SCREENING OF PINK PIGMENTED FACULTATIVE METHYLOTROPH (PPFM) ISOLATES FOR WATER STRESS TOLERANCE AND YIELD IN PADDY

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"SCREENING OF PINK PIGMENTED FACULTATIVE METHYLOTROPH (PPFM) ISOLATES FOR WATER STRESS TOLERANCE AND YIELD IN PADDY"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS AND SYMBOLS USED

AMS	Ammonium mineral salt
et. al.	And other co-workers
AW	Available water
cm	Centimetre
CMI	Cell membrane integrity
CSI	Chlorophyll stability index
CRD	Completely randomized design
CD	Critical difference
cm^3	Cubic centimetre
DAT	Days after transplanting
^0C	Degree celsius
DNA	Deoxyribo nucleic acid
DSI	Drought susceptibility index
FC	Field capacity
Fig.	Figure
FAO	Food and Agricultural Organisation
GA	Gibberellic acid
g	Gram
h	Hours
ha	Hectare
IRRI	International Rice Research Institute
LAI	Leaf area index
m	Metre
µg	Microgram
µL	Microliter
mg	Milligram
mL	Milliliter
mM	Millimolar

min	Minute
M	Molar
viz.,	Namely
nm	Nanomertre
NS	Non-significant
No.	Number
PTB	Pattambi
%	Per cent
PPFM	Pink Pigmented facultative methylotroph
PGPR	Plant growth promoting rhizobacteria
ROS	Reactive oxygen species
rpm	Rotations per minute
SVI	Seedling vigour index
sp. or spp.	Species (singular and plural)
SE (m)	Standerd error (Mean)
SOD	Super oxide dismutase
i.e.	That is

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Introduction

1. INTRODUCTION

In the present day climate change, crops are exposed frequently to a number of abiotic stresses *viz.*, drought, elevated temperature, salinity, submergence and nutrient deficiencies. Plant abiotic stress refers to environmental conditions that reduce growth and yield below optimum level. Crop production is considered to be one of the most vulnerable sectors susceptible to abiotic stresses. These stresses limit crop production. Drought is a major limiting factor in crop production which has a significant role in plant growth and development. FAO (2007) reported that 64 per cent of the global cropped area was affected by drought or water deficit and it shows the impact of abiotic stresses on crop production.

According to Widawsky and O'Toole (1990), water stress is considered as the most severe problem in rice production. Consumed by more than half of the world population, rice fulfills the caloric demands upto 23 per cent (Khush, 2003). Rice has semi- aquatic nature and grown under flooded condition conventionally to provide nutrient supply and large amounts of water. As a result of drought, half of the rice cultivating areas in the world do not maintain flooded conditions due to insufficient water, which ultimately results in reduced yield (Bernier *et al.*, 2008). Rice has very little adaptation for water stress and shows remarkable sensitivity to drought (Kamoshita *et al.*, 2008). In India on an average 23 Mha area of rice cultivation is affected by insufficient water availability thus affecting the crop production significantly (Pandey *et al.,* 2007). Drought conditions induces increased level of reactive oxygen species (ROS) (Sgherri *et al.*, 1996), which includes hydrogen peroxide (H_2O_2), hydroxyl free radical (OH), superoxide radical (O^-) and singlet oxygen resulting in denaturation of proteins, peroxidation of lipids, mutation of DNA and various types of cellular oxidative damage (Smirnoff, 1993).

Van Loon *et al.* (1998) reported that the bacterial inoculants that provide cross protection against both abiotic and biotic stress showed a better compatibility in sustainable agriculture system.

Induced systemic tolerance (IST), is a process which includes, production of antioxidants, bacterial production of cytokinins and degradation of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) by bacterial ACC deaminase through which plant growth promoting bacteria (PGPB) can mitigate the impact of abiotic stresses on plants. Rhizosphere colonizing bacteria have a significant role in stress tolerance (Sandhya *et al.,* 2011), but few studies were focused on phyllosphere bacterial amelioration of abiotic and biotic stress in plants.

Improved root colonizing capability, adaptability in catabolic processes and the ability to produce a large number of enzymes and metabolites are the important characteristics of PGPR like *Pseudomonas fluorescens* and *Bacillus subtilis* (Mayak *et al*., 2004; Saravanakumar and Samiyappan, 2007). These organisms have attracted attention as inoculants to withstand plants under varied biotic and abiotic stress conditions due to these characters. Phytohormones like cytokinin and auxins (Madhaiyan *et al*., 2005) were produced by phyllosphere colonizing *Methylobacterium* and they are also known to produce stress response enzyme ACC deaminase (Chinnadurai *et al.,* 2009).

