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Research Paper

Studies on the influence of germination on the activity of antioxidant enzymes of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides* seeds

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Abstract

Germination of seeds is an effective culinary process for centuries in India. The aim of the experiment was to study the effect of germination on the activity of antioxidant enzymes catalase, superoxide dismutase and peroxidase of *Vigna radiata,Vigna mungo* and *Pennisetum typhoides* seeds. The seeds were germinated for 24, 48 and 72h. After germination the seeds were homogenized in 0.1 M potassium phosphate buffer and used for the enzyme assay. Catalase activity was significantly ($p<0.001$) increased during the period of germination. Superoxide dismutase ($p<0.05$, $p<0.01$, $p<0.001$) and peroxidase ($p<0.001$) of germinated seeds were significantly increased compared to the dry seeds. *Pennisetum typhoides* showed a high level of catalase and peroxidase activity compared to *Vigna radiata* and *Vigna mungo*. Superoxide dismutase activity was comparatively high in *Vigna radiata* than the other two seeds. The activity of catalase and superoxide dismutase in *Pennisetum typhoides* did not show a significant change when compared to dry seeds in 24h germination, but the activity was highly significant in 48 and 72h when compared to dry seeds. The percentage of increase in enzyme activity during the period of germination for all the enzymes was high in *Vigna radiata* and *Pennisetum typhoides* seeds.

Keywords: Germination, antioxidant enzymes, *Vigna radiata,Vigna mungo* , *Pennisetum typhoides* seeds

Introduction

Germination (sprouting) of seeds is a simple and effective process practiced for centuries in Asian culinary preparations[1]. It improves the bioavailability of various minerals, vitamins and dietary fibres and thus improves the nutritional profile of seed grain[2] and decreased the levels of antinutritional factors present in legume seeds[3,4] . Germinated seeds have higher levels of amino acids, digestive protein and simpler carbohydrates and lower levels of non nutritive factors such as trypsin inhibitors, phytic acid and alpha galactosides over non germinated seeds [4,5]. Further health promoting benefits of germinated seeds are their richness in vitamins and minerals with important phytochemicals for disease prevention and activated enzyme processes for later growth stages[5,6]. Green gram, black gram and spiked millet / pearl millet are the food materials commonly used in Southern parts of India[7].

Green gram (*Vigna radiata* (L.) R. Wilczek) serves as an excellent source of protein as seed or sprout[8]. It belongs to the family Fabaceae commonly known as Mung bean and called as Pacchai Payaru/ Pasi payir in Tamil language. Black gram (*Vigna mungo* (L.) Hepper) is an important pulse

crop of India commonly used in Indian culinary preparations. It belongs to the family Fabaceae and is called as Ulundhu in Tamil language. It is an important source of protein and phosphorous. It possess significant hypolipidemic action[9,10]. *Pennisetum typhoides* (Burm f.) Stapf and C.E. Hubb belongs to Poaceae family commonly known as Pearl millet/spiked millet. It is called as Kambu in Tamil language. It is a staple food for people living in dry and rural regions of India[11]. Which is also important dietary source of Iron and Zinc. Supplementation of pearl millet products reduced anemia, increased the average IQ level and decline of moderate malnutrition of children[12].

During the process of germination, oxygen uptake and oxidative phosphorylation increases[13]and production of ROS. If the over production of ROS is not controlled it will cause reduced germination rate and seed death [14]. To scavenge the free radicals antioxidant mechanisms are present in plants includes enzymatic and non enzymatic antioxidants. Enzymes include SOD, catalase and peroxidases are involved in the scavenging of ROS in plant cells[15,16].

The increase in the nutritive quality of the germinated seeds compared to the dry seeds was already reported[17]. The main objective of the study was to determine the impact of germination on the activities of antioxidant enzymes catalase, superoxide dismutase and peroxidase of *Vigna radiata*, *Vigna mungo* and *Pennisetum typhoides*.

