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Effect of Salinity on Growth, Photosynthetic Pigments and Antioxidant Activity inWatermelon (Citrulluslanatus (L.))

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Abstract: ***The aim of this study was to examine the effect of different concentrations (0, 50, 100, 150, 200, 250, 300mM) of sodium chloride (NaCl)on growth response, photosynthetic pigments and major antioxidant enzyme activity in watermelon cv. Arkamanik.Salt stressed watermelon plants were analysedafter 15 days of treatment, for growth, photosynthetic pigments and antioxidant enzymes activity. The results indicated that growth traits such as plant height, leaf number, total fresh weight, total dry weight and tap root length were decreased, the photosynthetic pigment contents (chlorophyll a, chlorophyll b and carotenoid) also decreased and all the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR) in leaves were enhanced with increasing salt concentrations. These data showed that antioxidant enzymes appear to protect seedlings against stress-related damage, and play an important role in salinity tolerance of watermelon.***
Keywords: Salinity; Growth; Photosynthetic pigments; Antioxidants activity; Watermelon

I. NTRODUCTION

Crop plants are exposed to environmental stresses in field conditions, and about50% ofyield loss caused by biotic and abiotic stresses[1]. Plant development and productivity are adversely affected due to increase in salinization of lands. Overall 6 % of land throughout the world is affected by salinity,it is about 22 % of the total agricultural land [2].Plants exposed to drought or salinity environment results in considerable changes in physiological, biological and gene expression levels. Plants adopt numerous molecular mechanisms to prevent themselves from the negative impact of salt stress.

Development of salinity tolerant cultivars viagenetic engineering is an efficient approach to solve this environmentalproblem. However these salt-tolerant transgenic plantsare failed to survive under field condition with high salinity. To overcome this problem, it is necessary to understand the stress influencing factors for specific genotype during the development under field saline condition.With the development of newmolecular biology tools, it is feasible to understand the molecular mechanism behind plant response to salt stress.Hence, the knowledge of candidate salt tolerance genes is a prerequisite for utilization of modern techniques for development of salinity tolerant plants.

The prolonged exposure of plants to soil salinity leads to lethal effect due to osmotic stress and ionic toxicity ([3],[4],[5]). High salinity normallystimulate theexcess production of reactive oxygen species (ROS) ([6], [7]). These ROS are systematically regulated by both enzymatic and non-enzymatic antioxidants ([8], [9]). High salinity stress can weaken these regulatory system, results in the lethal effect of plants [10]. Salinitystress also diminish the activity of photosynthetic system which leads to reduction of chlorophyll contents, stomata number and stomatal conductance [11].

Watermelon (Citrulluslanatus L.), is an economically important fruit crops of high nutritious value worldwide and this plant is moderately sensitive to saltstress. The fruit contains 70% of water, due to large leaf area the plant consume more amount of water naturally ([12], [13],[14]).The cultivation of watermelon is heavily dependent on irrigation, especially during the fruit development stage [15]. China is the largest producer of watermelon with 1,839,750 hectares in 2013[16].

The plants grown in high salinity leads to limited growth and yield, which also affects the seed viability and germination, but it activates the antioxidant enzyme system. Till now,the physiological and biochemical aspects of salt tolerance in economically important crop plants are relatively unclear. There is not much information aboutthe effects of salinity on watermelon species in the current literature, with the exception of a few studies.Hence,the the present study was aimed to examine the growth response,photosynthetic pigment content and major antioxidant enzymes activity inwatermelon Arkamanik in response to different salt treatments.

II. MATERIALS AND METHODS

A. *Plant material*

The experiments were carried out in green house,Deparment of Biotechnology, Bharathidasan University, Tiruchirapalli. The seeds of watermelon cv. Arkamanik were obtained from IIHR, Bangalore, India.

B. *Salnity treatments*

Healthyseeds, were selected then washedunder tap water with soap solution (Tween 20).Twenty seeds were grown in each plastic pot, containing equal quantities of vermiculite and sand. The seeds were allowed to grow under greenhouse condition, 16/8 hrs (day/night), the temperaturewas 28–35°C in the daytime and 24–30°C at night and 60-70% relative humidity. Germinated seeds with same growth were transferred to plastic pots with one seed per potwith 1:1 (v/v) autoclaved mixture of sand and vermiculite.The seeds were grown for 4 weeks, the age of plantfor treatment was followed by [17],[18].

