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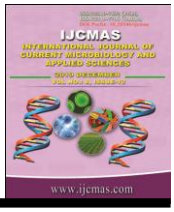

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Influence of Pink Pigmented Facultative Methylotrophs (PPFMs) and Plant Growth Promoters on Proline, Nitrate Reductase and Catalase Activity on Semi Dry Rice

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A B S T R A C T

Drought stress affects various physiological and metabolic processes in rice (*Oryza sativa* L.) plant. Non-availability of high-yielding varieties suitable for cultivation under drought condition lead to sharp decline in rice yield. The current study was aimed to carry out to assess the impact of PPFM and plant growth promoters on mitigating drought stress effects in semi dry rice. Field experiment was carried out in semi dry rice variety ADT 45 along with foliar spray different plant growth promoters like PPFM (500 ml ha^{-1}), brassinolide (1 ppm) and salicylic acid (100 ppm) under semi dry condition. The study indicated that the PPFM and plant growth promoters could effectively improve drought tolerance capacity of rice under semi dry condition. PPFM and different plant growth promoters was found to be superior in improving SPAD value and proline content. The antioxidant enzyme, catalase activity was enhanced by PPFM (500 ml /ha) and brassinolide (1 ppm) treatments which had the ability to protect the plant under abiotic stress by nullifying oxidative damage. Foliar spray of PPFM (500 ml ha^{-1}) was found effective in improving the NR activity followed by brassinolide (1ppm).

Introduction

Abiotic stress has been estimated that more than half of the yield potential of major crops is mainly affected due to unfavourable growing environments such as drought (Cortina and Culianez-Macia, 2005). The problem of drought is increasing day by day with reduction in production of crops (Qayyum and Malik, 1988). Drought stress disturbs water relations, causing a reduction in water-use efficiency. Several adaptive mechanisms have been developed within plants to counter the drought stress condition

including the accumulation of compatible solutes such as proline. There is evidence that higher levels of proline accumulation in plants are associated with greater tolerance in drought and stimulate oxidative stress tolerance by controlling antioxidant enzymes activities (Hayat *et al.,* 2010). The accumulation of proline also shows a role in plant growth regulation under drought stress. Drought has been shown to increase the activity of antioxidant enzymes such as Catalase (CAT) and Nitratereductase (NR). It was reported that antioxidant defence system components in plants were exaggerated differently depending on the degree of drought stress(Sharma and Dubey, 2005). There is a cumulative sign that physiological and biochemical attributes had been significantly affected under drought stress. Therefore, it is crucial for understanding the physiological and biochemical characteristics in plants to improve drought tolerance under abiotic stress. *Methylobacterium* species are a group of bacteria known as *pink-pigmented facultative methylotrophs*, or PPFMs (Green and Bousfield, 1983) and exogenous application of PPFM produces benefit in alleviating the adverse effects of drought stress and also improves germination, growth, development, quality and yield of crop plants (Hayat *et al.,* 2010). Van Loon *et al.,* (1998) reported that environmentally sustainable agricultural systems, the bacterial inoculants that provide cross protection against both biotic and abiotic stress would be highly preferable. Exogenously applied brassinolide alleviated the detrimental effects of drought in maize and remarkably improved the chlorophyll contents, protein, relative water contents (RWC), proline, and enzymatic antioxidants (Anjum *et al.,* 2011). Saruhan *et al.,* (2012) reported that the exogenous salicylic acid reduced the adverse effects of drought stress in maize and might have a key role in providing tolerance to stress by decreasing water loss and inducing the antioxidant system in plants with leaf rolling, an alternative drought protection mechanism. Application of cytokinin improves the physiological traits in terms of membrane stability index, relative water contents (RWC), and photosynthetic rate at the vegetative stage of wheat (Dwivedi *et al.,* 2014).

