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Effect of pink pigmented facultative methylotrophs (PPFM) on growth and growth attributes of rice (*Oryza sativa* L.)

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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). Among the PPFM isolates tested, PPFM 38 resulted in taller plants, more number of tillers per square metre, leaf area index, dry matter production and root shoot ratio. However, root volume of rice was observed to be higher with PPFM 16 and remained at par with PPFM 38. Treating rice seeds with PPFM at 1 per cent followed by foliar application (2%) at 30 and 50 DAS had significantly superior effect on the above mentioned growth attributes except root volume. Growth and growth attributes were significantly superior with the treatment combination PPFM 38 as seed treatment + foliar application. Between the controls, KAU POP was significantly superior to KAU POP + water spray.

Keywords: PPFM, *Oryza sativa*, pink pigmented

Introduction

Food and water are considered as the basic requirements for survival of human beings. Water, nowadays is becoming a precious commodity due to its excessive use for household, industry and agriculture. Rice, which is considered as the principal food crop of the world is semiaquatic in nature and its cultivation is usually undertaken in flooded conditions. About 75 per cent of the rice production is from the irrigated lowland systems, which accounts for 79 m ha. Irrigated rice consumes 3000 to 5000 L of water to produce 1kg of rice (Barker *et al.*, 1998) [3]. There are predictions that many Asian countries will face serious water crisis by 2025. On an average, 23 m ha area of rice is affected by insufficient water availability thus affecting the crop production significantly (Pandey *et al.*, 2007) [12]. It is at this juncture that aerobic rice can help farmers who are unable to cultivate conventional lowland rice due to water shortage.

Aerobic rice systems have been developed targeting the blending of drought tolerance and yield potential of upland rice and lowland rice respectively. It is of paramount importance that the drought tolerance behavior of crops need to be boosted so as to promote their satisfactory growth and productivity under water scarce situations. Apart from the use of drought tolerant aerobic rice varieties for cultivation the use of certain microorganisms has also shown improved crop production ability under water scarce or drought conditions. One such organism of paramount importance is the pink pigmented facultative methylotrophs (PPFM) which is capable of mitigating the effects of drought on crops and thereby improving its and growth and productivity. Considering the ability of PPFM to protect plants under drought stress condition, *in vitro* and *in vivo* screenings were conducted with several PPFM isolates at the Department of Agricultural Microbiology, College of Agriculture, Vellayani. Based on these studies, five isolates were identified for their ability in imparting moisture stress tolerance. The field efficacy of these five isolates was being tested in rice crop in the present study.

Materials and Methods

A field study was conducted 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] treatment replicated three times. 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 isolates were PPFM 16, PPFM 26, PPFM 35, PPFM 37 and PPFM 38 with two methods of application *viz.* seed treatment (1%) and seed treatment (1%) + foliar application (2%) at 30 and 50 DAS. Controls used were KAU POP and KAU POP + water spray at 30 and 50 DAS. In case of KAU POP irrigation was given once in three days upto 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 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) [5].

Result and Discussion

Plant Height and Tillers m⁻²

The results on the effect of PPFM and method of application on plant height and tillers per square metre of rice are presented in Table 1.

The PPFM isolates and method of application had no significant effect on plant height at 20 DAS, 40 DAS and 60 DAS. Among the five isolates tested, PPFM 38 recorded significantly taller (98.64 cm) plants at harvest, followed by PPFM 35. Tiller production of rice was observed to respond significantly to PPFM and method of application. Number of tillers per square metre was significantly more when treated with PPFM 38.

Between the two methods of application, seed treatment (1%) + foliar application (2%) at 30 and 50 DAS resulted in taller plants and more number of tillers per square metre. The treatment combination p₅m₂ (PPFM 38 as seed treatment and foliar application) was significantly superior at all the three above growth stages with respect to plant height and tiller count.

