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The effect of Vesicular Arbuscular Mycorrhiza on biochemical parameters under drought stress in Basmati Rice

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Abstract

The plants face so much stress which includes the biotic as well as abiotic. Abiotic stress like cold, drought, and heat stress is very common and these all are impacted the plants. The study of drought stress is the main and important component in improving plant health. The present study deals with the study of drought tolerance with the response of mycorrhiza on the rice plant. Drought tolerance is the mechanism in plants where the plant tolerates stress by producing antioxidants and develops some morphological changes. In this study, the rice plants were subjected to drought conditions, and various biochemical parameters were figured out from this research. The biochemical parameters like proline content where the AMF was more effective at the tillering phase of the crop rather than the reproductive phase of the crop. The antioxidants' activity like catalase and SOD were shown high content with the inoculation of AMF in stress conditions. Total soluble sugar, lipid peroxidation, and ascorbate acid content showed positive results with the symbiosis of AMF in the stress condition. Therefore this study supports the research of inoculation of AMF in rice in drought stress but using two different varieties of rice.

Keywords: *AMF, Antioxidants, Drought, Varieties.*

Introduction

The scarcity of water in the plants leads to abnormalities in the plants because it leads to some changes like morphological adaptations, and physiological and also biochemical changes . Rice is the water-loving crop and it requires 4000-5000 litres of water per kg of the grain produced and this crop has a less chance to survive in less water conditions. Kamoshita *et al*(2008).Rice which is more dependent on water survives only if there is water availability but the crop disturbed itself when there is a shortage of water Vallino *et al*(2009). To solve the problem mycorrhiza is one of the biofertilizer's which can protect plants from various types of stress. Studies have been conducted on mycorrhiza that the plants can overcome the stress of water when the application of VAM have done. The mechanism of vesicular Arbuscular mycorrhiza which do the mechanism of drought tolerance as well as increases the resistance of drought to plants (Augé R.M,2001)

The Arbuscular vesicular fungus Mycorrhiza acts as a symbiotic relationship between plants and fungi itself and it treats the condition as drought.It supports the plant against the stress condition in rice AMF is the fungus that is mutually related between plant roots and the fungus itself. (Auge,2001). In this research, the study was conducted on rice with various treatments to observe the effect of AMF on the growth of rice when the plants come in contact with stress like drought. The main objective of this research was to check the negative effects of abiotic stress like drought on the semi-aquatic plants like Rice and observe the biochemical parameters that were impacted on the rice crop in drought stress conditions.

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Materials and Method

For this experiment, a complete randomized design (CRD) with twelve treatments and four replication were used using two different varieties of rice. Nursery of both Pusa basmati 1121 and Pusa basmati 1718 was prepared after that the transplantation was done in pots. The treatments were chosen on the basis of the application of mycorrhiza. The drought stress was given for three to five days in all the treatments. The experiment was started with the sterilization of the soil in an autoclave at 120°with a pressure of 15 lbs. The two varieties of basmati were transplanted in the pot that was filled with mycorrhiza. For the determination of the water stress in soil, soil moisture was analyzed. The soil was saturated uniformly in all pots before the drought stress. To check the moisture percentage in the soil the sampling were done in drought stress treatments for up to three and five days.

Result And Discussion

Estimation of Proline Content in leaves of Rice

For determination of proline, leaf extract was prepared from all treatments in replicate. The final sample which was obtained appeared pink in colour and was observed at 520 nm in the spectrophotometer. Bates *et al*(1973)

Estimation of ascorbate acid content in leaf sample

For determination of ascorbate content in the leaf sample extract was prepared with 0.1 g of leaf sample which was crushed with 5 ml of 6 % TCA (trichloroacetic acid). Using with TCA spectrophotometer was calibrated at 530nm and other samples were observed at the same wavelength of 530 nm in the spectrophotometer. Mukherjee *et al*(1983)

Estimation of Malondialdehyde (MDA) content in leaves

For MDA determination content extract was prepared with 0.1g of plant material which was crushed with one ml of 5 % TCA (trichloroacetic acid). The intensity of the red colour gave the indication of MDA content and the absorbance was measured at 532 and 6000 nm in a spectrophotometer. Hodges DM *et al*(1999)

