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Oxidative stress in the seedlings of rice under water stress affected by *Azospirillum*

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Abstract
Oxidative stress is the common negative impact of water stress in the plant which severity disrupts the whole cellular activities of the plant. Rice seeds sown with treatment of *Azospirillum* under deficit of water. The oxidative stress caused by the water stress with the excess production of superoxide dismutase and hydrogen peroxide is scavenged by the superoxide dismutase, catalase and proxidase. *Azospirillum* treated seeds sown under water deficit stress induced by Polyethylene glycol (PEG) to analyze the tolerance against water stress at seedlings stage of rice. The four doses of *Azospirillum viz*. (0, 10, 15, 20) ml per kg of seeds interact with four level of PEG (0, 15, 20, 25) %, applied in soaking seeds. The treatment combination (*Azospirillum* 15 ml/kg + PEG 0%) and (*Azospirillum* 10 ml/kg + PEG 0%) were found better as compare to other treatment combination. The treatment combination (*Azospirillum* 15 ml/kg + PEG 25%) was found best among all the treated seeds in comparison to other treatment and control. Thus, *Azospirillum* is sustainable biofertilizers which keep the healthy seedlings of rice under water stress.

Keywords: *Azospirillum*, oxidative stress, water stress, rice, seedlings

Introduction

The abiotic stresses are the adverse environmental effects on crops which frequently occur in their life cycle. Sustainable approaches to mitigate the stresses in crops are needed in production of crops in natural farming. The effective plant growth-promoting rhizobacteria (PGPR) has to be identified that can induce crop tolerance to abiotic stresses especially drought, salinity, and heavy metal contamination. *Azospirillum* spp. are the PGPR significantly improved the growth and development of different crops particularly wheat under water stress (Arzanesh *et al*, 2011) [1]. They have capacity to fix atmospheric nitrogen in the rhizosphere, auxin production, phosphate solubilization and ACC deaminase activity. These abilities of *Azospirillum* make them more versatile and beneficial for plants growing in drought stress. *Azospirillum* spp. has capacities to fix nitrogen under low oxygen and nitrogen-deficient conditions due to presence of nitrogenase activity regulated at the transcriptional and posttranslational levels. Hence it can be developed as a powerful tool for sustainable agriculture prevention of soil erosion. Drought stress causes damage of cell organelles, proteins and lipids results in membrane damage, mutations, chromosomal aberrations, and eventually cell death due to production of excess reactive oxygen species (Huang *et al*, 2019) [2]. Disruption of metabolism compel to incomplete reduction of molecular oxygen that generate hydrogen peroxide (H_2O_2), superoxide radical (O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$). These ROS become very problematic in the cell if they present in excess for long time (Rajput *et al*, 2021) [4]. The metalloenzyme superoxide dismutase (SOD, EC 1. 15. 1. 1) is one of the effective ROS scavengers which act as important components of a plant cell's antioxidant defense system. It removes the superoxide radicals by catalyzing its conversion into oxygen and hydrogen peroxide. It is divided into three isoenzymes *viz.* Cu/Zn-SOD, Fe-SOD and Mn-SOD due to presence of these metal cofactors at its active site (Alscher *et al*, 2002) [3]. They are mostly present in chloroplasts, mitochondria, nuclei, peroxisomes, cytoplasm, and apoplasts (Mahanty *et al*, 2012) [5]. Catalases (CAT) are tetrameric heme-containing enzymes divided into three groups based on their structures in the plants. class 1 are found in photosynthetic tissues, class 2 are found in vascular tissues and class 3 are found in seeds and young plants where all the three classes have to convert hydrogen peroxide to water and oxygen. Peroxidase (POD) is a heme-containing enzyme catalyzes the oxidation of a wide spectrum of organic compounds in the presence of H_2O_2. (Kadkhodaie *et al*, 2014) [6]. Thus, enhancement antioxidative activities of these enzymatic antioxidants control the negative impact of oxidative stress in the seedlings of rice.

Materials and Methods

The experiment was conducted in a polyhouse situated at N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India. The average temperature was recorded as 31±10°C, and the relative humidity was approximately 80%.Seeds of the rice variety GNR-8, belonging to the indica subspecies, were chosen and sterilized using 0. 4% sodium hypochloride for 10 min. After sterilization, the seeds were washed thoroughly with demineralized water for 2-3 times to remove any residual sodium hypochloride. The seeds were then soaked in different concentrations of *Azospirillum* overnight. Following this, they were removed from the solution, gently wiped with tissue paper, and left to dry for half an hour. The treated seeds were sown in pots filled with a growing medium consisting of a 1:1 mixture of sand and peat. Water stress was applied through the use of different concentrations of polyethylene glycol, specifically 0%, 15%, 20%, and 25%.After 30 days of sowing, various

observations were made. The oxidative stress and antioxidative activities were studied following the induction of stress. Superoxide radical was estimated using the following procedure described by Das and Kar, (2018) [7].
It was expressed in nmol min^{-1} mg^{-1} protein. Hydrogen peroxide content of rice leaf samples from all the treatments was estimated as per the protocol Velikove *et al*. (2000). It was expressed in µmol g^{-1} fw. The SOD assay was based on the capacity of extracts to inhibit photochemical reduction of nitro blue tetrazolium (NBT) in the riboflavin-light-NBT system (Beauchamp and Fridovich, 1971) [8]. It was expressed in unit min^{-1} mg^{-1} protein. Catalase activity was estimated using the following procedure described by Barber (1980). It was expressed in µmol min^{-1} mg^{-1} protein. The peroxidase activity was estimated following a protocol described by (Perur, 1962) [10]. It was expressed in µmol min^{-1} mg^{-1} protein.