Different concentrations of sodium chloride (0, 50,100,150,200,250 and 300mM)were used to create the salinity condidtions. Pots were irrigated with nutrient solution (1x solution) in three days of interval for 4 weeksasdescribed in the earlier work [19]. The seedlings were separarted into seven groups, each group was treated with one concentration ofNaCl. The nutrient solution alone supplied for control plants. The treatment of concentrations were increased gradually from lower to higher upto the determined concentration of NaCl in nutrient solution. About 200ml of NaCl solution was given with three days of interval.Samples were collected on the 15th day from the start of treatment,the leaves from three seedlings in each group were analyzed.

C. *Growth measurements*

Growth measurements, for the plants exposed to saline treatments, were taken after 15 days of treatment. For each treatment three replicates were used to calculate the mean values. The following measurements were taken after treatment: Plant height (Shoot length), number of leaves in plant, fresh weight and dry weight (plants were dried at 70°C in hot air oven) and the tap root length.

D. *Photosynthetic Pigments*

The leaves were extracted with acetone for quantification of photosynthetic pigments [Chlorophyll a (Chl a), chlorophyll (Chl b), and total carotenoids (Caro)][20] with minor modifications as detailed by the earlier work[21]. The pigment concentrations were expressed as µg per g fresh weight.

E. *Antioxidant Activity*

The harvested leaves were frozen in liquid nitrogen, and used for crude protein extraction as described in the earlier literatutre.[22]. The specific activity of superoxide dismutase (SOD), catalase (CAT),ascorbate peroxidase (APX), peroxidase(POD), glutathione peroxidase (GPX), and glutathione reductase (GR), were quantified in the protein sample.SOD activity was determined by the inhibition of photoreduction of nitrobluetetrazolium (NBT), quantified by spectophotometer at 560 nm with the influence of riboflavin as superoxide radicals source [23].

The inhibition of 50% of NBT photoreduction was considered as one unit of SOD. CAT activity was measure byconsumption of H_2O_2 in the protein extract, which showed decrease in absorbance at 240 nm as described earlier [24]. The amount of enzyme required to decompose H_2O_2 (1µmol)per minute at optimum temperature of25ºC was calculated as one unit of CAT. The oxidation of ascorbate in the reaction was measured by decrease of absorbance (290 nm) under spectrophotometer; from these values the activity of APX was calculated [25].

The amount of enzyme required for consumption ofascorbate (1µmol)) per minute at optimum temperature of 25ºC was calculated as one unit of APX.The activityof GR was measured by the reduction in absorbance peak at 340 nm, due to the oxidation of NADPH [26].The one unit of GR was considered as the quantity of enzyme required to oxidize 1 µmol of NADPH per minute at optimum temperature of 25ºC. Specific POD activity was calculated by the oxidation of benzidine by spectrometric absorbance at 530 nm according to the previous report [27]. Glutathione peroxidase activity was assayed by the protocol followed by [28].The activities are expressed as µ g GSH consumed /minute/mg protein.

F. *Statistical Analysis*

All data obtained were analyzed by one-way analyses of variance (ANOVA) using SPSS 20.0 software. Significant difference fromcontrol among treatments were calculated by DMRT (Duncan's multiple range tests) (P=0.05).

III. RESULTS AND DISCUSSSION

A. Effect of NaclStress on Growth of Watermelon

The effect of salinity (NaCl) on growth parameters was analyzed by measuring the plant height, number of leaves, fresh weight and dry weight, and the tap root length of watermelon. The results showed that, the plant height, number of leaves, fresh and dry weight of plants were significantly reduced and the tap root length moderately while increasing the NaCl concentration from 50 to 300 mM(Figure.1).It was noted that the treatment of high salinity (300 mMNaCl) reduced plant growth parameters to more than 55% in comparison with control (Fig. 1).If the concentrations increased above 300mM of NaCl the plant growth stunted and died after treatment of 15 days (data not shown). Maximum growth inhibition was observed at 300 mMNaCl after 15 days of treatment with 68% of reduction in plant height with respect to control. There was a gradual decrease in plant height with growth inhibition percentage of 17%, 32%, 41%, 52%, 66% and 68% at50,100,150,200,250,300 mMNaCl treatments respectively.

The plant growth was drastically affected by abiotic stress (salinity), it decreasing the water potential and cause nutrient imbalances which leads to plant death ([29],[30],[31]).Therefore, investigating the molecular mechanism behind salt tolerance is prerequisite for plant breeding and genetic manipulation of plants to develop salinity tolerant plants[30]. The number of leaves reduced drastically with reduction percentage of 11%, 21%, 32%, 42%, 52% and 63% after exposed to 50,100,150,200,250,300 mMNaCl treatments respectively. The maximum number of leaves was reduced at 300 mM (63%, 7 leaves), when compared to control (19 leaves). The shoot fresh weight was decreased significantly by 20%, 34%, 46%, 53%, 73% and 80% at 50,100,150,200,250,300 mMNaCl respectively, with respect to control. The maximum fresh weight reduction occurred at 300 mM(80%, 3g FW) in comparison with control (15 g FW).

