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Short Communications

Activity of catalase and peroxidase in *Oryza sativa* treated with $CuSo_4$ and SA

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Abstract

Oryza sativa is an important food crop of the world and primary stable food for over two billion people. Catalase is unique among H_2O_2 degrading enzymes as it can degrade H_2O_2 without consuming cellular reducing equivalents. Hence, catalase provides the cell with a very energy-efficient mechanism to remove H_2O_2. Peroxidases are widely distributed in nature and are found in plants, micro-organisms and animals. H_2O_2 is a common end product of oxidative metabolism. Peroxidases serve to rid plant cells of excess H_2O_2 under normal and stress conditions. The catalase and peroxidase activity are strongly influenced by $CuSo_4$(copper sulphate) and SA(salicylic acid). Therefore, the present study was conducted to observe the effect of different concentrations of $CuSo_4$ and SA on catalase and peroxidase activity during seed germination in Oryza sativa at different time intervals of 3^{rd},6^{th} and 9^{th} day. Rice was chosen for this investigation because it has not been explored as much as for example wheat and barley. Spectrophotometric analysis of catalase and peroxidase activities were carried. Copper is an essential metal for normal plant growth and development. Excess copper inhibits plant growth and impairs important cellular processes. $CuSo_4$ treatment showed the increase in catalase and peroxidase activity with the control. SA plays a key role in plant growth,development and defence responses. On treatment with SA, the catalase activity was decreased as compared to control. On the other hand, SA showed increase in peroxidase activity with respect to control.

Introduction

Abiotic factors considerably effect plant growth and development. The effect each abiotic factor has on the plant depends on its quantity or intensity. Excess of Copper (Cu) in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (1). Many of these factors manifest their effect via oxidative stress and Reactive Oxygen Species (ROS). Exposure of plants to excess Cu generates oxidative stress and ROS (2). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (3). Copper toxicity affected the growth of *Alyssum montanum* (4), cucumber (5) and *Brassica juncea* (6). Abiotic factors affected adversely the germination, seedling length and number of lateral roots in Solanum melongena (7).

Salicylic acid (SA) may also cause oxidative stress to plants, partially through the accumulation of hydrogen peroxide (H_2O_2), the low concentrations of SA might have an acclimation effect, causing enhanced tolerance toward most kinds of abiotic stresses(8). The effect of exogenous SA depends on numerous factors such as the species and developmental stage of the plant, the mode of application, and the concentration of SA and its endogenous level in the given plant. Catalase is an important antioxidant enzyme (9). The typical catalase reaction is the

decomposition of two molecules of H_2O_2 to water and O_2. Peroxidases are heme-containing enzymes, composed of single peptide chain and omnipresent in plants. They are oxidoreductases that use several organic and inorganic substrates as hydrogen donors in the presence of hydrogen peroxide. Plant peroxidases are believed to be involved in many physiological and biological processes, including the cross linking of molecules in cell wall, auxin oxidation, oxidation of cinnamyl alcohols prior to their polymerization during lignin and subrin formation, and responses to biotic and abiotic stress (11, 12,13,14)

Oryza sativa (*Poaceae*) is a monocotyledonous angiosperm. It belongs to the genus Oryza, contains more than 20 species, cultivated in South-east Asian countries and Japan. *O. sativa* is the most important staple food in Asia. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population lives. Hybrid rice has to been developed in China since 1974 and now is planted in almost 40% of Chinese rice fields (15,16). In lieu of global warming and unpredictable whether there is heavy demand of developing stress -tolerant or resistant plant to ensure food security. With this background knowledge, the present study was conducted in *O. sativa.* The seedlings was treated with different concentration of copper (Cu) and lead (Pb) and peroxidase activity was monitored in response to these treatments.

Material and method;

Plant Material

Seeds of *Oryza sativa* (variety PR 123) were obtained from the local market of Jalandhar. Before start of experimentation seeds were thoroughly washed with water and surface-sterilized using 0.1% (*w/v*) solution of HgCl2 solution.

Germination of Seeds and imposition of stress

Surface sterilized seeds were germinated in petri plate by placing on moist filter paper. For $CuSO_4$ treatment nine petri plates were taken, three plates were treated with 0.3 %(*w/v*) $CuSO_4$, other three were treated with 0.6%(*w/v*) $CuSO_4$ and last three was treated with 0.9 %(*w/v*) $CuSO_4$.

For SA treatment nine petri plates were taken, three plates were treated with 1 %(*w/v*) SA , other three were treated with 2 %(*w/v*) SA and last three was treated with 3 %(*w/v*) SA. For the entire period of experimentation, plants were kept at 25°C with 8hr light and dark cycle.

Extraction of enzymes:

Tissue sample (1g) was homogenized in 5ml of 0.1M Sodium citrate buffer (pH 7). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C. Supernatant was collected carefully without disturbing the pellet and either immediately used for assay or kept at -20°C.