Hayat *et al.* (2010) reported that the exogenous application of PPFM improves germination, growth, development, quality and yield of crop plants there by counteracts the adverse effect of drought.

Sivakumar *et al.* (2017) reported that field application of PPFM are promising in enhancing photosynthetic rate, water status of the plant, compatible osmolytes like proline and anti-oxidant enzymes like catalase activity which protect the plant under drought stress condition in tomato. Chandrasekaran *et al.* (2017) reported that the PPFM (2%) and brassinolide (1 ppm) treatments were found superior in improving germination associated traits, stress tolerant index and anti-oxidant enzyme catalase activity which have the ability to protect the plant under abiotic stress condition. Gusain *et al.* (2015) also observed that the PGPR inoculation induced plants to produce higher amount of antioxidants under

drought stress which might be a basis for the lower accumulation of H_2O_2 in inoculated plants as compared to their respective control in rice. Kumar *et al.* (2017) reported that the application of *Bacillus altitudinis* FD48 and *Methylobacterium* sp. (PPFM) influenced the change in level of biochemical parameters of rice and helped them to improve tolerance to water stress. *Bacillus altitudinis* FD48 and *Methylobacterium* sp. (PPFM) proved to have an important role in improving plant performance under drought condition.

Considering the importance of PPFM to protect the plant under drought stress condition, an attempt was made to screen PPFM isolates for water stress tolerance based on *in vitro* and *in vivo* studies. The present study was undertaken with major thrust to screen the Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy.

Review of Literature

2. REVIEW OF LITERATURE

Drought is a recurring problem and is one of the major limiting factors that affect crop growth and productivity. Moisture stress is a major constraint for crop growth in arid and semi-arid regions, as the precipitation is low and uncertain in these areas. Efficient utilization of soil and water resources necessitates the adaptation of the appropriate water management techniques. In order to maintain water in the soil for longer period after an irrigation event, some additional materials such as organic matter, soil conditioners are added into the soil. Soil conditioners both natural and synthetic contribute significantly to provide a reservoir of soil water to plants on demand in the upper layers of the soil where the root systems normally develop.

Hanson *et al.* (1995) stated that drought is a meteorological term and is commonly defined as the inadequacy of water availability including period without significant rainfall that affects the crop growth and soil moisture storage capacity and it occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought is one of the greatest abiotic stresses to agriculture, inhibiting plant growth and thus reducing productivity (Zhang *et al.*, 2008).

Drought, a devastating natural hazard, affects a significant proportion of the global crop production. The percentage of the planet affected by drought has doubled in the last 40 years and in the same timespan droughts have affected more people worldwide than any other natural hazard. Agriculture bears much of the impact and in developing countries it is the most affected sector, damaging water availability, agricultural production, food security and rural livelihoods. With nearly 1.3 billion people – 40 percent of the world – relying on agriculture as the main source of income, drought is putting the livelihood of many at risk (FAO, 2018).

Water stress reduces plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Farooq, *et al.*, 2008; Jaleel, *et al.,* 2008a; Razmjoo, *et al.*, 2008).

According to the fifth assessment report of IPCC (2014), drought is the significant impact of current climate related extremes. In India, drought is a regular problem which affects agricultural production and life of animals and humans frequently. Water is the most limiting factor for plant growth. If plants do not receive adequate water, the resulting drought stress can reduce growth more than all other environmental stresses combined (Khan *et al.,* 2015). Drought is the most important environmental stress in agriculture and many efforts have been made to improve crop yield under drought.

Maharashtra faced a severe drought in 2018, in which 0 to 50 per cent yield loss of soybean crop has been reported from Latur district. In black cotton soil areas, the yield loss was up to 25 per cent. Whereas 65-70 per cent yield loss was reported from the old plantations of sugarcane in Latur (DownToEarth, 2018). In Kerala also drought is a major limiting factor which reduces the productivity of crops. In 2009, drought resulted in a huge crop loss of Rs.14.40 crores from 4,000 hectares (The Hindu, 2016). Forty seven per cent paddy cultivation was lost due to drought in 2016.

Rice (*Oryza sativa* L.) is one of the most important field crops after wheat in the world providing staple food to the millions. It is an indispensable source of calories for almost half of the population within Asia. More than 90 per cent of the world rice is produced and consumed in Asia, which is a native for 60 per cent of the earth's population. It is grown in all continents except Antarctica, occupying an area of 163 million ha and producing 755 million tones paddy (FAOSTAT, 2014-15). Improved production and access to this vital food crop is very important as it feeds more than half the world's population while providing income for millions of rice producers, processors and traders Plants are subjected

to several harsh environmental stresses that adversely affect growth, metabolism, and yield. Drought, salinity, low and high temperatures, flood, pollutants and radiation are the important stress factors limiting the productivity of crops (Lawlor, 2002).

