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Effects of salt stress on Biochemical & Antioxidant Activity in Mung Bean (*Vigna radiate*)

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Abstract

A significant food grain legume with considerable economic importance is the mungbean. For vegans in particular, it is a great source of dietary protein and offers nutritional health benefits. Due to its short lifespan, it plays a crucial part in important farming systems and promotes soil fertility. Due to the mungbean's vulnerability to many environmental stressors, production is still declining. Along with the growing global population and depleting natural resources, salt stress is one of the most pervasive abiotic stresses posing hazards to agriculture food crops. A lesser amount of work has been put on creating a better mungbean cultivar. The present review highlights the detrimental effects of salt stress and the physiological responses in mungbean. Abiotic stress such as salinity, drought, extreme temperature, chemical toxicity and oxidative stress are serious threat to agriculture and natural status of the environment. Salt affects growth of the crop, quality of the crop. Stress in environment which can be natural or induced by human. During salinity condition the length of the root, branches and number of roots hair are decrease. Salt stress cause chlorosis, necrosis & reduced the chlorophyll content in the plant. Salt decrease the osmotic potential of the soil solution. Salinity is the concentration of dissolved mineral salt electrolytes of cations and anions present in the soil and water.The effect of Nacl stress on growth, ion accumulation, content of protein and antioxidant enzymes activity. Nacl stress enhanced the activity of superoxide dismutase and Ascorbate peroxidase & Glutathione peroxidase. Antioxidant enzyme activity could be a response to cellular damage by Nacl. In this study specific mung genotype RMG-492 was analysed effect of different stress (moderate & severe) on plant growth. the impact of sodium stress on development, ion buildup, protein composition, and antioxidant enzyme function. Superoxide dismutase, ascorbate peroxidase, and glutathione peroxidase all had increased activity in response to a cation stress. Activity of antioxidant enzymes may be a reaction to Nacl-induced cellular damage. In this study, the effects of various stresses (moderate and severe) on the growth of a particular mung genotype, RMG-492, were examined.

Keywords: Mungbean, salt stress, photosynthesis, catalase, chlorophyll, Ascorbate peroxidise, Glutathione peroxidise, Superoxide dismutase

1. INTRODUCTION

Abiotic stressors, with salt being one of the key factors in lowering agricultural production, have significantly decreased food security due to the rise in global population and the trend toward shrinking arable land. Around the world, 800 million hectares of land (6% of the total land area) are impacted by salt [1]. The main cause of the rise in salinization issues is subpar irrigation, drainage, or agricultural methods [2]. The necessity to find solutions to improve agricultural productivity under salty circumstances is prompted by the sharp growth in the amount of land being affected by salinity. The balance of vital nutrients is disturbed by salinity's detrimental effects because of an increase in Na+ and Cl ions (with Cl being more hazardous) [3-4], stress that is hyperionic and hyperosmotic in nature. Damage to

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membranes, nutritional imbalances, changes in growth regulator levels, enzymatic inhibition, the formation of reactive oxygen species (ROS) that cause DNA damage, and the activation of programmed cell death are possible impacts [5–8]. Gibberellic acid (GA3) improves seedling growth and shoot-root biomass to mitigate the negative effects of salt [9]. By partially maintaining the quantities of RNA and protein, GA3 has a positive impact on the seedlings' hydration status. Exogenous GA3 administration to salt-affected soybean plants resulted in an increase in length and dry mass and a decrease in the oxidative stress marker proline. Additionally, it preserved regular growth and development and undid the negative effects of salt on seedling germination and growth in Arabidopsis. By raising sucrose, lowering sugar levels, the protein synthesis apparatus, and the activity of antioxidant enzymes, GA3 develops salt tolerance in plants [12,13]. In the vegetative tissues of many plants, including rice, exogenous polyamine administration is believed to boost endogenous PAs and repair salt stress damage [14-17]. Exogenous PA administration additionally encourages reproductive development in normal growth environments and provides reproductive structures with abiotic stress protection [18]. When Put is applied, the net buildup of Na+ and Cl ions in the several Atropa belladonna organs under salinity stress is reduced. Put simply, rice cultivars treated with salt grew larger and had more viable leaf tissue [14,18].