The dry weight of shoot decreased gradually by 9%, 20%, 32%, 52%, 62% and 77% at 50,100,150,200,250,300 mMNaCl treatments respectively, comparing to control. The maximum dry weight reduction observed at 300mM (77%, 0.8 g DW), compared to control (3.5 g DW).The tap root length was reduced dramatically by 11%, 22%, 38%, 50%, 62% and 67% at50,100,150,200,250,300 mMNaCl treatments respectively, compared to control. The treatment with 300 mMNaCl showed maximum percentage of root inhibition (67%, 6 cm) withrespect to control (18 cm).In the present study, the result conclude that increasing the concentrations of NaCl remarkably decreased the plant height, subsequently fresh and dry weight of the plants. Similar to our results, other studies done in moth bean *Vignaaconitifolia* L.[32], in radish plant, *Raphanussativus* L.[33], and in black gram *Vignamungo* L[34],concluded that increasing the concentrations of NaCl developed a decline in the lengths of the plants.InContrary to our results in maize Zea mays L.[35], in rice seedlings Oryza sativa L [36], in cowpea, *Vignaunguiculata* L [37] and in *Brassica campestris* L. [38] the researchers reported that the length of plants increased in lower concentration of NaCl treatment, at higher concentration the growth of plants drastically reduced.

B. PhotosyntheticPigments

The effect of different concentrations of salt stress, on the photosynthetic pigments(chlorophyll ‘a’, ‘b’ and carotenoids) of the watermelon plants was investigated. The results showed that an inversecorrelation between NaCl concentration and chl. ‘a’, and ‘b’ content (Figure. 2). Whenever the concentration of sodium chloride was increased, the chl. ‘a’, and ‘b’ content decreased and reached its lowest content, at 300mM, compared to control plant fresh weights, respectively.

The results showed that when compared to control chl.a content was significantly decreased by 6%, 10%, 13%, 17%, 20% and 25% at 50,100,150,200,250,300 mMNaCl treatments respectively. The maximum reduction of chl.a content was observed at 300 mM (25%, 96 µg/g FW) that the control (128 µg/g FW). Similarly, the chl.b content was gradually decreased by 6%, 15%, 24%, 31%, 35% and 41% at 50,100,150,200,250,300 mMNaCl treatments respectively. The maximum percentage of chl.b reduction was observed at 300 mM (41%, 32 µg/g FW), compared to control (54 µg/g FW).Ourresults showedthat a decrease in chlorophyll ‘a’, ‘b’, and carotenoids, agree with the earlier report [39], barley seedlings exposed to 0,120 and 240 mM of NaCl decreased the photosynthetic pigments. In addition to this, the studies inPaspalumvaginatum (L.) [40] and in Centauriumerythraea (L.) [41] explained that chlorophyll ‘a’, ‘b’ and total chlorophyll decreased with the increase of salt concentrations. A decrease in chlorophyll content under salt stress, is due to inhibition of Rubisco and PEP carboxylase enzymes, involved in biosynthesis of chlorophyll [42], and the chlorophyll degradation also occurs by the activation of chlorophyllase [43].In Contrary to our results, reported that salt stressed rice seedlings showed increase in the chlorophyll content of 15 days old seedlings[36]. Further it was confirmed in Beta vulgaris L inwhich the the treatment of increased concentration of NaCl, simultaneously increased the leaf total chlorophyll content [33].The effect of salinity stress on carotenoid content in leaves at different concentrations are given in Figure.2. The carotenoid content was decreased gradually, with the increasing concentrations of NaCl. There wasan inverse relationship between salt concentration and carotenecontenthave been observed from the present experiments (Fig 2). The carotenoid content was

gradually decreased by 8%, 16%, 24%, 28% 40%and 44% at 50,100,150,200,250,300 mMNaCl treatments respectively. The reduction percentage of carotenoids was maximum at 300 mM (44%, 28 µg/g FW), with respect to control (50 µg/g FW). The differences of carotene content in the statistical analysis were significant at all the used concentrations of sodium chloride.The reduction of carotenoid content in our study agree with results in barley seedlings showed drastic reduction of carotenoids [39]. On the other hand, contrary to our results, there wasan increase in carotene content in rice seedling exposed to salt stress[36].

