



Enhancement of Catalase Activity under Salt Stress in Germinating Seeds of *Vigna radiata*

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ABSTRACT

Catalase (CAT) (EC 1.11.1.6) is an important cellular antioxidant enzyme that defends against oxidative stress. Hydrogen peroxide, although not a free radical, is highly reactive. It serves to protect the cell from toxic effects of high concentrations of hydrogen peroxide by catalyzing its decomposition into molecular oxygen and water, without the production of free radicals. The effects of presoaking of seeds in salt solution on germination and catalase activity of *Vigna radiata* were studied. NaCl and CaCl₂ soaked seeds showed reduced germination (less than 1cm) as indicated by decreased shoot length when compared to controls. 5g seeds of *Vigna radiata* were presoaked for 24 hours in presence of NaCl (1, 5, 10, 15, 20 M) and CaCl₂ (0.5, 1, 1.5, 2.0 M). After two days of germination, the filtered water extracts (25ml) were tested for qualitative catalase activity. Further catalase assay of respective extracts was monitored in terms of decay in H₂O₂ concentration at 240 nm (Jasco-V-530) and compared with the decay in the absence of stress. There was nearly 40% increase in extent of decay of H₂O₂ at concentration of 1M NaCl and 33.9% increase in decay at 1M concentration of CaCl₂. Further study includes different combinations of NaCl and CaCl₂ to be tested followed by increase in germination period and catalase activity monitoring in partially purified and dialyzed extracts. There was an enhancement of catalase activity in presence of the salt stress.

Keywords: Sodium chloride, Calcium chloride, decay, Hydrogen peroxide

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1. INTRODUCTION

Mungbean (*Vigna radiata*) is an important traditional crop of India characterized by a relative high content of protein and is a short summer season crop. Moreover mungbean can be an export plant, but soil salinity is a major problem to legume production¹. Abiotic stress affect plant metabolism, disrupt cellular homeostasis and uncouple major physiological and biochemical processes^{2,3}.

Plants have developed a complex antioxidant system which migrate and repair the damage initiation by reactive O₂ species (ROS)^{4,5} towards enzyme synthesis,⁶ to protect the cellular and sub cellular systems from cytotoxic effects of active oxyfree radicals. The major ROS scavenging activities includes complex, non enzymatic (ascorbate, glutathione, alpha-tocopherol) and enzymatic antioxidants like catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) peroxidases (POX)⁷ etc.

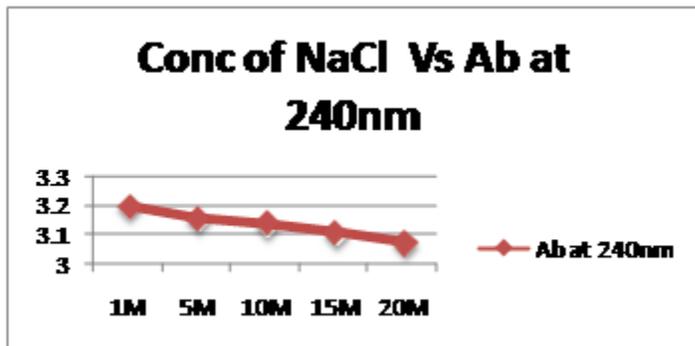
In the present study, the effect of presoaking of seeds in different concentration of salt likes NaCl and CaCl₂ solution on germination and catalase activity of *Vigna radiata* has been investigated by studying morphological changes, the decay of H₂O₂ concentration at 240 nm and compared the decay of H₂O₂ in the absence of salt stress.

2. MATERIALS AND METHODS

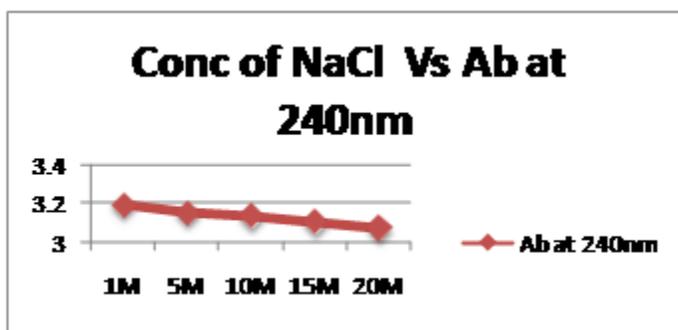
Mungbeans (*V. radiata*) weighing 5gms used in the study were obtained from local market. They were soaked in different concentrations of NaCl & CaCl₂ like (0.5, 1.0, 1.5, 2.0, 2.5M) & NaCl as (1.0, 5.0, 10.0, 15.0, 20.0 M) at normal conditions. After 24 hrs the weight of seeds was measured & kept for 2 days germination, then shoot length was measured. Then seeds were crushed through blender and extract with water (25ml) were tested for qualitative catalase activity. Further catalase assay of

respective extract was monitored in terms of decay in H₂O₂ concentration at 240nm (Jasco-V-530) and compared with the decay in absence of stress^{8,9}. For 2 minutes and is calculated by formula:

