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A COMPARATIVE STUDY ON THE EFFECT OF SALINE STRESS ON PEROXIDASE ACTIVITY IN GERMINATING *Vigna radiata* (L.)R. Wilczek and *Vigna mungo* (L.)Hepper SEEDS

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ABSTRACT :

Salinity is a serious concern globally as it is a major factor limiting agricultural productivity. Salinity stress triggers a wide range of plant responses affecting cellular structures and metabolism. The influence of increasing saline stress on peroxidase enzyme in Vigna radiata (L.)R. Wilczek and Vigna mungo (L.)Hepper seeds were investigated. The seeds were germinated in Hoagland's solution containing 25mM, 50mM, 75mM and 100mM NaCl. Samples were taken out at 24 hour intervals for 6 days and enzyme and protein assay was done. It was found that in both Vigna radiata and Vigna mungo the peroxidase activity increased as the duration and the concentration of NaCl was increased. The percentage of enzyme activity over control was also found to be increasing in both plants with increasing salt concentration and duration of treatment. The percentage of peroxidase activity over control was found to be more in Vigna radiata than in Vigna mungo. The amount of protein was found to be more in 100mM NaCl treated seedlings and the amount decreases as the duration increases in both Vigna radiata and Vigna mungo. Enzyme activity in units/mg protein was calculated and was found to be more in Vigna mungo. These results indicated that the peroxidase activity was clearly related to the ability of survival in these plants and the ability to detoxify H_2O_2.

KEYWORDS : *Vigna radiata, Vigna mungo, stress, salinity, peroxidase activity, protein concentration.*

Abbreviations: mg- milli gram, gfw- gram fresh weight.

INTRODUCTION

Salinity is one of the most important abiotic stresses, particularly relevant these days because of decreasing fresh ground water. Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone (Sangeetha and Subramani, 2014). Salinity reduces the ability of plant to take water and this causes reduction in growth rate along with a suite of metabolic changes (Arulbalachandran et.al, 2009). Increased salinity induces osmotic as well as toxic effects and hence affects growth and other physio-biochemical process including photosynthesis (Bhaskar and Bingru, 2014).

The Asiatic *Vigna* species consists of several grain legumes that are native to tropical regions of new world. A number of them are of considerable economic importance in many developing countries. Abiotic stresses such as high salinity is a major cause of their yield losses and significantly affect their sustainable production. *Vigna radiata* (L.)R. Wilczek (green gram) and *Vigna mungo* (L.)Hepper (black gram) are two of the most important grain legumes of *Vigna* genus grown in increasingly hostile soil, with salinity conditions both expected to worsen in future. Both green gram and black gram are classified as glycophytes (salt-sensitive). Despite its great importance, very little attention has been given to the improvement of salt tolerance in these crops. One of the major approaches for improving salt tolerance of crop species is the examination of variation within existing crop cultivars. The promising cultivars/lines can be exploited either for direct use in moderately saline soils, or for use in selection or breeding programmes to make further

advancement in salt tolerance. Exploitation of genetic variation of phenotypic traits through molecular breeding and plant translational genomics, that use knowledge and genes discovered in model plants, could facilitate improving tolerance in these two crops to salinity (Sagarika et.al, 2014).

Peroxidases are enzymes of hemoproteinaceous nature, that catalyse the oxidation of various organic compounds. Peroxidase is reported to consists of 300 amino acid residues, a couple of glucosamines, around 8 true sugars, 2-6 glycosylation sites, one protohematin and 2 bound calcium ions. Peroxidases are thermally stable and the isoelectric points range from 3.5 to 10. They are widely distributed in plants and play roles in growth, development and lignifications. Both negative and positive correlations have been shown between the soluble peroxidase level and the growth rate of tissues.

Peroxidase activity and/or its isoenzyme patterns alter with changes in plant development. Peroxidase have also been implicated in various types of stresses . Increased peroxidase activities has been cited as an indicator of physiological stress . While screening various plant families for salt tolerant phenotypes , peroxidase activity was investigated as an indicator of salt stress in young seedlings .