In mungbean, the reproductive stage has a greater negative impact on grain yield than any other stage. Growing salt concentrations decrease mungbean seed germination, fresh and dried biomass, shoot and root lengths, photosynthesis, and yield characteristics [2,]. Mungbean is a salt-sensitive crop. In light of this, the present study's primary objective is to investigate the toxic effects of salt stress on mungbean and how they might be mitigated by GA3 or Spm, with particular attention to morphological factors like overall plant growth, fresh and dry weight, root, shoot, and leaf length, as well as specific biochemical changes like chlorophyll content, stress-induced damage in the form of elevated malondialdehyde (MDA), proline, and hydrogen peroxide (H2O (SOD).

2. MATERIALS AND METHODS

2.1. Plant material

Plant material (seeds) are collected from Rajasthan agriculture research institute (RARI), Durgapura (Jaipur). Mung bean seeds were collected RMG 492.

2.2. Measurement of chlorophyll by spectrophotometer

For the determination of chlorophyll content, about 0.5 g of leaf samples from untreated or NaCl-treated seedlings were utilised [21]. In order to remove the chlorophyll, 80% (v/v) cooled alkaline acetone was used. Using the formula, the absorbance of chlorophyll b at 645 nm, chlorophyll an at 663 nm, and total chlorophyll was measured.

$$\text{Total chlorophyll (mg}^{-1}) = 20.2\ A_{645} + 8.02\ A_{663}$$

$$\text{Total chlorophyll (mg/g)} = \frac{\text{total chlorophyll (mg/)} \times \text{volume of 80\% acetone used}}{\text{sample weight}} \times \frac{1000}{}$$

ISSN: 0975-3583, 0976-2833 VOL04, ISSUE 03, 2013

2.3 Estimation of catalase enzyme activity In 0.1 M phosphate buffer pH 7.0, test seedlings were homogenised. Centrifuging the homogenate at 10,000 g for 20 min. at 4°C. The usual methodology [25] was modified slightly to carry out the catalase (CAT, EC 1.11.1.6) assay. The sets were incubated for 30 min at room temperature (25°C) in a standard reaction mixture that included 1 mL of enzyme extract, 10 mL of 0.1 M phosphate buffer pH 7.4, and 1 mL of 5% H2O2. Instead of 1 mL of enzyme extract as in the sample set, 1 mL of phosphate buffer was used in the blank set. The addition of 5 mL of 10% H2SO4 stopped the reaction. To test the residual H2O2, 0.02 N KMnO4 was used. Total H2O2 was estimated by converting the amount of KMnO4 ingested into H2O2 (1 mL of 0.02 N KMnO4=17 mg H2O2). Additionally, the amount of total soluble protein in the extract was measured. The CAT activity was calculated as mg H2O2 decomposed per mg total protein.

2.4 Estimation of Ascorbate peroxidase (APOX): Weigh 400 mg sample & homogenized it in 2 ml of homogenization buffer. Centrifuge the suspension at 14000 rpm for 30 min at 4 º.Take the supernatant for the enzyme assay. Absorbance is taken at 290nm.

2.5 Estimation of Glutathione peroxidise [GPOX]: Leaf sample homogenized in ice cold homogenization buffer. Centrifuge it at 8000 rpm for 10 minutes at 4º C. Supernatant was taken for enzyme assays. Absorbance is taken at 470nm.

2.6 Estimation of Superoxide dismutase (SOD):

In a 50 mM Tris-HCl buffer with a pH of 7.5, 0.1 mM EDTA, and 10% polyvinyl pyrrolidone, test seedlings were homogenised. Centrifuging the homogenate at 10,000 g for 20 min. at 4°C. The superoxide dismutase (SOD, EC 1.15.1.1). 2.5 mL of 80 mM Tris-HCl (pH 8.9) containing 0.12 mM EDTA and 10.8 mM TEMED, 0.1 mL of (3.3 109)% BSA, 0.1 mL of 6 mM NBT, 0.1 mL of 0.6 mM riboflavin in 5 mM KOH, and 0.1 mL of supernatant made up the standard reaction. The reaction mixtures were housed in glass tubes that were exposed to fluorescent light (40 W) at 25°C. The reaction was terminated by turning the light off. The increase in absorbance due to formation of formazon was read at 560 nm, and the enzyme activity was expressed as enzyme units (EU) min^{-1} mg^{-1} total protein.