In general, plant height is a genetically controlled growth attribute which plays an important role in productivity. Methylophs might have favoured an increase in plant height by the production of growth promoters like zeatin, auxins and cytokinins. High production of cytokinins in the apical tissues and rhizosphere soil has been reported by Poonguzhali *et al.* (2017) [13] as the reason for the increase in plant height of green gram in response to PPFM application. The coordination between auxins and cytokinins has been identified as crucial for promoting balanced growth of shoot and root system. The action of auxins help in the production of extensive root system and consequently cytokinins signals the shoot system to produce more tillers (Raghavendra and Santhosh, 2019) [14]. These results are in conformity with those of Holland (1997) [7] and Raja and Sundaram (2006) [15]. The effectiveness of supplementing seed treatment with foliar application of PPFM could be attributed to the efficacy of foliar sprays in easy and quick absorption as suggested by Umamageswari *et al.* (2019) [19]. The superiority of PPFM

treatments was also evident from significant variation in plant height and tiller production as compared to the two controls *viz.*, KAU POP (c₁) and KAU POP + water spray (c₂).

Leaf Area Index and Dry Matter Production

Leaf area index and dry matter production were found to be significantly superior on treating rice with PPFM 38 and with seed treatment (1%) followed by foliar application (2%) (Fig. 1 and 3). The interaction effect of the combination, p₅m₂ (PPFM 38 as seed treatment and foliar application) was significantly superior with respect to leaf area index and dry matter production (Fig. 2 and 4). Leaf area index of the treatments were significantly superior to both the controls. However, the mean dry matter production of the treatments and KAU POP (c₁) did not vary significantly. But the treatments were significantly superior to KAU POP + water spray (c₂).

The growth promoting substances produced by PPFM might have increased the number of leaves and the rate of leaf elongation as reported by Li *et al.* (2009) [9]. Similar increase in leaf area index due to foliar application of PPFM has been reported by Ajaykumar and Krishnasamy (2019) [1]. The higher leaf area index could be attributed to the higher tiller count in response to PPFM which led to more number of leaves and consequently higher leaf area index. Dry matter production is a function of leaf area index and radiation use efficiency. The higher dry matter production recorded in the treatments might be due to increase in plant height, more number of tillers per square metre and better root development. Further, the growth promoting activity mediated by PPFM might have also aided in the accumulation of photo assimilates in various sinks leading to higher dry matter production. Similar results have been reported by Thakur *et al.* (1995) [18], Singh *et al.* (2004) [16] and Li *et al.* (2009) [9]. The lack of significant variation in dry matter production between treatments and KAU POP might be because of the fact that the crop maintained under KAU POP received copious irrigation without stress at the critical growth stages.

Root Volume and Root Shoot Ratio

The results on the effect of PPFM on the root volume and root shoot ratio of rice are presented in Table 2.

The isolate PPFM 16, showed significantly superior effect on root volume and it was comparable with that of PPFM 38. However, significantly superior root shoot ratio was recorded with PPFM 38. Seed treatment (1%) followed by foliar application (2%) at 30 and 50 DAS (m₂) resulted in significantly higher root shoot ratio. The interaction effect of PPFM and methods of application revealed significantly higher root volume for the treatment combination p₁m₁ (PPFM 16 as seed treatment) which was on par with p₅m₂. The treatment combination p₅m₂ (PPFM 38 as seed treatment + foliar application) showed significantly superior root shoot ratio. Between the two controls root shoot ratio was higher for KAU POP (c₁) than KAU POP + water spray (c₂) whereas the root volume was significantly higher for KAU POP + water spray.

Root volume and root shoot ratio was significantly higher for the treatments when compared against both the controls.

Indole-3-acetic acid (IAA) is the main auxin that controls physiological processes during root formation and further development (Aloni *et al.*, 2006) [2]. The ability of *Methylobacterium* to produce IAA has been reported by Ivanova *et al.* (2001) [8]. This suggest that PPFM treatment can enhance IAA concentrations in plant and induce root

development. Ethylene is another compound that regulates root development (Madhaiyan *et al.*, 2007) [10] and this is in turn related to the biosynthesis pathway of auxin (Hardoim *et al.*, 2008) [6]. Ethylene concentration increases under stress and inhibits root elongation. Methylobacterium – plant interaction modulates the synthesis of ethylene. Sivakumar *et al.* (2018) [17] have also reported higher root volume in tomato with foliar spray of PPFM due to improved lateral root growth. The higher root volume recorded in KAU POP + water spray (c₂) might be due to the tendency of roots

to produce more lateral roots with increased diameter and surface area under stress conditions (Fenta *et al.*, 2014) [4] so that water and nutrients can be absorbed from the deeper layers of the soil for maintaining photosynthesis (Comas *et al.*, 2013). The higher root shoot ratio recorded in KAU POP as compared to KAU POP + water spray might be due to the higher root weight supported by moisture stress free condition. Similar observations were made by Nejad (2011) [11].