Materials and Methods

Dry seeds of *Vigna radiata, Vigna mungo,* and *Pennisetum typhoides* were purchased in Chennai, Tamilnadu, India. Imperfect seeds like broken and empty seeds were removed and washed with running tap water and also for disinfecting with 70% ethanol solution. Again they were washed thoroughly and soaked in distilled water for 4 h and transferred into petri plate containing moist filter paper and about 5ml of distilled water was added to it. Then the seeds were kept for germination under dark condition for 24, 48 and 72 h and used for the experiment.

Enzyme extraction: After germination 500 mg of sample was homogenized in 10ml of 0.1 M potassium phosphate buffer, pH 7.5 in ice cold condition. The homogenate was filtered through cheese cloth and centrifuged at 10000 rpm for 30 minutes at 4°C. The supernatant was collected and used for the assay of catalase[18], and superoxide dismutase[19], peroxidase [20] and protein[21].

Catalase: In a reaction mixture containing 1.0 ml of phosphate buffer (pH 7), 100µl of seed extract and 0.5 ml of 0.2M hydrogen peroxide solution, the reaction was stopped at 0 , 30 and 60 seconds by adding 2.0 ml of dichromate acetic acid reagent and then the reaction mixture was heated for 10 minutes and cooled and read spectrophotometrically at 570 nm against a plant extract hydrogen peroxide free blank. Catalase activity was expressed as µ moles of hydrogen peroxide decomposed/min/mg protein.

Superoxide dismutase: In a reaction mixture, 1.0 ml 125 mM of Sodium carbonate, 0.4 ml of 24 µM Nitro blue tetrazolium, 0.2 ml of 0.1 mM EDTA and 0.5 ml of seed extract were added. 0.4 ml of 1mM Hydroxylamine hydrochloride was added to initiate the reaction. The reaction was measured at 0 time and 5 minutes at 560nm spectrophotometrically. The control was measured without seed extract. Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50%.

Peroxidase: To the reaction mixture containing 1.0 ml of 0.1 M potassium phosphate buffer (pH 6.8), 20µl of 50mM guaiacol and 2.0µl of 0.2mM of hydrogen peroxide, 5.0µl of seed extract was added, the rate of change in absorbance per minute was used to quantify the enzyme in mixture at 470 nm. Blank reading was taken without the seed extract. The enzyme activity was expressed as nanomoles of guaiacol consumed/min/mg protein.

Statistical Analysis: All the experiment was conducted using three replicates in each group and the data were presented as Mean ± SEM. The student's t – test was used to compare the means of two groups.

Results and Discussion

The process of germination for 24, 48 and 72h for *Vigna radiata, Vigna mungo,* and *Pennisetum typhoides* was presented in figure 1. The original composition of the seeds changes during

germination by increasing the biological value of the sprout proteins and greater digestibility and by decreasing the amount of anti nutritive materials [22]. The decrease in the carbohydrate content and increase in the protein and ascorbic acid content of the germinated seeds of *Vigna radiata, Vigna mungo,* and *Pennisetum typhoides* was reported by Maneemegalai and Nandakumar [17].

The present study showed an increase in the nutritive quality with the increase in the antioxidant enzymes. Gloria *et al*[23] observed that germination of pea seeds for 3 days significantly improves palatability of seeds and the nutritive utilization of protein and carbohydrates . Seed germination initiates several metabolic changes and in turn increased the activity of various enzymes including alpha amylase, beta amylase, acid phosphatase and proteases [11]. The generation of many antioxidant phytochemicals and vitamins by sprouted seeds caused the elevation in their antioxidant potential [24].