3. RESULTS

3.1 Chlorophyll estimation

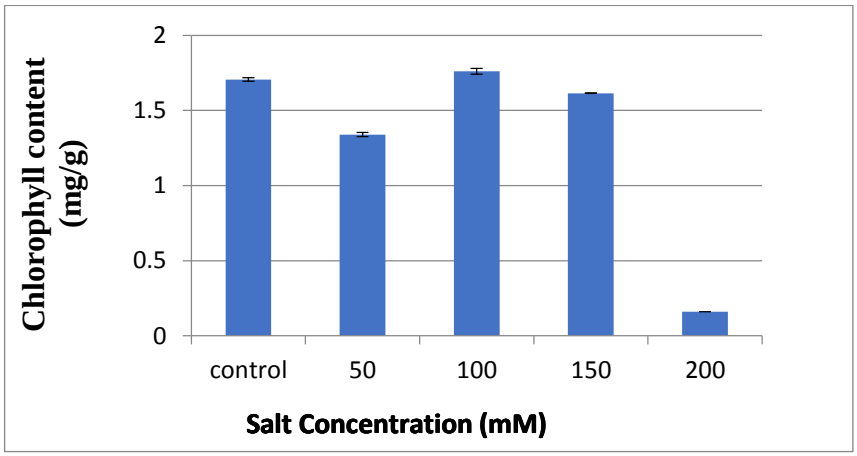
Salt stress produced changes in the ratio of chlorophyll and carotenoid. Chlorophyll content is positively associated with photosynthetic rate. As the salt concentration increases the chlorophyll decreases, this shows that the chlorophyll and carotenoid content in variety RMG-492 is increases as the salt concentration decreases.

ISSN: 0975-3583, 0976-2833 VOL04, ISSUE 03, 2013

Table: 1 Analysis of chlorophyll content in leaves (*in-vivo study*):

Salt con.	0mM	50mM	100mM	150mM	200mM
Chlorophyll	1.706188	1.33973	1.761151	1.615005	0.161501

Graph: 1 Analysis of chlorophyll content



3.2 . Catalase content in leaves and roots (*in-vivo* study)

In roots catalase shows maximum activity at 100mm concentration whereas in leaves catalase activity is increases in salt stress. Leaves are more affected from salt stress as compared to roots. In variety RMG-492 the leaves are more affected by salt stress given to plants grown *in-vivo.*

Table: 2 Analysis of Catalase content in leaves and roots (*in-vivo study*):

Salt conc.	0mM	50mM	100mM	150mM	200mM
Leaves	0.0864	30.690	992.208	134.987	35.191
Roots	36.406	33.136	42.131	42.189	64.688

3.3 APOX content in leaves and roots (*in-vivo* study):

In roots APOX shows maximum activity at 100mm concentration & in leaves APOX shows maximum at 0mm concentration. Leaves were more affected as compared to roots of RMG-492.

Table: 3 Analysis of APOX content in leaves and roots (*in-vivo study*):

Salt conc.	0mM	50mM	100mM	150mM	200mM
Leaves	2.346	3.660	15.601	5.506	0.745
Roots	6.088	1.686	1.235	3.566	0.960

ISSN: 0975-3583, 0976-2833 VOL04, ISSUE 03, 2013

3.4. GPOX content in leaves and roots (*in-vivo* study):

In roots GPOX shows maximum activity at 100mm salt concentration & in roots GPOX shows maximum activity at 50 mm concentration. Roots were more affected as compared to leaves of RMG-492.

Table: 4 Analysis of GPOX content in leaves and roots (*in-vivo study*):

Salt conc.	0mM	50mM	100mM	150mM	200mM
Leaves	118.055	164.707	5608.740	63.017	256.330
Roots	92.630	649.359	482.565	285.284	139.853

3.5 SOD Content in leaves and roots (*in-vivo* study):

SOD shows maximum activity at 150mm salt concentration and at 200mm salt concentration SOD shows minimum activity.