C. Antioxidant enzymatic assay

We observed thesignificant changes in the antioxidant activity including SOD, CAT, POD, APX, GR and GPX under salinitystress.In the present study, there were significant differences in leaf SOD,POD, GPX, GR, APX and CAT activity under different NaCl concentrations . In addition, all the antioxidant enzyme activity of the leaf was increased gradually with respect to saltconcentration,those values were higher in all salt treated plants than that in the control.

The activity of CAT and SOD under salinity stress of different concentrations was presented in Figure.3. There was a significant difference in superoxide dismutase and catalase activity at 50 to 300 mM of salt stressed and untreated seedlings. Compared with control (without salt treatment), CAT activity gradually increased by 4%,10%,19%,24%,31% and 42% after treated with 50,100,150,200,250,300 mMNaCl respectively. The activity of CAT was higher at 300 mMNaCl treatment with increased percentage of 42% (145 U $g^{-1}min^{-1}$ FW), than that of control plant (102 U $g^{-1}min^{-1}$ FW).CAT is an efficient antioxidant produced during stress conditions, it prevents the plants from oxidative damage by degrading the hydrogen peroxide ([44],[45]). During unfavourable stress conditions the catalase activity was improved to act as a protective agent against oxidative stress caused by toxic effect of H_2O_2,produced throughoutthe metabolic processes of plant cells ([45],[46],[47]). Similar to our results, in Ipomoea pescaprae the catalase activity increased with increasing the concentrations of NaCl [48]. Increasing in catalase activity under different salt stress was reported in Cassia angustifolia, maize, wheat and Sesamumindicum ([49],[50],[51],[52]). SOD is an important antioxidant, with the capability to repair oxidative damage caused by ROS. Hence, SOD act as efficient enzyme which maintain the physiological conditions and the regulation of intercellular levels of ROS under oxidative stress [44].In the present study, NaCl stress to watermelon increased the SOD activity with increasing the concentrations. The control leaves showed low level of enzyme activity than the salt treated leaf samples. The SOD activity gradually increased by 9%,16%,24%,29%,42% and 49% after treated with 50,100,150,200,250,300 mMNaCl respectively. The activity of SODpeaked at 300 mMNaCl with increased percentage of 49% (142 U $g^{-1}min^{-1}$ FW), than that of control plant (95 U $g^{-1}min^{-1}$ FW).Similar to our report, others observed that significant increase in SOD activity due to salt stress, increase in ROS (O_2^-) simultaneously increase SOD activity, because this enzyme act as scavenger of O_2^-[53].

The effect of NaCl on the APX and POD activity in the leaves of watermelon at various concentrations was presented in Figure.4. Ascorbate peroxidase and peroxidase activity also showed a similar increasing trend up to maximum concentration of 300mM of NaCl. There was a gradual increase in activity of POD by5%,15%,20%,25%,35% and 45% after treated with 50,100,150,200,250,300 mMNaCl respectively, when compared to control. The higher activity of POD was observed at 300 mMNaCl(45%,29 U $g^{-1}min^{-1}$ FW) with respect to control plant (20 U $g^{-1}min^{-1}$ FW).Similar to our results, in mangrove such as Aegicerascorniculatum there was a significant increase in peroxidase activity under saline stress [54]. During salt stress increase in peroxidase activity indicates that the formation of huge amount of hydrogen peroxide (H_2O_2) and which could release from membrane structure [55]. The peroxidase enzyme involved in the decomposition of co-substrates like phenolic compounds and antioxidants. The isomers of peroxidase consume H_2O_2 and phenolic compounds for the synthesis of secondary metabolites necessary for plant growth, development and differentiation [56]. In the present study, POD activity was increased in all the stressed plants than the control plants, it was agreed with other researchers report that increase in POD activity under salinity stress [57]. Salinity condition induced the production of ROS [6], which can damagecellular proteins, lipids and nucleic acids ([58],[59],[60]).

In our experiments, the APX enzyme activity was higher under salt treatment than in the control leaves, this concluded that APX activities showed better relation with salinity treatment. The APX activity increased progressively in a concentration dependent manner with 5%,13%,18%,21%,26% and 31% after treated with 50,100,150,200,250,300 mMNaCl respectively. The activity of APX was higher at 300 mMNaCl(31%, 50 U $g^{-1}min^{-1}$ FW),compared to control (38 U $g^{-1}min^{-1}$ FW).There are several reports which confirmed the increase of antioxidant enzyme activiy during salinity treatments. In pea plant, the antioxidants ascorbate peroxidase (APX), dehydroascorbatereductase (DHAR) and monodehydroascorbatereductase (MDHAR) were increased under salt stress conditions. During stress conditions the cellular metabolism was regulated by APX and GR, and the cellular redox state was maintained properly which enables the plants to tolerate against environmental stresses[61].