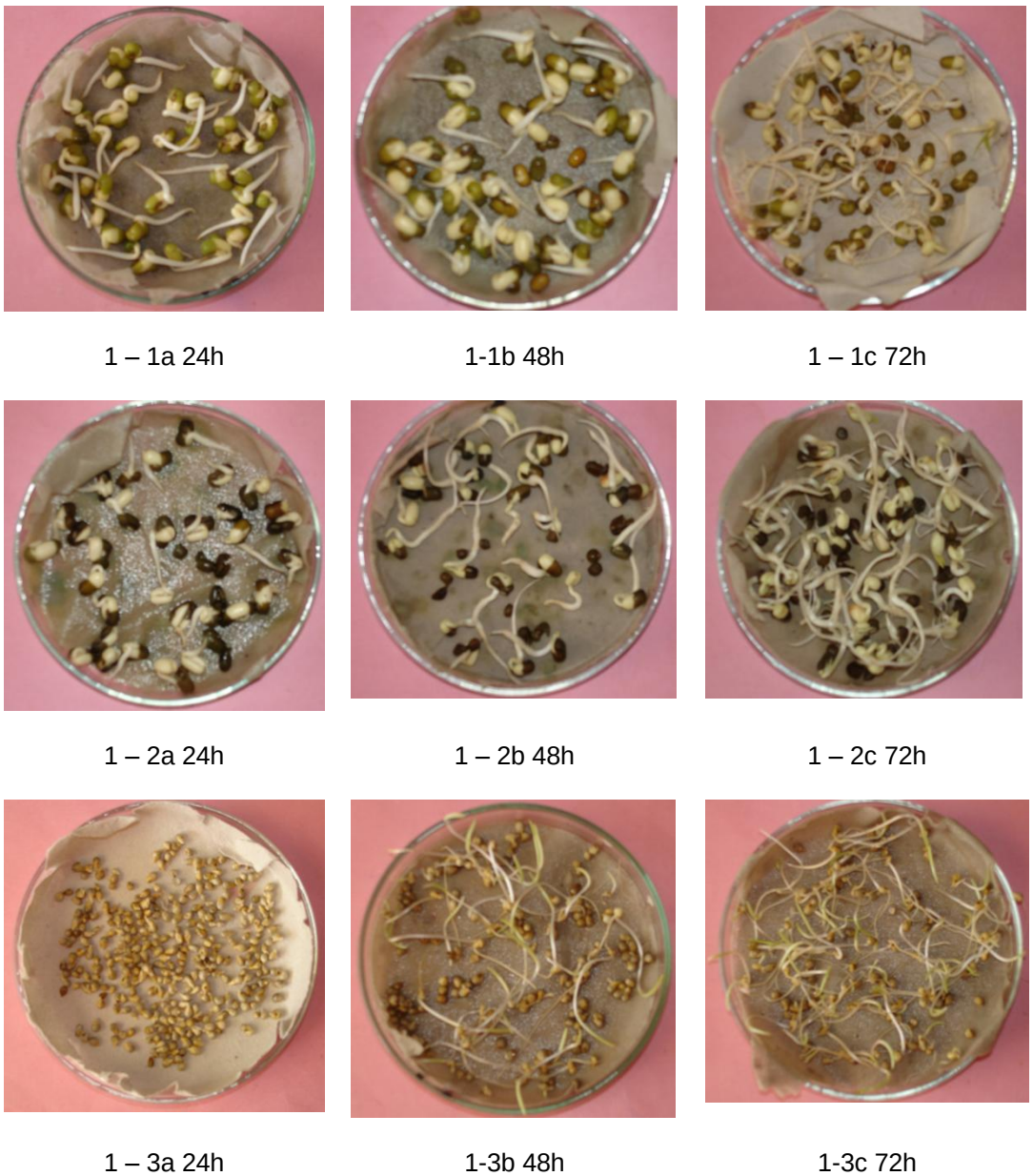

Figure 1: Process of germination for 24h, 48h and 72h time duration. 1-1a,b,c , *Vigna radiata*, 1-2a,b,c, *Vigna mungo,* 1-3a,b,c, *Pennisetum typhoides* seeds

The activity of catalase enzyme was observed in figure 2. The enzyme activity was seen significantly increased (p<0.001) from the 24h of germination compared to the dry seeds of *Vigna radiata* (green gram) and *Vigna mungo* (black gram). In the case of *Pennisetum typhoides* (spiked millet), the

catalase activity was found to be increased from the second day. Overall the level of enzyme activity was found to be high in the seeds of spiked millet. Superoxide dismutase activity of dry and germinated seeds of green gram, black gram and spiked millet were represented in figure 3. The enzyme activity was increased significantly (p<0.05) for the green gram seed in 24h of germination, there was no much change in the activity for black gram and spiked millets in the first 24h. In 48h of germination there was a rise in the activity of the enzyme in all three seeds. It was highly significant (p<0.001) for spiked millet compared to black gram and green gram (p<0.01). The activity of superoxide dismutase was elevated significantly (p<0.001) in 72 h of germination of all three seeds. The high level of activity was observed in green gram seeds compared to black gram and spiked millet.