Morphological description of plant cultivars often present problems in clear cut identification because of the phenotypic differences within species / varieties are too minute to discriminate . Ideally the differences between cultivars should be based on the gene differences but direct comparison of genes is difficult and time consuming. However, the differences can be measured by comparing the products of gene activity, that is by using proteins as genotype markers. Genetic studies have demonstrated that plant isoenzymes, like many morphological and physiological traits, are conditioned by one or few genes. The study of protein polymorphisms through isozyme analysis provides a powerful tool for assessing the genetic variability.

MATERIALS AND METHODS:-

PLANT MATERIAL: The plant materials chosen for this study were seeds of *Vigna radiata* and *Vigna mungo*.

GROWTH CONDITIONS: The seeds were surface sterilized with 0.1% mercuric chloride and soaked overnight in water. The seeds were allowed to germinate on a wire mesh on a beaker containing Hoagland's solution (Hoagland and Arnon, 1938) and were grown upto 6 days. Sodium chloride was used as the salt stress. Seeds were grown in Hoagland's solution containing 25, 50, 75 and 100mM concentrations of NaCl. The germinated seeds were taken out at 24 hour intervals from 1^{st} to 6^{th} day and protein estimation and enzyme assays were performed.

ENZYME EXTRACTION : One gram of fresh plant material (seedling) containing both shoots and roots were homogenized in 10ml of 0.1M Tris HCl buffer, pH 7.5 at 4^0C. The homogenate was strained through muslin cloth and centrifuged at 5000rpm for 20 minutes. The supernatant was collected and used as the crude enzyme preparation. This is used as a source for enzyme assay and protein estimation.

ASSAY OF PEROXIDASE: To 0.5ml of the enzyme extract 3.0ml of 0.1M sodium phosphate buffer (pH 7.0) solution and 0.1 ml of 20mM guaiacol solution was added. Using the assay mixture autozero was adjusted at 430nm. Then 20µl of H_2O_2 (30% v/v) was added to the mixture and time taken for the absorbance to increase by 0.05 (Δt) was recorded. The enzyme activity was expressed in terms of rate of increased absorbance per unit time per litre of the sample. (Malik C. P and Singh M. B, 1980).

One unit of enzyme activity is defined as the amount of enzyme which produces a change of 0.05 absorbance at 430nm/minute of incubation.

QUANTIFICATION OF PROTEIN: Protein concentration was estimated by the method of Lowry et al., (1951), using Bovine Serum Albumin (BSA) as standard.

RESULTS AND DISCUSSION:-

Antioxidant enzyme, peroxidase has been the focus of present investigation to understand the mechanism of salt tolerance at biochemical level. The endogenous level of peroxidase was assayed in both control and treated seedlings. It was observed that the peroxidase activity increased over the control in 25mM, 50mM, 75mM and 100mM NaCl treated seedlings of both *Vigna radiata* and *Vigna mungo* as the

duration of the treatments were increased. The maximum enzyme activity was obtained in 75mM and 100mM NaCl treated seedlings on the sixth day in both *Vigna radiata* and *Vigna mungo.*

