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Importance of antioxidants enzymes in the survival of rice seedlings after desubmergence

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Abstract
The influence of submergence on Survival and antioxidant capacities in four rice genotypes Swarna-Sub1, NDR8610-2, Lalkadhan and Sambha were evaluated under control, simulated complete submergence and subsequent re-aeration. Maximum SOD and Peroxidase activity were recorded in NDR 8610-2 with highest survival percent after desubmergence over control. The result indicated that survival percent is associated with antioxidative enzymes.

Keywords: SOD, Peroxidase, Antioxidant, Submergence and Rice

Introduction
Rice is the major staple food for more than half of the world population and 90% of it is being produced and consumed in Asia. It is the major crop in most flood- prone areas of South and South-East Asia (Ismail, 2013) [10]. According to the Asian condition irrigated paddy occupies the vast area of paddy lands. Rain-fed lowland and deep-water rice together account for approximately 33% of global rice farmlands (Serres *et al.*, 2010). Submergence substantially decreases the rate of gas diffusion, limiting oxygen uptake andcompelling carbon inefficient anaerobic metabolism (Bailey-Serres and Colmer, 2014) [2]. Continuous anaerobic metabolism can result in the accumulation of phytotoxic end-products (Bailey-Serres and Voesenek, 2008) [3, 13]. When floodwaters subside, submerged plants encounter the rapid entry of oxygen, causing oxidative damage through overproduction of reactive oxygen species (ROS) and toxic oxidative products (Crawford 1992, Fukao *et al.*, 2011) [4]. Likewise, sudden exposure to higher light can induce photooxidative damage to photosystem II reaction centers, leading to reduced photosynthetic capacity (photoinhibition) (Ruban,2011). Desiccation of leaves following desubmergence is also observed due to a marked reduction in hydraulic conductivity in shoots (Setter *et al.*, 2010). Flooding/submergence and reoxygenation can induce oxidative stress, causing an increased production of reactive oxygen species (ROS) (Ella *et al.*, 2003; Fukao *et al.*, 2011). The ROS acts as a cellular indicator of submergence stress and as secondary messenger involved in the stress response signal transduction pathway (Fukao and Bailey-Serres, 2004) [8]. Plants have active oxygen-scavenging systems consisting of several antioxidant enzymes. Among all the antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) play key roles in protecting plants from oxidative stress damage (Damanik *et al.*, 2010) [6].

Material and Methods
The experiment was conducted in earthen pots and submerged into cemented pond at the experimental site of the Department of Crop Physiology N.D.U.A.&T., Kumarganj, Faizabad (U.P.). Bold and healthy seeds of 4 rice genotypes (Swarna Sub-1, Sambha, NDR8610-2 and Lalkadhan) were sown in the pot. Submergence was given to 35 days old seedlings at vegetative stage in pot culture for 15 days. At the time of submergence, water level was maintained at 30cm. The leaves were submerged in pond water. The level of pond water was maintained through water pipe. Control was kept without any submergence. Plant survival % was recorded after 20 days of de-submergence. Survival was indicated by the capacity of the plants to produced new leaves. The difference between the number of plants per pot before and 20 days after de-submergence were used for calculation of survival percent.

The activity of peroxidase (enzyme unit g^{-1} fresh weight) was determined by the method at Curne and Galston (1959) [5].
Phosphate buffer 0.1 M (pH 6.0), Pyrogallol (0.1 N), H_2O_2 (0.02 %). 200 mg fresh leaf material was homogenized in 10 ml of 0.1 M phosphate buffer (pH 6.0) and centrifuged at 10,000 rpm for 30 minutes at low temperature (4 ^{0}C). 2 ml enzyme extract was taken in a test tube to which 2 ml phosphate buffer (pH 6.0), 1.0 ml Pyrogallol and 0.2 ml H_2O_2 was added. The mixture was shaken and kept in a water bath at 37 ^{0}C for ten minutes for formation of purpurogallin. The intensity of colour was measured at 430 nm on spectronic 20.
Estimation of SOD (enzyme unit g^{-1} fresh weight) spectrophotometrically according to the methods of Asada *et al.* (1974) [1].

