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In vitro Screening of PPFM Isolates for Water Stress Tolerance in Paddy

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A B S T R A C T

Rice (*Oryza sativa* L.) is one of the most important field crop after wheat in the world providing staple food to the millions. Drought is the most important environmental stress on rice and many efforts have been made to improve crop yield under drought. The present study reveals the response of rice for the drought stress at germination to seedling stages. Mannitol was selected for inducing osmotic stress. For *in vitro* screening of PPFM isolates for water stress tolerance, 20 isolates of PPFM from paddy were selected from the previous study of M.Sc. (Ag.) thesis work conducted in the Department of Agricultural Microbiology, Vellayani during 2015-2017 on the basis of carotenoid pigment production, IAA production, proline content, seedling vigour index and yield. These isolates were screened by paper towel method for water stress tolerance under *in vitro* conditions using mannitol for inducing osmotic stress. There were 21 isolates (20 KAU isolates of PPFM and one TNAU isolate) and four water stress levels (1%, 2%, 3% mannitol and control).Osmotic stress was higher in 3 per cent mannitol treatment. Seeds treated with PPFM 26 recorded the highest germination percentage, shoot length and seedling vigour index. The highest root length and shoot dry weight were observed with the isolate PPFM 15 whereas the highest root dry weight was recorded with PPFM 9. Scoring was done to assess the best five isolates and those with higher ranks were selected as better water stress tolerance capacity. Consequently, PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 which secured ranks from 1to 5 were selected as better water stress tolerance.

Introduction

Rice is one of the greatest water user among cereal crops, consuming about 80% of the total irrigated fresh water resources in Asia. In Asia, with relatively more suitable growing conditions for rice, production has declined due to increasing water stress (Tao *et al.,* 2004). Drought is one of the greatest abiotic stresses to agriculture, inhibiting plant growth and thus reducing productivity (Zhang *et al.,* 2008).

Rice is more vulnerable to drought due to its semi aquatic phylogenetic origin. Bartels and Souer (2004) reported that the response of plants to water stress depends on the

duration and severity of the stress and the developmental stage (Zhu *et al.,* 2005). In the case of rice, the sensitive period is flowering stage, resulting in severe yield losses (Liu *et al.,* 2006). The physiological processes during flowering stage will be negatively affected by water stress and it will lead to decreased spikelet fertility.

Blum (2011) reported that drought is the insufficiency of soil moisture content to meet plant water requirements resulting in reduced growth and development of the plant and hence low yield. Sokoto and Muhammad (2014) observed that at cellular level, drought results in impaired cell division and cell elongation due to decrease in turgor pressure.

*Methylobacterium*spp. are a group of bacteria known as pink-pigmented facultative methylotrophs, or PPFMs (Austin and Goodfellow, 1979; Patt *et al.,* 1976; Green and Bousfield, 1982, 1983), which are classified as alpha-*Proteobacteria* and are capable of growth on one-carbon compounds such as formate, formaldehyde, methanol, and methylamine as well as on a variety of C2, C3 and C4 compounds (Lidstrom, 2001).

PPFMs excrete auxins and cytokinins, plant growth hormones that influence germination and root growth and play critical roles in a plant's response to water stress (Doronina *et al.,* 2002; Madhaiyan *et al.,* 2005).

Polyethylene glycol and Mannitol has been used to stimulate osmotic stress and these neutral polymers are being widely used to impose water stress in plants (Zgallai *et al.,* 2005). Polyethylene glycol and mannitol have significant effect on per cent germination. Increase in polyethylene glycol and mannitol concentration linearly decreased the percent germination of canola, cauliflower and tomato.

The minimum germination was observed at highest concentration of polyethylene glycol or mannitol. The mannitol highly reduced the germination rate compared to the PEG effect (Hadi *et al.,* 2014).

Mannitol was found to be more efficient and selective than polyethylene glycol (PEG) as osmotic agent (Anber, 2010). Mannitol is an organic compound often used for drought tolerance studies (Mohamed *et al.,* 2000; Hassanein and Dorion, 2006). Since previous studies reported that PEG had a toxic effect on plant cells (Bhojwani and Razdan, 1996; Hassanein *et al.,* 2009), hence in the present investigation, mannitol was selected for inducing osmotic stress at three levels along with water control. Here the result of 3 per cent mannitol which induced maximum water stress was selected.