In India, rice is the premier food crop and foremost cereal and therefore, national food security systems largely depend on the production and productivity of rice ecosystem. More than 70% of the Indian people consume rice. Among the rice growing countries, India stands first in area and second in production next only to China. In India rice alone is cultivated in 43.9 million ha with production of around 106.77 million tones and a productivity of 22.03 q ha^{-1} (GOI, 2014). This productivity is among the lowest in the developing countries which need to be improved. India alone would need about 122 million tonnes of rice for domestic consumption.

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).

2.1 Impact of Drought on Rice Production

Drought stress is a major constraint to rice production, particularly in water-limited environments (Bernier *et al.*, 2008; Mishra *et al.*, 2014) such as those for upland rice cultivation. Large areas of lowland and upland rainfed rice occupy 31% and 11% of the global rice-growing area, respectively (Murty and Kondo, 2001; Kamoshita *et al.*, 2008).

Evenson *et al.* (1996) reported an average annual global reduction of rice production due to drought of 18 Mt.

In India, the droughts of 1987 and 2002-2003 affected more than 50 per cent of the crop area in the country (Wassmann *et al.*, 2009).

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 and ultimately yield reduction.

2.2 Microorganisms and Drought Mitigation

Van Loon *et al.* (1998) reported that in environmentally sustainable agricultural systems, the bacterial inoculants that provide cross protection against both biotic and abiotic stress would be highly preferable.

Beneficial, symbiotic interactions of plants with microbes can shield plants from biotic and abiotic stresses (Mascher, 2007).

PGPR like *Pseudomonas fluorescens* and *Bacillus subtilis*, recently have obtained attention as inoculants to help withstand plants under varied biotic and abiotic stress conditions because of their excellent root colonizing ability, versatility in their catabolic activity and their capacity to produce a wide range of metabolites and enzymes (Mayak *et al.*, 2004; Saravanakumar and Samiyappan, 2007).

Hayat *et al.* (2010) opined that exogenous application of PPFM improves germination, growth, development, quality and yield of crop plants there by counteracts the adverse effect of drought.

The ROS content reduced in plants colonized with AM fungi under various abiotic stresses as studied in wide range of species like maize, lettuce, rice, chickpea and wheat (Li *et al.*, 2011). It might be due to the protective role of bio-inoculants under abiotic stress.

Shukla *et al.* (2012) reported that *Trichoderma harzianum* significantly increased the ability of rice plants to tolerate drought stress and increase rice

water holding capacity. Out of 43 isolates of *T. harzianum*, only five isolates were able to colonize well on cow dung at low moisture content of 10-20 percent. Two isolates, Th 56 and Th 75, grew even at 5 percent moisture content. They also investigated the impact of endophytic fungus *T. harzianum* on rice response to drought stress. Among test isolates of *Trichoderma*, Th 56 induced maximum drought tolerance as treated rice plants recorded only 20-40 percent wilting even at 9 days drought stress. *Trichoderma*-colonized rice seedlings were slower to wilt in response to drought.

2.3 Pink Pigmented Facultative Methylotrophs (PPFMs)

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). They can be easily isolated from plant tissues using selective media containing methanol as the sole carbon source (Corpe, 1985) and identified by their pink color, which distinguishes them from other unrelated methylotrophic organisms normally encountered on plant tissue.

2.3.1 Impact of PPFM on Drought Stress Alleviation in Plants

Increased incidence of abiotic and biotic stresses affecting productivity in major crops are being witnessed all over the world. Among these drought stress is the major threat to principal crops. The problem of drought is increasing continuously with reduction in production of crops (Qayyum and Malik, 1988). The tolerance of plants to drought stress needs to be improved in order to allow growth of crops that satisfy food demands under limited water resourse availability.

Madhiyan *et al.* (2006) reported the presence of ACC deaminase in *Methylobacterium fujisawaense* and its lowering of ethylene levels and promotion of root elongation in canola seedlings under gnotobiotic conditions.

Hayat *et al.* (2010) reported that exogenous application of PPFM produces some benefit in alleviating the adverse effect of drought stress and also improves germination, growth, development and yield of crop plants.

Gawad *et al.* (2015) investigated the effect of PPFM bacteria on the antioxidant enzymes, growth and yield of snap bean plants. Results revealed that application of plants with PPFM individually or combined with methanol changed the level of antioxidant enzymes including polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and super oxide dismutase (SOD). This study proved the positive effect of PPFM on the growth and yield of snap bean plants.

Sivakumar *et al.* (2017) assessed the impact of PPFMs and plant growth regulators on alleviating the drought stess effects in tomato. The study indicated that the PPFMs and PGRs could effectively improve drought tolerance capacity of tomato crop under drought. Among the three different concentrations of PPFM used, PPFM (2%) was found to be superior in improving relative water content, photosynthetic rate, soil plant analytical development (SPAD) value and proline content of tomato plants.

