Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 2920-2923
Received: 04-07-2020
Accepted: 09-08-2020

Aswathy JC
Department of Agronomy, Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

P Shalini Pillai
Department of Agronomy, Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Jacob John
Kerala Agricultural University, Integrated Farming System Research Station, Karamana, Thiruvananthapuram, Kerala, India

KS Meenakumari
Department of Agricultural Microbiology, Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Corresponding Author:
Aswathy JC
Department of Agronomy, Kerala Agricultural University, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala, India

Physiological parameters of rice (*Oryza sativa* L.) as influenced by pink pigmented facultative methylotrophs (PPFM)

Aswathy JC, P Shalini Pillai, Jacob John, KS Meenakumari

Abstract
A field experiment was conducted to assess the effect of PPFM on the growth of aerobic rice, at Integrated Farming System Research Station, Karamana, Thiruvananthapuram, Kerala, during summer, 2019 -2020 with the medium duration rice variety MO16 (Uma). The experiment was laid out in factorial randomised block design with [(5 x 2) + 2] treatments replicated thrice. The treatments comprised of five promising isolates of PPFM (P) obtained from the Department of Agricultural Microbiology, College of Agriculture, Vellayani, two methods of application (M) compared against two controls (C). The physiological parameters *viz.* chlorophyll content and proline accumulation had positive effect on application PPFM. The isolate PPFM 38 resulted in higher chlorophyll content and proline accumulation at both panicle initiation stages. Method of application did not show any significant difference in the chlorophyll content of rice whereas, the proline accumulation was found to be significantly higher with seed treatment with PPFM 38. The combined effect of PPFM and method of application was observed to be superior for the above mentioned physiological parameters significantly higher content at p_5m_2 both at panicle initiation and flowering stages.

Keywords: Pink pigmented facultative methylotroph, chlorophyll, proline

Introduction
Rice is the most extensively consumed staple food of more than half of the world's population, providing 21 per cent of global human per capita energy and 15 per cent of per capita protein (Khush, 2005). In the world, rice (*Oryza sativa* L.) is one of the most important field crops that provide staple food to millions after wheat. It is considered as an indispensable source of calories for majority of the population within Asia. Asia which is the native of 60 per cent of Earth's population produces and consumes about 90 per cent of the rice in the world. Rice occupies an area of 167.13 m ha with a production of 782 million tonnes, covering all continents except Antarctica (FAOSTAT, 2018) [4].

Plants are subjected to several harsh environmental stresses like drought, salinity, low and high temperatures, flood, pollutants, and radiation adversely affect growth, metabolism, and yield and ultimately the productivity of crops (Lawlor, 2002) [12]. Enhancing rice production assumes paramount importance despite the constraints and challenges faced by the rice farmers. India ranks first in area and production among all the rice growing countries in the world next to China. In India rice alone is cultivated in 43.9 m ha with production of around 110.15 million tonnes and a productivity of 2.50 t ha^{-1}(GOI, 2016) [5].

Though being the largest producer in rice, the productivity is the lowest among the developing countries which need to be improved. India alone would need about 122 million tonnes of rice for domestic consumption. Among cereal crops, rice consumes about 80 per cent of the total irrigated fresh water resources. The rice production in Asia has declined due to increasing water stress (Tao *et al*., 2004).

In ecologically sustainable agricultural systems, the bacterial inoculants that provide cross protection against both biotic and abiotic stress would be extremely desirable (van Loon *et al.*, 1998) [19]. Proline is one of the most important amino acids, which occur widely in higher plants that accumulates in large quantities normally in response to environmental stress. The concentration of proline has been shown to be generally higher in stress-tolerant than in stress-sensitive plants and the accumulation is mainly correlated with stress tolerance. It has been suggested by Madhusudhan *et al*. (2002) [14] that under conditions of water stress accumulation of proline contributes to maintain proper balance between extra and intra-cellular osmolarity.

Proline serves as a compatible solute and a protective agent for cytoplasmic enzymes and structures.

In plant stress tolerance, proline accumulation is believed to play an important role (Ashraf and Foolad, 2007) [2]. Water stress induces a significant decrease in the metabolic factors such as decrease in the chlorophyll content and an increase in the proline accumulation.

