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# A COMPARATIVE STUDY ON THE EFFECT OF SALINE STRESS ON PEROXIDASE ACTIVITY IN TWO VARIETIES OF GERMINATING RICE (POKKALI) – *Oryza sativa* var.*V1* and var.*V2*

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***Abstract:*** **The effect of increasing saline stress on peroxidase enzyme was investigated in two varieties of rice (Pokkali), *Oryza sativa* var.*V1* and *Oryza sativa* var.*V2*. The seeds were germinated in Hoagland's solution containing 50mM, 100mM and 150mM NaCl. Samples were taken out at 24 hour intervals for 10 days and enzyme and protein assay was done. It was found that the peroxidase activity increased in both *Oryza sativa* var.*V1* and *Oryza sativa* var.*V2* with the increase in the duration and the concentration of NaCl. The percentage of enzyme activity over control was also found to be increasing in both varieties. The percentage of peroxidase activity over control was found to be more in *Oryza sativa* var.*V1*. The amount of protein was found to be more in 150mM NaCl treated seedlings and the amount decreases as the duration increases in both. Enzyme activity in units/mg protein was calculated. Among these two varieties, *Oryza sativa* var.*V1* was found to be more tolerant towards salt stress. These results indicated that salt-tolerant varieties exhibit protection mechanism against increased radical production by maintaining the activity of antioxidant enzyme peroxidase.**

***Keywords: Oryza sativa*, Pokkali, salinity, antioxidant enzymes, peroxidase.**

## I. INTRODUCTION

Soil salinity is a major threat to global food security. Up to 20% of the world's irrigated land, which produces one third of the world's food is influenced by salt stress. *Oryza sativa* L. is one of the most important food crops globally and is considered to be the primary staple food for half of world's population [21]. Salt tolerance is an important constrain for rice, which is generally categorized as a typical glycophyte. Soil salinity is one of the major problems affecting rice production worldwide, especially in the coastal areas [7].

Rice plants are sensitive to salt stress, particularly at the seedling and reproductive stages. However, rice genotypes differ in their sensitivity to salt stress [14]. Extensive research is being carried out worldwide for further improvement of rice cultivars to adapt according to the environment with high yield. Exploring the physiological and biochemical mechanism of salinity could be helpful in the selection of rice cultivar for the agriculturists as well as breeders. Variation in terms of salt tolerance is a common phenomenon in rice [21].

The term Pokkali refers to a salt tolerant traditional 'rice cultivar' grown in the coastal saline soils of Kerala, South India. Pokkali tracts are low lying marshes located near the estuaries of rivers and are close to the sea. The rice is cultivated

from June to early November when the salinity level of the water in the fields is low. The organically-grown Pokkali is famed for its peculiar taste and its high protein content.

Increasing respiration induced by salt stress enhances destructive ions production in mitochondria. To protect against these toxic oxygen intermediates, plant cells and its organelles like chloroplast, mitochondria and peroxisomes employ antioxidant defense systems. A great deal of research has established that the induction of the cellular antioxidant machinery is important for protection against various stresses [8], [19]. Many researches proved that plants are equipped with a diverse array of antioxidant enzymes [12].

Salinity affects plant growth and development in two ways. First, it imposes osmotic stress by reducing the soil water potential leading to limiting the water uptake. Second, it causes excessive uptake of ions particularly Na+and Cl−that ultimately interferes with various metabolic processes. Reactive oxygen species (ROS) are regarded as the main source of damage to cells under biotic and abiotic stresses [11], [20]. ROS's are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration [11], [12]. However, plants have their own complex antioxidant system in both enzymatic and non-enzymatic response mechanisms as a defence strategy to combat and repair the damage caused by ROS [2], [6], [18],[21].

Over the past two decades increasing attempts have been made to develop salt resistant varieties , which are able to grow under saline conditions. The effect of salinity on plant processes are three fold in terms of water stress from the osmotic effects, mineral toxicity of the salt and interruptions to the mineral nutrition of the plant. The elevated peroxidase activity is commonly associated with the variety of stresses in plants including hypogravity, hypergravity, vibration, hypoxia, chilling, freezing, radiation and infection.