Estimation of catalase (CAT) activity in leaves

For determination of catalase activity in the plant, sample extract was prepared with 0.1 g of leaf samples which were crushed in five ml of 0.1 M phosphate buffer. The absorbances of the samples were measured in the spectrophotometer at 240 nm of wavelength. Aebi *et al* (1984)

Estimation of Superoxide Dismutase activity in leaves

For estimation of SOD in leaf samples extract was prepared with 0.1g of leaf samples which was crushed with five ml of 0.1M phosphate buffers. The readings of the samples were observed at 560 nm of wavelength in a spectrophotometer. Dhindsa *et al*(1981)

Statistical analysis

Two-way analysis of variance (ANOVA) was carried out at a 0.05 level of significance on the data and SPSS version 13.0 was used. The values corresponded to each table in the results K.A. Gomez et al(1984)

Result and discussion

Effect of AMF on Proline content in drought stress

Proline content was estimated at 45 and 90DAT during drought stress. During 45 DAT the highest proline content was found in T7 which was 580.96 (µg/g) followed by T1 which was significantly different from all other treatments. The lowest proline content was observed in absolute control which was T2 53.61 (µg/g) followed by T4. During 90 DAT all the treatments with AMF or without AMF in drought stress were shown significant changes.

Effect of AMF on Catalase activity in drought stress

The catalase content was estimated at 45 DAT where CAT activity was higher only in one variety of PB1718 in drought stress by using AMF. On the other hand, at 90 DAT both varieties of basmati i.e. PB1121 and PB1718 showed better results in the higher catalase activity.

Effect of AMF on Superoxide dismutase (SOD) activity in drought stress

The overall comparative study showed that at 45 DAT the higher SOD activity was determined in

AMF treatments and also without AMF in drought stress conditions. Both varieties i.e. PB1121 and PB 1718 well respond to AMF and also without AMF in drought stress. But at 90 DAT the AMF showed variation and increment of SOD activity in only one treatment of drought stress. The result of this study signifies that SOD activity was higher by using AMF in drought stress Per *et al*(2016).

Effect of AMF on Malondialdehyde content (MDA) in drought stress

The MDA content at 45 DAT showed significant changes. The highest MDA content was recorded from T6 which led to 44nmoles where there was the stress of three days and was significantly different from absolute control. On the other hand, MDA content at 90 DAT showed positive changes in decreasing MDA content in treatments where AMF was symbiotically associated with host plants in drought stress as compared to the plants without AMF in drought stress.

Effect of AMF on Ascorbate acid content in **drought stress**

Ascorbate acid content was estimated both at 45 and 90 DAT where AMF brings a variation in treatments which had AMF in drought stress.At 45 DAT the highest ascorbate acid was found in T8 which was 67.1026 microgram/ml which was significantly different. At 90DAT highest ascorbate content was found in T11(36.4615microgram/ml) which was significantly different from all other treatments while the lowest content was estimated in T2 (4.4872microgram/ml)

Conclusion

When the proline accumulated in plants this result in minimising the osmotic potential of plants which sustain the plants to maintain the photosynthetic apparatus by maintaining turgid pressure (Ruiz-Lozano *et al* 1996; Wang *et al* 2004; Kandowangko *et al* 2009). In the present study, the overall discussion stated that AMF showed a better response at 45 DAT but was not much effective at 90 DAT in drought stress. The Proline content was increased in 45 DAT and show high proline content in AMF plants in drought stress. This result was also the same with the *Oryza sativa* where AMF-mediated plants in drought stress progressively increase proline content. The lower proline accumulation in mycorrhiza plants as compared to non amf plants are correlated with several studies in *Pistacia vera* (Abbaspour *et al* 2012 ; Aroca *et al* 2008 ; Fan and Liu 2011;Hong *et al* 2000). The results of other study were similar to our result because it signifies that when proline accumulates in plants during stress, the host plant has a better capacity for osmotic adjustment as compared to non-AMF plants. Catalase activity was higher at the tillering phase of the crop and was significant at 45 DAT which stated that AMF only responds to PB-1718 in drought stress. But at 90 DAT both varieties i.e. PB1121 and PB1718 showed significant changes in catalase activity in response to AMF in the stress of drought. These results were consistent with the study by Huang *et al* (2014) where CAT activity is enhanced with the responses of AMF in drought stress. At 45 DAT the higher SOD activity was determined in AMF treatments and also without AMF in drought stress conditions.