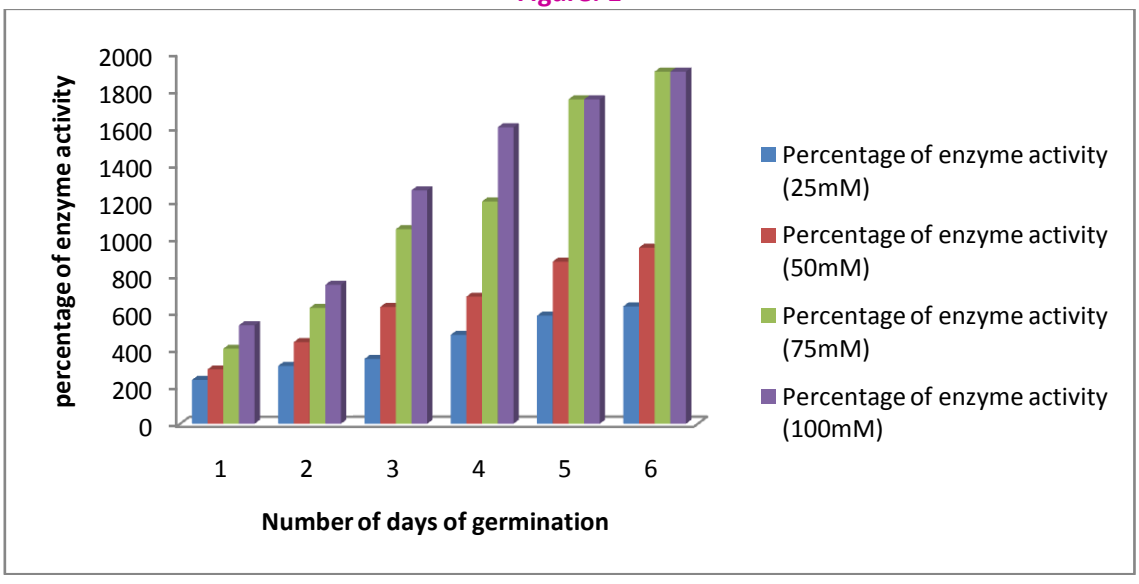
The percentage of enzyme activity over control was also found to be increasing in both plants with increasing salt concentration and duration of treatment. But a deviation was found in 75mM and 100mM NaCl treated seedlings. The percentage of enzyme activity was found to be the same for both 75mM and 100mM treated seedlings on the sixth day in *Vigna mungo* where as in *Vigna radiata*, it was found to be the same on 5^{th} and 6^{th} days of treatment (figure 1 and 2). When the percentage of enzyme activity over control was compared between *Vigna radiata* and *Vigna mungo,* the former showed a significant increase over the other in all salt concentrations and durations of treatment (figure 3,4,5 and 6). Hence, this investigation showed that NaCl stress tolerance was more in *Vigna radiata* rather than *Vigna mungo.*

These results indicated that the peroxidase activity was clearly related to the ability of survival in each plant and the ability to detoxify H_2O_2. These results correlates with the findings of Guru devi et. al (2012) in *Vigna mungo* and Papiya et. al (2010) in *Vigna radiata.*

The amount of protein (mg/gfw) was found to be more in *Vigna radiata*. It was observed that the amount of protein was more in treated seedlings than the control in both *Vigna radiata* and *Vigna mungo*. The amount of protein was more in 100mM NaCl treated seedlings. This may be due to decreased proteolysis caused by salinity leading to slower depletion of reserve proteins and not as a result of enhanced protein synthesis. (Dubey and Manju, 1989; Mohammed, 2016). But as the duration increases the protein concentration decreases in control and 50mM, 100mM and 150mM NaCl treated seedlings. This is in confirmation with the findings of Arvinder and Matta (2014).

