[42,43]. The data for fresh weight, number of shoot per culture vessel and shoot length of plant are

shown in fig. 1(a,b,c) and fig. 2, from the graph it is evident that all these parameters were significantly affected by the NaCl and NaCl+AA medium. At 0 and 0+1mM, an increase in mean value from 0.56 to 0.62 mg in Co 0238 and 0.42 to 0.48 mg in var. Co 0118 was observed in fresh weight. The average number of shoot in the presence of AA was also found to be increased by 8.6 to 10.2 for Co 0238 and 7.8 to 9 for Co 0118 at 0mM and 0+1mM itself. Similarly, in case of shoot length there was an increment of 7.7 cm to 9 cm in Co 0118 and 8.4 cm to 9.7 cm in Co 0238. Although, results indicate a significant decline in fresh weight, number of shoot and shoot length with subsequent increase of NaCl, variety Co 0238 in the presence of AA give better result than Co 0118 in all the concentration with the presence and absence of AA. Plant survival rate decrease severely, browning and necrosis of plants was observed after 80 mM NaCl containing medium in Co 0118 and after 160 mM in Co 0238. The inhibitory effects of salt stress on plant growth and biomass production are well known, accumulation of high amounts of toxic salts in the leaf and other tissues leads to dehydration and turgor loss, and hence death of leaf cells and tissues[44]. Exogenous application of AA appreciably increased the growth of sugarcane plants under both the salt-stressed and non stressed conditions e.g. shoot length, shoot diameter, and leaf area of sugarcane plant[27,34]. Similarly, a significant improvement in the salt tolerance of wheat[45,46] and sorghum plants[47] were found on applying AA externally. AA application on *Khaya senegalensis* greatly increased the leaf area, stem height, and diameter of the plant[48]. In the present study, presence of AA to the NaCl containing medium appreciably enhanced the fresh weight, shoot length and number of shoots per explants of sugarcane plants when compared to non treated plants. Similarly, on applying foliar spray of AA on *Solanum melongena* L. under salinity stress, biomass production of *Brassica* plants was also improved[26,49] . There are much more evidence that proves the importance of AA in plant development in stressed and non stressed condition whether supplied exogenously or by endogenous itself.

Catalase and Peroxidase activity

Here, activities of antioxidant enzymes (peroxidase and catalase) in sugarcane plants were found to be increased under salt stress as well as after AA application. In this study, 1 mM concentration of AA enhanced the oxidative capacity and hence protects the plant from oxidative stress. Multiple antioxidant enzyme systems are involved in the enzymatic scavenging of ROS, catalase scavenge hydrogen peroxide by breaking it directly to form water and oxygen and an increase in its activity is related to an increase in stress tolerance. Peroxidases decompose H_2O_2 by oxidation of phenolic compounds and its activities increased under salt stress[50,51,52,53]. Catalase and peroxidase, both parameters shows significant increase in stressed and non stressed medium as shown in fig1. (d, e). An increase of 8.62 units/ml to 9.62 units/ml enzyme at 0 and 0+1mM in var. Co 0118, while 9.2 units/ml to 10.04 units/ml in Co 0238 was found. Maximum value of 14.36 units/ml was observed at 40+1 mM and 12.4 units/ml at 50+1mM in Co 0238. On the other hand, in variety Co 0118 maximum value of 13.18 units/ml was observed at 40+1 mM and 11.44 units/ml in 20 mM alone. From the graph, it is evident that the value of enzymatic action increase upto a limit of 40 mM and then decrease with the increase concentration of NaCl and NaCl+AA medium significantly. An increase in the activity of antioxidant enzymes helps the plants to maintain their growth under stress conditions and signifies itself as an indicator of salinity tolerance[54,55]. Presence of higher concentrations of antioxidants in plant cell is directly related to resistance against the oxidative stress caused by salinity[56]. Positive effect of POD activity in response to NaCl and NaCl +AA medium was recorded in all the concentration of MS medium. Like catalase, peroxidase activity was also found to be increase efficiently from 0, 0+ 1mM to 40+1mM in both the varieties. No significant difference is found in between 40+1mM (.039 mg) and 80+1mM (.036 mg) in variety Co 0238, while in Co 0118, 0.36 mg is the value for 40+1 mM and 0.024 mg at 80+1mM. In the absence of AA, the value was found 0.030 mg for Co 0118 and 0.033 mg for Co 0238 at 40 mM NaCl containing medium. Least value for POD at 200 mM NaCl induced medium was found as 0.00926 mg for the variety Co 0118 and 0.013mg for Co 0238. Although the value seems to be decreasing effectively after 40 mM in both the varieties yet Co 0238 proves itself better than Co 0118 in all the concentrations. The activity of catalase enzyme increased significantly with the increment in NaCl stress[57], thus regulating the level of other peroxidases necessary for preventing peroxidation of organelle and cell membrane[58]. Upon exogenous application of ascorbic acid on potatoes, the activities of antioxidant enzymes, such as superoxide dismutase, catalase and peroxidase, were found to be increased significantly under the influence of NaCl stress and it also enhances the plant resistance to survive under environmental stress[59]. While studying the effect of exogenous application of AA on *Vicia faba* seedlings, addition of AA (4 mM) in the NaCl containing medium induced a beneficial and statistically significant increase in the percentage resistance to salt stress and the growth[60]. Addition of 1 mM ascorbic acid significantly increased seed germination, carotenoid,

