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Research Article

Influence of Biological Seed Priming on Pigments and Superoxide Dismutase Activity of Maize Seedlings Under Drought Stress

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Abstract The study was conducted to find the changes in physiological responses of drought facing maize seedlings when inoculated with phosphate solubilizing Paenibacillus polymyxa. Drought stress was imposed by withholding of water supply for 3 days. Stress caused decreased in photosynthetic pigment content but enhanced carotenoid content. Superoxide dismutase activity is reported higher in seedlings undergo biological seed priming. The results suggest the positive effect of seed priming on growth of maize seedlings under mild water stress.

Key words: drought stress, superoxide dismutase (SOD), photosynthetic pigments, reactive oxygen species (ROS), biological seed priming, PGPR.

Introduction

Drought stress is a type of abiotic stress, which is an unavoidable situation in any plant's life. Plant experience scarcity of water due to successive decrease in ground water level, low rainfall, high transpiration rate due to high temperature, etc. Limitations of water supply can be considered as first signal of stress (Zhu, 2016, Skirycz and Inze 2010). Every plant device some mechanism to cope up with the adverse effects caused by drought, up to some extent. But if scarcity of water persist for a longtime, biochemical reactions alters which consequently leads to death of cell (Jaleel et.al, 2009).

Drought causes hyperosmotic stress or also called as osmotic stress (Rubio et. al, 2002; Zhu, 2002). Osmotic stress can effects sub-cellular components, almost all the cell organelles get affected (Walter and Ron 2011). Different types of reactive oxygen species (ROS) are generated in response to oxidative stress. Superoxide radicals and hydrogen peroxide are the commonest ROS produce during stress (Giortti, 1990; Smirnoff, 1993). Lipid peroxidation, membrane leakage, decreased photosynthetic pigments (Singh et al. 2010) and distortion in proteins are common consequences of oxidative stress (Hou et.al, 2016; Neill et.al, 2002, Moller et.al, 2007). To combat with the deleterious effects caused by ROS mediated oxidative stress, production of superoxide dismutase (SOD)enzyme and its activity increased under different abiotic stress (Rubio, et.al, 2002; Singh et.al,2009; Singh et.al, 2015).

Plant-growth promoting rhizobacteria (PGPR) are recognised as a potential mean in increasing plant growth and overall development (Timmusk et al., 1999; Singh et.al, 2009). PGPR enhanced plant growth by their diverse function. PGPR provide nitrogen and solubilizes phosphates (Singh et al, 2010), and other nutrients.

Maize plant is selected as test crop due to its high economic importance. It also known as 'corn' or 'bhutta', widely grown crop in North India and in many part of world. It belongs to grass family Poaceae. Maize is susceptible to water deficits, intolerant of nutrient-deficient soils, due to its shallow root system only 1-2 inches deep. Present study conducted to find out the growth and biochemical changes in maize seedlings under drought stress. Also investigates the influence of surface seed inoculation with PGPR.

Materials and Methods

Growth Inoculation and Stress Condition

The seeds of test plant i.e. maize (Zea mays), were surface sterilized with 30% ethanol and divided into two

major groups C (control) and T (treatment) which further divided into two sub groups -b and +b representing without and with Paenibacillus polymyxa respectively. Seeds were surface inoculated with PGPR (108 CFU mL-1) for 30 min then air dried in shade. Inoculated and non-inoculated seedlings were raised up to 20 days. After 20 d one set (T) was subjected to drought stress by withholding of water supply for 3 d. Stressed plants then rewatered to check recovery status.Leaves were harvested from 23 d old seedlings from each set for RWC and other biochemical analysis.

Measurement of Relative Water Content

For the measurement of relative water content (RWC) leaves samples were cut into a definite number of discs of uniform size by means of cork borer fitted with a piston. Fully expanded leaves were selected which showing no sign of senescence. Freshly cut leaf discs were weighed for a fresh weight (FW) and then they were immediately floated on distilled water at 25°C in darkness and allowed to maintain turgidity. After 24 h leaf-discs were taken out from water and the excess water was removed with filter paper. Then the turgid discs were weighed for turgid weight (TW). The discs were dried in oven at 80°C for 48 h for the dry weight (DW). The RWC was calculated following Bars and Weatherley (1962):

RWC (%) = (FW-DW)/ (TW-DW) x 100

Determination of pigments : The pigments, viz. chlorophyll a, chlorophyll b and carotenoids from leaf were extracted with 80% acetone and quantify following Lichtenthaler (1987).