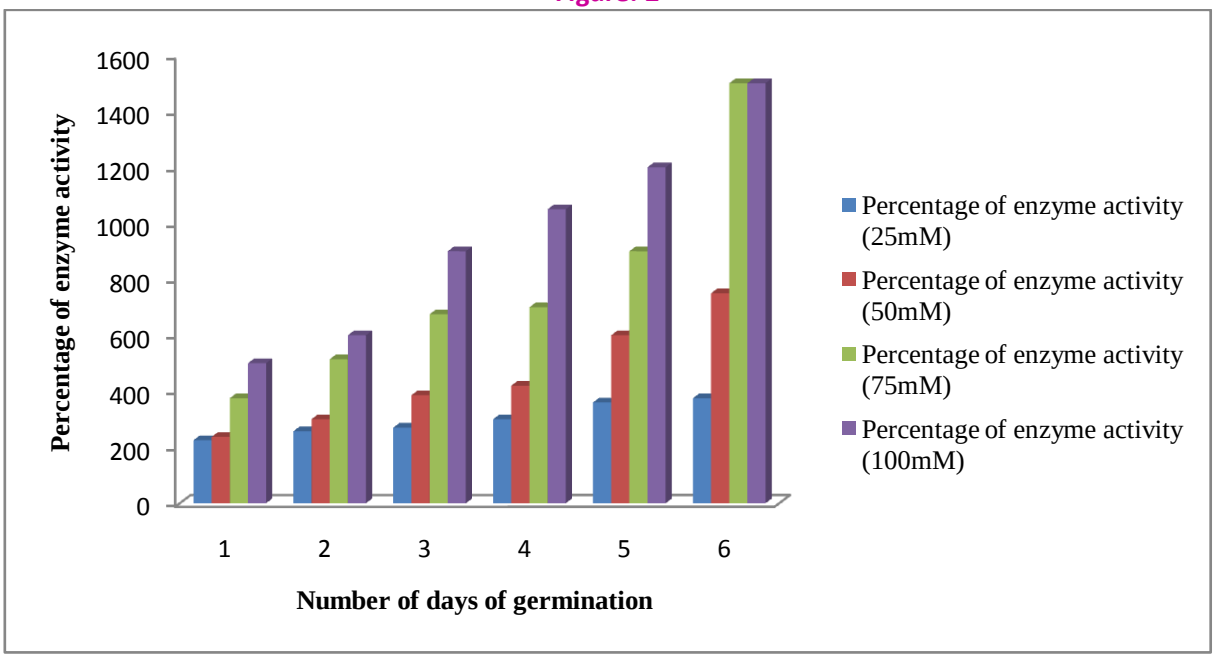
Enzyme activity in units/mg protein was calculated and was found to be more in *Vigna mungo* than *Vigna radiata* (figure 7and 8). In the case of *Vigna radiata* the enzyme activity decreased from 0.0595 to 0.0581 units on the fifth day and from 0.1389 to 0.1351 units on the sixth day of 100mM NaCl treatment when compared with the 75mM treated seedlings (figure7). In the case of *Vigna mungo* the enzyme activity (units/mg protein) decreased from 0.3846 to 0.3333 units only on the sixth day in the 100mM NaCl treatment when compared with the 75mM NaCl treatment (figure 8). This decline in activity may be due to lower tolerance to salt-induced oxidative stress with advancing age.

Figure: 1



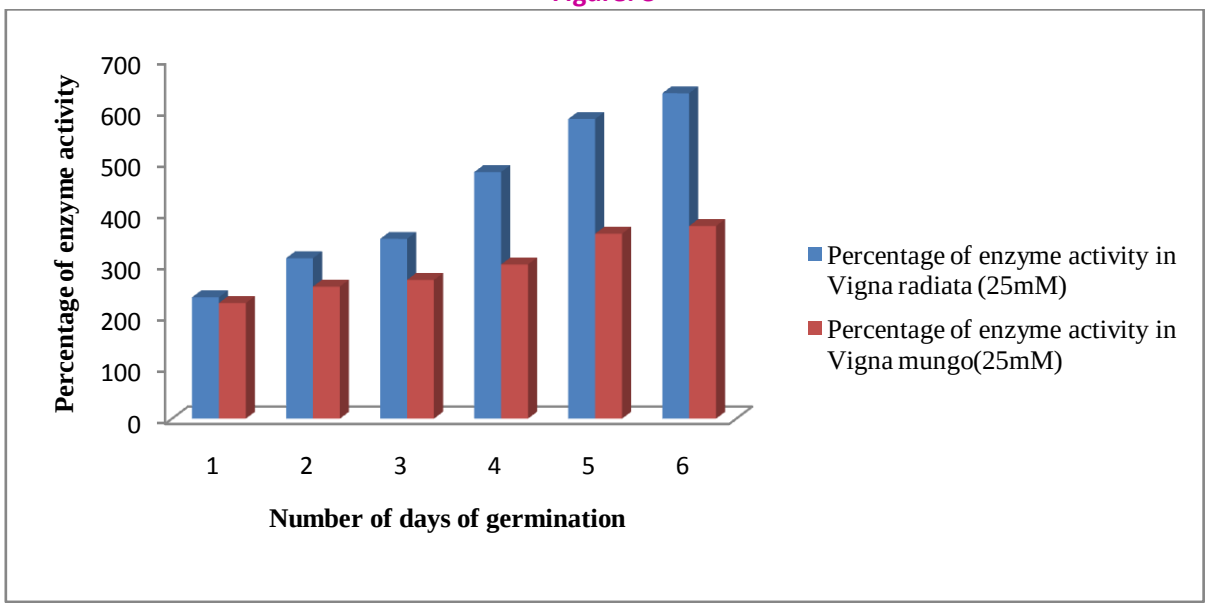
Comparison of percentage of enzyme activity in germinating *Vigna radiata* seeds treated with 25mM, 50mM, 75mM and 100mM NaCl