Enzymes assay

The assay mixture contained 200µl of guaiacol, 250µl Tris-HCL, 50µl enzyme extract and 500µl of H_2O_2. The reaction was started with the addition of H_2O_2 and the absorbance at 470nm was recorded up to 5 min. The difference in absorbance was divided by the molar extinction coefficient 26.6/mM/cm and specific activity of enzyme is expressed as µmol of H_2O_2 oxidized/min/mg protein.

In gel assay

Native page was performed in 10% gel. After electrophoresis, gel was immersed in 30mM sodium acetate buffer (pH 6.0).The gel was immediately incubated in the 1.5ml H_2O_2 and 330µl guaiacol solution for 5 mins. The brown color bands were developed. The intensity of these bands was the direct index of peroxidase activity.

Result

Effect of SA on activities of peroxidase and catalase:

Measurent of peroixdase and catalase was used as scavenging effect index of antioxidant under SA treatment. Exposure of SA resulted in time dependent increase in activity of peroxidase. Maximum increase in activity was observed at 1% SA on 9th day of treatment (Fig.1). This value was 0.049 µmol/ min/mg protein, which was almost double than respective control (0.029). Activity of peroxidase was found higher upon initial and shorter exposure of SA at all the concentration used in the experimentation with respect to control. These values were 0.007, 0.023 and 0.034 on third day of treatment at concentration SA (1%), SA (2%) and SA (3%) respectively. These values were significantly higher than respective control (0.004). It was observed that lower concentration (1%) was most effective in enhancing the activity of peroxidase during the entire period of experimentation. However, higher concentration (3%) increased the peroxidase activity only for short period (Day 3) and after longer period activity fall to 0.008 µmol/ min/mg protein, which was 3 times less than control. These results are in accordance to earlier report (10), where in SA suppressed total peroxidase activity, but did not inhibit the peroxidase with pI ~9.8. A novel chitin-specific peroxidase with pI ~3.5 appeared after the SA treatment. (10). Suppression of total peroxidase and appearance of pI specific peroxidase might be responsible for fluctuation in peroxidases activity. SA had been shown to involved in responses to abiotic stresses, such as ozone (17,18, 19), UV-B (20, 21), drought (22), heat (23) , cold (24) and metal stress (25,26,27). The elevation in peroxidase activity could be due to either ionic micro environment or tissue specific environment.

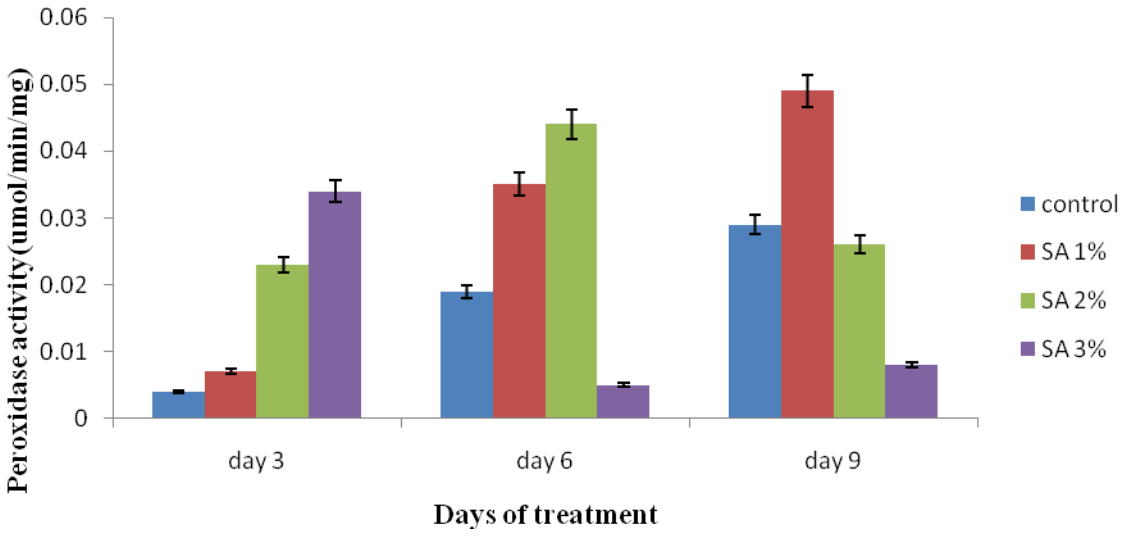

Fig. 1: Effect of SA treatment on peroxidase activity. Seedling of *Oryza sativa* were treated with different concentrations of SA; SA(1%), SA(2%) and SA(3%) and peroxidase activity were recored at 3rd, 6th and 9th day of germination Vertical bars represent Standard deviation.