Materials and Methods

The *in vitro* screening experiment was conducted at the Department of Agricultural Microbiology, Vellayani during 2017-2018 with rice variety Harsha. For *in vitro* screening of PPFM isolates for water stress tolerance, 20 isolates of PPFM from paddy were selected from the previous study of M.Sc. (Ag.) thesis work conducted in the Department of Agricultural Microbiology, Vellayani during 2015-2017 on the basis of carotenoid pigment production, IAA production, proline content, seedling vigour index and yield. These isolates were screened by paper towel method for water stress tolerance under *in vitro* conditions using mannitol for inducing osmotic stress. There were 21 isolates (20 KAU isolates of PPFM and one TNAU isolate) and four water stress levels (1%, 2%, 3% mannitol and control).The experiment was laid out in completely randomized block design with two replications.

Preparation of PPFM Inoculum

The PPFM broth culture was prepared by inoculating 72 h old log phase PPFM culture into AMS broth (Whittenburry *et al.,* 1970). The flasks were kept in a temperature controlled shaker at 25±2^{0}C for 10 days.

Soaking of Paddy Seed

Rice seeds (variety Harsha) were soaked overnight in 1 per cent liquid culture of 10 days old PPFM isolates.

Paper Towel Method

The isolates of PPFM were screened by paper towel method for water stress tolerance under *in vitro* conditions using mannitol for inducing osmotic stress (Yaklich, 1985).

Germinability of the seeds were determined in the laboratory at room temperature (30±2°C). One hundred seeds were randomly taken from the paddy seeds and 8 treated seeds were placed between a pair of moist paper towels. The towels were rolled and the ends were closed by threads and covered by polyethylene paper to prevent drying. After 14 days of incubation period observations were taken.

Observations

Germination Percentage

Germination percentage was calculated after14 days. Germination percentage was calculated by using the equation:

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

Shoot Length

Shoot length was measured from the collar region to the tip of the longest leaf at 14 days of growth. It was expressed in centimeter.

Root Length

Root length was measured from base of the stem to the tip of the root at 14 days of growth and was expressed in centimeter.

Shoot Dry Weight

The shoot dry weight was taken after drying the shoot samples at 60°C in a hot air oven. It was expressed in mg.

Root Dry Weight

The root dry weight was taken after drying the root samples at 60°C in a hot air oven. It was expressed in mg.

Seedling Vigour Index

Seedling vigour index was calculated by using the equation proposed by Abdul-Baki and Anderson (1973).

Seedling vigour index = (Root length + Shoot length) x Germination percentage

Results and Discussion

In the present study, effect of PPFM isolates on paddy seed germination and seedling growth was tested and the results revealed that the PPFM inoculated seeds under water stress condition showed a significant increase in germination percentage and other seedling parameters. Maximum germination percentage, shoot length and seedling vigour index of 87.50 per cent (Table 1), 9.47 cm (Table 2) and 2143.25 (Table 6) respectively were recorded in PPFM 26 treated seeds at maximum water stress levels of 3 per cent mannitol. Seeds treated with PPFM 15 recoded the maximum root length (18.38 cm) (Table 3) and shoot dry weight (7.40 mg) (Table 4) at maximum water stress levels of 3 per cent mannitol. This treatment was found to be significantly superior which secured 55.56 per cent increase in germination over water treated control. Maximum root dry weight of 4.50 mg was recorded in seeds treated with PPFM 9 (Table 5) at maximum osmotic stress of 3 per cent mannitol. Holland (1997) reported that PPFMs could be used as seed coatings designed to enhance germination and vigour index. The advantage of PPFM bacteria is the rich supply of plant hormones, as most of the metabolic products of the methanol released by plants are lost from leaves during leaf expansion that is catalyzed by pectin methylesterase (Dourado *et al.,* 2015). PPFMs have been reported to influence seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins and auxins. Seeds treated with methylotrophic strains improved seed germination, seedling vigour index and biomass of rice seedlings. In vegetative stages, methylotrophic population in the treated seedlings increased compared to seedling stages. Treated seedlings showed a higher accumulation of plant hormones *viz*., trans-zeatin riboside, isopentenyladenosine, and indole-3-acetic acid than untreated seedlings (Lee *et al.,* 2006). Moreover, some aerobic methylotrophs also synthesize this important phytohormone (Doronina *et al.,* 2001; Ivanova *et al.,* 2001), and PPFMs effectively enhance seed germination (Anitha, 2010; Meena *et al.,* 2012). Moreover, some aerobic methylotrophs also synthesize this important phytohormone (Doronina *et al.,* 2001; Ivanova *et al.,* 2001), and PPFMs effectively enhance seed germination (Meena *et al.,* 2012; Anitha, 2010). Similar observations were also reported by Chandrasekaran *et al.,* (2017) where in seeds treated with PPFM (2%) showed higher germination percentage (73.53%) than control (55%) followed by salicylic acid (71%) under drought created by PEG 6000 in tomato. Presoaking with PPFM (2%) treatment enhanced germination up to 33.69 per cent when compared to control. This may be due to production of various compounds by PPFMs which enhance the seed germination. PPFM bacteria stimulate plant growth (Basile *et al.,* 1969) presumptively as a result of turn out plant growth regulators (Freyermuth *et al.,* 1996) and vitamin B complex (Basile *et al.,* 1985).