Sivakumar *et al.* (2017) [18] suggested that the positive effect of PPFM might be due to the increment of osmolytes like proline that enhances the water uptake and maintain the water status of the plant. Phyllosphere isolates were observed to increase the content of proline, total sugars and total amino acids under drought conditions induced by poly ethylene glycol (PEG), as compared against stress-free condition (Kumar *et al*., 2017) [11]. Riyas (2019) [15] observed significantly higher proline content in PPFM 37 treated plants under different moisture levels. At FC, the maximum proline content of 90.18μg g^{-1} tissue was recorded with the PPFM 37 isolate.

Keeping the above information on hand, the present study was undertaken to assess the impact of PPFM on the physiological parameters such as chlorophyll content and proline accumulation in rice crop.

Materials and Methods

A field study was conducted at Integrated Farming System Research Station, Karamana, Thiruvananthapuram, Kerala, during summer, 2019 -2020. The variety selected for the study was MO16 (Uma), a medium duration one. The experiment was laid out in factorial randomised block design with [(5 x 2) + 2] treatments, replicated three times. The treatments comprised five promising isolates of PPFM (P) made available from the Department of Agricultural Microbiology, College of Agriculture, Vellayani and two methods of application (M) compared against two controls (C). The isolates included PPFM 16, PPFM 26, PPFM 35, PPFM 37 and PPFM 38 whereas, the methods of application used were seed treatment (1%) and seed treatment (1%) + foliar application (2%) at 30 and 50 DAS. Controls used were package of practices recommendation of Kerala Agricultural University (KAU POP) and KAU POP + water spray at 30 and 50 DAS. In the case of KAU POP, irrigation was given once in three days up to panicle initiation stage and thereafter continuous irrigation was given. Wherever foliar application and water spray were provided, irrigation was stopped one week before and after the treatment. The soil of the experimental site was moderately acidic in reaction (pH - 6.05) with normal electrical conductivity (0.24 dS m^{-1}). The data generated were statistically analysed using analysis of variance technique (ANOVA), as applied to randomized block design (Gomez and Gomez, 2010) [6].

Result and Discussion

Chlorophyll Content

The variation in the chlorophyll content with PPFM and method of application is presented in Table 1a and 1b.

The isolate PPFM 38 resulted in higher chlorophyll content at both panicle initiation (1.109 mg g^{-1} of leaf tissue) and flowering (1.151 mg g^{-1} of leaf tissue) stages. However, the effect of PPFM 38 was comparable with PPFM 37 at both the growth stages. Method of application did not show any significant difference in the chlorophyll content of rice.

The interaction effect of PPFM and method of application was higher for PPFM 38 as seed treatment followed by foliar application at both panicle initiation (1.27 mg g^{-1} of leaf tissue) and flowering (1.29 mg g^{-1} of leaf tissue) stages. It was at par with p_5m_1 and p_4m_2 during both the above growth stages. KAU POP (c_1) was found to show significantly higher chlorophyll content when compared to KAU POP + water spray (c_2) at panicle initiation and flowering stages.

The treatments when compared against the controls revealed significance at panicle initiation stage, with KAU POP (c_1) recording higher chlorophyll content. However there was no significant difference between the treatment effect and controls at flowering stage.

The effect of PPFM in increasing the chlorophyll content has been reported by several workers. Methylobacterium inoculation has been reported to increase the photosynthetic activity by increasing the stomatal count, chlorophyll content and malic acid content in crops (Madhaiyan *et al*., 2004) [13]. Satyan *et al*. (2018) [17] have also observed an increase the chlorophyll stability index of small cardamom due to PPFM inoculation. In the present study also, a similar trend was observed as evidenced by the higher chlorophyll content in the treatments compared to the controls. Between the two controls, KAU POP + water spray recorded a lower chlorophyll content. This is possibly due to the effect of periodical moisture stress imposed. Moisture stress induces reduction in chlorophyll level, stomatal conductance and photosynthesis (Sanchez *et al*., 1983) [16]. The decrease in chlorophyll in response to stress has been attributed to enhanced catabolism of the chlorophyll complex or due to severe retardation in chlorophyll synthesis. Greening under rewatering was observed to be inhibited by at least 50 per cent even under mild moisture stress (Alberte *et al*., 1975) [1].

Proline Accumulation

Treating rice with PPFM 38 (p_5) resulted in significantly higher proline accumulation of 0.87 μg mol^{-1} each at panicle initiation and flowering stages (Table 1a). Seed treatment with PPFM (m_1) recorded a significantly higher proline content when compared to seed treatment followed by foliar application (m_2) (Table 1b).