Figure: 2



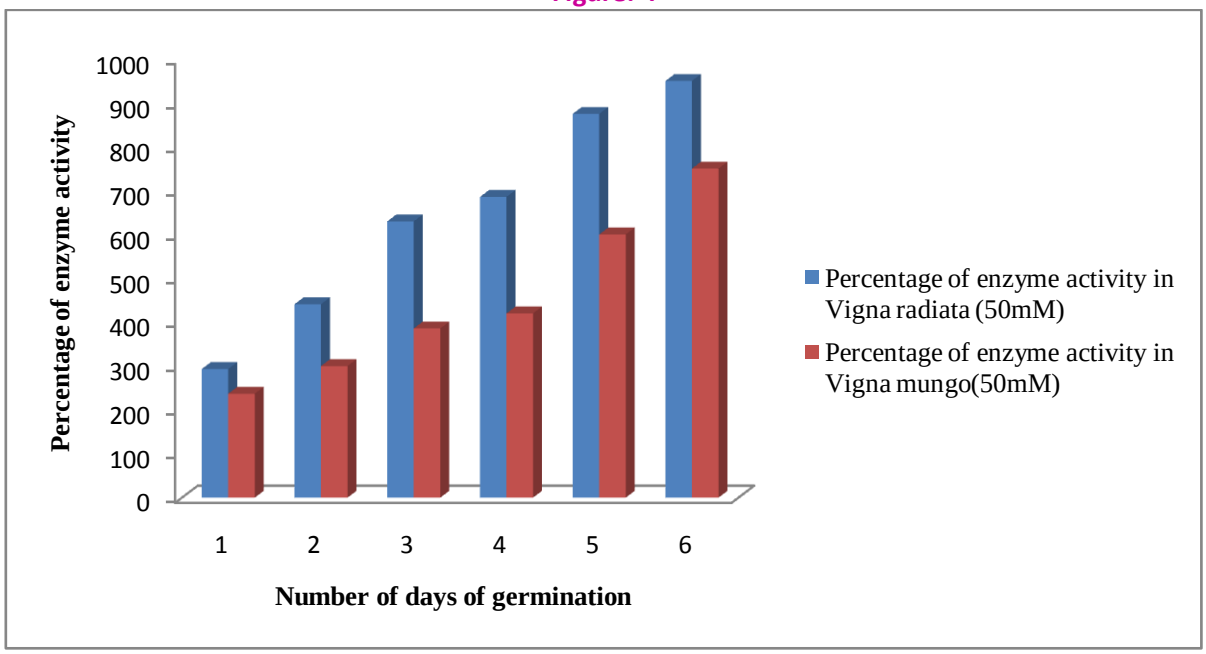
Comparison of percentage of enzyme activity in germinating *Vigna mungo* seeds treated with 25mM, 50mM, 75mM and 100mM NaCl