Sivakumar *et al.* (2018) studied that PPFM and PGRs on alleviating the drought stress effects on tomato through root characters, yield and quality. Among the three different concentrations of PPFM used, PPFM (2%) was found to be superior in improving root characters, yield, highest specific leaf weight and highest lycopene content, PPFM (2%) has the ability to protect the plant under drought.

Chandrasekaran *et al.* (2017) reported that the PPFM (2%) and brassinolide (1 ppm) treatments were found superior in improving germination associated traits, stress tolerant index and anti-oxidant enzyme catalase activity which have the ability to protect the plant under abiotic stress condition.

Kumar *et al.* (2017) reported that the application of *Bacillus altitudinis* FD48 and *Methylobacterium* sp. (PPFM) helped to improve tolerance to water stress in rice.

2.3.2 Effect of Osmotic Stress on Seed Germination and Seedling Growth

The germination of seeds and early seedling growth are considered the most crucial phases for seed establishment, determining successful crop production (Uniyal *et al.*, 1998). 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 (12%) or mannitol (2.5%). Mannitol highly reduced the germination rate compared to the PEG effect (Hadi *et al.*, 2014). Maximum shoot length was recorded in control while lowest shoot length was observed in maximum PEG or mannitol for all of the three plant species. These findings demonstrate that mannitol highly reduced the shoot length of canola, cauliflower and tomato as compared to polyethylene glycol treatments (Hadi *et al.*, 2014).

Polyethylene glycol and mannitol showed a significant effect on root length and the highest root length was noted in control C while lowest was found in maximum PEG or mannitol. The data on root length showed reduction with increasing level of polyethylene glycol and mannitol and mannitol greatly decreased the root length as compare to polyethylene glycol (Hadi *et al.*, 2014).

Both PEG and mannitol significantly reduced the fresh biomass of canola, cauliflower and tomato. Fresh biomasses were adversely affected with increasing PEG and mannitol concentration and the maximum fresh biomass was found in control C while lowest was found in T5 (maximum PEG or Mannitol) (Hadi *et al.*, 2014).

Mannitol strongly reduced the canola seedling dry biomass compared to PEG treatments. The highest dry biomass of canola was found in control C while lowest dry biomass was found in T5 (maximum PEG or mannitol). PEG showed slight effect on dry biomass of cauliflower and tomato. Mannitol strongly decreased the dry biomass of three plant species as compared to PEG treatments (Hadi *et al.*, 2014).

Using mannitol for inducing osmotic stress was found to be more selective than PEG (Anber, 2010).

Seed vigour index is also an important component that can influence crop plant density and yield (Siddique and Wright, 2004).

2.3.3 Effect of PPFM on Seed Germination and Seedling Growth under Water Stress Condition

Holland (1997) reported that PPFMs could be used as in seed coatings designed to enhance germination and vigour index. The advantage for PPFM bacteria is a rich supply of plant hormones, as most of the metabolic products of the methanol released by plants are lost from leaves during leaf expansion, which is catalyzed by pectin methylesterase (Dourado *et al.,* 2015).

PPFM (2%) showed higher germination percentage (73.53%) when compared to control (55%) followed by salicylic acid (71%) under drought created by PEG 6000 in tomato. Presoaking with PPFM (2%) treatment enhance the germination up to 33.69 per cent when compared to control (Chandrasekaran *et al.*, 2017). This may be due to different compounds produced by PPFMs which can enhance seed germination. PPFM bacteria stimulate plant growth (Basile *et al.,* 1969) presumably because they produce plant growth regulators (Freyermuth *et al.,* 1996) and vitamin B_{12} (Basile *et al.,* 1985). This increment may have been due to the Gibberellin (GA_3) which improved the synthesis and secretion of hydrolytic enzymes from aleurone cells. These enzymes then mobilize the endosperm storage reserves that are fuel for germination and growth (Cirac *et al.,* 2004).

Seed soaking with PPFM (2%) enhances the shoot length (5.67 cm) followed by gibberellic acid (5.40 cm) and salicylic acid (4.91 cm) under drought created by PEG 6000 in tomato (Chandrasekaran *et al.*, 2017).

Chandrasekaran *et al.* (2017) observed that PPFM (2%) showed higher root length (3.72 cm) compared to control followed by gibberellic acid (3.61 cm) and salicylic acid (2.86) under drought created by PEG 6000 in tomato. This increment might be due to the ability of *Methylobacterium* 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.

PPFM (2%) recorded highest value of vigour index (690.45) followed by gibberellic acid (617.28) and salicylic acid (551.67) under drought created by PEG 6000 in tomato (Chandrasekaran *et al.*, 2017).