The main objective of this study was to investigate the response of two Pokkali rice varieties, *Oryza sativa* var.*V1* and *Oryza sativa* var.*V2* to saline stress by assessing their peroxidase enzyme activities during vegetative growth stage.

## II. MATERIALS AND METHODS

**1. PLANT MATERIAL:** The plant materials chosen for this study were seeds of *Oryza sativa* var.*V1* and *Oryza sativa* var. *V2* (Pokkali ) and were obtained from the Rice Research Institute, Vytilla, Kerala.

**2. 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 [5] and were grown upto 15days. Sodium chloride was used to induce the salt stress. Seeds were grown in Hoagland's solution containing 50mM, 100mM and 150mM concentrations of NaCl. The germinated seeds were taken out at 24 hour intervals for 10 days from 6 $^{th}$ to 15$^{th}$ day and protein estimation and enzyme assays were performed.

**3. 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 $4^0$C. 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 was used as a source for enzyme assay and protein estimation.

**4. 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 [10].

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.

**5. QUANTIFICATION OF PROTEIN:** Protein concentration was estimated by the Lowry's method using Bovine Serum Albumin (BSA) as standard [9].

## III. RESULTS AND DISCUSSION

Antioxidant enzyme, peroxidase has been the focus of present investigation to understand the mechanism of salt tolerance at biochemical level. In both control and treated seedlings the endogenous level of peroxidase was assayed.

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The enzyme activity (units/litre) was found to be the maximum on the 15th day in both the control and 50, 100, and 150mM NaCl treated samples. In *Oryza sativa* var.*V1* the maximum enzyme activity obtained was 263.16 units/litre in 150mM NaCl treated seedlings on the 15th day and in *Oryza sativa* var.*V2* it was found to be 250.00 units/litre. It was observed that the peroxidase activity significantly increased over the control in 50mM, 100mM and 150mM NaCl treated seedlings as the duration of the treatments were increased in both *Oryza sativa* var.*V1* and *V2*. When the enzyme activities (units/litre) of *Oryza sativa* var.*V1* and *V2* were compared it was found to be more in *V2* in all the treatments except 150mM treatment. In 150mM treated seedlings from the 10th day of treatment onwards, the enzyme activity was found to be more in *Oryza sativa* var.*V1*.

With the increasing salt concentration and duration of treatment, the percentage of enzyme activity over control was also found to be increasing in both varieties. In both significant difference was seen between 100mM and 150mM NaCl treated seedlings (figure 1 and 2). The percentage of enzyme activity was found to be more in *Oryza sativa* var.*V1* than *V2* in all salt concentrations and durations of treatment. But a deviation from this result was seen in 50mM NaCl treated seedlings. During the sixth and seventh days of treatment in 50mM NaCl treated seedlings, the percentage of enzyme activity over control was found to be more in *Oryza sativa* var.*V2*.(figure 3, 4, and 5). These results clearly showed that *Oryza sativa* var.*V1* was more tolerant towards salt stress during this study.

All 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 Swapna (2003) in Pokkali, M148 and Jyoti [17], Djanaguiraman et.al (2004) in Pokkali, Co43, ADT38 and IR50 [3] and Sankhalkar and Vernekar (2016) in Jyoti [16].

Protein estimation was done for both the control and treated samples for 10 days from 6 th to 15th day. The amount of protein (mg/gfw) was found to be more in *Oryza sativa* var.*V1*. It was observed that the amount of protein was more in treated seedlings than the control. The amount of protein was more in 150mM 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. [4], [13]. But as the duration increases the protein concentration decreases in control, 50mM, 100mM and 150mM NaCl treated seedlings. This is in confirmation with the findings of Arvinder and Matta [1].

The enzyme activity in units/mg of protein is calculated for both the control and the treated samples. The enzyme activity (units/mg protein) was found to be increasing in both *Oryza sativa* var.*V1* and *V2* with the increasing salt concentration and with the duration of treatment. The enzyme activity (units/mg protein) was found to be more in *Oryza sativa* var.*V2* (figure 6 and 7).