Table.1 Effect of PPFM isolates on germination percentage of paddy seeds

Treatments	Germination percentage (%)			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	43.75	87.50	68.75	93.75
PPFM 3	62.50	68.75	43.75	68.75
PPFM 4	43.75	75.00	81.25	62.50
PPFM 6	87.50	62.50	62.50	75.00
PPFM 9	62.50	81.25	37.50	62.50
PPFM 11	81.25	81.25	81.25	93.75
PPFM15	62.50	68.75	75.00	62.50
PPFM 16	68.75	43.75	68.75	81.25
PPFM 17	75.00	43.75	56.25	68.75
PPFM 19	68.75	62.50	50.00	62.50
PPFM 22	56.25	87.50	56.25	56.25
PPFM 24	62.50	87.50	68.75	50.00
PPFM 26	68.75	56.25	87.50	50.00
PPFM 32	75.00	81.25	50.00	68.75
PPFM 34	43.75	68.75	75.00	81.25
PPFM 35	62.50	62.50	75.00	68.75
PPFM 37	81.25	43.75	81.25	50.00
PPFM 38	43.75	68.75	68.75	50.00
PPFM 42	62.50	93.75	50.00	43.75
PPFM 46	62.50	93.75	37.50	62.50
PPFM 47 (TNAU)	75.00	56.25	68.75	75.00
0.5% Methanol	68.75	68.75	68.75	68.75
AMS	68.75	56.25	62.50	81.25
Water	87.50	50.00	56.25	56.25
SEm (±)	6.751	10.206	8.169	9.288
CD (0.05)	19.821	29.967	23.985	27.271

Table.2 Effect of PPFM isolates on shoot length of paddy seedlings

Treatments	Shoot length (cm)			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	10.21	8.61	6.44	9.40
PPFM 3	8.45	9.48	8.24	8.90
PPFM 4	9.10	9.05	7.11	9.91
PPFM 6	9.17	7.96	7.85	8.50
PPFM 9	8.95	7.65	6.84	8.79
PPFM 11	9.20	8.99	8.88	8.88
PPFM15	9.32	7.02	9.39	9.93
PPFM 16	9.84	8.84	8.36	10.63
PPFM 17	8.27	8.65	6.65	8.73
PPFM 19	9.11	7.89	5.25	8.90
PPFM 22	9.51	6.07	8.06	9.90
PPFM 24	8.82	8.21	7.10	11.15
PPFM 26	9.11	8.20	9.47	10.51
PPFM 32	7.16	5.62	5.64	7.96
PPFM 34	7.52	10.88	6.20	7.13
PPFM 35	9.68	10.51	6.98	9.86
PPFM 37	7.76	5.76	7.02	12.37
PPFM 38	5.38	8.15	8.65	9.51
PPFM 42	8.97	7.33	5.51	9.83
PPFM 46	7.54	6.23	6.25	11.90
PPFM 47 (TNAU)	9.72	7.03	6.35	9.31
0.5% Methanol	9.20	7.08	7.49	10.18
AMS	6.32	7.72	7.78	8.35
Water	8.80	7.26	6.86	8.90
SEm (±)	0.653	0.877	0.808	0.680
CD (0.05)	1.918	2.574	2.372	1.998