Dry and germinated seeds of green gram, black gram and spiked millets peroxidase activity were presented in figure 4. The activity of the enzyme was highly significant (p<0.001) from the first day of germination in all three seeds. Spiked millets showed an elevated level of activity compared to green gram and black gram.

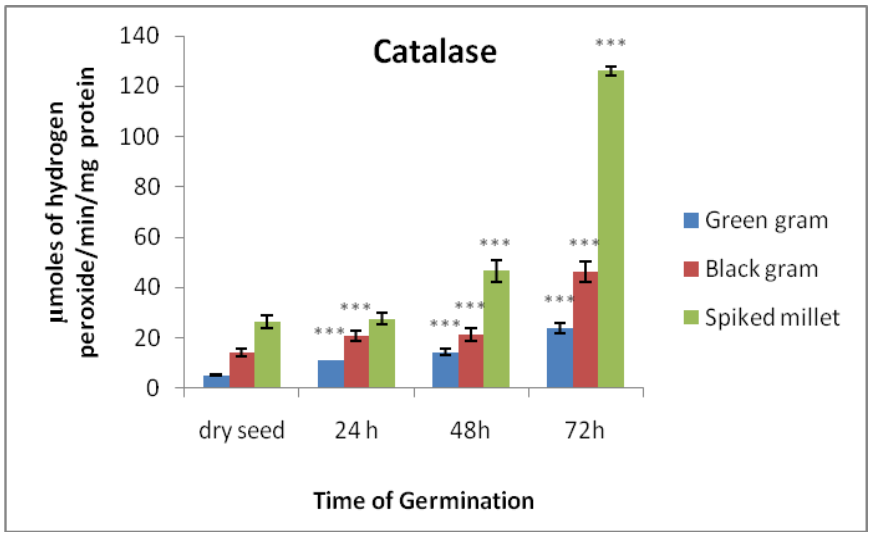

Figure 2: The activity of Catalase in germinated seeds. Values are expressed as mean±SEM of three replicates

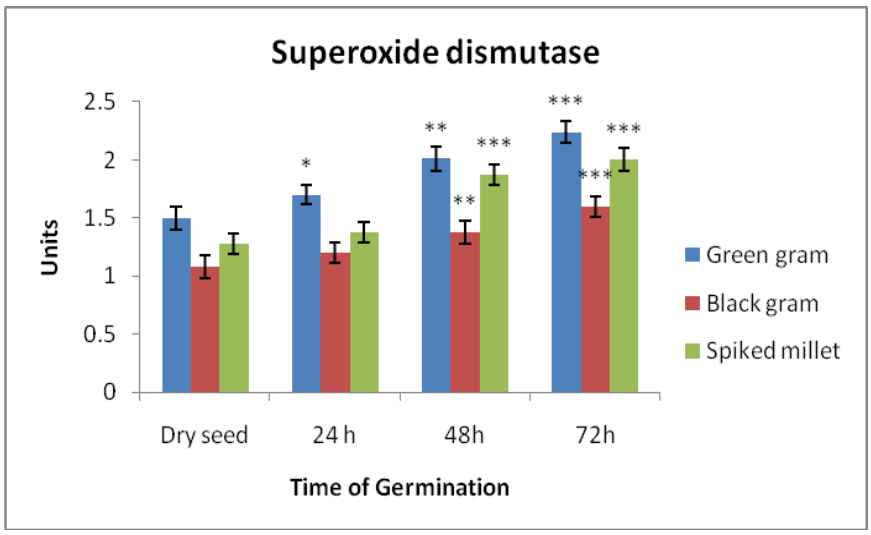

Figure 3: The activity of Superoxide dismutase in germinated seeds. Values are expressed as mean±SEM of three replicates

The percentage of increase in enzyme activity during the period of germination for all the enzymes was high in *Vigna radiata* and *Pennisetum typhoides* seeds.