Both varieties i.e. PB-1121 and PB 1718 well respond to AMF and also without AMF in drought stress. But at 90 DAT the AMF had shown variation and increment of SOD activity in only one treatment of drought stress. From the comparison of the two varieties at 90 DAT, PB1718 performed much better as compared to PB1121. The result of this study signifies that SOD activity was higher by using AMF in drought stress Per *et al(*2016) The analysis of the results was correlated in the research of soya bean where only shoot SOD activities showed a positive interaction with mycorrhiza and observed significant changes with *G. mosseae* Porcel *et al* (2003). In water-deficient conditions AMF symbiosis plants had less content of MDA content in plants and PB1718 was more effective both at 45 and 90 DAT. This result was correlated that AMF cam decreases the overall damage to cell membranes.F. Zhao(2012).

When plants are subjected to drought stress oxidative stress damage the membrane of cell and causes a high amount of MDA Q. S. Wu *et al(*2004) .In moisture stress conditions , oxidative stress are caused due to the misbalance between the production of MDA as well as scavenging of ROS . This phenomenon cause the lipid peroxidation of the cell .The MDA production results in the degradation of the membrane (Abbaspour *et al* 2012; Lacan et *al* 1998). In the present study of basmati, less content of MDA was correlated with the work of (M. Ruiz Sanchez *et al* 2010). Similar study where less content of MDA was estimated where the maize plants were associated with AMF in stress conditions. Zhu, X.C *et al*(2010)

Table 1: Analysis of variance of data for the Biochemical parameters like CAT, SOD and Proline of Basmati Rice variety PB1121 and PB1718 by AMF

Treatments	Catalase mg/g 45DAT	Catalase mg/g 90DAT	SOD (EU/g) 45DAT	SOD(EU/g) 90DAT	Proline(µg/g) 45DAT	Proline(µg/g) 90DAT
T1=R1+WRC	14.76±.36^{c,d}	40.52±.17^{b}	0.41±.00^{d}	0.57±.01^{d}	263.54±2.34^{f}	651.87±1.19^{a}
T2=R2+WRC	24.65±.35^{f}	72.30±1.70^{d,e}	0.42±.00^{d,e}	0.41±.00^{a,b}	53.61±3.92^{a}	236.96±2.38^{d}
T3=R1+A+WRC	25.46±.85^{f,g}	32.66±.02^{a}	0.34±.003^{b}	0.36±.005^{a,b}	162.18±2.81^{c}	459.05±3.92^{f}
T4=R2+A+WRC	24.89±.72^{f}	61.51±1.32^{c}	0.27±.001^{a}	0.55±.005^{c,d}	99.11±6.25^{b}	798.26±6.06^{i}
T5=R1+(WRC+3Days)	12.61±.39^{a,b}	73.11±1.02^{d,e}	0.41±.003^{dd}	0.35±.04^{a}	161.28±5.01^{c}	297.13±3.76^{e}
T6=R2+(WRC+3Days)	26.31±.28^{g}	34.75±.02^{a}	0.44±.01^{e}	0.73±.02^{e}	192.36±6.30^{d}	205.42±1.56^{c}
T7=R1+(WRC+5Days)	11.92±.02^{a}	35.00±.09^{a}	0.44±.005^{e}	0.40±.08^{a,b}	580.96±4.75^{h}	520.77±2.50^{g}
T8=R2+(WRC+5Days)	14.77±.04^{c,d}	61.20±1.69^{c}	0.38±.003^{c}	0.36±.003^{a,b}	360.85±4.12^{g}	1093.81±1.19^{j}
T9=R1+A+(WRC+3Days)	13.60±.31^{b,c}	42.42±.08^{b}	0.57±.008^{h}	0.35±.01^{a}	226.15±3.92^{e}	206.87±1.40^{c}
T10=R2+A+(WRC+3Days)	26.49±.02^{g}	86.43±1.69^{f}	0.46±.003^{f}	0.34±.02^{a}	361.75±5.64^{g}	135.51±3.03^{a}
T11=R1+A+(WRC+5Days)	15.15±.25^{d}	75.07±1.34^{e}	0.58±.008^{h}	0.46±.03^{b,c}	102.71±1.35^{b}	155.57±1.46^{b}
T12=R2+A+(WRC+5Days)	19.60±.05^{e}	70.89±.05^{c}	0.50±.003^{g}	1.96±.008^{f}	101.81±1.19^{b}	210.48±2.82^{c}