The combined effect of PPFM and method of application was observed to be superior for proline content with significantly higher content at p_5m_2 both at panicle initiation and flowering stages. It was followed by p_4m_1. Between the controls, c_2 (KAU POP + water spray) showed significantly higher proline content at both the stages compared to c_1 (KAU POP).

Proline content was observed to be significantly higher in the treatments than the controls, at panicle initiation and flowering stages. Proline is a non-protein amino acid that has been observed to accumulate in plants when subjected to moisture stress (Yoshiba *et al*., 1997) [20]. Increase in proline content in response to moisture stress might be the result of the transcriptional activation of P5CS (delta-1 pyrroline-5 carboxylate synthase) as reported by Babiychuk *et al*. (1996) [3] and the associated increase in protease activity, which causes the degradation of proteins under water stress conditions (Jain *et al*., 1996) [8]. Proline is switter ionic and highly hydrophilic in nature and hence acts as a compatible solute, stabilizes sub cellular structures and help in the solubility of proteins and other bio polymers. This in turn aids in maintenance of turgour pressure of both root and shoot even when plant is under moisture stress. Similar findings have been reported by Gowri (2005) [7] and Jinsy (2014) [9].

The higher proline accumulation observed with KAU POP + water spray might be due to the moisture stress imposed before and after the spraying water.

Table 1a: Effect of PPFM and method of application on chlorophyll content and proline accumulation

Treatment	Chlorophyll content (mg g^{-1} of leaf tissue)		Proline accumulation (µg mol^{-1} of fresh weight)	
	Panicle initiation	Flowering	Panicle initiation	Flowering
PPFM (P)				
p_1 – PPFM 16	0.620	0.687	0.551	0.529
p_2 – PPFM26	0.517	0.552	0.403	0.404
p_3 – PPFM 35	0.494	0.506	0.705	0.702
p_4 – PPFM 37	0.790	0.862	0.716	0.697
p_5 – PPFM 38	1.109	1.151	0.870	0.870
SE m (±)	0.164	0.163	0.006	0.012
CD (0.05)	0.3437	0.3421	0.0137	0.0267
Method of application (M)				
m_1 - seed treatment (1%)	0.655	0.698	0.673	0.656
m_2 - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	0.757	0.805	0.624	0.625
SE m (±)	0.164	0.163	0.006	0.012
CD (0.05)	NS	NS	0.0087	0.0169

Table 1b: Effect of PPFM and method of application on chlorophyll content and proline accumulation

Treatment	Chlorophyll content (mg g^{-1} of leaf tissue)		Proline accumulation (µg mol^{-1} of fresh weight)	
	Panicle initiation	Flowering	Panicle initiation	Flowering
PPFM (P) x Method of application (M)				
p_1m_1 (PPFM 16 - seed treatment)	0.692	0.727	0.678	0.656
p_1m_2 (PPFM 16 - seed treatment + foliar application)	0.547	0.647	0.423	0.402
p_2m_1 (PPFM 26 - seed treatment)	0.400	0.430	0.394	0.390
p_2m_2 (PPFM 26 - seed treatment +foliar application)	0.634	0.674	0.413	0.420
p_3m_1 (PPFM 35 - seed treatment)	0.510	0.524	0.689	0.682
p_3m_2 (PPFM 35 - seed treatment +foliar application)	0.478	0.487	0.720	0.722
p_4m_1 (PPFM 37 - seed treatment)	0.720	0.789	0.915	0.880
p_4m_2 (PPFM 37 - seed treatment +foliar application)	0.859	0.926	0.516	0.513
p_5m_1 (PPFM 38 - seed treatment)	0.952	1.011	0.689	0.672
p_5m_2 (PPFM 38 - seed treatment +foliar application)	1.266	1.290	1.051	1.067
Control (C)				
c_1 - KAU POP	1.048	1.080	0.205	0.246
c_2 – KAU POP + water spray	0.876	0.915	0.367	0.320
SE m (±)	0.150	0.150	0.020	0.010
CD (0.05)	0.4497	0.4580	0.0446	0.0341
Treatment *vs*. Control1	S	NS	S	S
Treatment *vs*. Control 2	NS	NS	S	S

Conclusion

Chlorophyll content and proline accumulation estimated at panicle initiation and flowering stages exhibited remarkable variation with PPFM treatment. The present study revealed that the application of PPFM 38 as seed treatment (1%) followed by foliar application (2%) at 30 and 50 DAS was found to show a positive effect on the physiological parameters (chlorophyll content and proline accumulation) in rice.