The GPX and GR activity against different concentrations of NaClwas presented in Figure.5. There was a significant increase in the glutathione peroxidase and glutathione reductase activity up to 300mM of NaCl. The Compared with control, the GR activity dramatically increased by 16%,33%,38%,50%,55% and 66% after treated with 50,100,150,200,250,300 mMNaCl respectively. The activity of GR peaked at 300 mMNaCl with increased percentage of 66% (0.30 U $g^{-1}min^{-1}$ FW), than that of control plant (0.18 U $g^{-1}min^{-1}$ FW).In the present study, we found that GPX activity significantly increased by 11%,16%,27%,33%,38% and 55% after treated with 50,100,150,200,250,300 mMNaCl respectively. The activity of GPX was higher at 300 mMNaCl(55%, 2.8 U $g^{-1}min^{-1}$ FW), compared with control (1.8 U $g^{-1}min^{-1}$ FW).Our results were inagrement with the reports of other researchers, that the activities of APX and GR were increased in A. portulacoides[62], Salicorniabrachiata[63] and B. parviflora[64] under salinity stress. Similarly in L.stocksii the activity of antioxidants plays a key role in detoxification of ROS under salt stress [8].Plants exposed to salinity were prone to oxidative stress because of the formation of ROS such as O_2 −, H_2O_2 and OH ([65],[66]). These ROS can affect the integrity of cellular membranes, enzymes activities and the plant photosynthetic apparatus ([8], [67]). The antioxidants enzymes are abundantly present in plant cells, it act as a scavenger to protect the cells from damage caused by oxidative stress. The plant defense mechanism plays a vital role in plant stress tolerance, it majorly depends on the activity of SOD, CAT and POD antioxidant enzymes [68],[69].

IV. CONCLUSION

In conclusion, watermelon growth was decreased at higher concentration of NaCl and the levels of photosynthetic pigments also reduced. This study also indicate that the increase in antioxidant enzyme activity provides the detoxification mechanism, which may improves the salinity tolerance in watermelon.Salt-induced enhancement of antioxidant enzymes was the most important mechanisms for its stress tolerance. Further investigation on analyzing the expression level of antioxidant genes to salt stress will provide basic regulatory mechanism behind the salt tolerance. These candidate genes will be highly useful to developplants with field salt tolerance.

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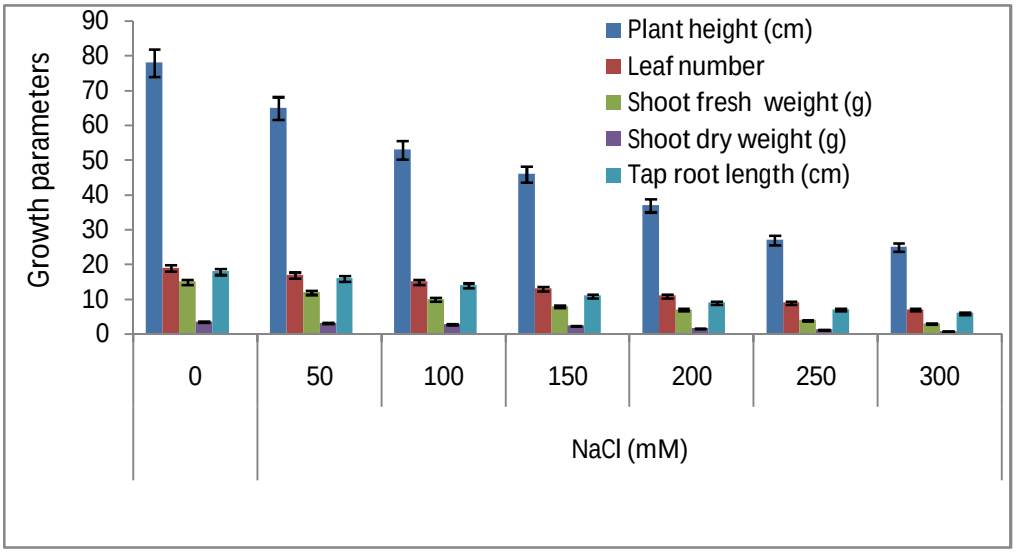

Figure 1.Effect of different concentrations of NaCl on growth parameters of watermelon cv. Arkamanik.

