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Antioxidant Assay of *Bryophyllum gastonis bonnieri* at Salt Stress

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Abstract

Bryophyllum gastonis bonnieri is a folk medicine as it consists of many antioxidants that protects against oxidative damage, and include compounds to remove or repair damaged molecules. The work is carried out for qualitative and quantitative phytochemical responses of plant to salt stress. When the plant is subjected to various strengths of NaCl (0, 25, 50, 75 & 100 mM) the growth of the plant is reduced due to stress induced. In salinity stress the plants responses by the accumulation of enzymatic antioxidants which react against the free radicals produced (Kanika Patel et al, 2011). Bryophyllum gastonis bonnieri subjected to 7, 14, 21 and 28 days of salt stress has shown reduction in heightening of plant, amount of leaves and relative growth rate was reduced with increased in sodium chloride treatments. During salinity stress free radical level increased dramatically resulting in oxidative damage to cells. The plant overcame this damage by increased accumulation of antioxidants which neutralize free radicals.

Keywords: Bryophyllum, Antioxidants, Phytochemical, Oxidative, Salinity.

Introduction

Bryophyllum gastonis bonnieri is rich in alkaloids, flavonoids, phenolic compounds, tannins, saponins [1]. As per the various ethnopharmacological record of gastonis bonnieri, many research class have conducted studies to prove their pharmacological or biological properties. In addition, some researchers have carried out phytochemical studies that resulted in the findings of various types of secondary metabolites, along with different ingredients, especially from their leaves and aerial parts [2].

Salt stress is said to be a more unfavorable factor can minimize leaf water potential, which leads to less stiffness and some other reactions, and eventually lower crop productivity in arid and semi-arid zones. It is clear that plant salt stress tolerance needs the activation of complex metabolic activities including antioxidative pathways, particularly reactive oxygen species (ROS) and macrophage which contributes to continued growth under water stress [3]

Considering the destructive effect of salt stress on plants, one of the tasks for plant biologists is to find the ways that can develop salt tolerance in crop plants. In fact, salt tolerance is a multigenic trait that governs different morphological and physiological factors [4]. Salt stress causes various physiological changes, like interruption of membranes, nutrient imbalance, change in detoxification of reactive oxygen species (ROS), differences in the activity of antioxidant enzymes and alteration in photosynthetic activity, and lowering of aperture of stomata [3, 5]. Salt stress is one of the kind stress given to plant.

Salinity tolerance is directly related to scavenging capacity of biocatalyst, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), and glutathione reductase (GR) and by collection of non-enzymatic antioxidant compounds [6,7]. Gill et al. and Tuteja et al. [8] the studies have revealed the presence of helicase proteins (e.g., DESD-box helicase and OsSUV3 dual helicase) functions during salt stress on plant showed modifying the photosynthetic and antioxidant machinery. Taking merits of the novel progress in the area of genomic, transcriptomic and metabolic methods. Life scientist are concentrating on the modified changes of a total countor of genes, proteins, and secondary metabolites that are accountable for different mechanisms during salt tolerance in different medicinal vegetation species.

Materials and Methods

Selection of plants for salt stress studies

The plants were divided into five groups and put through four individual salt stress (25, 50, 75,100mM) and one as control.

Preparation of plant extract

Fresh leaves are collected and crushed using different solvents like ethanol, chloroform and water. The crude solution was separated using whatmann filter paper and this filtrate was used for phytochemical findings.

Preparation of concentrate for enzymatic antioxidants

2g of fresh leaves are crushed with 3ml of phosphate buffer using pestle and mortar until slimy paste was obtained and paste is transferred to centrifuge tubes. The sample of interest is centrifuged at 10,000rpm for 10 minutes and upper supernatant solution was collected.

Total phenolic content

Aliquots of the extract were taken in glass tube and

add 2.5 ml of FCR reagent to the each test tubes. Then add 2 ml of sodium bicarbonate to each test tube and make up the volume10 ml by adding the distilled water. A blue color was developed. The test solutions were warmed for a minute make it cold and optical density measured around 760nm.

Total tannin content

Aliquots of the extract were taken in glass tube and add 0.5 ml of FD reagent to the each test tubes. Then add 1ml of sodium bicarbonate to each test tube and made up to 10 ml by adding the pure water. The test solutions were kept for incubation for half an hour at room temperature. Optical density was measured at 760nm

Enzyme activity assay

Enzyme assay :