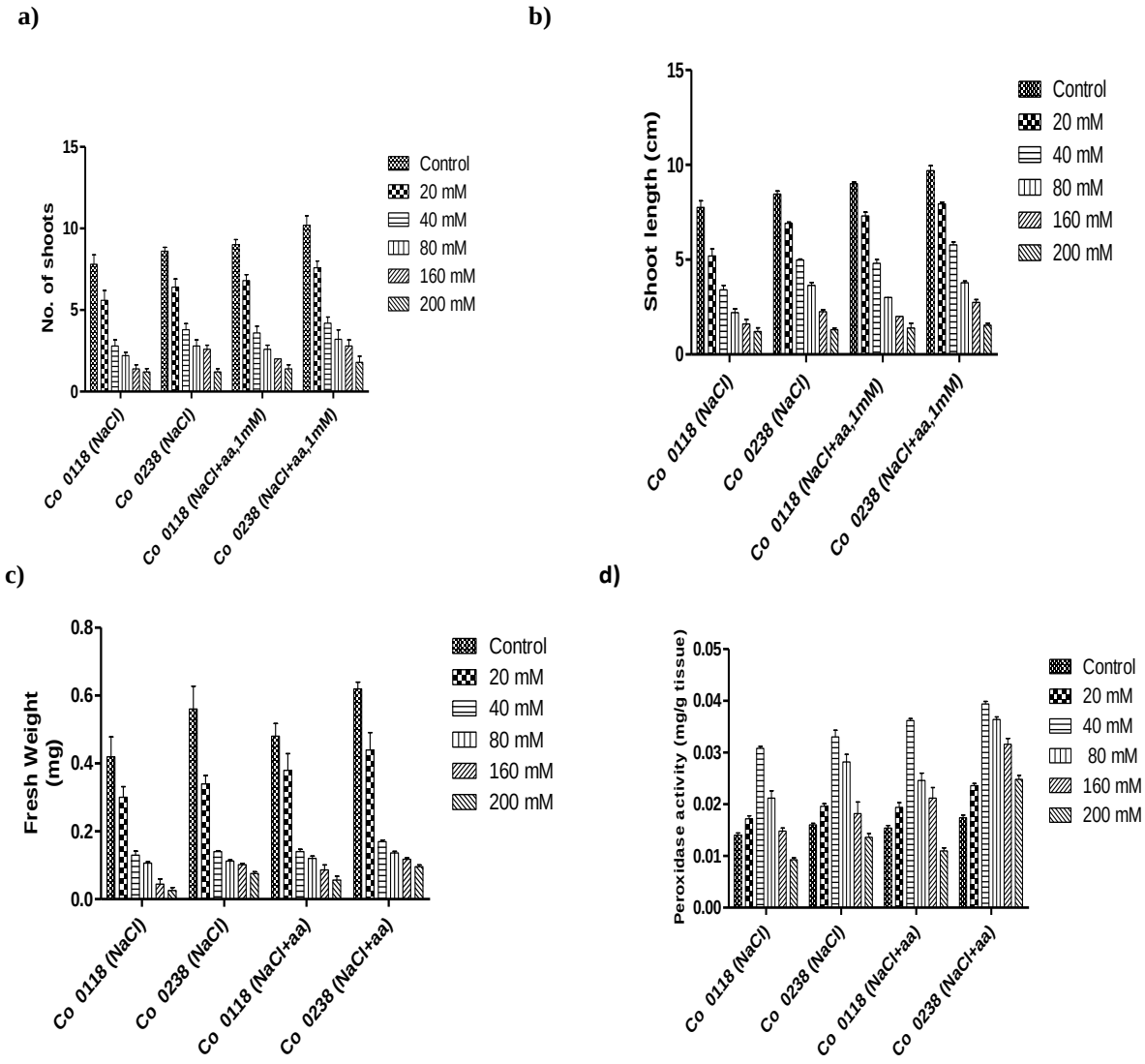
chlorophyll contents and dry mass of Egyptian clover (*Trifolium alexandrinum* L.) seedlings grown in NaCl induced medium[61]. Similarly, in case of rice also addition of 1mM AA to different concentration of NaCl induced medium enhances the chlorophyll content and hence the photosynthetic ability of plant, resulting in their improvement of salinity tolerance[62]. NaCl in the medium generate ROS and addition of AA increase the inhibition of oxidative stress through the development of enzymatic antioxidants responsible for organizing the ROS in Barley plant [63].