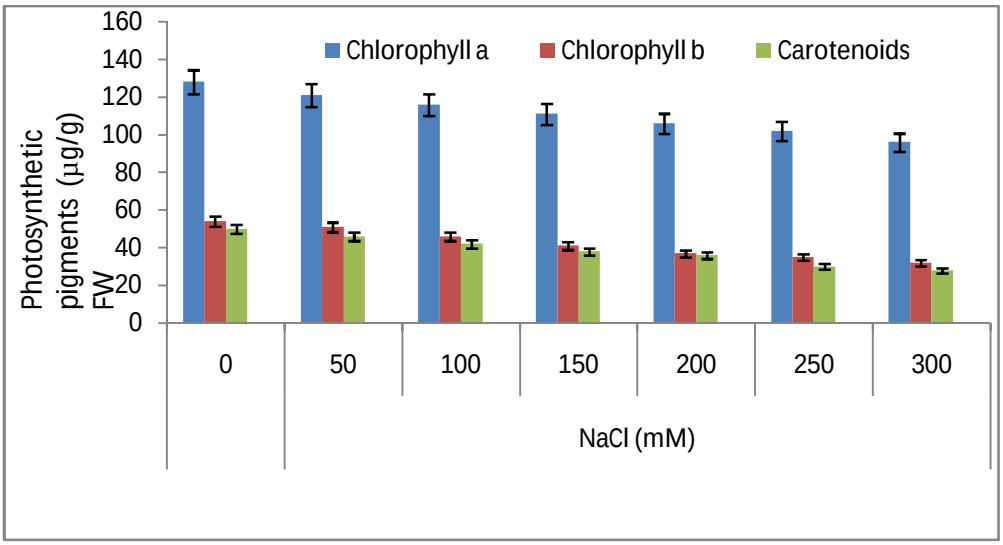

Figure 2.Effect of different concentrations of NaCl on photosynthetic pigments in leaves of watermelon cv. Arkamanik.

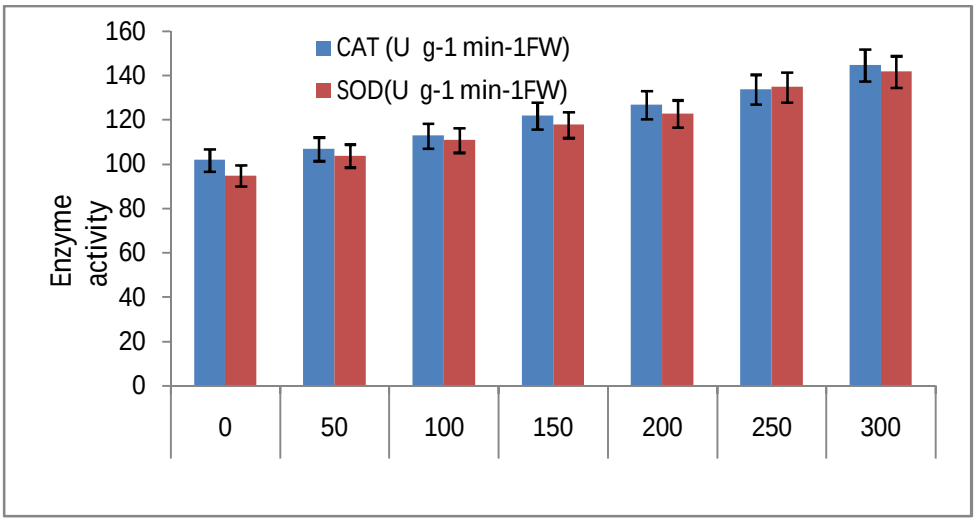

Figure 3.Effect of different concentrations of NaCl on CAT and SOD activity in leaves of watermelon cv. Arkamanik.