Madhaiyan *et al.* (2004) reported that PPFM inoculation has resulted in increased seedling vigour, dry matter production and yield in rice.

Copeland and McDonald (1995) reported that vigour of seedlings relates with their ability upon germination to grow rapidly and well.

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).

Madhaiyan *et al.* (2004) reported that the treatment of three strains of *Methylobacterium* sp. like PPFM-Os-07, *M. extorquens* AM1 and *M. extorquens miaA* mutant enhance rice seed germination.

Kumar *et al.* (2017) reported that rice germination was decreased as the concentration of PEG increased, that is, 0 to 25%. However, the effect of PEG was greatly reduced by treating rice seeds with bacterial cultures viz., *B. altitudinis* FD48, *B. pumilus* FS20, *B. aquimaris* MD02 and *Methylobacterium*

spp. (PPFM). At higher concentration of PEG (25%), highest shoot length was observed in seedlings treated with *B. altitudinis* FD48 (9.5 cm) which was significantly superior to *Methylobacterium* spp. (PPFM) (8.51 cm). The uninoculated control recorded the lowest shoot length (5.23 cm) while higher root length was recorded in *B. altitudinis* FD48 treated seedlings (15.23 cm) followed by *Methylobacterium* spp. (PPFM) treated seedlings (14.01 cm). The least root length was observed in control (6.76 cm). The root dry weight was the highest in *B. altitudinis* FD48 treatment (3.77 mg) followed by *Methylobacterium* spp. (PPFM) treatment (3.37 mg). The least root dry weight was observed in control (1.75 mg). *B. altitudinis* FD48 (5.11 mg) showed the highest shoot dry weight followed by *Methylobacterium* spp. (PPFM) (4.43 mg). The control recorded least shoot dry weight (2.89 mg).

The study of Nysanth (2018) also revealed that the germination percentage of PPFM inoculated seeds showed a significant increase compared to uninoculated control. Maximum germination percentage of 100 % was recorded in seeds treated with PPFM 35.

2.3.5 Effect of PPFM on Biometric Parameters of Plants under Stress Condition

Basile *et al.* (1985) found that the PPFMs influence plant growth by production of phytohormones, such as IAA, cytokinins and vitamins.

These results clearly indicated that the production and release of important growth promoting substances by non-pathogenic *Methylobacteria* which might have been involved in the regulation of plant growth and highly correlated with drought tolerance (Sivakumar *et al.*, 2017).

Drought stress reduced the plant height, leaf number, size and tillers which finally lowered the dry matter production (Khan and Abdullah, 2003).

The PPFM mediated hormonal activity might be attributable for the increase in leaf area, crop growth rate and other growth parameters (Ajaykumar and Krishnasamy, 2018).

Nysanth (2018) proved that the PPFM inoculation in paddy had a significant effect on growth parameters such as plant height, tiller production and leaf area compared to uninoculated control.

Combined inoculation of PPFMs and *Rhizobium* in groundnut cultivar Co(Gn)4 gave significant increase in plant growth, biomass production and yield parameters of groundnut (Reddy *et al.*, 2002).

Higher crop growth rate (CGR) was noticed under PPFM (1%) during *rabi* 2016-17, 2017-18; summer 2017 and 2018 at panicle initiation to flowering stages of rice. Lesser crop growth rate was observed under control at both the year of experiments. This might be due to the result of increased leaf area index. CGR had positive association with leaf area index. The PPFM mediated hormonal activity might be attributed for the increase in leaf area, crop growth rate and other growth parameters (Ajaykumar and Krishnasamy, 2018).

Methylobacterium sp. strain PPFM-Os-07-treated plants showed increased numbers of tillers and plant height when compared to untreated control (Madhaiyan *et al.*, 2004).

2.3.5 Effect of Water Stress on Physiological Parameters of Plants

2.3.5.1 Leaf Rolling Score and Leaf Drying Score

Chang, *et al.* (1974) reported that in rice, leaf rolling character and death of leaves are good criteria found in assessing drought tolerance levels in a large scale screening.

Leaf rolling is one of the drought avoidance mechanism to prevent water loss during drought stress (O'Toole and Cruz, 1980).

Turner *et al.* (1986) reported that leaf rolling can be used as a criteria for scoring drought tolerance in tall and semi dwarf rice cultivars. Also, they observed that rice varieties differ in their ability to roll leaves under similar water deficit condition.

2.3.5.2 Leaf Temperature

Sobarado (1987) reported that as the temperature of the leaves increases, the stomata become close and the rate of transpiration decreases considerably with leaf rolling.