Enzyme assay: Superoxide dismutase (EC 1.15.11) activity was determined by the nitroblue tetrazolium (NBT) photochemical assay method following Beyer and Fridovich (1987). About 0.2 g fresh leaf tissue was homogenized in 1% polyvinyl pyrrolidone (PVP) prepared in 50 mM potassium phosphate buffer (pH 7.0) and centrifuged at 15,000 g for 30 min at 40C. The reaction mixture contained 0.5 ml clear supernatant, 2 ml 0.15 mM ethylene di-amine tetra acetic acid (EDTA), 20 mM methionine, 0.12 mM NBT and 0.5 ml 11.96 lM riboflavin, 0.5 ml PVP and determined spectrophotometrically against blank at 560 nm. One unit of enzyme was defined as the amount of enzyme which caused 50% inhibition of NBT reduction.

Statistical Analysis

Treatments were arranged in randomised block design with three replications. Data were analysed using analysis of variance (ANOVA).

Result and Discussion

RWC

Water is a determining factor for plant survival (Bradford and Hsaio, 1982) and its limitation directly interferes plant growth and their physiological activities (Bartels and Sunkar 2005, Osakabe et.al., 2014). Measurement of relative water content (RWC) of leaves is a significant technique in determining actual stress condition even in mild drought. Both the control set with and without inoculation exhibited RWC above 90%, there is no significant difference between C-b and C+b treatment. However lowest RWC was recorded in 3dS plant without inoculation (Table 1). Low RWC of leaves minimise CO2 intake as it affect stomatal opening and ultimately affects rate of photosynthesis (Lawlor, 2002).

Photosynthetic Pigments

Chl a, chl b and accessory pigment carotenoids were recorded for different sets. Highest chl a content was recorded in control plant with inoculation (C+b) and lowest in 3dS plants without inoculation. Rewatering of plants definitely helped them in maintaining their photosynthetic pigments. Chl a content of plants under 3dSR+b treatment, is nearly equal to that of control plant without inoculation. Chl b content doesn't exhibited any significant difference among stressed and recovered plants with or without inoculation. However carotenoids were recorded highest in stressed plants without inoculation and lowest in non-inoculated re-watered plants (Table 2). Decrease in chlorophyll content in stressed plants explained the fact that stress caused its degradation (Latakowska et al. 2006) and also promotes the conversion of chlorophyll content into carotenoids, which was in agreement of results. Carotenoids also help plant to tolerate stress condition as it function as osmolytes (Singh et al. 2009).

SOD

Increased SOD activity recorded in plants subjected to water stress. Minimum SOD activity was found in control plants with inoculation. Rewatering of plants minimise SOD activity which was still greater than control (Table 1). High SOD activity in water stressed plants suggest that WS caused formation of ROS which triggers the expressions of antioxidative enzyme. Antioxidative enzymes serve as defense system of plant. SOD is capable in removing excess free radicals by converting it into H2O2. SOD level increased in plants in stress condition to combat excessive ROS and minimise its deleterious effects (Reddy et al. 2002, Singh et al. 2010).

Inoculation with P. polymyxaameliorated the effects of drought stress as seen in different parameters. P. polymyxa help plant to maintain good health by solubilizing phosphate, also it is reported for secretion of some hormones and vitamins (Ahmad et al. 2008, Singh et al. 2010).

Table 1: Effect of water stress on relative water content (RWC) and SOD activity of *Zea mays* seedlings with and without inoculation.

Treatments	RWC	SOD (EU/gFW)
C-b	91.40 ± 0.21	15.6 ± 0.07
C+b	94.63 ± 0.13	12.6 ± 0.18
3dS-b	70.11 ± 3.71	25.6 ± 0.72
3dS+b	75.92 ± 1.13	22.5 ± 0.13
3dSR-b	81.64 ± 2.31	20.2 ± 0.16
3dSR+b	85. 95 ± 0.33	18.9 ± 0.05

Mean ± (SE) values of 3 replicates of each treatment. C=control, C+b= control with inoculation, 3dS= 3 day stress, 3dSR= 3day stress recovery (rewatered plants), +b= with inoculation, -b=without inoculation.

Table 2: Effect of water stress on photosynthetic pigments of *Zea mays* seedlings with and without inoculation.

Treatments	Chl a	Chl b	Total Chlorophyll	Carotenoids
C-b	4.50±0.02	1.04±0.07	5.54±0.05	1.15±0.03
C+b	5.25±0.24	1.12±0.09	6.37±0.09	1.35±0.09
3dS-b	2.82±0.07	0.39±0.05	3.21±0.23	2.07±0.04
3dS+b	3.94±0.18	0.45±0.18	4.39±0.31	1.45±0.28
3dSR-b	3.78±0.13	0.51±0.38	4.29±0.27	0.73±0.06
3dSR+b	4.22±0.72	0.61±0.07	4.83±0.12	0.83±0.11

Mean ± (SE) values of 3 replicates of each treatment. C=control, C+b= control with inoculation, 3dS= 3 day stress, 3dSR= 3day stress recovery (rewatered plants), +b= with inoculation, -b=without inoculation.