Results and discussion

The higher superoxide radical was observed in A3P3 (*Azospirillum*20 ml/kg + PEG 25%) when compared to all other treatments. The lowest superoxide radical was recorded in A2P0 (*Azospirillum*15 ml/kg + PEG 0%). The higher hydrogen peroxide was observed in A0P3 (*Azospirillum* 0 ml/kg + PEG 25%) while the lowest hydrogen peroxide was recorded in A2P0 (*Azospirillum* 15 ml/kg + PEG 0%) as compared to all other treatments. The higher superoxide dismutase, catalase and peroxidase activities were observed in A2P3 (*Azospirillum*15 ml/kg + PEG 25%) while the lowest activities were recorded in A0P0 (*Azospirillum*0 ml/kg + PEG 0%) in comparison to all other treatments. Shah *et al*. (2001) [11] studied the effect of increasing concentration of Cd on superoxide anion generation in rice seedlings as a higher level of superoxide anion production was noticed in Cd-treated seedlings throughout the growth period (5-20 days), as compared to non-stressed plants. Mendez *et al*, (2016) reported that reduce oxidative stress by enhancing the antioxidative activities in wheat. The similar results were shown by El-Ballat *et al*, (2023) [13] where *Azospirillum brasilense* enhance the antioxidatns, osmolytes and reduce the oxidative stress. Alharbi *et al*, (2022) [14] studied that the application of combined PGPRs and nano-Si resulted in increased antioxidant enzymes activity such as CAT, SOD, and POD, which decreased hydrogen peroxide (H_2O_2) under soil salinity in rice plants compared to control. Gureeva *et al*, (2023) [13] deciphered the molecular mechanisms of *Azospirillum spp*. to combat the abiotic stress by minimizing the oxidative stress and enhancing the antioxidative properties. Rizvi *et al*, (2019) [16] reported similar finding with treatment of *Bacillus subtilis* in winter wheat under heavy metal stress. It has been reported that *Azospirillum brasilense* applied at sowing or by leaf spray increased the activities of CAT, SOD and POD (Gureeva *et al*, 2025) [17].
Similar results reported in wheat plants in presence of the water stress inoculated plants showed increased activities of CAT, SOD and POD as compared to non-inoculated control (Agami *et al*, 2017). Salem and Alhamd (2009) [18, 19] reported that the activities of antioxidant enzymes peroxidases (POD) increased significantly compared to control due to the treatment with *Azotobacter*. Fukami *et al*. (2018) [20] has been shown that *Azospirillum brasilense* cells applied at sowing or by leaf spray increased the activities of CAT, SOD and peroxidase and decreased the oxidative stress in comparison to control.

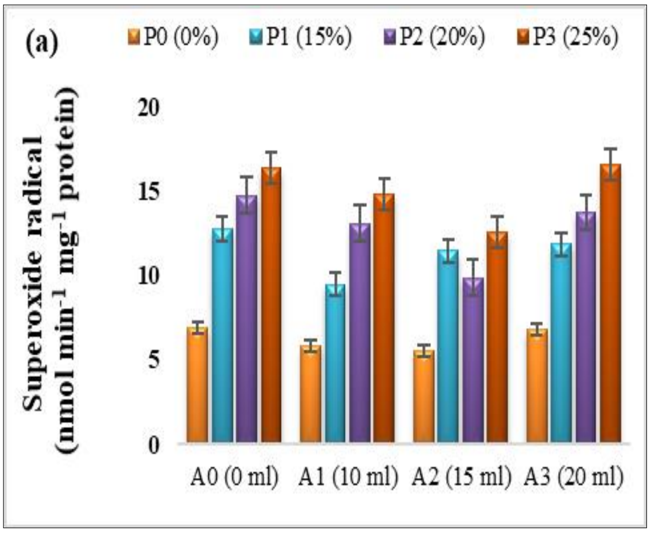

Fig 1: (a) Superoxide radical as affected by *Azospirillum* under water deficit stresses and control. ± Bars indicate SD (n = 10)