Table 1: Effect of PPFM and method of application on plant height and tillers m⁻² of rice

Treatment	Plant height (cm)				Tillers m ⁻² (nos.)			
	20 DAS	40 DAS	60 DAS	At harvest	20 DAS	40 DAS	60 DAS	At harvest
PPFM (P)								
p ₁ – PPFM 16	34.17	46.65	56.67	96.19	70.33	299.00	440.50	463.83
p ₂ – PPFM 26	35.23	46.49	56.68	94.38	73.00	295.67	446.33	463.00
p ₃ – PPFM 35	37.28	47.79	57.97	96.26	70.10	296.33	435.83	443.33
p ₄ – PPFM 37	34.43	45.88	55.29	93.13	67.33	292.67	438.00	442.83
p ₅ – PPFM 38	35.78	48.20	58.86	98.64	78.33	306.33	447.00	497.83
SE (m) ±	1.19	0.56	0.86	0.64	2.70	2.54	0.67	3.25
CD (0.05)	NS	NS	NS	1.347	5.678	5.339	1.406	6.842
Method of application (M)								
m ₁ - seed treatment (1%)	34.94	46.69	56.97	95.32	72.20	296.53	442.46	466.07
m ₂ - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	35.79	47.32	57.17	96.32	71.47	299.53	442.47	458.27
SE m (±)	1.19	0.56	0.86	0.64	2.70	2.70	0.67	3.25
CD (0.05)	NS	NS	NS	0.851	NS	NS	0.889	4.328
PPFM (P) x Method of application (M)								
p ₁ m ₁ (PPFM 16 - seed treatment)	33.72	46.27	56.85	97.08	72.00	301.67	445.33	486.00
p ₁ m ₂ (PPFM 16 - seed treatment + foliar application)	34.63	47.03	56.28	95.30	68.67	296.33	435.67	441.67
p ₂ m ₁ (PPFM 26 - seed treatment)	35.53	46.44	57.30	96.72	71.67	296.00	444.33	461.00
p ₂ m ₂ (PPFM 26 - seed treatment + foliar application)	34.92	46.54	56.05	92.03	74.33	295.33	448.33	465.00
p ₃ m ₁ (PPFM 35 - seed treatment)	37.15	47.87	58.27	97.07	76.67	294.67	435.67	446.00
p ₃ m ₂ (PPFM 35 - seed treatment + foliar application)	37.32	47.71	57.66	96.17	63.67	298.00	436.00	440.67
p ₄ m ₁ (PPFM 37 - seed treatment)	33.06	45.05	54.37	88.29	63.33	282.00	429.67	440.67
p ₄ m ₂ (PPFM 37 - seed treatment + foliar application)	35.80	46.71	56.22	97.96	71.33	303.33	446.33	445.00
p ₅ m ₁ (PPFM 38 - seed treatment)	35.26	47.82	58.08	97.15	77.33	308.00	448.00	496.67
p ₅ m ₂ (PPFM 38 - seed treatment + foliar application)	36.29	48.59	59.64	100.13	79.33	304.66	446.00	499.00
Control (C)								
c ₁ - KAU POP	33.49	43.71	53.08	85.83	53.33	288.67	427.67	407.67
c ₂ – KAU POP + water spray	33.83	44.40	54.00	86.21	65.33	285.33	425.67	407.33
SEm (±)	1.30	0.60	0.78	0.66	2.49	2.38	0.36	4.15
CD (0.05)	NS	1.766	2.277	1.931	7.310	6.976	1.997	12.198
Treatment vs. Control 1	NS	S	S	S	S	S	S	S
Treatment vs. Control 2	NS	S	S	S	S	S	S	S

Table 2. Effect of PPFM and method of application on root volume and root shoot ratio of rice.

