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Assessing the Impact of Bio-Inoculants on Biochemical Activity in Wheat (*Triticum aestivum* L.) Under Varying Soil Moisture Regimes

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Abstract—*Wheat is a major cereal crop, widely cultivated under varied agro-climatic condition. Wheat crop needs water for the whole growth period, but there are some stages, which are more vulnerable to water shortage and any water shortage during this period may result in significant yield losses. Crops demonstrate various morphological, biochemical and molecular responses to tackle drought stress resulting growth inhibition. In plants, metabolism of reactive oxygen species (ROS), such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) is kept in dynamic balance. Under water stress conditions, this balance is disturbed and antioxidant systems are needed to decrease the damage to tissues. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of the enzymatic as well as non-enzymatic antioxidants. Antioxidative system or Avoidance of oxidative stress through preventing ROS accumulation as the most effective approach used by mycorrhizal plants to cope with drought stress. Biochemical parameter (total soluble protein, total soluble sugar, hydrogen peroxide, peroxidase activity and proline content showed decreasing values with increased irrigation levels, whereas, catalase activity was found to be maximum in three irrigation and decreased with restricted irrigation. Inoculations of AM fungi play an important role in orchestrating antioxidant activities in shoot and roots of associated plants in the process of drought tolerance. These results indicate the different capacity to efficiently detoxify ROS in the production site according to activity of microbial inoculants applied. Antioxidants status like hydrogen peroxide, catalase, peroxidase, total soluble sugar, proline content, The mycorrhizal inoculation significantly increased the contents of some metabolites (proline; free amino acids; total soluble and crude proteins; total soluble sugars) in wheat and enhanced the activities of some antioxidant enzymes such as peroxidase (POX) and catalase (CAT) at heading stage of growth. The similar response was observed with bio-inoculants having AM fungi with recommended dose of fertilizers.*

Keywords*: antioxidant activity, drought, ROS.*

1. INTRODUCTION

Wheat is one of the world's most important food crop providing 20% of all calories and protein in developing and developed countries. It is major source of starch, protein, sugar and provides food for human population. India held at second rank in wheat production by contributing 12% in global wheat production. To fulfill increasing demand of food for growing population 1.6% annual increase is required with limited sources and climate change. According to the most recent, the global population has quadrupled, there are 7.3 billion people and we may reach 9.7 billion by 2050 (Schierhorn, 2016). Wheat covers 30% of cereal area and act as staple food for 36% of world's population (Cossani and Reynolds, 2012). Global food security is being haunted by the rapid increase in population and drastic changes in the climate (Lesk *et al*., 2016). Food demand is expected to increase from 59% to 98% by 2050. So, 1.6% annual increase is required to fulfill increasing demand of food for growing population. The growing population will result in considerable additional demand for food and it will also contribute towards changing climate, which is an alarming issue to the world's food safety. Water availability is one of the major limiting factors that enhance the growth of plant, this condition occur when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation causes drought. Drought condition affects many aspects of plant physiology and tends to shut down plant growth and reduction of photosynthetic activity (Lipiec *et al.*, 2013). At present, drought stress has become the main one in abiotic stresses, and could restrain growth and yield of wheat (Gong *et al.,* 2014). Now, it becomes an increasingly severe problem in many regions of the world (Hossain *et al*., 2013). Mainly abiotic stresses negatively influence survival, biomass production and crop yield but crop loss due to abiotic stress is the primary source of decreased agricultural productivity. Mainly water is considered as one of the most important components for plants to perform biological activities (Lizarazo *et al*., 2016). Much effort is being made by agricultural researchers around the globe to reduce water use by crops to address the challenges that especially affect farmers in drought-prone environments across the developing world.

To alleviate the problem of drought stress, there are many strategies like nitrogen fertilizers, seed hardening, development of resistant genotype and use of antitranspirants and alcohol (Gangadhar *et al.,* 1999). The integrated nutrient management strategies involving chemical fertilizers and bio-fertilizers have been suggested to enhance the sustainability of crop production (Rigi *et al.,* 2014). Bio-fertilizers are products of different type of microorganisms which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Kaur *et al.,* 2014). Biological fertilizers may supply wheat plant with all nutrients needed for plant growth and metabolism without all hazards occurred when applying chemical fertilizers under water stress condition. So, this is a method of farming system aimed to reduce this environmental pollution and develop a more profitable and sustainable farming in which arbuscular mycorrhizal fungi is an efficient and new way to enable plants to grow well in drought-prone environments (Gholamhoseini *et al.,* 2013).