IV. Conclusions

There are a number of reports indicating that ascorbic acid is an important factor involved in biological defence mechanisms[64,65,34]. Likewise in our study, ascorbic acid in the medium improved the growth of sugarcane plants as indicated by morphological as well as biochemical characteristics, by combating the harmful effect of oxidative damages. These higher levels of antioxidant enzymes might be attributed to their property to help develop the plant's resistance against ROS caused due to NaCl. So, here we can conclude that an increase in the activities of antioxidant enzymes of NaCl+AA treated sugarcane plants than NaCl alone, as observed during the present work is in line with the earlier findings cited above in the paper.

Acknowledgment

Authors are thankful to the Vice-chancellor and Director Research SVPUA&T, Meerut, Uttar Pradesh for providing the required facilities to conduct the present research work.



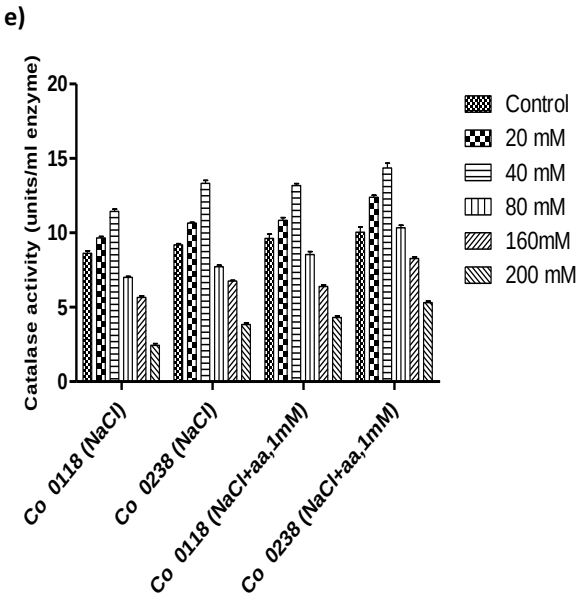
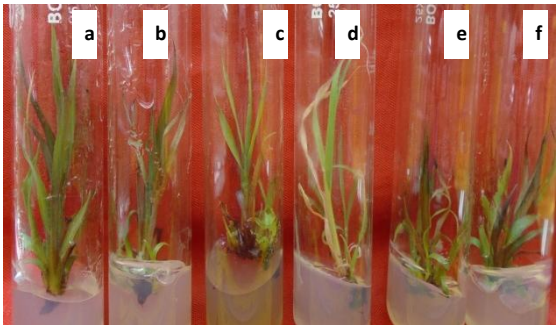
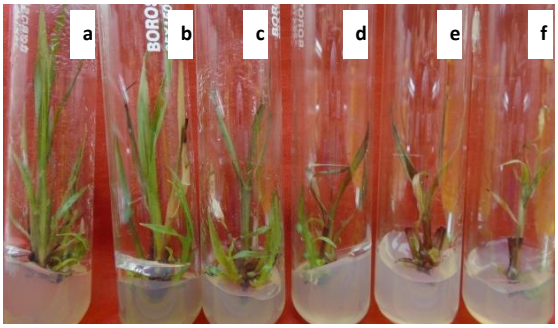