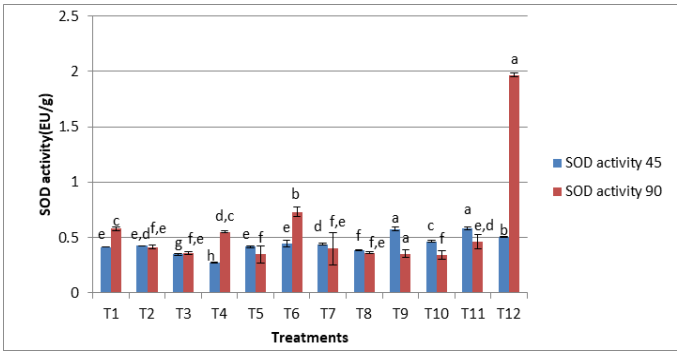
Arbuscular Mycorrhiza Fungi,, R1-PB1121, R2-PB1718 , WRC -Water at recommended critical stages , T-Treatments, SOD-superoxide dismutase ,
Values are means ± SE, n=3, the mean followed by a similar letter(s) are not significantly different at p=0.05, according to DMRT (Duncan's Multiple Range Test)

Table 2: Analysis of variance of data for the Biochemical parameters like Malondialdehyde (MDA) and ascorbate acid content were measured under drought stress of PB1121 and PB1718 by AMF

Treatments	MDA 45DAT (nmoles)	MDA 90DAT (nmoles)	Ascorbate acid 45DAT(µg/ml)	Ascorbate acid 90DAT (µg/ml.)
T1=R1+WRC	1.46±.21^{a}	6.00±2.51^{a}	14.02±.84^{a}	18.12±.16^{f}
T2=R2+WRC	3.70±.98^{a}	7.00±1.52^{a,b}	20.88±.22^{b}	4.48±.23^{a}
T3=R1+A+WRC	6.50±1.72^{a}	11.33±2.60^{a,b,c}	21.07±.29^{b}	11.14±.23^{e}
T4=R2+A+WRC	3.96±.27^{a}	12.00±1.52^{a,b}	36.01±2.08^{e}	9.28±.33^{d}
T5=R1+(WRC+3Days)	30.00±2.88^{g}	65.66±3.38^{e}	26.25±1.77^{c}	9.53±.29^{d}
T6=R2+(WRC+3Days)	44.00±7.02^{d}	51.00±3.60^{f}	39.41±.72^{f}	7.80±.11^{c}
T7=R1+(WRC+5Days)	38.33±7.26^{f,g}	61.66±1.20^{e,f}	34.28±.33^{e}	5.75±.23^{b}
T8=R2+(WRC+5Days)	40.00±2.88^{e,f}	55.66±3.48^{e,f}	67.10±1.18^{h}	21.94±.97^{g}
T9=R1+A+(WRC+3Days)	16.66±2.40^{b,c}	24.66±2.60^{c,d}	11.52±.33^{a}	9.08±.27^{d}
T10=R2+A+(WRC+3Days)	13.06±1.55^{b}	23.00±4.35^{a,b,c}	24.85±.38^{c}	7.32±.15^{c}
T11=R1+A+(WRC+5Days)	19.66±.88^{c}	32.66±2.60^{d}	62.42±.22^{g}	36.46±.40^{h}
T12=R2+A+(WRC+5Days)	19.00±2.3^{c}	32.66±3.84^{d}	30.75±.67^{d}	9.87±.12^{d}

A-Arbuscular Mycorrhiza Fungi, R1-PB1121, R2-PB1718, WRC -Water at recommended critical stages, T-Treatments, MDA-Malondialdehyde,
Values are means ± SE, n=3, the mean followed by a similar letter(s) are not significantly different at p=0.05, according to DMRT (Duncan's Multiple Range Test