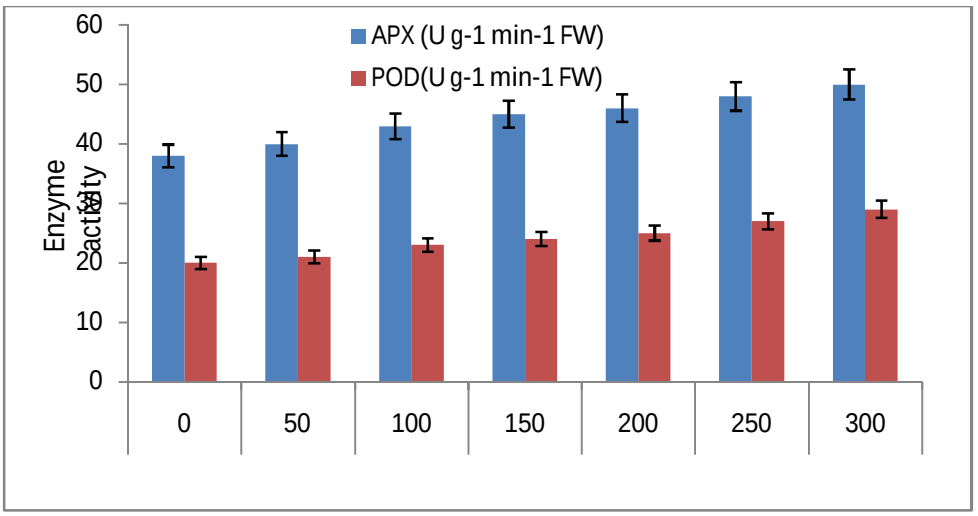

Figure 4.Effect of different concentrations of NaCl on APX and POD activity in leaves of watermelon cv. Arkamanik.

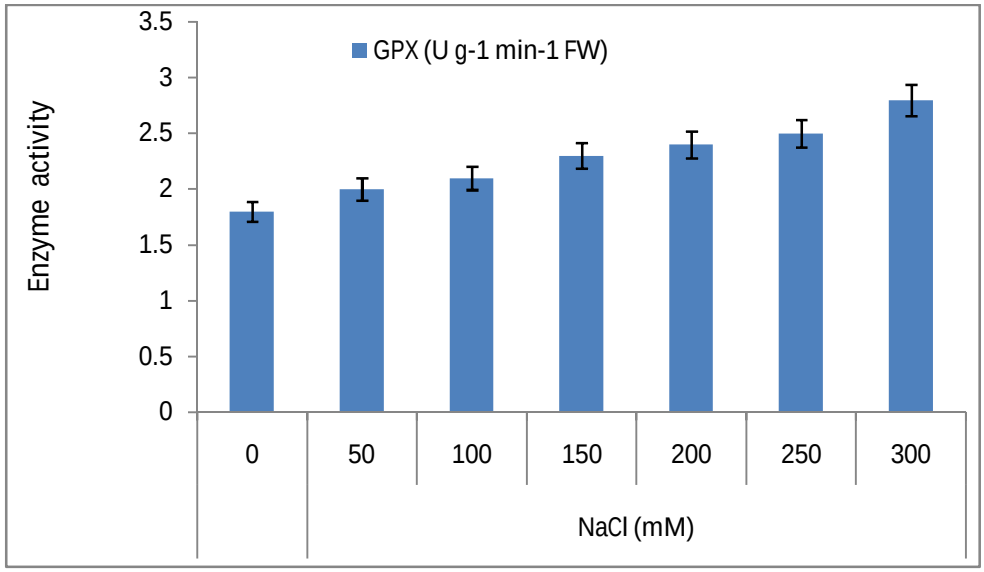

Figure 5.Effect of different concentrations of NaCl on GPX activity in leaves of watermelon cv. Arkamanik.