Sensing the infrared radiation emitted by the leaf is one way of measuring water stress. Blum *et al.* (1978) observed a rise in leaf temperature associated with the decrease of transpiration rate, reflecting the degree of water stress in sorghum and indicated the possibility of selecting for drought tolerance based on the leaf temperature.

As water becomes limiting, leaf temperature increases above air temperature because transpiration is reduced. Differences in canopy temperature among rice cultivars are known to be related to drought avoidance based mainly on the potential to maintain transpiration under stress and canopy temperature was shown to be negatively co-related with biomass and grain yield under stress in rice (Blum, 1988). Plants with deeper root system would maintain cooler canopy temperature and ultimately higher yield under drought. Canopy temperature was found to have a positive correlation with leaf rolling and leaf drying and negative correlation with root thickness in rice (Babu *et al.*, 2003).

2.3.5.3 Cell Membrane Integrity

Cell membrane integrity is a physiological index widely used for the evaluation of drought and temperature tolerance (Blum and Ebercon, 1981). This method was developed for a drought and heat tolerance assay in sorghum and measure the amount of electrolyte leakage from leaf segments (Sullivan, 1972). Lower membrane stability or higher injury reflects the extent of membrane lipid

peroxidation, which in turn is a consequence of higher susceptibility to oxidative stress due to various environmental stresses including drought (Leibler *et al.*, 1986). The movement of molecules across membranes is accelerated by heat stress and thereby loosening chemical bonds within molecules of biological membranes. This make the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids (Savchenko *et al.*, 2002).

Limiting watering caused a loss in membrane stability in untreated rice plants and treated plants. However, *B. altitudinis* FD48 treated plants significantly improved membrane stability (69.32%) compared to *Methylobacterium* spp. (PPFM) treated plants (68.55%) and control (60.42%) (Kumar *et al.*, 2017).

Drought stress caused a disturbance in membrane permeability and expressed by an increase in solute leakage (Premchandra *et al.*, 1990; Deshmukh *et al.*, 1991). The results on membrane stability index showed a decreasing trend as the time without water prolonged. The leakage was higher in untreated plants than *B. altitudinis* FD48 treated plants indicating severe membrane damage in the former under drought stress (Kumar *et al.*, 2017).

The higher leakage of solutes was probably due to enhanced H_2O_2 accumulation and lipid peroxidation under oxidative stress (Sese and Tobita, 1998). The plasma membrane is generally protected from desiccation induced damage by the presence of membrane compatible solutes, such as sugars and amino acids. Therefore, a link may exist between the capacity for osmotic adjustment and the degree of membrane protection from the effect of dehydration. Accumulation of antioxidant enzymes may also result in protecting membrane stability.

2.3.5.4 Relative Water Content

Relative water content is considered as a measure of water status of plant, indicating the metabolic activity in tissues. It can be used as the most meaningful

index for dehydration tolerance. The capacity to maintain higher relative water content (RWC) under moisture stress condition is obviously a resistance mechanism in rice (O'Toole and Moya, 1978). Relatively high RWC have been reported in drought tolerant cultivar of rice. Fischer (1989) found that RWC was directly related to soil water content. A substantial decrease in relative water content, leaf water potential and transpiration rate, and a simultaneous increase in leaf temperature were observed when rice plants were exposed to drought stress (Akram *et al.*, 2013).

Haloi and Baldev (1986) revealed that the productivity of the crops may be related to physiological attributes like photosynthetic rate and relative water content.

Sivakumar *et al.* (2017) reported that RWC decreased up to 32.69 per cent in plants under drought compared to absolute control. Among the PGRs and PPFM used, PPFM (2%) treatment gave statistically superior relative water content of 64.42 per cent followed by brassinolide (62.66%) and salicylic acid (61.24%) at 60 DAT in tomato plant. Higher RWC indicates better water status of plant, which in turn cause rapid early growth and maintenance of RWC at reasonably higher level during reproductive phase greatly influences the tolerance under water stress conditions. Foliar spray of PPFM (2%) was found to be superior in improving relative water content, photosynthetic rate, SPAD value, proline content which ultimately improve the drought tolerance capacity in tomato.

Relative water content (RWC) of plants decreased in response to drought condition. However, culture treated plants were observed to have more RWC compared to control under induced drought condition. *B. altitudinis* FD48 treated plants showed 69.38% RWC followed by *Methylobacterium* spp. (PPFM) treated plants (68.61%) whereas the control recorded the lowest RWC (60.53%) (Kumar *et al.*, 2017).

Under drought stress, relative water content (RWC) declined in inoculated and uninoculated seedlings. However, bacterial inoculation did help seedlings to maintain their Relative water content (RWC) during drought periods. Similar report was made on the use *Pseudomonas* spp. inoculation to help the maize plants to maintain their relative water content under drought condition (Sandhya *et al.*, 2010). The mechanism behind the increased Relative water content (RWC) when treated with PGPB is yet to be elucidated. Some studies predict that this may be a result of bacterial absicisic acid which results in closure of stomata (Casanovas *et al.*, 2002).