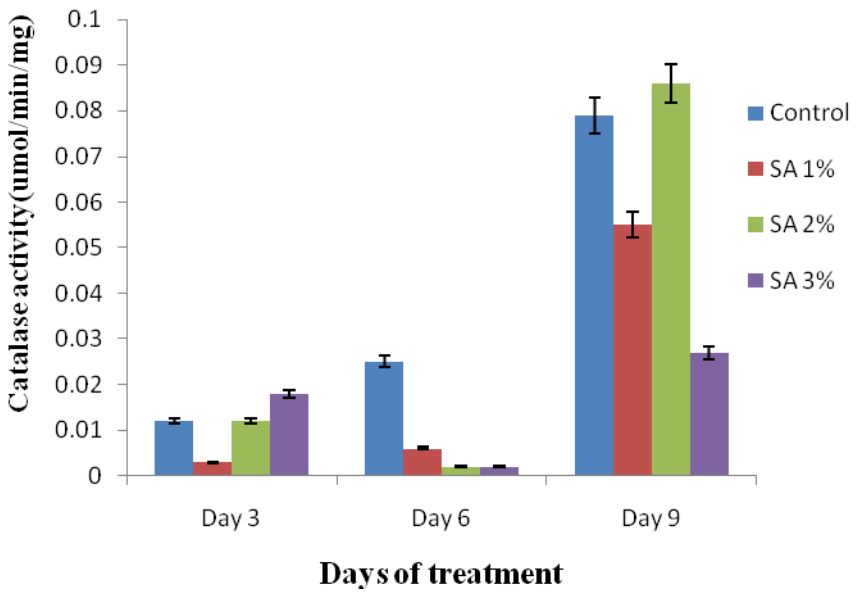

Fig 2: Effect of SA treatment on catalase activity. Seedling of *Oryza sativa* were treated with different concentrations of SA; SA(1%), SA(2%) and SA(3%) and peroxidase activity were recored at 3rd, 6th and 9th day of germination Vertical bars represent Standard deviation.

Catalase was the first antioxidant enzyme to be discovered and characterized, (9). The typical catalase reaction is the dismutation of two molecules of H_2O_2 to water and O_2. The measureable change in catalase activities was recorded on 6th day as compared to respective control (Fig.2). The recorded values on 6th were 0.006, 0.002 and 0.002 for SA(1%), SA(2%) and SA(3%) respectively, which was far lower than control (0.025) . The was no appreciable change in catalase activity on 3rd day when compared with control. The previous findings by different researcher throw considerable light on elucidation of the SA signal transduction mechanism. In tobacco, SA binds a soluble 280-kDa SA-binding protein (SABP) whose binding specificity and affinity are consistent with a receptor function (28, 29, 30). Analysis using different analogues with those of SA and its active derivatives suggests that these compounds share several features that may allow or facilitate binding by SABP/catalase and inhibition of its enzymatic activity. SA appears to be a defense-mediating signal in *Nicotania tobaccum* (31), including *Cucumis sativus* (32) and *Arabidopsis thaliana* (33). Our results in the present studies are in-line with these findings.

Effect of $CuSo_4$ on activities of peroxidase and catalase:

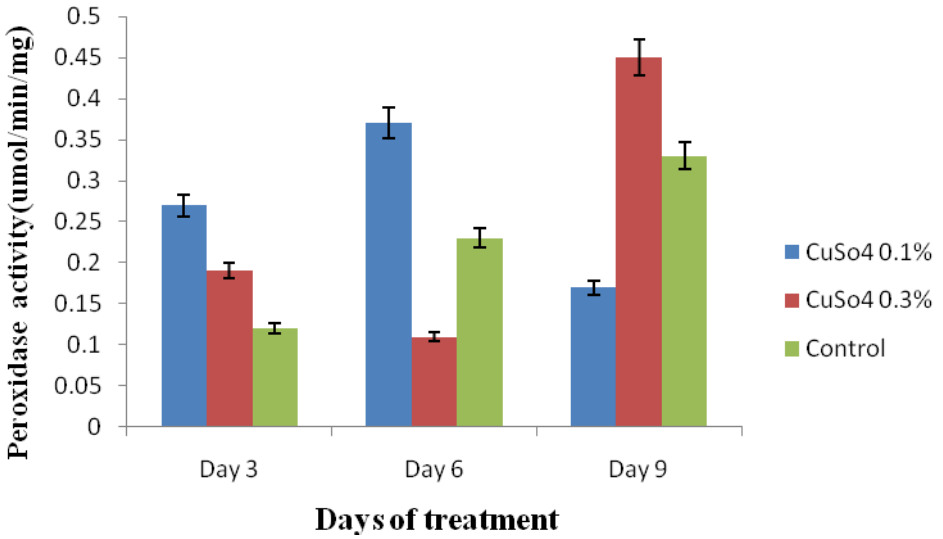

Fig. 3: Effect of $CuSo_4$ treatment on peroxidase activity. Seedling of *Oryza sativa* were treated with different concentrations of SA; SA(1%), SA(2%) and SA(3%) and peroxidase activity were recored at 3rd, 6th and 9th day of germination Vertical bars represent Standard deviation.