Materials and Methods

The field experiments were conducted in sandy clay loam soil of farmers field, Therkutheru, Melur Block, Madurai during 2017-18 with the short duration variety ADT 45. The experiment was conducted on the basis of factorial randomized block design with three replication. The experiment was conducted with the following treatments *viz*., Factor 1: Soil Application S_1: 100% Recommended Dose of Fertilizer (120:40:40 kg ha^{-1} N, P_2O_5 and K_2O), S_2: 100 % RDF + *Azospirillum* (soil application @ 2 kg ha^{-1}), S_3: 100 % RDF+*Azophos* (soil application @ 2 kg ha^{-1}) and S_4: 100 % RDF+ *Azotobacte*r (soil application @ 2 kg ha^{-1}), Factor 2: Foliar application F_1: PPFM (Foliar application 500 ml ha^{-1}), F_2: Brassinolide (1 ppm),F_3:Salicylic acid (100 ppm) and F_4: Control

The estimation of proline content was adopted by using the protocol of Bates *et al.,* (1973) and amount of proline in the sample is expressed in mg g^{-1}. 0.5 g leaf sample was homogenized with 10 mL of 3 per cent sulphosalicylic acid and centrifuged at 3000 rpm for 10 minutes. Two mL of the supernatant was taken and 2 mL of glacial acetic acid, 2 mL of ortho phosphoric acid and 2 mL of acid ninhydrin mixture were added.

The contents were allowed to react at 1000℃ for 1 hour under water bath and then it is incubated on ice for 10 minutes to terminate the reaction. The reaction mixture was mixed vigorously with 4 ml toluene for 15 to 20 seconds. The chromophore containing toluene

was aspired from the aqueous phase, warmed to room temperature and optical density was read at 520 nm.

Nitrate reductase activity was estimated in fully expanded functional leaves following the method of Nicholas *et al.,* (1976) by using assay medium (Phosphate buffer + KNO_3 + Iso propanol), sulphanilamide and N(1-Naphthylamino)ethylamine dihydrochloride (NEDH). The enzyme activity was expressed as µmol NO2 $g^{-1}h^{-1}$ using KNO_2 as a standard. Catalase activity was determined by following titration method using potassium permanganate (Gopalachari, 1963) and expressed as µg H_2O_2 g^{-1} min^{-1}. The data on various parameters were analyzed statistically as per the procedure suggested by Gomez and Gomez (1984).

Results and Discussion

Impact of PPFM and plant growth promoters on proline

Proline is believed to protect plant tissues against stress by acting as nitrogen storage, osmo regulator and protectant for enzymes and cellular structure. It is one of the important amino acids, is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stress (Ali *et al.,* 1999).From the current study, it was noted that the higher accumulation of proline was found in semi dry condition compared to control. The maximum proline content, *Azophos* @ 2 kg ha-1and PPFM ranged from 1.25, 3.48 and 4.76 µ mol g^{-1} followed by 1.21, 3.34 and 4.62 µ mol g^{-1} were though consolidating brassinolide @ 1 ppm at 30 DAS, 60 DAS and at harvest stage exclusively (Table 1). This might be due to the fact that PPFM and plant growth promoters reduced the impact of stress leading to high level of proline accumulation. However, the proline content is higher than control in all the PPFM and plant growth promoters treated plants. Our results are agreeable with the findings of Uyprasert *et al.,* (2004), who stated that proline acts as a compatible solute and a protective agent for cytoplasmic enzymes and structures. The role of ameliorants such as PPFM and brassinolide was significant in increasing the content of proline in the stressed plants (Aruna *et al.,* 1999).

These bioregulators could increase the hydrolysis of macro- molecules into the simpler ones like mono and disaccharides and amino acids especially proline etc. and consequently higher osmolyte concentration resulting in favourable osmoregulation process during water stress conditions.

Impact of PPFM and plant growth promoters on nitrate reductase and catalase activity

NR activity is vital for the metabolic and physiological status of plants and can be used as a biomarker of plant stress including drought, since, nitrate reductase activity decreases in plants exposed to water limitation (Azcon*et al.,* 1996). In the present study too the data showed a concomitant increment concerning maximum nitrate reductase activity, *Azophos* @ 2 kg $ha^{-1}$and PPFM ranged from 54.9, 57.8 and 55.1 µg NO_2 g^{-1} h^{-1} followed by 52.8, 56.1 and 53.3 µg NO_2 $g^{-1}h^{-1}$were though consolidating brassinolide @ 1 ppm at 30 DAS, 60 DAS and at harvest stage exclusively (Table 2 and 3). The optimistic role of PPFM for the protection of NR activity under drought was observed in the present study. However, the physiological basis for the protection of NR activity by PPFM will be elucidated.