2.3.5.5 Chlorophyll Stability Index

Sathyan *et al.* (2018) studied the effect of pink pigmented facultative methylotrophic bacteria and synthetic materials on small cardamom (*Elettaria cardamomum* Maton.) under drought and reported a significant increase in the chlorophyll stability index in the PPFM treated (60.3%) over control (15.90%).

Water stress induced a significant decrease in metabolic factors such as the decrease in chlorophyll content in canola plants (Sakova *et al.*, 1995).

Sivakumar *et al.* (2017) reported that the foliar application of BAP, brassinolide and PPFM prevent the chlorophyll breakdown under drought leading to retention of chlorophyll and delay of senescence.

Meenakshi and Savalgi (2009) found high chlorophyll content in treatment, which received both seed inoculation and foliar spray of *Methylobacterium*.

Madhaiyan *et al.* (2004) observed higher photosynthetic activity in rice cultivar Co-47 that received *Methylobacterium* and attributed the effect due to enhancement of chlorophyll concentration, maleic acid content and increased number of stomata.

Chlorophyll stability index (CSI) of plants decreased in response to drought condition. Drought stressed plants inoculated with *B. altitudinis* FD48 showed 69.23% CSI followed by *Methylobacterium* spp. (PPFM) inoculation (68.32%). The chlorophyll stability index of control plants were the lowest (55.4%) under drought condition (Kumar *et al.*, 2017).

Chlorophyll stability index is a function of temperature, the property of chlorophyll pigments can be correlated with drought tolerance/susceptibility of the crop plants. Prolonged drought stress reduced the chlorophyll stability index in all treatments. But *B. altitudinis* FD48 treated plants showed more Chlorophyll Stability Index (CSI) when compared to *Methylobacterium* spp. (PPFM) and control (Kumar *et al.*, 2017).

2.3.5.6 Root Traits (Rooting Depth, Root Weight, Root Volume, Root Dry Weight)

The possession of deep and thick root system which allows access to water deep in the soil profile is crucially considered important in determining drought tolerance in upland rice and substantial genetic variation exist for this trait (O'Toole, 1982; Yoshida and Hasegawa, 1982; Ekanayake *et al.,* 1985; Chang *et al.*, 1986 and Fukai and Cooper, 1995).

Among the treatments, PPFM (2%) marked the highest root length of 25.90 cm, followed by brassinolide (25.20 cm) and salicylic acid (22.93 cm). Root length was increased up to 26.34 per cent by PPFM (2%) higher than control followed by brassinolide (22.93%) in tomato under drought condition. The maximum root length was recorded in absolute control (27.90 cm) and minimum in control of 13.50 cm (Sivakumar *et al.*, 2018).

Chandrasekaran *et al.* (2017) found that the treatment with PPFM (2%) recorded higher root length followed by gibberellic acid and salicylic acid in tomato under drought condition. This increment by PPFM might be due to, *Methylobacterium* which are capable to generate plant growth regulators such as

auxin and cytokinin (Ivanova *et al.,* 2000) which induce cell division and cell elongation.

Highest root volume was observed in absolute control (122.80 cm^3) whereas in control (97.90 cm^3) recorded lowest in tomato under drought condition (Sivakumar *et al.*, 2018). The foliar spray of PGRs and PPFM helped to alleviate drought by improving the lateral root growth which increased the root volume.

Response of root growth to drought can be variable; root growth can be greater under moderate moisture stress, because of increased partitioning of carbohydrates to roots, whereas, reduction in root growth were observed in severe drought. Drought stress increases the concentrations of ABA in the root, which in turn maintain root growth and increase root hydraulic conductivity, which can postpone development of water stress by increase in water uptake (Gowda *et al.*, 2011).

Secondary traits such as deep, thick, coarse and highly branched roots as well as higher root to shoot ratio are reported in rice as drought adaptation (Blum, 2011).

Niones *et al.* (2015) reported that lateral root production in response to varying soil water content has been demonstrated as an important trait in maintaining dry matter production and grain yield.

2.4.5.7 Shoot Dry Weight

Boyer (1985) reported that increased root to shoot ratio was observed in plants during soil moisture deficit as a result of reduced shoot dry weight. Sharp *et al.* (1994) observed that abscisic acid influences the relative growth rates of plant parts such as an increase in the root to shoot dry weight ratio, inhibition of leaf area development and production of prolific and deeper roots. Prasad *et al.* (2006) observed mild drought stress changes pattern of resource allocation and they generally noticed more root growth than shoot growth. Wahid (2007) reported that

high temperatures caused significant decline in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane.