Figure: 3



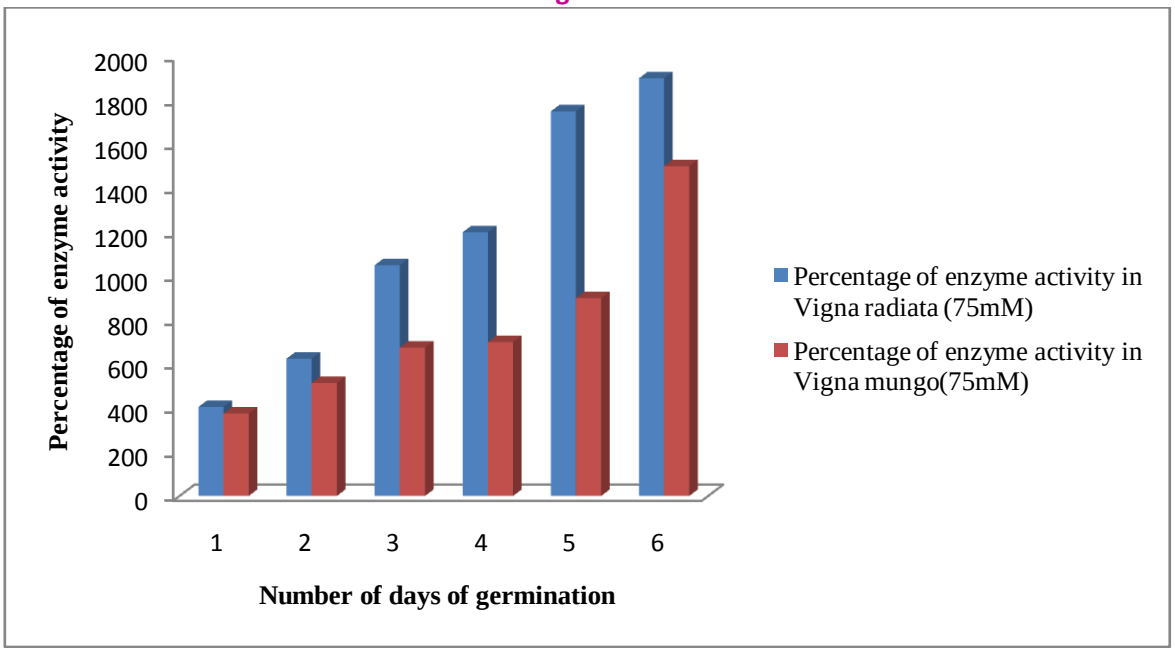
Comparison of percentage of enzyme activity in germinating *Vigna radiata* and *Vigna mungo* treated with 25mM NaCl

Figure: 4



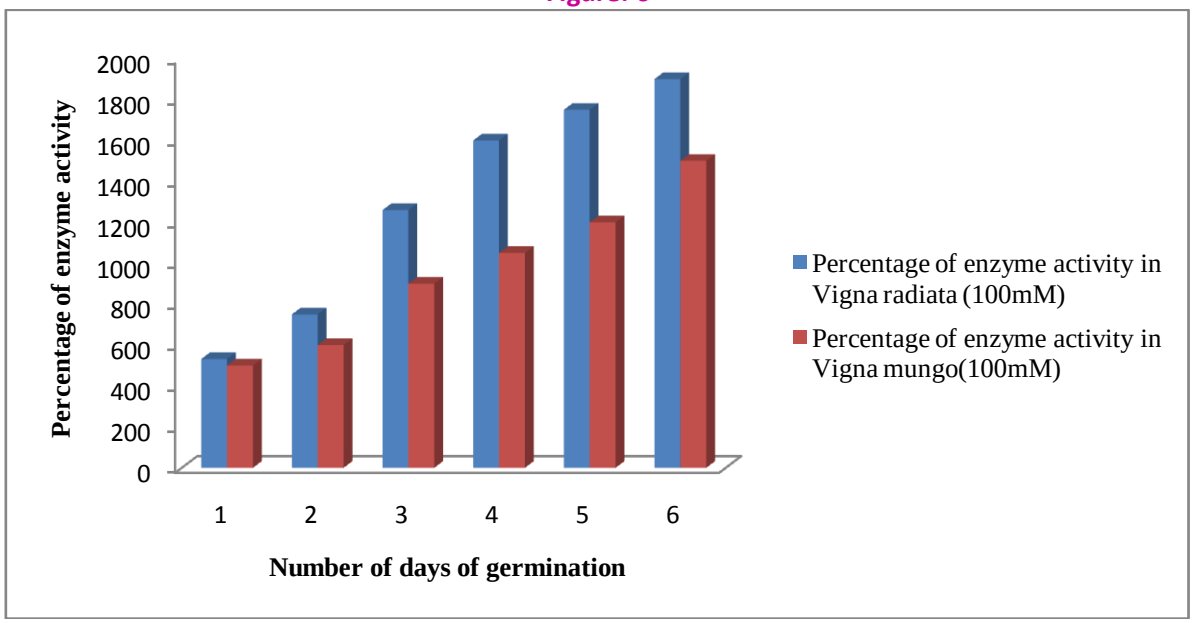
Comparison of percentage of enzyme activity in germinating *Vigna radiata* and *Vigna mungo* treated with 50mM NaCl