Analysis of SOD activity and CAT activity on the rice



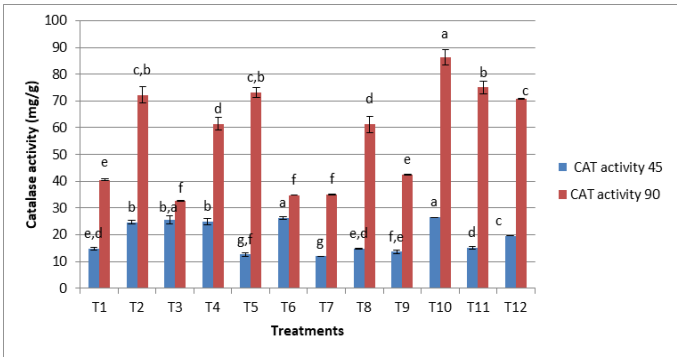
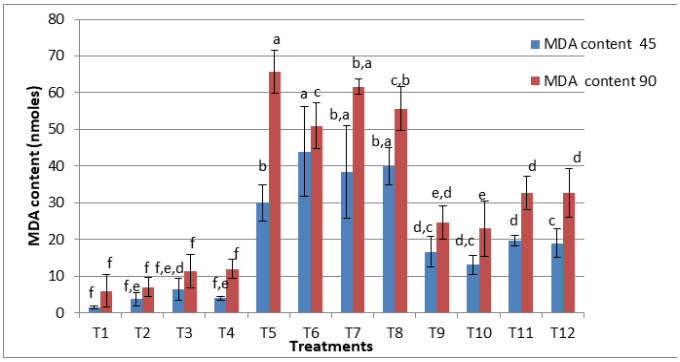
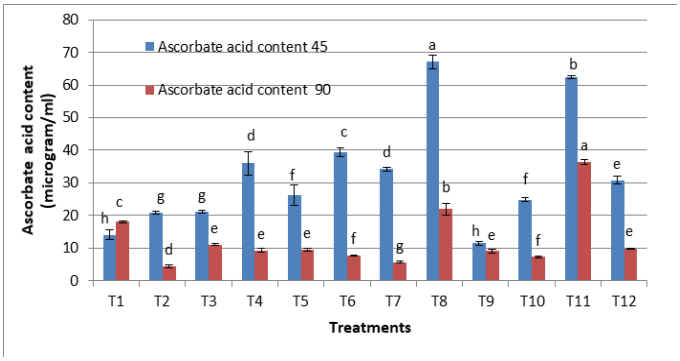


Fig 1: Effect of SOD activity and Catalase activity in drought stress.

Analysis of Ascorbate acid content, MDA content and Proline content on the rice





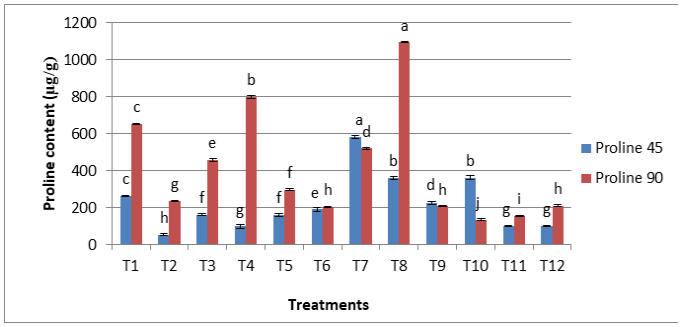


Fig 2: Effect of ABA, MDA and Proline content in drought stress

As the present study of Basmati rice in drought stress conditions with VAM were shown positive and significant changes with two different varieties at 45 and 90DAT. The present study showed that PB1718 brings more ascorbate acid content with AMF in drought stress conditions at 45 DAT and PB1121 was more effective at 90 DAT. Ascorbate is an antioxidant molecule which is useful in tolerating stress in host plants (Miguel *et al* 2006; Khan *et al* 2010; Naz *et al* 2016). In the present study, the accumulation of more ascorbate in drought stress leads to the protection of host plants from the reactive oxygen species. (Latif *et al* 2016; Mukhtar *et al* 2016; Naz *et al* 2016)

Conflict of interest: Nill

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