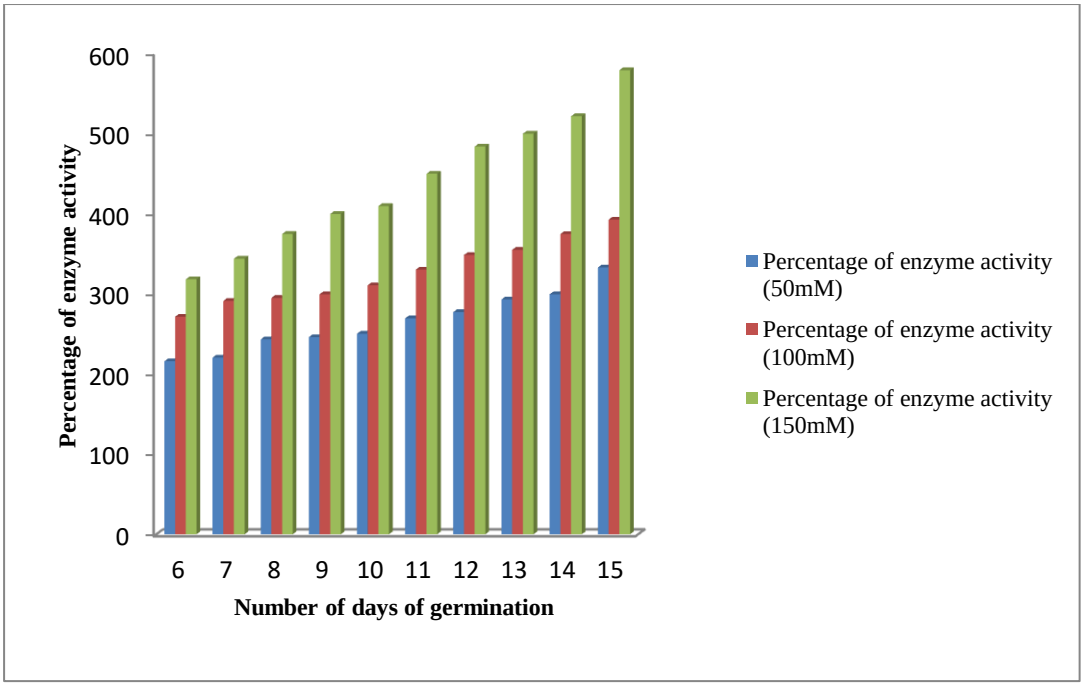


**Fig. 1. Comparison of percentage of enzyme activity in germinating *Oryza sativa* var.*V1*seeds treated with 50mM, 100mM and 150mM NaCl**

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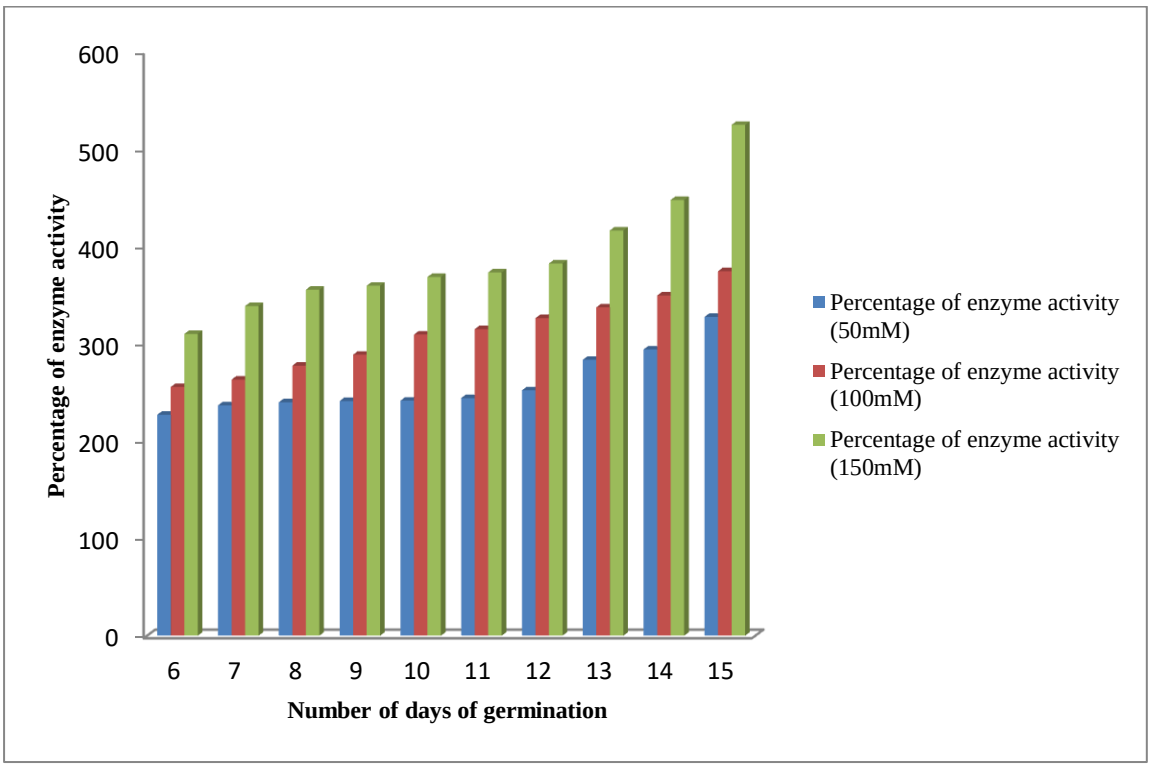