Peroxidase activity was increased at 0.37 on 6th day and 0.27 on 3rd day, respectively as compared to control at $CuSo_4$ (0.1%) treatment. However it decreased to 0.17 on 9th day as compared to control under $CuSo_4$ (0.1%) treatment. Also on treatment with $CuSo_4$ (0.3%), peroxidase activities were increased to 0.45 and 0.19 on 9th and 3rd day respectively but decreased to 0.11 on 6th day of treatment as compared to control. Copper had been known to induce several alterations in plant cells in concentration dependent manner. At higher concentrations it is toxic and adversely affect photosynthetic and respiratory process, protein synthesis and development of plant organelles (34,35). Excessive concentration causes chlorosis, inhibition of root growth and damage to membrane permeability leading to ion leakage (36, 37) and induced mineral deficiency (37, 38). As consequences Copper causes an oxidative burst (39) and the damage might be alleviated by action of antioxidative enzymes such as peroxidase, catalase and superoxide dismutase (40, 41). Peroxidases detoxify the cell by removing free radicals produced as a result of metal stress. This might be reason for the observe trend during experimentation.

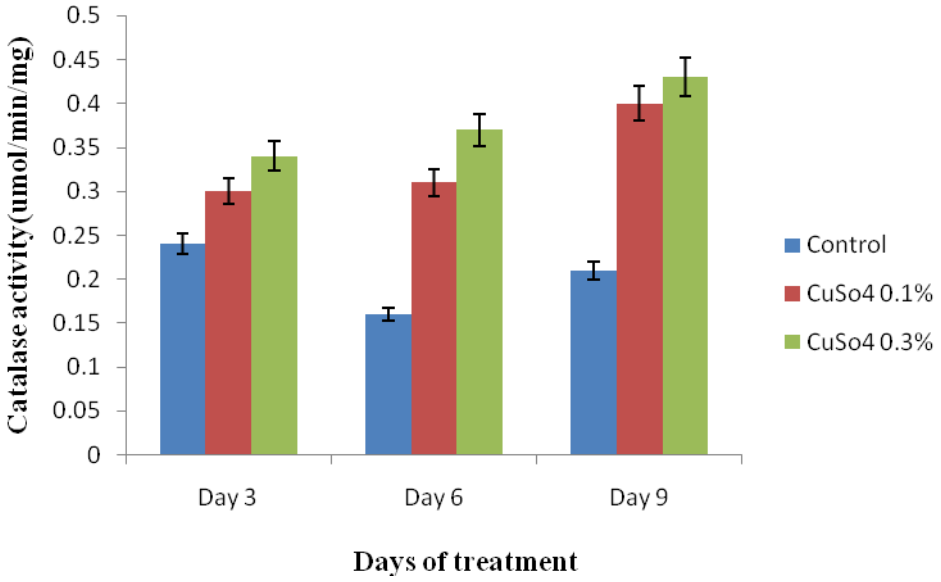

Fig. 4: Effect of $CuSo_4$ treatment on catalase activity. Seedling of *Oryza sativa* were treated with different concentrations of SA; SA(1%), SA(2%) and SA(3%) and peroxidase activity were recored at 3rd, 6th and 9th day of germination Vertical bars represent Standard deviation.

Treatment with $CuSo_4$ (0.1%) increased the catalase activity to 0.40 on 9th day, 0.31 on 6th day and 0.30 on 3rd day as compared to control. $CuSo_4$ (0.3%) increased the catalase activity to 0.43 on 9th day, 0.37 on 6th day and 0.34 on 3rd day as compared to control. In general there was significant increase observed in response to treatment with $CuSO_4$. Activity of catalase (CAT), responsible for the degradation of H_2O_2, therefore it might be possible that alleviate effect of $CuSO_4$ catalase activity increased for the degradation of H_2O_2 to H_2O. Lyubenova, et, al. 2014 reported this type of increase in *Typha latifolia* (42). It is also part of detoxification mechanism. It is well known that catalase and peroxidase play an important role in preventing oxidative stress by catalyzing the reduction of hydrogen peroxide (43). Devi and Prasad (1998) found that catalase and peroxidase activities were increased on copper treatment, suggesting that excess copper may increase the production of hydrogen peroxide (44). H_2O_2 is a necessary substrate for the cell wall stiffening process catalyzed by cell wall peroxidase (45), which is considered to be one of the mechanisms resulting in growth inhibition.Catalase is an enzyme involved in antioxidant defense that eliminates hydrogen peroxide

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