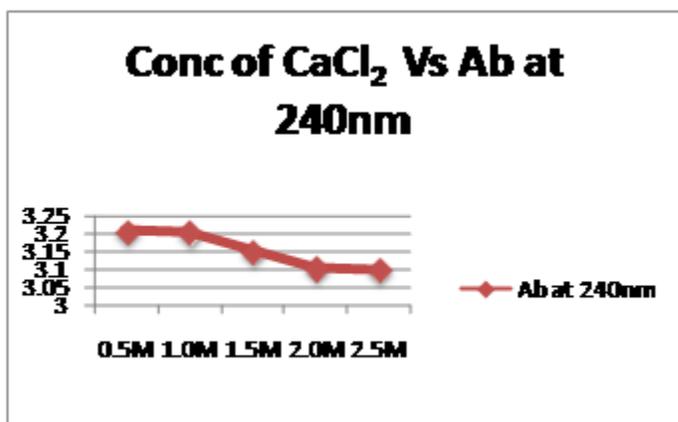
Activity in terms of K = 69/DT * log (A1/A2)



Graph 1



Graph 2



Graph 3

NaCl Concentration (M)	Weight after soaking (gm)	Weight after germination (gm)	Absorbance at 240nm	H ₂ O ₂ decay (%)
1	8.865	5.919	3.1985	43.41
5	5.399	5.058	3.1569	48.16
10	5.175	5.042	3.1376	50.77
15	5.160	5.055	3.1069	68.09
20	5.151	4.974	3.0755	85.83
Control Water	9.879	6.822	2.939	43.01

Table 1

NaCl Concentration (M)	Weight after soaking (gm)	Weight after germination (gm)	Absorbance at 240nm	H ₂ O ₂ decay (%)
0.5	8.973	6.791	4.991	55.33
1.0	8.191	6.013	4.820	55.50
1.5	8.643	5.988	4.088	55.76
2.0	8.516	5.565	3.979	55.96
2.5	8.464	4.179	3.797	57.25

Table 2

CaCl ₂ Concentration (M)	Weight after soaking (gm)	Weight after germination (gm)	Absorbance at 240nm	H ₂ O ₂ decay (%)
0.5	8.095	6.000	3.2087	45.67
1.0	8.144	5.718	3.2040	46.67
1.5	7.676	5.690	3.1514	48.85
2.0	7.660	5.188	3.1036	51.23
2.5	6.109	4.109	3.0989	51.43

Table 3

Catalase activity:-

Catalase activity was measured according to method of LizaA, Ewe, Acbi (1984). The assay mixture contain 2.9ml of 50mM Potassium phosphate buffer containing 10.3mM H₂O₂ of PH 7.0, 100µl of extract (~2-4mg/ml protein) and decomposition of H₂O₂ was monitored for 2 minutes against blank (2.9ml, 59mM phosphate buffer without H₂O₂).

3. RESULTS AND DISCUSSION:

The effects of different concentrations of NaCl and CaCl₂ on germination of mung beans are shown in Table no 1, 2 and 3. The graph of concentrations Vs absorbance shows that there is increase in concentration of NaCl and CaCl₂ with decrease in percentage of germinations and with increase in catalase activity in terms of decay of H₂O₂. The reduction in shoot and root lengths in different plants species with progressive increase in salinity stress has been reported.^{10, 11, 12, 13}. In the present study it was found that salinity caused a significant effect on the normal growth and development of mungbean seedlings. Salinity results in enhanced generation of the reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical; hydrogen peroxide. Plants under stress shows some defense mechanisms to protect themselves against the harmful effect of salt stress. In this salt stress altered the antioxidant enzyme activity as compared to control. The significant increase in catalase activity may be due to H₂O₂ which is one of the ROS produced in response to different environment stress including salt and ionic stress and there is significant reduction of shoot lengths caused by salts stress. These antioxidant enzymes show a role in imparting tolerance against salt stress and any type of environmental stress¹⁴.

4. CONCLUSION:

It has been revealed that at different concentrations of NaCl and CaCl₂ shows enhancement of catalase activity and 0.5M, 1.0M shows maximum activity than other concentrations against control water.

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Conflict of Interest: None Declared