**Fig. 2. Comparison of percentage of enzyme activity in germinating *Oryza sativa* var.*V2* seeds treated with 50mM, 100mM and 150mM NaCl**

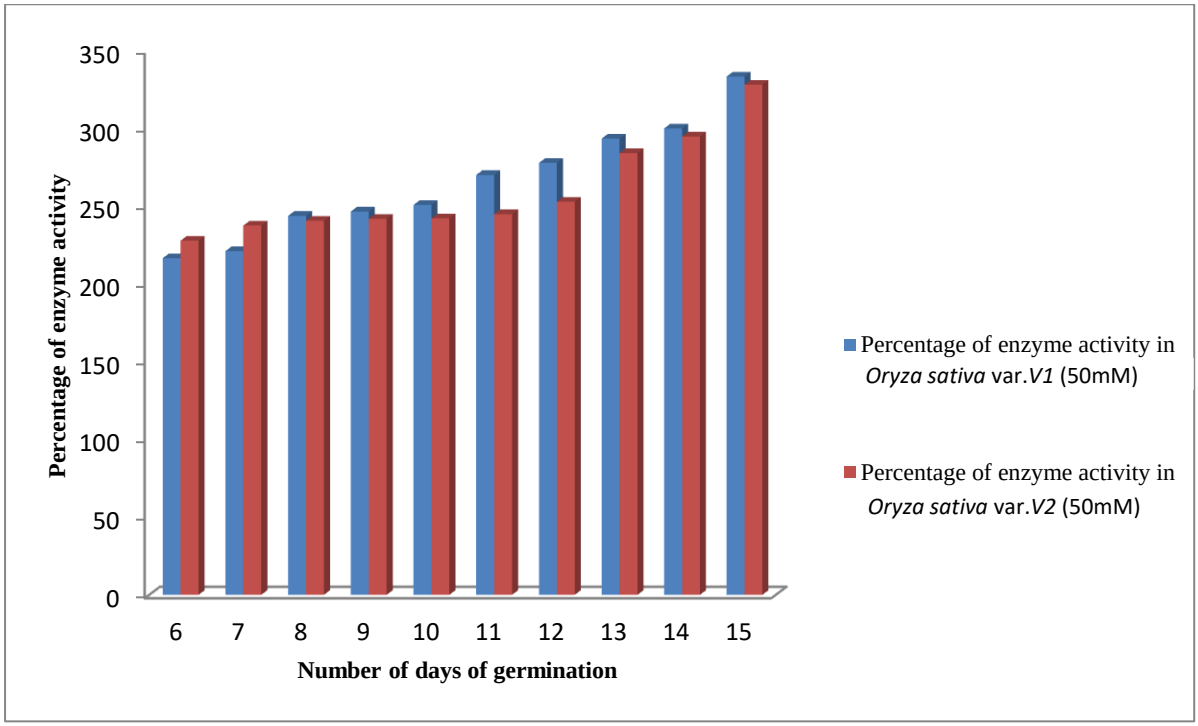


**Fig.3. Comparison of percentage of enzyme activity in germinating *Oryza sativa* var. *V1* and *V2* treated with 50mM NaCl**