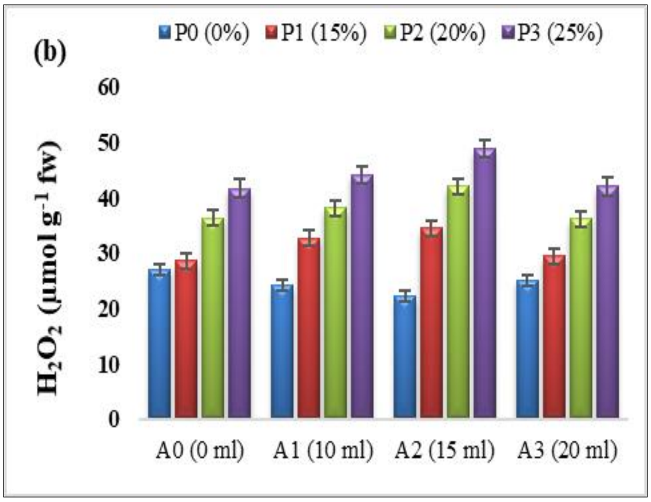

Fig 1: (b) Hydrogen peroxide as affected by *Azospirillum* under water deficit stress and control. ± Bars indicate SD (n = 10)

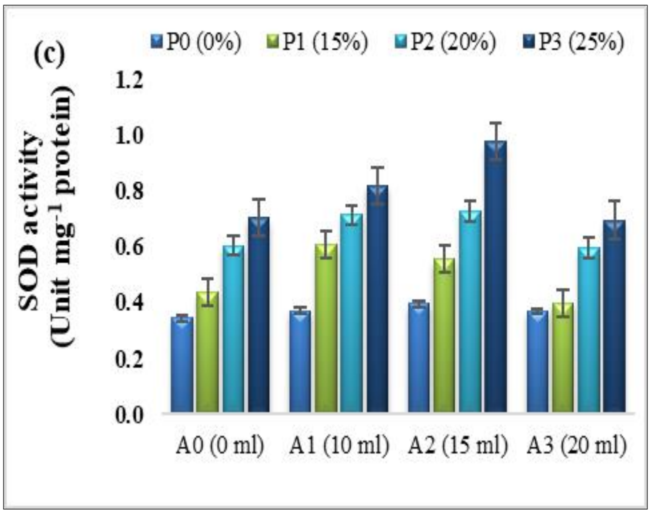

Fig 1: (c) SOD activity as affected by *Azospirillum* under water deficit stress and control. ± Bars indicate SD (n = 10)

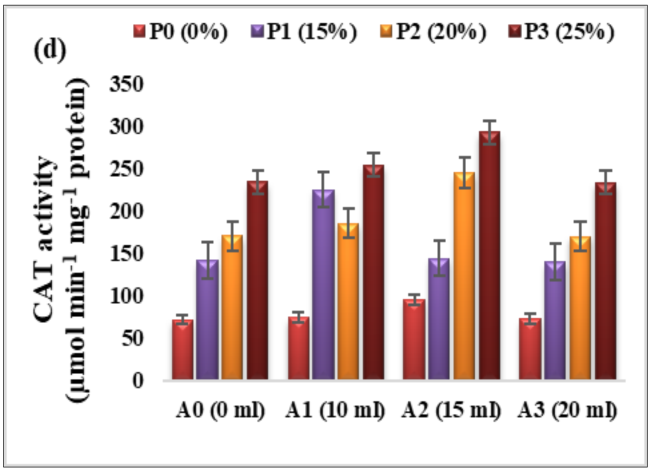

Fig 1: (d) CAT activity as affected by *Azospirillum* under water deficit stress and control. ± Bars indicate SD (n = 10)

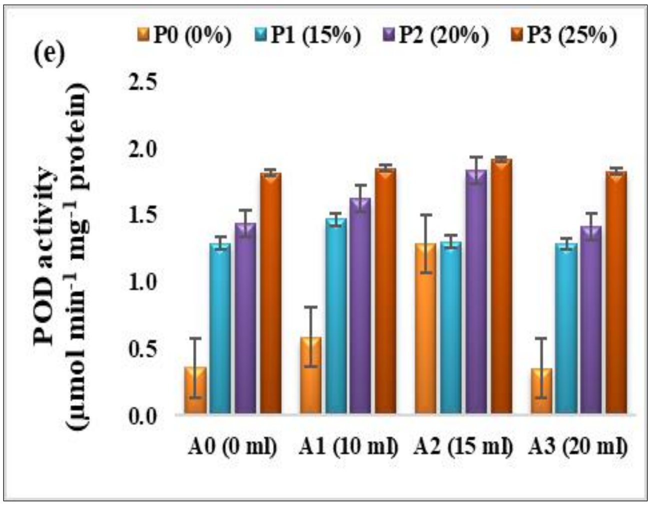

Fig 1: (e) POD activity as affected by *Azospirillum* under water deficit stress and control. ± Bars indicate SD (n = 10).

Conclusion

PGPR are the right choice of application for biofertilizers and abiotic stress management in the plants. *Azospirillum* spp. (15 ml/kg) treated rice seed showed the enhanced antioxidative enzymes and reduced oxidative stress in growing seedlings of rice. Thus, Seed treatment with *Azospirillum* (15 ml/kg) might be a useful strategy to protect the plant from water deficit stress.

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