Table: 5 Analysis of SOD content in leaves and roots (*in-vivo study*):

Salt conc.	0mM	50mM	100mM	150mM	200mM
Leaves	70.624	1126.138	3859.5	2444.793	98.003
Roots	179.945	3696.366	3256.083	4364.934	1241.452

4. DISCUSSION

The most critical stage in seedling establishment is seed germination that determines fruitful crop production. Acceptable growth of plants in arid and semi-arid lands which are under exposure of salinity stress is related to the ability of seeds for best germination under unfavourable conditions, so necessity of evaluation of salinity tolerant genotypes is important at primary growth stage. Increasing salinity levels during mungbean seed germination significantly reduced germination characters and seedling characters with varying responses for mungbean cultivators. Salinity may affect mungbean seed germination by producing an outside osmotic potential that avoids water uptake or due to toxic effects of Na+ and Cl- ions during seed germination. On various phases of plant growth and development, salinity is known to have a number of harmful morphological impacts. Under salt stress, both growth and metabolism are impacted [28,29]. Low osmotic potential and nutritional imbalance are linked to the effects of salinity on plant growth. Different plant species have already been found to respond to salt stress by decreasing root and branch development [2,].

Salinity caused swelling of membranes in chloroplasts of sensitive plants which affects their chlorophyll content, or due to excess ion in leaves which induced loss of chlorophylls. The reduction in pigment contents may be due to the inhibitory effect of the accumulated ions (Na^+ and $Cl^{-)}$ on the biosynthesis of the different pigment fractions and/or/on their degradation or due to the effect of NaCl on chloroplast structure. Earlier researchers reported salinity induced decrease in chl. a, chl. b, carotenoids and consequently photosystem II electron transport activity contents of mungbean leaf. High chlorophyll content under stress

ISSN: 0975-3583, 0976-2833 VOL04, ISSUE 03, 2013

especially in the later stages of growth helps in maintaining the leaves to remain active for longer periods to supply photosynthetic resulting in more seed yield per plant. Germination percentage, relative water content, relative growth rate and photosynthetic pigments (chlorophyll a, b, carotenoids and chlorophyll fluorescence intensity) decreased with the increasing concentration of NaCl treatment in all species of *Vigna* In this respect, the obtained data showed variations in pigment content depending on salt stress. In quite a few cases, the chlorophyll content was paralleled by changes in the chl a/b ratio, which is an indicator of the antenna size of PS I and PS II. The case antenna contains only chl a, whereas the enter antenna contains both chlorophyll a and chlorophyll b. A higher chlorophyll a/b ratio therefore indicates, a smaller antenna size and a lower ratio show a larger antenna size. The effect of NaCl stress on growth, ion accumulation, and contents of protein, proline, and antioxidant enzymes activity was investigated. Exposure of callus to NaCl decreased growth in a concentration dependent manner. NaCl treated callus accumulated Na and declined in K, CA and Mg contents. Na/K ratio increased steadily as a function of external NaCl treatment. NaCl induced significant differences in quality and quantity of proteins, whereas, proline accumulation remained more or less constant with treatment. NaCl stress enhanced the activity of superoxide dismutase (SOD) and peroxidase and glutathione peroxidase (GPOX). NaCl strongly induced activity of SOD 50mm, 100mm, 150mm, 200mm NaCl concentrations. Increase in antioxidant enzymes activity could be a response to cellular damage induced by NaCl. This increase could not stop the deleterious effects of NaCl, but it reduced stress severity and thus allowed cell growth to occur.

5. Conclusion

The application of NaCl negatively impacted the growth, defensive mechanisms, and metabolism of mungbean seedlings, it can be inferred from the current study. In order to characterise the numerous biochemical reactions that take place in response to salt stress, various research methodologies and genetic approaches are applied. Salt tolerance is a complex phenomenon in plants. High salt concentrations cause stunted growth, a decrease in chlorophyll content, and oxidative damage by changing the antioxidant system, which damages membranes through lipid peroxidation. Applications of phytohormones like GA3 and PAs like Spm at low quantities in the presence of NaCl at high concentrations have an antagonistic effect on salt uptake. The genetically varied accessions resistant to salt stress may aid in understanding the mechanism underlying salt tolerance. The salinity breeding programme can employ the resistant accessions as genetic resources to increase the genetic diversity of mungbean and other related crops.

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