Figure: 5



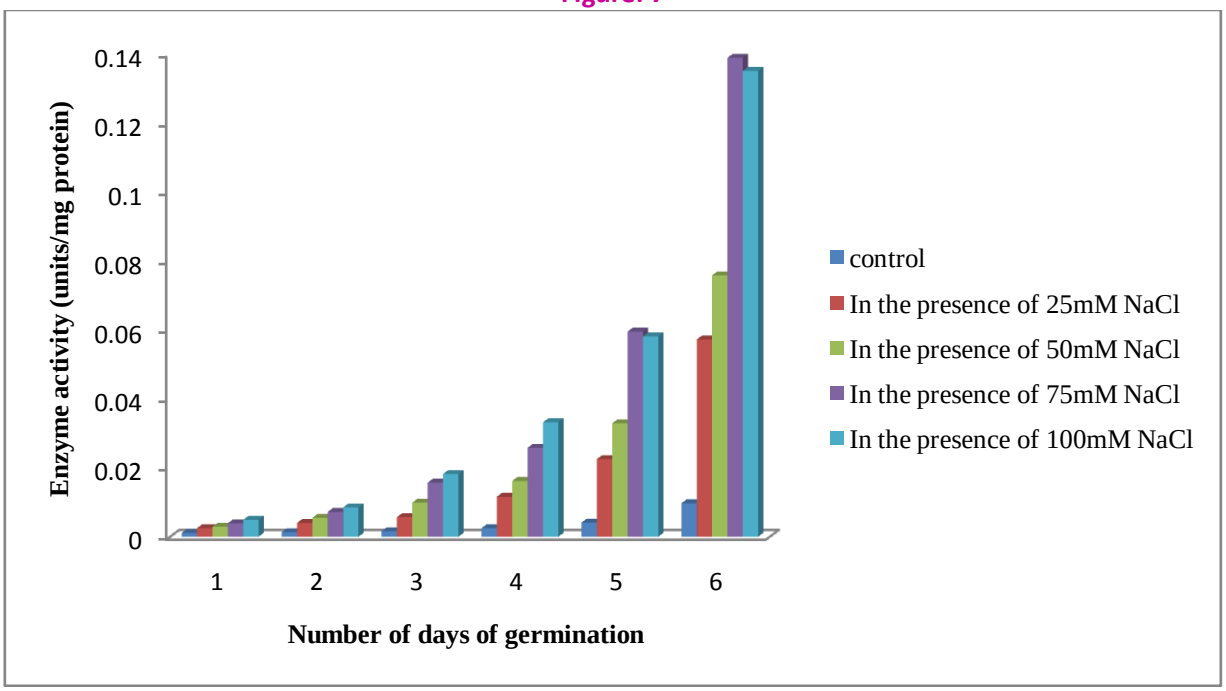
Comparison of percentage of enzyme activity in germinating *Vigna radiata* and *Vigna mungo* treated with 75mM NaCl