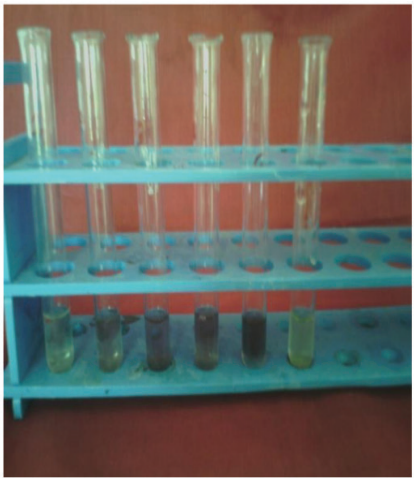

Figure 1: SOD Activity

SOD Activity

SOD activity was determined by reduction in absorbance of superoxide nitro blue tetrazolium compound by the enzyme of reaction mixture containing 50 mM potassium phosphate buffer 1580µl , 100 mM methionine 390µl , 10mM EDTA 30µl, 10 mM NBT 50µl, 1M Sodium bicarbonate 750µl , 200µl enzyme was taken in duplicates for all the samples. Control tubes were taken for experiment. Reaction starts by adding 10 mM riboflavin 0.6 µl and placing the tubes below a light source of florescent lamps for 15 min. By switching off the light and covering the tubes with black cloth the reaction was stopped. Tubes without enzyme developed maximal colour. The blank did not show any colour development. OD was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme, which decreased the absorbance of the samples to 50 percent in comparison with blank tubes that was lacking enzymes.

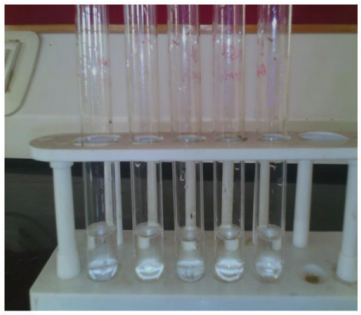

Figure 2: Peroxidase Activity

SOD ACTIVTY = (Sample O.D/Control O.D) × 100 = X

Peroxidase Activity

Peroxidase activity was measured by taking buffer (KH_2PO_4 buffer 1.35 ml, 50mM), Guicol (0.4 ml, 20mM) and 0.2ml of enzyme extract (3 ml). Reaction was started by adding hydrogen peroxide (0.2 ml, 10 mM) and OD was recorded at 470 nm for every 30 seconds.

POX ACTIVITY = (Total reaction mixture × 100)/ (6.39×0.1×Δt)

Results and Discussion

Total Phenol Content

On treatment of Nacl of varying concentration (25mM, 50mM, 75mM & 100mM) at 7th, 14th, 21st and 28th days the content of phenol gradually increased.

NaCl concentration	7th DAY µgm/ml	14th DAY µgm/ml	21st DAY µgm/ml	28th DAY µgm/ml
25mM	850	910	920	970
50mM	880	930	950	1012
75mM	900	950	980	1108
100mM	930	970	1102	1302

Table no 1: Effect of salt stress on Phenol content

Total Tannin Content

NaCl concentration	7th day µg/ml	14th day µg/ml	21st day µg/ml	28th day µg/ml
25mM	340	380	600	900
50mM	420	60	820	1200
75mM	420	740	980	1220
100mM	900	920	1200	1400

Table no 2 : Effect of salt stress on Tannin content

NaCl concentration	7th DAY µmol/mg/ml	14th DAY µmol/mg/ml	21st DAY µmol/mg/ml	28th DAY µmol/mg/ml
25mM	1.56	2.73	4.9	1.12
50mM	2.16	3.59	7.32	4.77
75mM	2.33	4.84	9.79	6.15
100mM	1.82	3.78	6.24	5.02

Table no 3 : Effect of salt stress on SOD activity

NaCl concentration	7th DAY Units/ml	14th DAY Units/ml	21st DAY Units/ml	28th DAY Units/ml
25mM	5.63	6.94	8.92	59.15
50mM	5.63	55.86	46.02	125.82
75mM	94.8	64.78	85.44	237.5
100mM	79.8	24.78	24.4	69.48

Table no 4 : Effect of salt stress on Peroxidase activity

Total Tannin Content

On treatment of Nacl of varying concentration (25mM, 50mM, 75mM & 100mM) at 7th, 14th, 21st and 28th days the content of tannin gradually increased.

Enzyme assay:

SOD Activity

There was gradual increase in SOD activity upto 21st days later there was decrease in the activity after 22nd day of treatment, this was observed upto 28th days. Even the higher concentration of about 100mM NaCl showed decrease after 14th day of treatment of saline solution which suggested that high concentration of salinity will inactivate the SOD activity which was observed upto 28 days.

Peroxidase Activity

The peroxidase activity was increased upto 28 day of treatment with the varying concentration of NaCl (25mM, 50mM, 75mM & 100mM) at 7th, 14th, 21st and 28th days, the activity of peroxidase enzyme increased gradually.

Conclusion

The metabolic pathways of plant as well as the structure gets effected. Salinity develops cellular adjustment with a significant understanding of cell structure and metabolism due to ionic and osmotic effects. Proteins show an important role in salt tolerance and cellular adaptation. There was proportionate increase in the accumulation of antioxidants as the salt stress was increased and there was slightly reduction in the growth of Bryophyllum gastonis bonnieri plant. Altogether, the observation done in various Phytochemical studies prove that medicinal plants reciprocate to salt stress.

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