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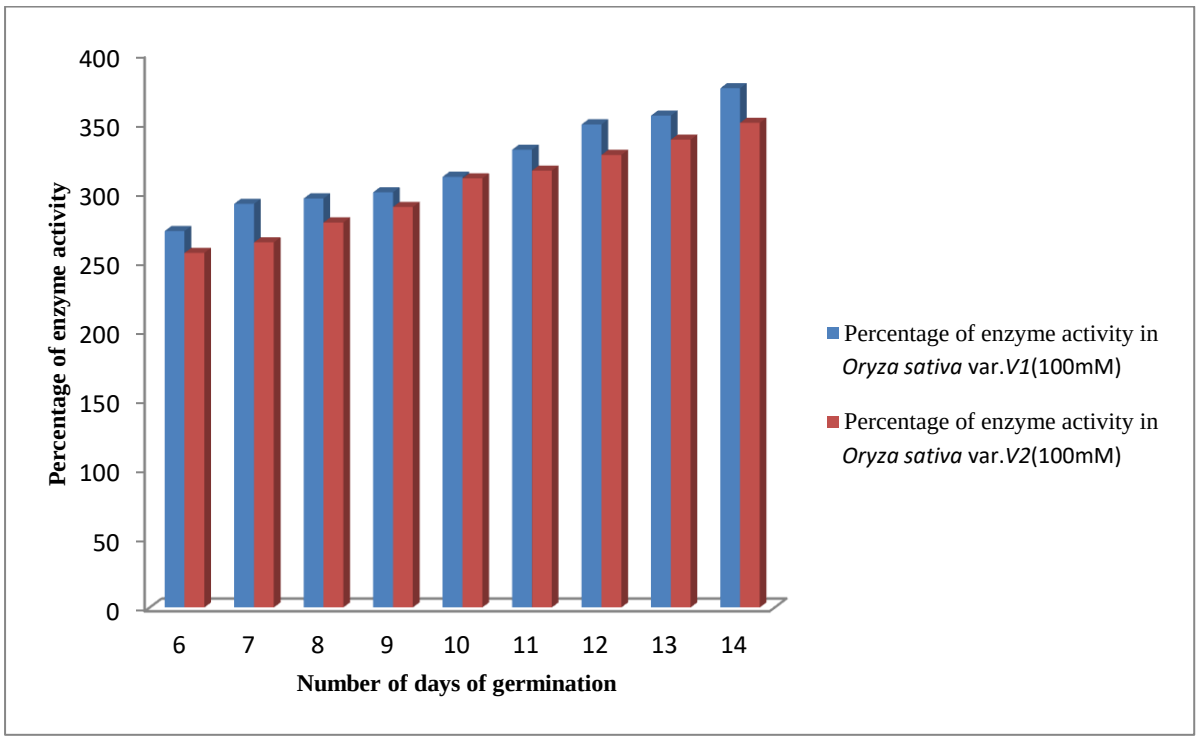


**Fig.4. Comparison of percentage of enzyme activity in germinating *Oryza sativa* var. *V1* and *V2* treated with 100mM NaCl**