Treatment	Root volume (cm ³ hill ⁻¹)	Root shoot ratio
PPFM (P)		
p ₁ – PPFM 16	33.33	0.19
p ₂ – PPFM 26	28.33	0.38
p ₃ – PPFM 35	21.67	0.34
p ₄ – PPFM 37	26.67	0.29
p ₅ – PPFM 38	31.67	0.47
SE m (±)	3.57	0.02
CD (0.05)	7.492	0.049
Method of application (M)		
m ₁ - seed treatment (1%)	28.00	0.32
m ₂ - seed treatment (1%) + foliar application (2%) at 30 and 50 DAS.	28.67	0.35
SE m (±)	3.57	0.02
CD (0.05)	NS	0.038
PPFM (P) x Method of application (M)		
p ₁ m ₁ (PPFM 16 - seed treatment)	36.67	0.17
p ₁ m ₂ (PPFM 16 - seed treatment + foliar application)	30.00	0.21
p ₂ m ₁ (PPFM 26 - seed treatment)	26.67	0.39
p ₂ m ₂ (PPFM 26 - seed treatment + foliar application)	30.00	0.37
p ₃ m ₁ (PPFM 35 - seed treatment)	20.00	0.49

p ₃ m ₂ (PPFM 35 - seed treatment + foliar application)	23.33	0.20
p ₄ m ₁ (PPFM 37 - seed treatment)	23.33	0.27
p ₄ m ₂ (PPFM 37 - seed treatment + foliar application)	30.00	0.31
p ₅ m ₁ (PPFM 38 - seed treatment)	33.33	0.46
p ₅ m ₂ (PPFM 38 - seed treatment + foliar application)	30.00	0.67
Control (C)		
c ₁ - KAU POP	16.66	0.24
c ₂ - KAU POP + water spray	20.00	0.21
SE m (±)	3.45	0.02
CD (0.05)	10.104	0.068
Treatment vs. Control1	S	S
Treatment vs. Control 2	S	S

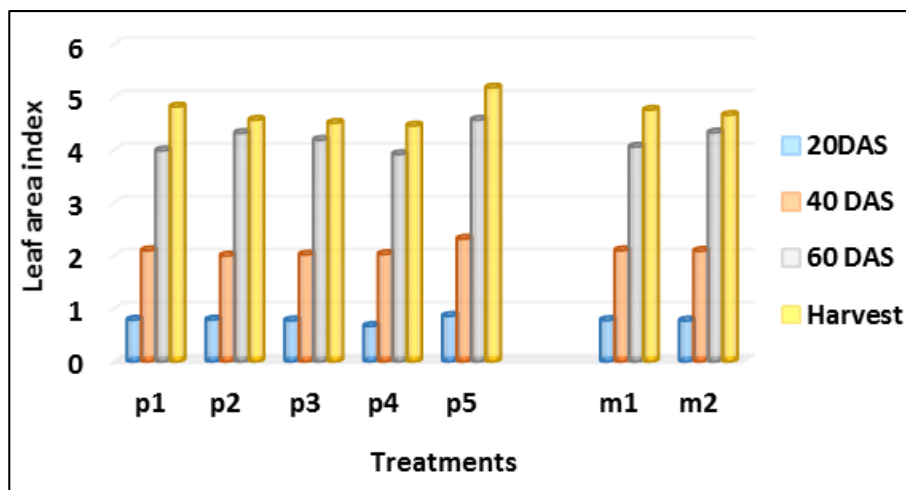


Fig 1: Effect of PPFM and method of application on leaf area index.