The enzyme catalase involved in the detoxification of reactive oxygen species, especially hydrogen peroxide.

Table.1 Effect of PPFMs and plant growth promoters on proline content (µ mol g^{-1}) of semi - dry rice (pooled analysis for two years)

Soil application	Proline content (µ mol g^{-1})														
	30 DAS					60 DAS					At harvest				
	Foliar application					Foliar application					Foliar application				
	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean
S1	0.71	0.66	0.62	0.54	**0.63**	2.35	2.21	2.13	2.00	**2.17**	3.47	3.37	3.25	3.07	**3.28**
S2	1.12	1.07	0.94	0.58	**0.92**	3.00	2.87	2.74	2.04	**2.65**	4.27	4.16	4.02	3.11	**3.89**
S3	1.25	1.21	1.16	0.60	**1.05**	3.48	3.34	3.23	2.08	**3.03**	4.76	4.62	4.44	3.16	**4.24**
S4	0.87	0.78	0.74	0.56	**0.74**	2.68	2.61	2.49	2.04	**2.45**	3.86	3.77	3.62	3.09	**3.58**
Mean	**0.99**	**0.93**	**0.86**	**0.57**		**2.88**	**2.76**	**2.65**	**2.06**		**4.09**	**3.98**	**3.83**	**3.11**	
	S	F	S x F			S	F	S x F			S	F	S x F		
SEd	0.01	0.01	0.02			0.03	0.03	0.06			0.04	0.04	0.09		
CD (P=0.05)	0.02	0.02	0.03			0.07	0.07	0.13			0.09	0.09	0.18		

Treatment details

S1 : 100 % RDF (75:25:37.5 kg ha^{-1}N, P_2O_5, K_2O)	**F1 : PPFM (Foliar application 500 ml ha^{-1})**
S2 : 100 % RDF + Azospirillum (Soil application @ 2 kg ha^{-1})	**F2 : Brassinolide (1 ppm)**
S3 : 100 % RDF + Azophos (Soil application @ 2 kg ha^{-1})	**F3 : Salicyclic acid (100 ppm)**
S4 : 100 % RDF + Azotobacter (Soil application @ 2 kg ha^{-1})	**F4 : Control**

Table.2 Effect of PPFMs and plant growth promoters on catalase activity (µg of H_2O_2 g^{-1} m^{-1}) of semi - dry rice (pooled analysis for two years)

Soil application	Catalase activity (µg of H_2O_2 $g^{-1}m^{-1}$)														
	30 DAS					60 DAS					At harvest				
	Foliar application					Foliar application					Foliar application				
	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean
S1	23.7	24.2	24.4	26.6	**24.7**	27.4	27.6	27.9	30.2	**28.2**	25.3	25.6	25.8	30.2	**26.7**
S2	21.7	21.8	21.9	26.3	**22.9**	26.5	26.7	26.8	29.9	**27.5**	24.9	25.1	25.2	28.9	**26.1**
S3	20.2	20.3	20.4	25.7	**21.6**	26.2	26.4	26.5	29.4	**27.1**	24.5	24.7	24.9	28.0	**25.5**
S4	22.4	22.5	22.7	23.5	**22.8**	26.4	26.6	26.8	29.9	**27.4**	24.9	25.1	25.2	29.5	**26.2**
Mean	**22.0**	**22.3**	**22.4**	**25.5**		**26.6**	**26.8**	**26.9**	**29.8**		**24.9**	**25.1**	**25.3**	**29.2**	
	S	**F**	**S x F**			**S**	**F**	**S x F**			**S**	**F**	**S x F**		
SEd	0.22	0.22	NS			0.34	0.34	NS			0.28	0.28	NS		
CD (P=0.05)	0.44	0.44	0.89			0.71	0.71	1.42			0.59	0.59	1.18		