Table.3 Effect of PPFM isolates on root length of paddy seedlings

Treatments	Root length (cm)			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	20.28	17.44	14.38	23.46
PPFM 3	23.26	20.82	16.79	20.96
PPFM 4	19.30	22.91	15.53	24.17
PPFM 6	19.85	19.38	16.33	20.35
PPFM 9	20.82	18.17	15.14	18.49
PPFM 11	16.63	20.91	14.93	19.97
PPFM15	15.12	18.48	18.38	20.25
PPFM 16	18.23	21.95	16.11	21.00
PPFM 17	19.13	16.85	14.40	22.46
PPFM 19	17.23	20.37	15.35	16.62
PPFM 22	14.74	18.65	14.28	22.68
PPFM 24	13.99	21.38	15.75	22.08
PPFM 26	16.33	15.70	15.38	21.51
PPFM 32	13.04	15.06	11.67	17.95
PPFM 34	21.33	19.86	14.61	16.90
PPFM 35	19.17	15.10	15.36	22.89
PPFM 37	19.97	14.72	18.07	23.45
PPFM 38	10.90	18.96	15.28	20.68
PPFM 42	11.94	15.04	7.08	19.81
PPFM 46	13.01	14.10	15.19	21.80
PPFM 47 (TNAU)	13.40	9.13	17.26	19.24
0.5% Methanol	13.27	15.43	16.76	20.24
AMS	13.90	17.43	14.33	19.03
Water	16.99	14.31	17.13	21.04
SEm (±)	1.135	2.209	1.579	1.334
CD (0.05)	3.332	6.487	4.637	3.917

Table.4 Effect of PPFM isolates on shoot dry weight of paddy seedlings

Treatments	Shoot dry weight (mg)			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	6.15	6.60	4.60	6.05
PPFM 3	7.05	8.05	6.40	6.15
PPFM 4	7.55	7.45	6.05	7.30
PPFM 6	6.00	6.55	6.30	6.50
PPFM 9	7.50	7.60	5.95	6.40
PPFM 11	6.45	7.15	6.25	6.20
PPFM15	6.20	5.45	7.40	6.30
PPFM 16	5.70	8.25	6.35	6.70
PPFM 17	6.45	6.20	5.80	8.05
PPFM 19	6.10	6.75	4.85	5.55
PPFM 22	7.65	6.00	5.80	8.85
PPFM 24	7.20	7.00	5.55	5.35
PPFM 26	5.25	7.30	7.00	5.85
PPFM 32	4.55	5.10	4.80	5.60
PPFM 34	6.10	7.20	5.05	5.30
PPFM 35	6.80	7.05	5.80	6.35
PPFM 37	7.00	4.35	6.75	6.50
PPFM 38	4.60	5.95	6.95	6.15
PPFM 42	5.80	5.05	4.00	4.25
PPFM 46	5.05	4.55	5.15	5.20
PPFM 47 (TNAU)	6.05	5.55	5.20	5.80
0.5% Methanol	6.25	5.65	6.00	6.35
AMS	5.05	5.90	6.25	5.10
Water	6.00	5.80	4.95	6.15
SEm (±)	0.581	0.646	0.539	0.507
CD (0.05)	1.706	1.896	1.583	1.489

Table.5 Effect of PPFM isolates on root dry weight of paddy seedlings

Treatments	Root dry weight (mg)			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	3.35	2.40	2.90	3.10
PPFM 3	3.30	3.70	3.20	3.25
PPFM 4	3.65	5.35	2.05	3.75
PPFM 6	2.75	3.60	2.45	4.35
PPFM 9	3.70	2.70	4.50	3.30
PPFM 11	2.80	3.25	2.55	3.00
PPFM15	2.95	3.05	3.00	2.80
PPFM 16	2.40	4.05	3.05	3.45
PPFM 17	3.35	2.80	4.25	2.45
PPFM 19	3.15	3.30	4.20	2.80
PPFM 22	2.95	2.70	3.60	4.60
PPFM 24	3.35	3.85	2.70	2.45
PPFM 26	2.85	4.35	3.75	2.75
PPFM 32	1.95	2.40	2.60	2.30
PPFM 34	2.85	3.65	2.85	2.35
PPFM 35	2.85	3.95	3.90	3.00
PPFM 37	2.80	3.00	3.20	3.05
PPFM 38	2.15	5.05	3.80	3.35
PPFM 42	1.90	2.60	2.60	3.35
PPFM 46	2.30	2.25	3.85	3.40
PPFM 47 (TNAU)	2.65	1.90	3.25	2.30
0.5% Methanol	2.50	2.75	2.65	2.65
AMS	2.55	3.80	3.00	2.35
Water	2.55	3.20	2.75	3.00
SEm (±)	0.399	0.511	0.447	0.320
CD (0.05)	0.996	1.502	1.313	0.939