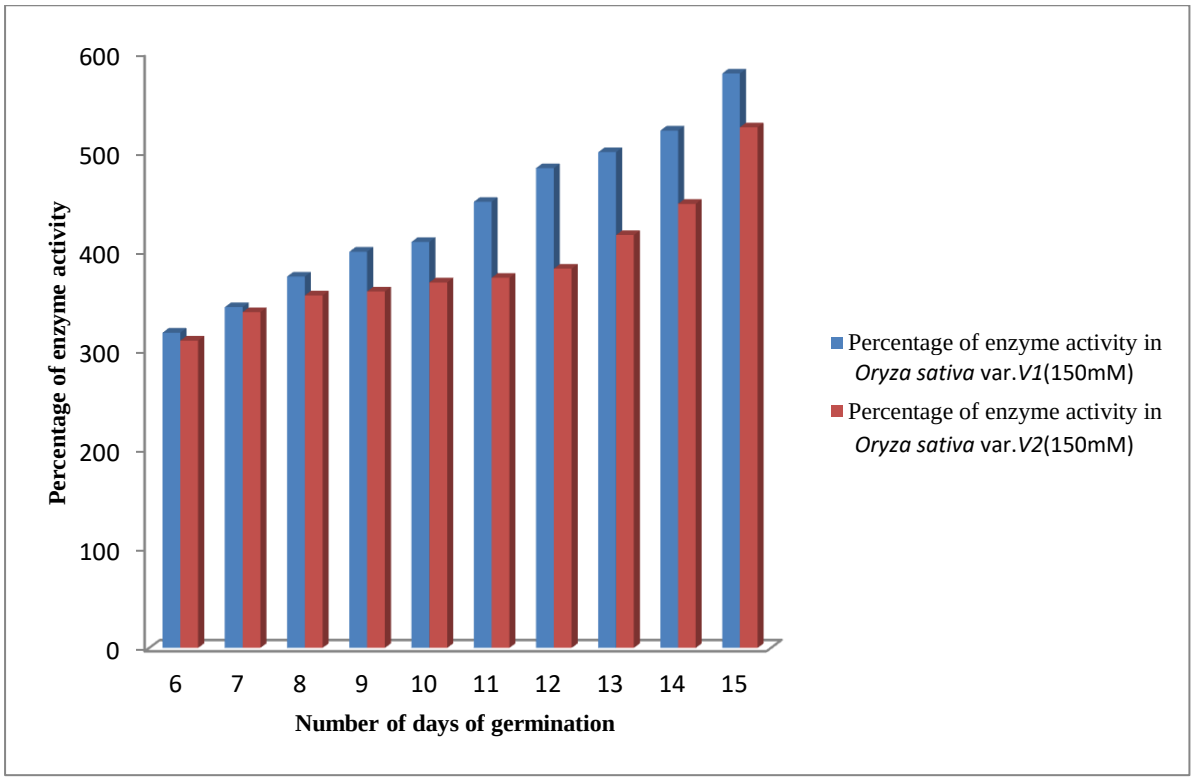


**Fig.5 Comparison of percentage of enzyme activity in germinating *Oryza sativa* var. *V1* and *V2* treated with 150mM NaCl**

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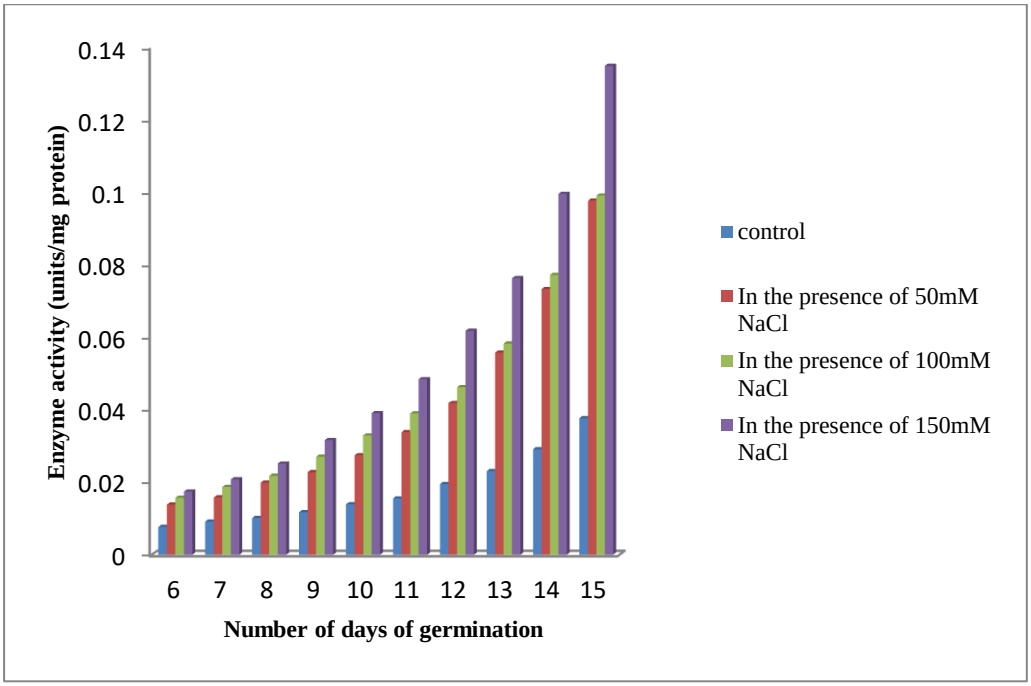