Under drought conditions, plant requires antioxidant systems would able to work in a coordinate form to provide the best scavenging systems and plant defense (Blokhina *et al.,* 2003). Oxidative stress arises from an imbalance in generation and utilization of reactive oxygen species (ROS). Prolonged exposure to the conditions that cause excess excitation energy can result in an increase in the generation of ROS such as $^1O_2$, $^{\cdot}O_2^-$ and H_2O_2. Ant oxidative system or Avoidance of oxidative stress through preventing ROS accumulation as the most effective approach used by mycorrhizal plants to cope with drought stress (Fouad *et al*., 2014). Among the antioxidant enzymes, SOD constitutes the first line of defense against ROS, it converts $O_2^{\cdot-}$ into H_2O_2 which is then eliminated by catalase (CAT), peroxidase (POX) that dismutase H_2O_2 into water and oxygen (Sharma *et al*., 2012).

Lipid peroxidation is expressed as malondialdehyde (MDA) content which is a decomposition product of polyunsaturated fatty acids like hydrogen peroxides, has been utilized very often as a suitable biomarker for lipid peroxidation, which is an effect of oxidative damage activity (Ma *et al.,* 2017). Catalase (CAT) is one of the H_2O_2 detoxifying enzymes and it is found abundantly in plant tissue but is absent in chloroplast and most of the catalase activity is associated with peroxisomes where it removes the H_2O_2 formed during photorespiration. Catalase activity has been reported to change under drought (Oktem *et al*., 2008). SOD converts $O_2^{\cdot-}$ into H_2O_2 which is then eliminated by catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) that dismutate H_2O_2 into water and oxygen (Sharma *et al*., 2012). Peroxidases are non chlorplastic enzymes that detoxify H_2O_2 in the cytosolic part of cell and are non-specific in utilizing electron donor for oxidation of H_2O_2. Peroxidase activity has been shown to be stimulated under drought stress (Oberoi *et al.,* 2014). Total soluble sugar is one amongst the most important cyto-solutes and accumulates in higher plants during the adaptation to various abiotic stresses especially during drought stress (Vendruscolo *et al.,* 2007). The increase of the soluble sugars in water deficient in wheat could be attributed to the stimulate the conversion of starch into sucrose at the carbon dioxide compensation concentration presumably for osmotic adjustment helping the movement of water and it may also contributes to maintain the size of metabolic pools of the photosynthetic carbon reduction cycle (Shao *et al.,* 2009).

Recently, it has been observed that the symbiotic interaction of plants with AM fungi, being important from the agricultural and ecological point of view (Yang *et al.,* 2008), it could be a sustainable mitigation practice for water deficit stress (Aroca *et al.,* 2013). AM fungi are especially important for sustainable farming systems because AM fungi are efficient when nutrient availability is low and when nutrients are bound to organic matter and soil particles (Rigi *et al.,* 2014). Many important agricultural crops can benefit from AM fungi, including wheat, maize, potato, sunflower, onion and soybean, especially under conditions where nutrient availability is limiting plant growth. Moreover, AM fungi not only can promote via directs effects, but there are also a number of indirect effects such as a stimulation of soil quality and the suppression of organisms that reduce crop productivity (Dodd *et al.,* 1983).

Inoculations of AM fungi play an important role in orchestrating antioxidant activities in shoot and roots of associated plants in the process of drought tolerance. These results indicate the different capacity to efficiently detoxify ROS in the production site according to activity of microbial inoculants applied. It might be due to the protective role of bio-inoculants under abiotic stress (Kadam *et al.,* 2017). The ROS content reduced in plants colonized with AM fungi under various abiotic stresses i.e drought, salinity, as studied in wide range of species like maize, rice, chickpea and wheat (Li *et al*., 2012). The mycorrhizal inoculation significantly increased the contents of some metabolites (proline; free amino acids; total soluble and crude proteins; total carbohydrate; total soluble and insoluble sugars) in wheat and enhanced the activities of some antioxidant enzymes such as peroxidase (POX) and catalase (CAT) at heading stage of growth. Catalase activity was significantly higher in drought tolerant cultivar (Zeinolabedin *et al.,* 2017).