Bailly[25] have reported that seed germination and post germination seedling growth are well regulated processes that involve high metabolic activity and generation of reactive oxygen species in the cell. This ROS are highly reactive and unstable. This ROS play an important role in seed desiccation, germination and ageing. The generated ROS lead to oxidative stress and cellular damage resulting in seed deterioration. The presence of antioxidant compounds and enzymes scavenge the ROS and participate in seed survival.

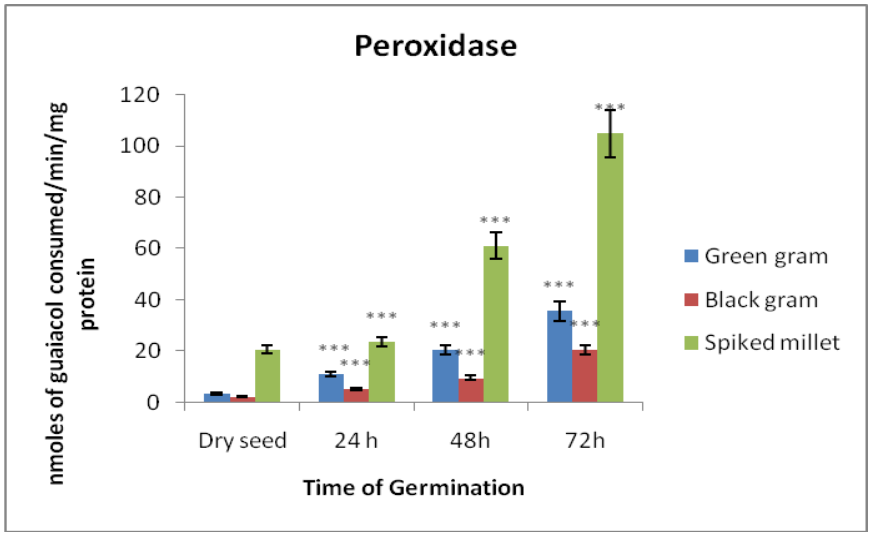

Figure 4: The activity of Peroxidase in germinated seeds. Values are expressed as mean ± SEM of three replicates

Photini et al.[26] observed that ROS induced antioxidant gene expression and caused a substantial increase of the respective enzymatic activities of catalases and super oxide dismutases in developing and germinated maize seeds. Super oxide dismutase reduces superoxide radical to hydrogen peroxide and catalase reduces hydrogen peroxide to water and dioxygen thus formation of highly reactive hydroxyl radical prevented which can cause lipid peroxidation, protein denaturation and mutations [27-29]. Antioxidant enzymes such as superoxide dismutase, Peroxidase, and catalase are considered to be the main protective enzymes in scavenging free radicals and ROS [30-32] and SOD acts as the first line of defense system. It was also reported that the germination of oats and green gram caused elevated levels of antioxidant activity[33,34] and thereby increased the health benefits. Sarita and Ekta Singh[35] have reported that Millets have nutraceutical properties in the form of antioxidants which prevent deterioration of human health. The present study showed a increased level of antioxidant enzymes in the germinated seeds of *Vigna radiata,Vigna mungo* and *Pennisetum typhoides* was also in row with the other reports of germination studies thereby endorsing the beneficial effect of consuming germinated seeds.

Conclusion

Germination of seeds resulted in the elevated activity of antioxidant enzymes catalase, superoxide dismutase and peroxidase in seeds of *Vigna radiata, Vigna mungo* and *Pennisetum typhoides* thereby increasing the antioxidant potential. *Pennisetum typhoides* showed a high level of catalase and peroxidase activity in 48 and 72h of germination. Overall in the present study elevation in the activity of antioxidant enzymes was observed throughout the period of germination. *Vigna radiata* and *Pennisetum typhoides* showed a significant level of increase in the activity of enzymes during germination compared to dry seeds.

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