Reference

1. Alberte RS, Fiscus EL, Naylor AW. Effects of water stress on the development of the photosynthetic apparatus in greening leaves. Plant Physiol. 1975; 55:317-321.
2. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 2007; 59(2):206-216.
3. Babiychuk E, Kushnir SV, Bellis-Boix E, Montagu MV, Inze D. *Arabidopsis thaliana* NADDH oxidoreductase homologs confer tolerance of yeasts towards the thiol-oxidising drug diamide. J Biol. Chem. 1996; 270:26224-26231.
4. FAOSTAT [Food and Agricultural Organization Corporate Statistical Database]. 2018. FAO home page [on-line]. Available: http://faostat.fao.org [18 October, 2018].
5. GOI [Government of India]. *Economic Survey of India: 2016-2017*. Finance Division, Economic Advisory Wing, New Delhi, India, 2016, 21.
6. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research. John Wiley and Sons, Inc., New York, 1984, 67-215.
7. Gowri S. Physiological studies on aerobic rice (*Oryza sativa* L.). MSc(Ag) thesis, Tamil Nadu Agricultural University, Coimbatore, 2005, 145.
8. Jain S, Agarwal RM, Tiwari HS. Seedling growth of rice (*Oryza sativa* L. var. Kranti) under polyethylene glycol 6000 induced water stress. Physiol. Mol. Bio. Plants. 1996; 2:71-74.
9. Jinsy VS. Productivity analysis of aerobic rice (*Oryza sativa* L.) and its impact on greenhouse gas emission. Ph D thesis, Kerala Agricultural University, Thrissur, 2014, 183.
10. Khush G. Productivity improvements in rice. Nutr. Rev. 2005; 61:114-116.
11. Kumar AS, Sridar R, Uthandi S. Mitigation of drought in rice by a phyllosphere bacterium *Bacillus altitudinis* FD48. Afr. J Microbiol. Res. 2017; 11(45):1614-1625.

12. Lawlor DW. Limitation to photosynthesis in water stressed leaves: stomata *vs*. metabolism and the role of ATP. Ann. Bot. 2002; 89:1-15.
13. Madhaiyan M, Poonguzhali S, Senthilkumar M, Sundaram S, Heekyung C, Jinchul Y *et al*. Growth promotion and induction of systemic resistance in rice cultivar Co-47 (*Oryza sativa* L.) by Methylobacterium spp. Bot. Bull. Acad. Sin. 2004; 45:315-324.
14. Madhusudhan KV, Giridharakumar S, Ranganayakulu GS, Reddy PC, Sudhakar C. Effect of water stress on some physiological responses in two groundnut (*Arachis hypogoea* L.) cultivars with contrasting drought tolerance. J Plant Biol. 2002; 29:199-202.
15. Riyas NK. Screening of pink pigmented facultative methylotroph (PPFM) isolates for water stress tolerance and yield in paddy. MSc (Ag.) thesis, Kerala Agricultural University, Thrissur, 2019, 199.
16. Sanchez RA, Hall AJ, Trapani N, de Hunau RC. Effects of water stress on the chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. Photosynthesis Res. 1983; 4:35-47.
17. Satyan T, Mohanan AK, Dhanya MK, Aswathy TS, Preethy TT, Murugan M. Impact of pink pigmented facultative methylotrophic bacteria and synthetic materials on small cardamom (*Elettaria cardamom* Maton.) under drought. Pharma Innovation J. 2018; 7(1):1-4.
18. Sivakumar R, Nandhitha GK, Chandrasekaran P, Boominathan P, Senthilkumar M. Impact of pink pigmented facultative methylotroph and PGRs on water status, photosynthesis, proline and NR activity in tomato under drought. Int. J of Curr. Microbiol. Appl. Sci. 2017; 6(6):1640-1651.
19. Van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopath. 1998; 36:453-483.
20. Yoshiba Y, Kiyosue T, Nakashima K, Shinozaki YK, Shinozaki K. Regulation of levels of proline as an osmolyte in plants under water stress**.** Plant Cell Physiol. 1997; 38:1095-1102.