**Fig.6 Developmental patterns of peroxidase in *Oryza sativa* var. *V1* during seed germination**

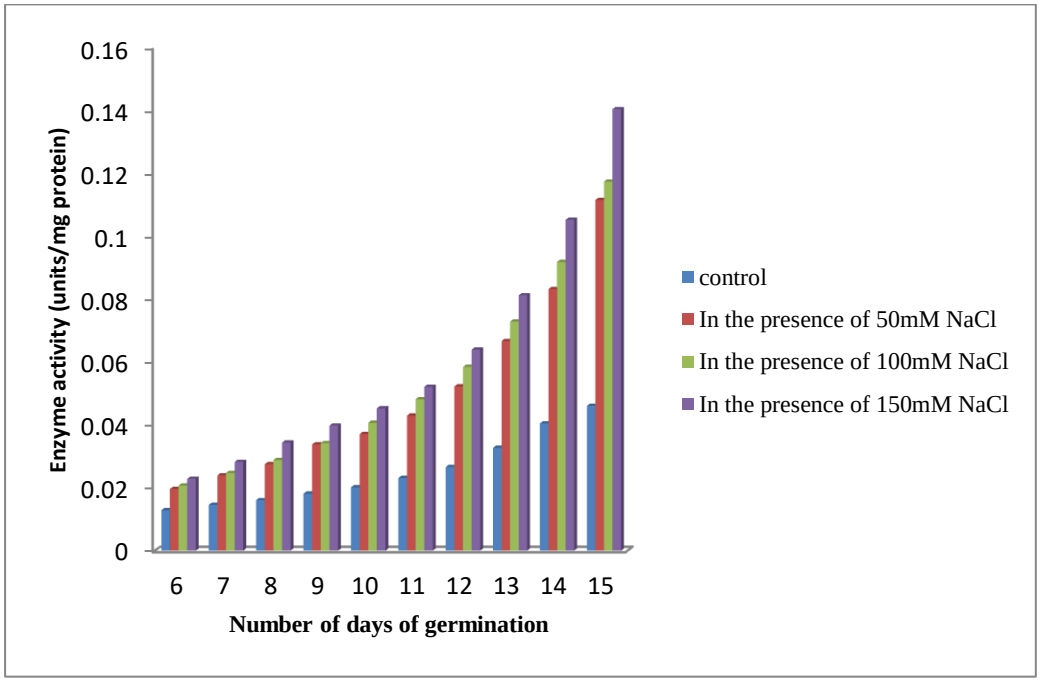


**Fig.7 Developmental patterns of peroxidase in *Oryza sativa* var. *V2* during seed germination**

## IV. CONCLUSION

From the above study it was concluded that the environmental stress such as salinity resulted in varied production of the antioxidant enzyme peroxidase. Among the two rice varieties, Oryza *sativa* var.*V1* showed better defence mechanism against salt stress by the elevated levels of peroxidase activity. The increase in the level of the enzyme activity could be due to the fact that the basal level of peroxidase activity is insufficient to combat the increased hydrogen peroxide under stress conditions. This may be the reason for these varieties to successfully establish itself under saline conditions. However, further study is necessary to understand the regulatory mechanism of the antioxidant enzyme, peroxidase at gene level. Isolation and identification of salt tolerant genes from traditional non hybrid rice varieties can thus be a better tool for plant breeding and genetic engineering program.

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