Conclusion

Present study may help in understanding the plant tolerance mechanism towards drought stress, also help in recognizing the role of P. polymyxa as potential biofertilizer and its contribution in increasing plant tolerance against oxidative stress caused by scarcity of water. This would definitely improve plant growth by decreasing dependencies on high need of irrigation.

References

Ahmad F, Ahmad I and Khan MS. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiology Research 163:173-181.

Bars HD and Weatherly PE. 1962. A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biology Science.15:413-428.

Bartels D and Sunkar R. 2005. Drought and salt tolerance in plants. Critical reviewin Plant Science 24: 23-58.

Beyer WF and Fridovich I. 1987. Assaying superoxidase dismute activity: some large consequences of minor changes in condition. Anal Biochem 161:559-566.

Bradford KJ and Hsiao TC. 1982. Physiological responses to moderate water stress. Physiological Plant Ecology11:263-324.

Giortti AW. 1990. Photodynamic lipid peroxidation in biological systems. Photochem. Photobiol. 51: 497-509.

Hou Q, Ufer G, Bartels D. 2016. Lipid signalling in plant responses to abiotic stress. Plant cell environment. 39: 1029-1048.

Jaleel CA, Manivannan P, Wahid A, Farooq M, Al-Juburi HJ, Somasundaram R and Panneerselvam R. 2009. Drought stress in plants: A review on morphological characteristics and pigment composition.International Journal of Agriculture andBiology 11:100-105.

Latakowska E, Lechowski Z, Bialczyk J, Pilarski L (2006) Photosynthesis and water relations in tomato plants cultivated long term in media containing (+)-usnic acid. Journal of Chemical Ecology 32:2053- 2066. doi:10.1007/s10886-006-9128-6.

Lawlor DW. 2002. Limitation to photosynthesis in water stresses leaves: Stomata vs. metabolism and the role of ATP. Annals of Botany. 89 (7):871-885.

Lichtenthaler HK. 1987. Chlorophyll and carotenoids: pigments of photosynthetic bio-membranes. In: Packer L, Douce lR (eds) Methods Enzymol. Academic Press, Sandiego, pp 350-382

Miller IM, Jensen PH, Hansson A. 2007. Oxidative modifications to cellular components in plants. Annual Review of Plant Biology. 58: 459-481.

Neill SJ, Desikan R, Hancock JT. 2002. Hydrogen peroxide signaling. Current Opinion Plant Biology.5: 388-395.

Osakabe Y, Osakabe K, Shinozaki K and Tran lam-son P. 2014. Response of plants to water stress. Front. Plant Sci. 5 (Article 86): 1-8.

Reddy AR, Chaitanya KV and Vivekanandan M. 2002. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. Journal of PlantPhysiology. 161:1189-1202.

Rubio MC, Gonzalez EM, Minchin FR, Webb KJ, Arrese-Igor C, Ramos J, Becana M. 2002.Effect of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa over expressing superoxide dismutases. Physiologia Planatarum. 115: 531-540.

Singh NB, Singh A and Singh D. 2010. Autotoxicity of maize and its mitigation by plant growth promoting rhizobacterium Paenibacillus polymyxa. Allelopathy Journal 25 (1): 195- 204

Singh NB, Singh D, Singh A. 2009. Modification of physiological responses of water stressed Zea mays seedlings by leachate of Nicotiana plumbaginifolia. General andapplied plant physiology 35(1-2): 51-63.

Singh NB, Singh D, Singh A. 2015. Biological seed priming mitigates the effects of water stress in sunflower seedlings. Physiology and Molecular Biology of Plants 21(2): 207-214.

Skirycz A and Inze D. 2010. More from less: Plant growth under limited water. Current opinion in Biotechnology. 21(2): 197-203.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist.125: 27-58.

Timmusk S, Wagner EGH 1999. The plant growth promoting rhizobacterium Paenibacillus polymyxa induces changes in Arabidopsis thaliana gene expression: A possible connection between biotic and abiotic stress responses. Molecular Plant- Microbe Interactions. 12: 951-959.

Walter P and Ron D. 2011. The unfolded protein responses: from stress pathway to homeostatic regulation. Science. 334: 1081-1086.

Zhu JK 2002. Salt and drought stress signal transduction in plants. Ann. Rev. plantBiol. 53: 247-273.

Zhu JK 2016. Abiotic stress signalling and responses in plants. Cell 167(6): 313-324.