Treatment details

S1 : 100 % RDF (75:25:37.5 kg ha^{-1}N, P_2O_5, K_2O)	**F1 : PPFM (Foliar application 500 ml ha^{-1})**
S2 : 100 % RDF + Azospirillum (Soil application @ 2 kg ha^{-1})	**F2 : Brassinolide (1 ppm)**
S3 : 100 % RDF + Azophos (Soil application @ 2 kg ha^{-1})	**F3 : Salicyclic acid (100 ppm)**
S4 : 100 % RDF + Azotobacter (Soil application @ 2 kg ha^{-1})	**F4 : Control**

Table.3 Effect of PPFMs and growth promoters on nitrate reductase activity (µg of NO_2 g^{-1} h^{-1})of semi - dry rice (pooled analysis for two years)

Soil application	Nitrate reductase activity (µg of NO_2 $g^{-1}h^{-1}$)														
	30 DAS					60 DAS					At harvest				
	Foliar application					Foliar application					Foliar application				
	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean	F1	F2	F3	F4	Mean
S1	40.9	39.4	38.0	35.3	**38.4**	43.9	42.4	40.9	37.9	**41.3**	42.2	40.5	39.2	35.6	**39.3**
S2	49.9	48.2	47.3	36.1	**45.4**	52.8	51.4	48.4	39.3	**47.9**	50.5	48.9	47.5	36.7	**45.9**
S3	54.9	52.8	51.3	36.4	**48.8**	57.8	56.1	54.5	40.0	**52.0**	55.1	53.3	51.9	37.6	**49.4**
S4	45.5	43.9	42.6	35.9	**41.9**	48.5	46.9	45.4	38.6	**44.8**	46.5	45.0	43.5	36.4	**42.8**
Mean	**47.8**	**46.2**	**44.7**	**35.9**		**50.7**	**49.2**	**47.3**	**38.9**		**48.5**	**46.9**	**45.4**	**36.5**	
	S	F	S x F			S	F	S x F			S	F	S x F		
SEd	0.34	0.34	0.68			0.48	0.48	0.97			0.44	0.44	0.88		
CD (P=0.05)	0.70	0.70	1.40			0.99	0.99	1.99			0.90	0.90	1.81		

Treatment details

S1 : 100 % RDF (75:25:37.5 kg ha^{-1}N, P_2O_5, K_2O)	F1 : PPFM (Foliar application 500 ml ha^{-1})
S2 : 100 % RDF + Azospirillum (Soil application @ 2 kg ha^{-1})	F2 : Brassinolide (1 ppm)
S3 : 100 % RDF + Azophos (Soil application @ 2 kg ha^{-1})	F3 : Salicyclic acid (100 ppm)
S4 : 100 % RDF + Azotobacter (Soil application @ 2 kg ha^{-1})	F4 : Control

In the present study also the data showed a concomitant increment concerning catalase activity by the combined application of soil and foliar application recorded the minimum catalase activity ranged from of 20.2, 26.2 and 24.5 µg of H_2O_2 g^{-1} m^{-1}respectively, which was followed by 20.3, 26.4 and 24.7µg of H_2O_2 g^{-1} m^{-1}were though joining brassinolide @ 1 ppm at the harvest stage. Among the enzymes, catalase (CAT) is an important and most powerful antioxidant enzyme under abiotic stress condition to nullify the effect of H_2O_2 and protects the plants under stress condition. This enzyme is generally regarded as H_2O_2 scavenger involved in the reduction of damage by oxidation function (Reddy *et al.*, 2004). Abd El-Gawad *et al.*, (2015) found that the antioxidant enzymes like catalase and SOD activity were increased by the PPFM in snap bean.

Agricultural production has been declined year by year due to many abiotic stresses especially drought. Of the various management practices available, mitigation through plant growth promoters and bio-products like PPFM are promising measures to enhance water status of the plant, photosynthetic rate, compatible osmolytes namely, proline, nitrate reductase and antioxidant enzyme like catalase activity which protects the plant under any abiotic stress condition. Therefore, these results have practical field application to protect the plant under water stress condition especially PPFM. Among the treatment combinations RDF + *Azophos*@2 kg ha^{-1} with PPFM @ 500 ml ha^{-1}haveregistered maximum proline content, nitrate reductase and catalase activity on semi dry rice.

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