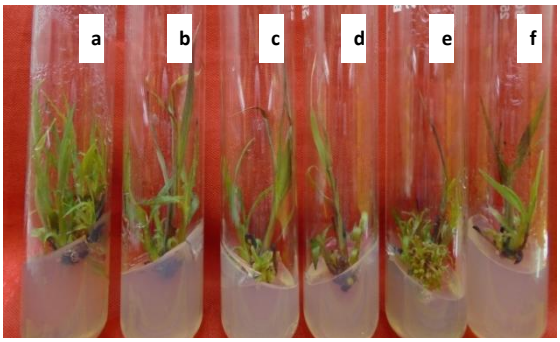
Fig. 1 Effect of different concentration of NaCl and NaCl+ AA medium on (a) number of shoot per culture vessel, (b) shoot length (c) fresh weights, (d) peroxidase activity and (e) catalase activity in *in vitro* grown sugarcane plants after 4th week of treatment.



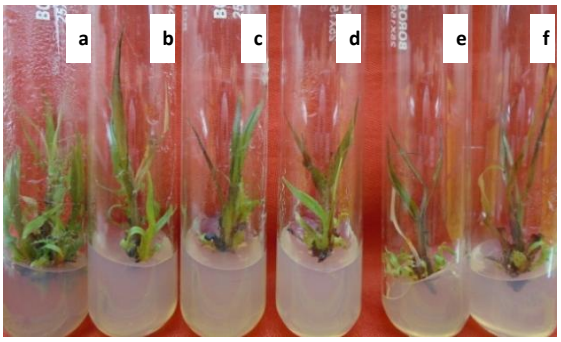
i) Co 0238 (NaCl+AA)



ii) Co 0118 (NaCl + AA)



iii) Co 0238 NaCl



iv) Co 0118 NaCl

Fig. 2 Effect of different concentration of NaCl and NaCl +AA medium on plant multiplication rate of Co 0118 and Co 0238 under *in vitro* condition after 4th week of treatment. i: Co 0238 (NaCl+AA) ii: Co 0118 (NaCl + AA) iii: Co 0238 NaCl iv: Co 0118 NaCl {a: 0 (control) mM, b: 20 mM, c: 40 mM, d: 80 mM, e: 160 mM and f: 200 mM in NaCl supplemented medium, and a: 0+1(control) mM, b: 20+1 mM, c: 40 +1 mM, d: 80+1 mM, e: 160+1 and f: 200+1 mM in NaCl and ascorbic acid supplemented medium.}

References

[1]. Cordeiro G, Amouyal O, Eliott F, Henry R, Sugarcane. In: Kole C (ed) Genome mapping and molecular breeding in plants, Pulses, sugar and tuber crops. Springer, Berlin. 2007; 3: 175–203.

[2]. Rao VP, Sengar RS, Singh S and Sharma V, Molecular and metabolic perspectives of sugarcane under salinity stress pressure. Progressive Agriculture. 2015; 15 (1) : 77-84.

[3]. Arencibia A, Gene transfer in Sugarcane. In: Biotechnology of food crops in developing countries (Eds Hohn.T and Leisinger KM) Springer-Verlug. New York. 1998; 71-104.

[4]. Sengar RS, Sengar K and Garg SK, Biotechnological approaches for high sugarcane yield. Plant Sciences Feed. 2011; 1: 7.

[5]. Lingle SE and Weigand CL, Soil salinity and sugarcane juice quality. Field Crop Research. 1997; 54 :259-268.

[6]. Gomathi R, Thandapani TV, Salt stress in relation to nutrient accumulation and quality of sugarcane genotypes. Sugar Tech. 2005; 7: 39–47.

[7]. Sairam RK, Tyagi A, Physiology and molecular biology of salinity stress tolerance in plants. Current Science. 2004; 86: 407-412.

[8]. Ashraf M , Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances. 2009: 27(1): 84-93.