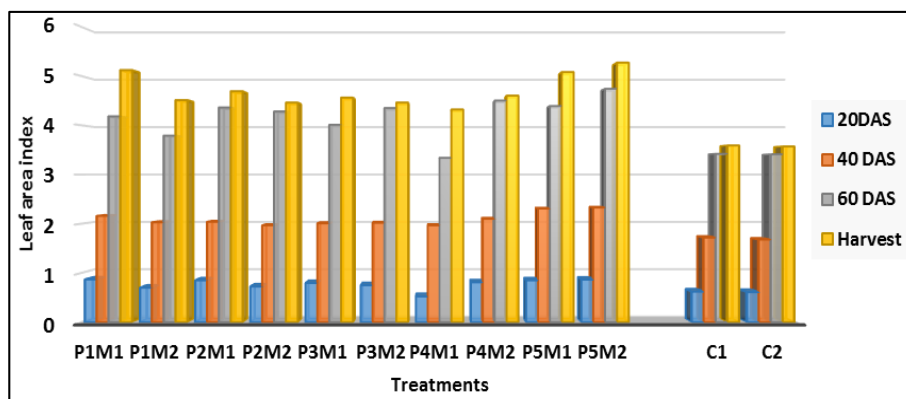


Fig 2: Effect of PPFM x method of application on leaf area index.

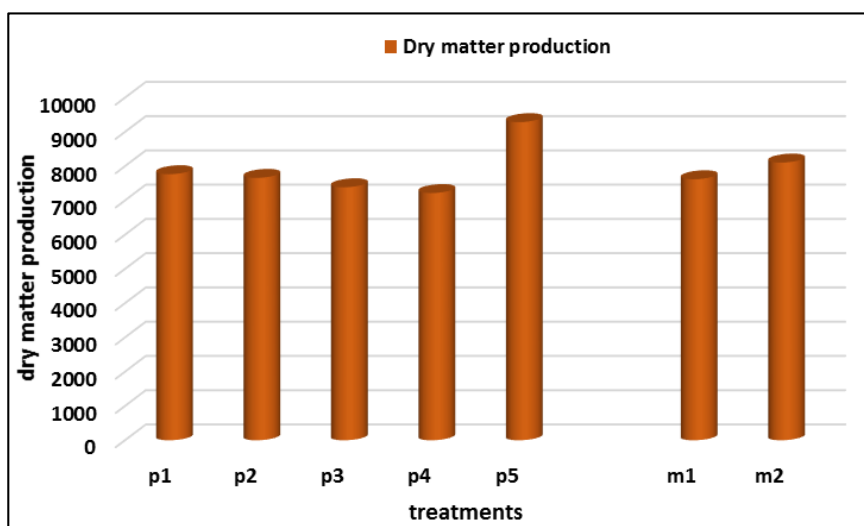


Fig 3: Effect of PPFM and method of application on dry matter production, kg ha⁻¹.

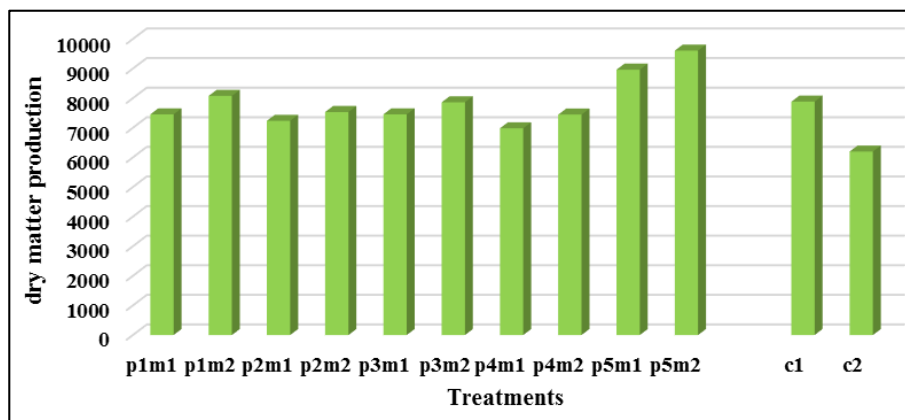


Fig 4: Effect of PPFM x method of application on dry matter production, kg ha⁻¹.

Conclusion

The present study revealed that seed treatment (1%) followed by foliar application (2%) of PPFM 38 (p_{5m2}), twice, at 30 and 50 days after sowing enhanced the growth of aerobic rice.

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