Figure: 6



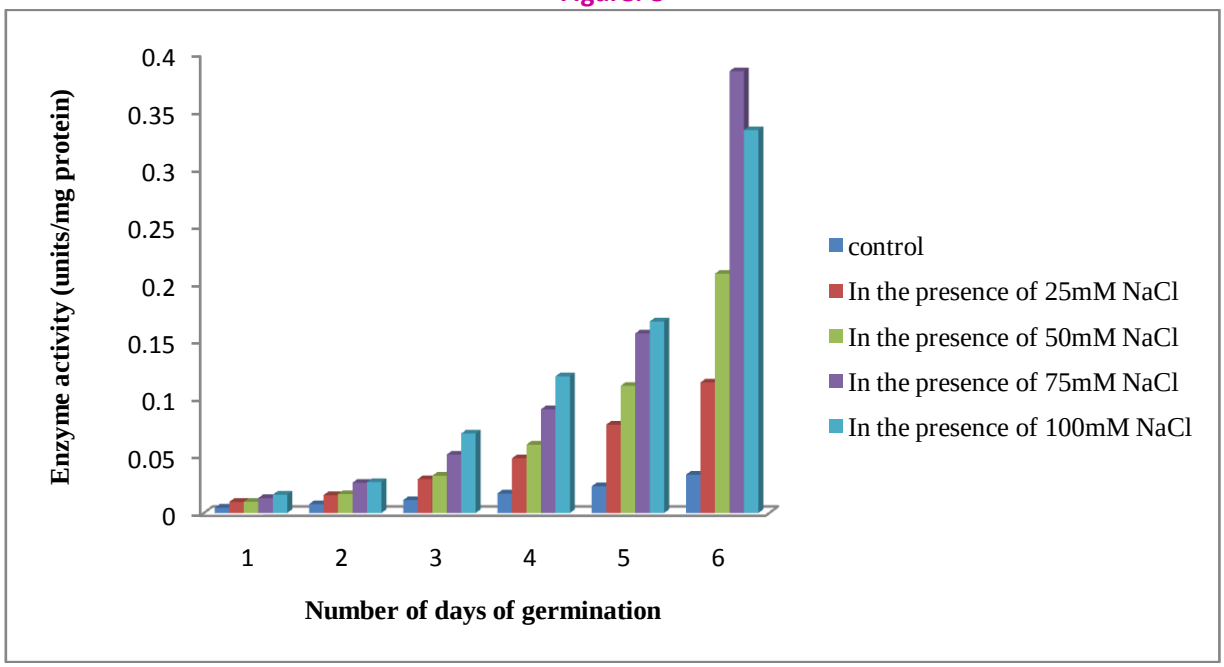
Comparison of percentage of enzyme activity in germinating *Vigna radiata* and *Vigna mungo* treated with 100mM NaCl

Figure: 7



Developmental patterns of peroxidase in *Vigna radiata* during seed germination

Figure: 8



Developmental patterns of peroxidase in *Vigna mungo* during seed germination

CONCLUSION:

The study concludes that environmental stress such as salt greatly influence the activity of the enzyme peroxidase. Salinity cause oxidative stress which affect the biochemical and enzymatic components in plant cell. All these results may possibly be due to the salt tolerant mechanism adapted by the plants to combat efficiently the increased quantity of oxygen radicals produced under stress. The enhanced activity of antioxidant systems play a role in the ability of plants to withstand high salt stress. However, further study is necessary to understand the regulatory mechanism of the antioxidant enzyme, peroxidase at gene level that would give a possible insight in intercellular and intracellular molecular interaction to salinity stress. Development of salt tolerant green gram and black gram could play an important role in ensuring dietary protein supply, improving human health and nutrition, and enhancing ecosystem resilience, especially in developing countries of Asia.

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