[9]. Lutts S, Majerus V, Kinet JM NaCl effects on proline metabolism in rice (*Oryza sativa*L.) seedling. Physiologiae Plantarum. 1999; 105: 450–458.

[10]. Suriyan Cha-um, Chuencharoen S , Mongkolsiriwatana C, Ashraf M , Kirdmanee C, Screening sugarcane (*Saccharum* sp.) genotypes for salt tolerance using multivariate cluster analysis. Plant cell, Tissue and Organ Culture. 2012; 110(1): 23–33.

[11]. Patade VY, Bhargava S and Suprasanna P, Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. Agriculture, Ecosystem & Environment. 2009; 134(1): 24-28.

[12]. FAO, Global network on integrated soil management for sustainable use of salt affecteds soils. Rome, Italy: FAO Land and Plant Nutrition Management Service. 2008; http://www.fao.org/ag/agl/agll/spush.

[13]. Tester M, Davenport R, Na+ Tolerance and Na+ Transport in Higher Plants. Annals of Botany. 2003; 91: 1-25.

[14]. Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R, Control of sodium transport in durum wheat. Plant Physiology. 2005; 137: 807–818.

[15]. Giri B, Kapoor R, Mukerji KG, Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass, and mineral nutrition of *Acacia auriculiformis*. Biology and Fertility of Soil. 2003; 38: 170–175.

[16]. Hameed A, Dilfuza E, AbdAllah EF, Hashem A, Kumar A, Ahmad P, "Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: M. Miransari (Ed.), Use of Microbes for the Alleviation of Soil Stresses, Springer Science+Business Media, New York. 1. 2014; 139-159.

[17]. Ahanger MA, Hashem A, Abd Allah EF, Ahmad P, Arbuscular mycorrhiza in crop improvement under environmental stress. In: Ahmad P, Rasool S. (Eds.), Emerging Technologies and Management of Crop Stress Tolerance. 2014; 2: 69-95.

[18]. Wu QS, Zou YN, Abd_Allah EF, Mycorrhizal Association and ROS in Plants. In: P. Ahmad (Ed): Oxidative Damage to Plants. Elsevier Inc. 2014; 453- 475.

[19]. Porcel R, Aroca R, RuizLozano JM, Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agronomy for Sustainable Development. 2012; 32: 181–200.

[20]. Sarwat M, Hashem A, Ahanger M A, Abd_Allah EF, Alqarawi AA, Alyemeni MN, Ahmad P, Mitigation of NaCl Stress by Arbuscular Mycorrhizal Fungi through the Modulation of Osmolytes, Antioxidants and Secondary Metabolites in Mustard (*Brassica juncea* L.). Frontiers in Plant science. 2016; 7: 869.

[21]. Borland A, Elliott S, Patterson S, Taybi T, Cushman J, Pater B and Barnes J, Are the metabolic components of crassulacean acid metabolism up-regulated in response to an increase in oxidative burden? Journal of Experimental Botany. 2006; 57: 319-328.

[22]. Pourcel L, Routaboul JM, Cheynier V, Lepiniec L and Debeaujon I, Flavonoid oxidation in plants: From biochemical properties to physiological functions. Trends in Plant Science. 2007; 12: 29-36.

[23]. Arrigoni O, Zacheo G, Arrigoni-Liso R, Bleve-Zacheo T and Lamberti F, Relationship between ascorbic acid and resistance in tomato plants to *Meloidogyne incognita*. Phytopathology. 1979; 69: 579-581.

[24]. Beltagi MS, Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chick pea (*Cicerarietinum L.*) plants. African Journal of Biology, 2008; 5: 423-427.

[25]. Salama KHA, Amelioration of NaCl-induced alterations on the plasma membrane of *Allium cepa* L. by ascorbic acid. Australian journal of Basic and Applied Sciences. 2009; 3: 990-994.

[26]. Khan A, Iqbal I, Shah A, Nawaz H, Ahmad F and Ibrahim M, Alleviation of adverse effects of salt stress in brassica (*Brassica campestris*) by pre-sowing seed treatment with ascorbic acid. American-Eurasian Journal of Agricultural & Environmental Sciences. 2010; 7: 557-560.

[27]. Ejaz B, Sajid ZA, Aftab F, Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of *Saccharum* spp. hybrid cv. HSF-240 under salt stress. Turkish Journal of Biology. 2012; 36: 630-640.