Result and Discussion

Among four genotypes maximum SOD activity and Peroxidase activity was found in NDR8610-2 followed by Swarna sub-1 and minimum was recorded in Sambha after desubmergence over control (Fig. 1&2). Maximum Survival percent occur in NDR8610-2 followed by Swarna sub-1 and minimum was occur in Sambha. This result presented in Figure: 3. Result showed that relationship between survival percent and antioxidative enzymes (Fig: 3). Genotypes which had maximum survival percent gave maximum antioxidative enzyme activity. This result agreed with the H2O2 accumulation and its concomitant effects in the plants resulting oxidative degradation have also been documented in plants including rice and other cereals (Vijayalakshmi *et al.*, 2014) [16]. H2O2, though not a free radical, also possess the characteristic phenomena to lyse the tissue beyond its cellular threshold concentration. SOD is marked as the first line of defense which lyses O2 - into H2O2 and O2 (Sarkar *et al.*, 2001) [12]. It is interesting to note that the rice varieties are not tolerant to O2 -. Also, their defense to this ROS was also not enough and thereby becomes more prone to oxidative damages. Moreover, the variation in SOD activity as compared to control is almost alike root and shoot. Thus, in cells, a number of reactions involving O2 - are featured for elevation of oxidative deterioration finally leading to tissue death (Sarkar *et al.*, 2001) [12]. Submergence being an important facet of oxidative stress is of no exception for ROS. In a number of cases, the activity of SOD are found to be minimal or intermediate to experience the shock of oxidative burst in tissues. A number of tolerant species may differ from susceptible members to vary in SOD activity during the exposure of submergence after entering the post anoxic condition on receding of water level. It is being well acclimatized for tolerant varieties to other courses of ROS activities in succession, particularly, which causes many serious oxidative damages at post submergence period (Steffens *et al.*, 2013) [15].

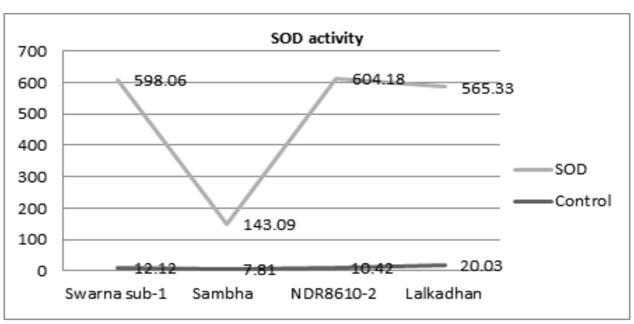

Fig 1: SOD activity under control and after desubmergence

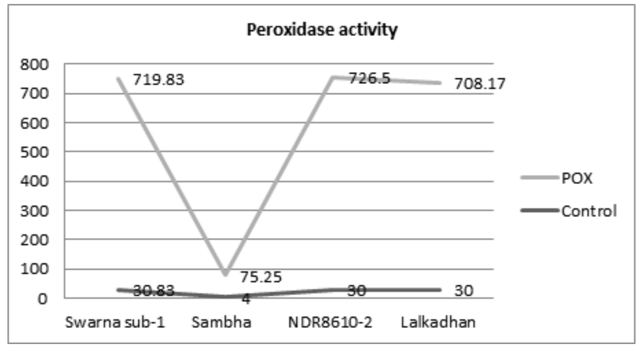

Fig 2: Peroxidase activity under control and after desubmergence

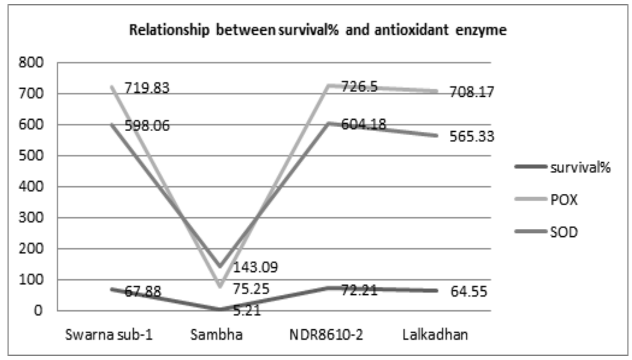

Fig 3: Relationship between survival% and antioxidant enzyme

Conclusion

It is unquestionable that ROS activity and its amelioration are regulated at different levels varying according to species. Conclusively, it is well perceived that survival percent was positively correlated with SOD activity and Peroxidase activity under submergence condition in rice genotypes. Highest survival percent was found in NDR8610-2 with highest survival percent. Antioxidant enzymes protect plant from oxidative damage.

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