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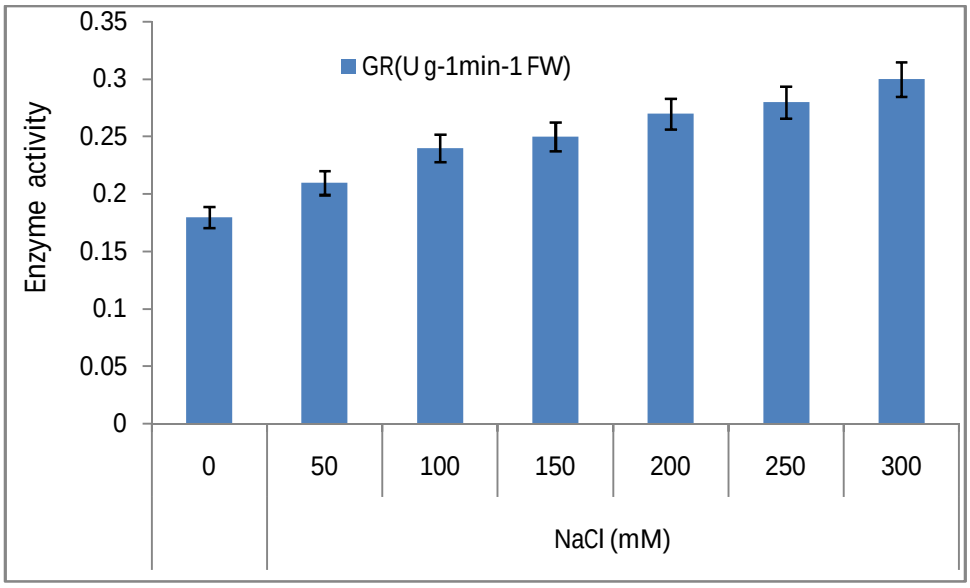
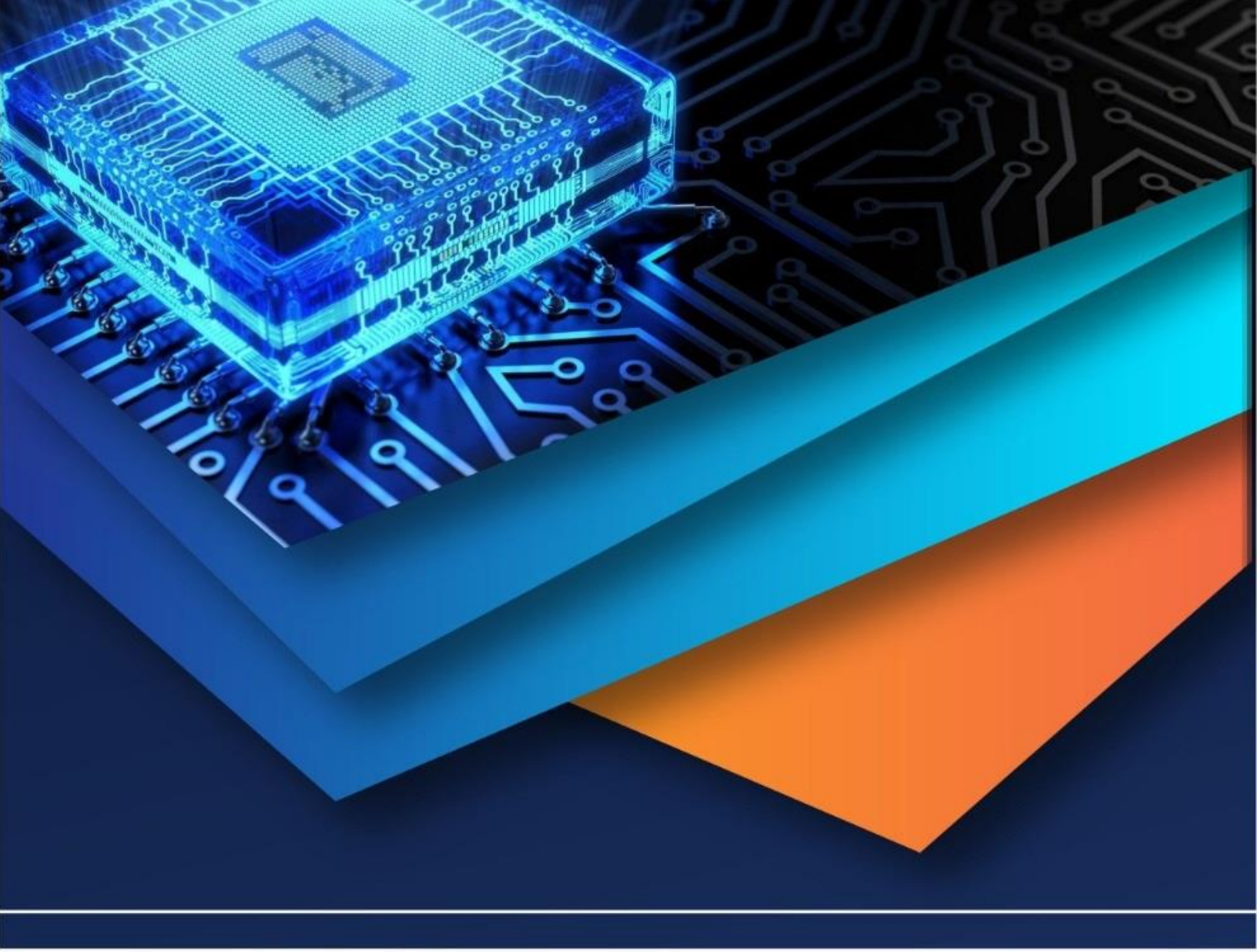


Figure 6.Effect of different concentrations of NaCl on GR activity in leaves of watermelon cv. Arkamanik.



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