[28]. Stevens R, Buret M, Garchery C, Carretero Y, Causse M, Technique for rapid, small-scale analysis of vitamin C levels in fruit and application to a tomato mutant collection. Journal of Agricultural and Food Chemistry. 2006; 54: 6159–6165.

[29]. Arfan M, Athar H, Ashraf M, Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress. Journal of Plant Physiology. 2007; 164: 685–694.

[30]. Abbasi M , Faghani E, Role of salicylic acid and ascorbic acid in the alleviation of salinity stress in wheat (*Triticum aestivum* L.). J. Bio. & Env. Sci. 2015; 6: (2) 107-113.

[31]. Hayat Q, Hayat S, Irfan M and Ahmad A (2010) Effect of exogenous salicylic acid under changing environment: a review. Environmental and Experimental Botany. 68 (1): 14–25.

[32]. Gallie DR, The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. Journal of Experimental Botany. 2013; 64 (2): 433-443.

[33]. Lisko KA, Aboobucker SI, Torres R, Lorence A, Engineering elevated vitamin C in plants to improve their nutritional content, growth, and tolerance to abiotic stress. Recent Advances in Phytochemistry, Springer. 2014; 109-128.

[34]. Munir N, Naz S, Aslam F, Shahzadi K, Sumera Javad, Effect of various levels of ascorbic acid pretreatment on alleviation of salt stress in salt sensitive sugarcane genotype SPF-213 J. Agricultural Research. 2013; 51(3).

[35]. Murashige T and Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum. 1962; 15: 473-497.

[36]. Sinha AK, Colorimetric assay of catalase. Analytical biochemistry. 1972; 47 (2): 389-394)

[37]. Kumar, KB and Khan PA, Peroxidase and polyphenol oxidase in excised ragi (Eleusine corocana cv PR 202) leaves during senescence. Indian Journal of Experimental Biology. 1982; 20 (5): 412-416.

[38]. Latif M, Akram NA, Ashraf M, Regulation of some biochemical attributes in drought-stressed cauliflower (*Brassica oleracea* L.) by seed pre-treatment with ascorbic acid. The Journal of Horticultural Science and Biotechnology. 2016; 91: 129–137.

[39]. Mukhtar A, Akram NA, Aisha R, Shafiq S, Ashraf M, Foliar-applied ascorbic acid enhances antioxidative potential and drought tolerance in cauliflower (*Brassica oleracea* L. var. Botrytis). Agrochimica. 2016; 60: 100–113.

[40]. Naz H, Akram NA, Ashraf M, Impact of ascorbic acid on growth and some physiological attributes of cucumber (*Cucumis sativus*) plants under water-deficit conditions. Pakistan Journal of Botany. (2016) 48: 877–883.

[41]. Akram NA, Shafiq F, and Ashraf M, Ascorbic Acid-A Potential Oxidant Scavenger and Its Role in Plant Development and Abiotic Stress Tolerance. Frontiers in Plant Science. 2017; 8: 613.

[42]. Rasool S, Ahmad A, Siddiqi TO, Ahmad P, Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiologiae Plantarum. 2013; 35: 1039-1050.

[43]. Hashem A, Abd Allah EF, Alqarawi AA and Egamberdieva D, Induction of salt stress tolerance in cowpea [Vigna unguiculata (L.) Walp.] by arbuscular mycorrhizal fungi. Legume Research. 2015; 38 (5): 579-588.

[44]. Munns R, Comparative physiology of salt and water stress. Plant Cell and Environment. 2002; 25: 239-250.

[45]. Athar HR, Khan A and Ashraf M, Inducing salt tolerance in wheat by exogenously applied ascorbic acid through diff erent modes. Journal of Plant Nutrition. 2009; 32: 1799-1817.

[46]. Athar HR, Khan A, Ashraf M, Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environmental and Experimental Botany. 2008; 63: 224-231.

[47]. Arafa AA, Khafagy MA, El-Banna MF, The effect of glycinebetaine or ascorbic acid on grain germination and leaf structure of sorghum plants grown under salinity stress. Aust J Crop Sci. 2009; 3: 294-304.