2. MATERIAL AND METHOD

Wheat variety WH 1142 was selected to study the response of biofertilizers under varying level of soil moisture. The seeds were obtained from Wheat Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. The AM fungi obtained from Mycorrhizal Section, The Energy and Resources Institute (TERI), New Delhi, while the *Azotobacter* biofertilizers (azoteeka) and PSB biofertilizers (phosphoteeka) were obtained from Department of Microbiology, CCS Haryana Agricultural University, Hisar. Before sowing the seeds were inoculated with AM fungi, *Azotobacter* and PSB (Phosphate Solubilizing Bacteria). Strains used for AM Fungi and *Azotobacter* were *Glomus* *mosseae* and *Azotobacter croococum,* respectively. Recommended dose of fertilizers and crop protection measures was adopted as per packages and practices.

The experiment was conducted in field at Crop Physiology Research Area, Department of Agronomy, CCS Haryana Agricultural University, Hisar. The plot size for each treatment was 2.7 x 5 m with 12 rows of 5 m length at 22.5 cm spacing. Research area is situated in semi-arid and sub-tropical region at 29º-10º N latitude and 75º-46º E longitudes with an altitude of 215.2 m above mean sea level. The experiment was laid out in split plot design with three replications. Samples from each plot were collected for various biochemical parameters were recorded at two stages *i.e*., anthesis and 20 days after anthesis (in flag leaf only). Flag leaves were used for preparation of extract. The sample materials were processed in such a way that only specific portion of leaf material was chosen by removing upper and lower portion along with midrib. All biochemical studies were carried out under laboratory conditions in the Department of Botany and Plant Physiology, CCS Haryana Agricultural University, Hisar. Biochemical activities were done by following methods-

1. Proline content was estimated by using the method of Bates *et al.* (1973).
2. Total soluble carbohydrates were determined with the method of Yemm and Willis (1954).
3. Total soluble proteins were determined by Bradford (1976) method using supernatant.
4. MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modifications of the method of Heath and Packer (1968).
5. Hydrogen peroxide (H_2O_2) and catalase were estimated by the method of Sinha (1972).
6. The peroxidase enzyme was assayed by adopting the method of Shannon *et al.* (1966).

3. SUMMARY AND CONCLUSION

- Biochemical parameter (total soluble protein, total soluble sugar, hydrogen peroxide, peroxidase activity and proline content showed decreasing values with increased irrigation levels, whereas, catalase activity was found to be maximum in three irrigation and decreased with restricted irrigation. The similar response was observed with bio-inoculants having AM fungi with recommended dose of fertilizers.
- Total soluble sugar content was low in normal irrigation as compare to restricted irrigation condition at both the stages. It was 40.4 % and 35 % higher in three irrigations and 21.8 % and 19.5 % higher in two irrigations over one irrigation environment during both seasons, respectively.
- Total soluble protein content decreased in one irrigation as compared to three irrigation environments at both the stages but decrease was more pronounced at 20DAA. Bioinoculants treatment of AM fungi maintained significantly higher soluble protein content and lowest was with 75 % RDF.
- Hydrogen peroxide activity was recorded maximum in one irrigation environment during both years respectively, while catalase activity was higher in normal irrigation as compare to restricted irrigation environment. Hydrogen peroxide activity was higher with 75 % RDF with AM fungi while lowest activity was observed in RDF with AM fungi treatment.
- Peroxidase activity was recorded maximum in one irrigation environment and minimum in three irrigation environments. The application of 75 % RDF bioinoculants showed maximum activity of POX in one irrigation environment.
- Catalase enzyme was higher in normal irrigation as compare to restricted irrigation environment while lowest activity was observed with AM fungi and 75 % of RDF over RDF at both the stages.
- Proline content increased significantly in one irrigation environment as compare to normal irrigated environment. The magnitude of accumulation of proline was higher at 20 DAA as compare to anthesis. The level of proline content at both the growth stages showed maximum by reduced amount of fertilizers to 75 % RDF with AM fungi followed by 75 % RDF.

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