Table.6 Effect of PPFM isolates on seedling vigour index of paddy seedlings

Treatments	Seedling vigour index			
	Water stress levels			
	1% Mannitol	2% Mannitol	3% Mannitol	Water
PPFM 2	1,326.32	2,293.75	1,443.75	3,080.63
PPFM 3	1,982.82	2,085.44	1,083.75	2,058.75
PPFM 4	1,247.63	2,396.63	1,832.63	2,123.75
PPFM 6	2,538.38	1,684.75	1,490.25	2,118.50
PPFM 9	1,860.32	2,069.88	824.065	1,705.00
PPFM 11	2,097.00	2,435.82	1,939.25	2,702.07
PPFM15	1,527.50	1,751.94	2,070.82	1,886.25
PPFM 16	1,932.00	1,353.25	1,671.00	2,559.19
PPFM 17	2,054.63	1,122.82	1,262.63	2,122.44
PPFM 19	1,822.25	1,704.38	1,028.76	1,595.00
PPFM 22	1,355.19	2,162.57	1,247.44	1,832.19
PPFM 24	1,443.38	2,588.26	1,562.63	1,677.76
PPFM 26	1,756.25	1,356.88	2,143.25	1,560.07
PPFM 32	1,514.63	1,667.44	865.00	1,782.00
PPFM 34	1,270.07	2,101.69	1,517.19	1,950.82
PPFM 35	1,806.13	1,492.94	1,675.13	2,246.19
PPFM 37	2,256.00	921.63	2,038.32	1,790.75
PPFM 38	718.57	1,851.25	1,638.50	1,524.01
PPFM 42	1,306.57	2,079.44	629.25	1,275.07
PPFM 46	1,271.50	1,903.94	803.815	2,105.94
PPFM 47 (TNAU)	1,733.63	935.875	1,633.63	2,150.75
0.5% Methanol	1,552.00	1,560.82	1,653.63	2,054.13
AMS	1,414.38	1,405.25	1,381.88	2,227.19
Water	2,256.63	1,078.00	1,327.63	1,677.63
SEm (±)	214.132	264.211	186.030	259.425
CD (0.05)	628.730	775.771	546.216	761.718

Table.7 Ranking of PPFM isolates based on *in vitro* screening

Isolates	Index rank
PPFM 2	18
PPFM 3	7
PPFM 4	10
PPFM 6	14
PPFM 9	11
PPFM 11	8
PPFM15	2
PPFM 16	6
PPFM 17	12
PPFM 19	17
PPFM 22	13
PPFM 24	9
PPFM 26	1
PPFM 32	19
PPFM 34	15
PPFM 35	5
PPFM 37	4
PPFM 38	3
PPFM 42	20
PPFM 46	16

This increment may be due to the gibberellin (GA_3) which improves the synthesis and secretion of hydrolytic enzymes from aleurone cells. These enzymes then mobilize the endosperm storage reserves serve as fuel for germination and growth (Cirac *et al.,* 2004).

Chandrasekaran *et al.,* (2017) observed that PPFM (2%) resulted in higher root length (3.72 cm) compared to control followed by gibberellic acid (3.61 cm) and salicylic acid (2.86 cm) under drought created by PEG 6000 in tomato. This increment might be due to, *Methylobacterium* which are capable to grow on carbon compounds such as methanol and generate plant growth regulators such as auxin and cytokinin (Ivanova *et al.,* 2000) which induce cell division and cell elongation.

In rice seedlings, the increase in root and shoot length and their dry weight may be due to the plant growth promoting activities of the isolates. The isolate *B. altitudinis* FD48 and *Methylobacterium*sp. (PPFM) also supported the germination of riceseeds under different PEG concentration (Kumar *et al.,* 2017). It has beensuggested that production of betaine, an osmolyte by certain bacteria provides a barrier against dehydration (Sleator and Hill, 2002).

Maximum stress level of 3% mannitol was selected for calculating the weighted average of PPFM isolates. Based on the results of *in vitro* screening experiment, ranking of PPFM isolates was done taking into consideration germination percentage, shoot length root length, shoot dry weight and seedling vigour index of paddy seedlings. The isolates PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 having top weighted average rankswhich secured ranks from 1 to 5 were selected as better water stress tolerance.

Author Contributions

N. K. Riyas: Investigation, formal analysis, writing—original draft. K. S. Meenakumari: Validation, methodology, writing—reviewing. Elizabeth K. Syriac:—Formal analysis, writing—review and editing. R. Beena: Investigation, writing—reviewing. P. Shalini Pillai: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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