[48]. Abd El-Aziz NG, Mazher AAM and El-Habba E, Effect of foliar spraying with ascorbic acid on growth and chemical constituents of *Khaya senegalensis* grown under salt condition. American-Eurasian J. Agric. Environ. Sci. 2006; 1: 207-214.

[49]. El-Tohamy WA, El-Abagy HM and El-Greadly NHM, Studies on the effect of putrescine, yeast and vitamin C on growth, yield and physiological responses of eggplant (*Solanum melongena*L.) under sandy soil conditions. Australian journal of Basic and Applied Science. 2008; 2: 296-300.

[50]. El–Bassiouny HMS and Bekheta MA, Effect of salt stress on relative water content, lipid peroxidation, polyamines, amino acids and ethylene of two wheat cultivars. International Journal of Agriculture and Biology. 2005; 7(3): 363-368.

[51]. Khattab H, Role of glutathione and polyadenylic acid on the oxidative defense systems of two different cultivars of canola seedlings grown under saline conditions. Australian journal of Basic and Applied Science. 2007; 1(3): 323-334.

[52]. Agarwal S and Shaheen R, Stimulation of antioxidant system and lipid peroxidation by a biotic stresses in leaves of *Momordica charatia*. Brazilian Journal of Plant Physiology. 2007; 19 (2): 149-161.

[53]. El-Bassiouny HMS and Sadak MS, Impact of foliar application of ascorbic acid and α-tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. Acta biologica Colombiana. 2015; 20 (2): 209-222.

[54]. Zheng Y, Jia A, Ning T, Xu J, Li Z, Jiang G, Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance. Journal of Plant Physiology. 2008; 165: 1455-1465

[55]. Azevedo RA, Carvalho RF, Cia MC, Gratao PL, Sugarcane under pressure: An overview of biochemical and physiological studies of abiotic stress. Tropical Plant Biology. 2011; 4: 42-51.

[56]. Meloni DA and Martinez CA, Glycinebetaine improves salt tolerance in vinal (*Prosopis ruscifolia* Griesbach) seedlings. Brazilian Journal of Plant Physiology. 2009; 21: 233-241.

[57]. Bor M, Ozdemir M and Turkan I, The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Science, 2002; 164: 77-84.

[58]. Lin C and Kao CH (1999) Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regulation. 30 : 151-155.

[59]. Sajid ZA and Aftab F, Amelioration of salinity tolerance in *Solanum tuberosum* L. by exogenous application of ascorbic acid. In vitro Cell and Developmental Biology- Plant. 2009; 45: 540-549.

[60]. Younis ME, Hasaneen MNA and Kazamel AMS, Exogenously applied ascorbic acid ameliorates detrimental effects of NaCl and mannitol stress in *Vicia faba* seedlings. *Protoplasma*, 2010; 239: 39-48.

[61]. Aly AA, Khafaga AF and Omar GN, Improvement the adverse effect of salt stress in Egyptian clover (*Trifolium alexandrinum* L.) by AsA application through some biochemical and RT-PCR markers. Journal of Applied Phytotechnology in Environmental Sanitation. 2012; 1: 91-102.

[62]. Alhasnawi AN, Kadhimi AA, Isahak A, Mohamad A, Yusoff WMW and Zain CRCM, Exogenous Application of Ascorbic Acid Ameliorates Detrimental Effects of Salt Stress in Rice (MRQ74 and MR269) Seedlings. Asian Journal of Crop Science. 2015; 7 (3): 186-196.

[63]. Agami RA, Applications of ascorbic acid or proline increase resistance to salt stress in barley seedlings. *Biologia Plantarum*, 2014; 58: 341-347.

[64]. Loewus FA and Helsper JPFG, Metabolism of L-Ascorbic acid in plants. In: Ascorbic Acid, Chemistry, Metabolism and Uses. Postharvest Physiology and pathology of vegetables. 1982; 249-261.

[65]. Noctor G and Foyer CH, Ascorbate and glutathione: Keeping active oxygen under control. Annual Review of Plant Physiology and plant Molecular Biology. 1998; 49: 249-279.

Ranjana Singh. " Effect of Ascorbic acid in combination with NaCl for mitigating the salinity stress in sugarcane (Saccharum officinarum L.) variety Co 0118 and Co 0238 under in vitro condition ." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 5.4 (2019): 42-48.