2.3.5.8 Root Shoot Ratio

Boyer (1985) reported that increased root to shoot ratio was observed in plants during soil moisture deficit.

Cruz *et al.* (1986) presented that mild stress condition during vegetative stage in rice can cause more reduction in root dry weight than shoot dry weight and thereby decreasing root to shoot ratio.

Nysanth (2018) reported that root shoot ratio of seedlings showed significant increase when seeds were treated with PPFM isolates. Maximum root shoot ratio of 0.62 was observed when seeds were treated with PPFM 26 and PPFM 35.

2.3.5.9 Drought Susceptibility Index

Drought index is an important criterion for selection for stress environment, which provides a measure of drought based on loss of yield under drought condition in comparison to moist condition and has been used for screening of drought tolerance genotypes (Brukner and Frohberg, 1987).

Pink-pigmented facultative methylotrophic (PPFM) bacteria are predominant and explored largely for their ability to release plant-growth regulation molecules (Dourado *et al.,* 2015) and thereby increasing the tolerant capacity of plants under drought conditions.

According to studies of Grzesiak *et al.* (2012), drought susceptibility indices (DSIGY) for maize and triticale genotypes were calculated by determining the changes in grain yield (GY) under two soil moisture levels (irrigated and drought). Variation of DSIGY for maize ranges from 0.381 to 0.650 and for triticale from 0.354 to 0.578.

2.3.5.10 Proline Content

Proline is believed to protect plant tissues against stress by acting as nitrogen storage, osmoregulator and protectant for enzymes and cellular structure. It is one of the important amino acids, known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stress (Ali *et al.,* 1999).

According to Anjum *et al.* (2000), proline is a scavenger of OH^- radical and plays an important role in osmotic adjustment during oxidative stress. It reduces the damaging effect of ROS to the membrane lipid and protein, enzymes and DNA. Proline has an important role to sustain root growth under water stress condition.

Uyprasert *et al.* (2004) stated that proline acts as a compatible solute and a protective agent for cytoplasmic enzymes and structures. And also they confirmed that the rice genotypes exhibiting high proline accumulation had a marked effect on the ability to maintain water status consequently delayed tissue death and leaf senescence in rice under water stress.

It has been suggested that accumulation of proline contributes to maintain proper balance between extra and intra-cellular osmolarity under conditions of water stress (Madhusudhan *et al.*, 2002). Accumulation of proline under stress in many plant species has been correlated with stress tolerance and its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants.

PPFMs exude osmoprotectants (sugars and alcohols) on the surface of host plants (Trostenko *et al.*, 2001).

The positive effect of PPFM might be due to the increment of osmolytes like proline and enhance the water uptake and maintained the water status of the plant (Sivakumar *et al.,* 2017). These osmolytes might increase the osmotic

pressure of cytoplasm and enhance water flow into the different plant organs and tissues.

Sivakumar *et al.* (2017) reported that the foliar application of PPFM (2%) increased the proline content by 11.34 per cent followed by brassinolide (8.34%) and salicylic acid (7.89%) compared to absolute control.

The role of ameliorators such as PPFM and brassinolide was significant in increasing the content of proline in the stressed plants (Aruna *et al.,* 1999).

Kumar *et al.* (2017) studied that proline content was significantly influenced by both drought stress and culture treatments. A substantial increase in the amount of free proline was observed in all treatments due to drought stress. However, it was interesting to note that *B. altitudinis* FD48 treated rice plants produced the highest concentration of proline (5.73 μ mol g^{-1}fresh weight) relative to *Methylobacterium* spp. (PPFM) treated plants (5.11 μ mol g^{-1}fresh weight) and control (3.16 μ mol g^{-1} fresh weight).

The phyllosphere isolates showed increased content of proline, total sugars and total amino acid under PEG induced drought stress condition when compared with non-stressed condition (Kumar *et al.*, 2017).

Under stress conditions, energy flow of the cells is directed towards protection mechanisms to synthesize osmolytes (sugars, proline, etc.) to protect them against fluctuations in osmotic conditions (Timmusk, 2003) and these osmolytes accumulate to higher levels to alleviate stress effects (Rasanen *et al.*, 2004). The accumulated osmolytes enhance the stability of proteins and membrane under water-limiting environments (Kogut and Russell, 1987).

Azospirillum and arbuscular mycorrhizal inoculation increased the shoot proline content in rice under drought condition when compared to control (Sanchez *et al.*, 2011).

The inoculation also increased proline content under drought stress compared to control which may be due to up regulation of proline biosynthesis pathway to keep proline in high levels, which helps in maintaining cell water status, protects membranes, and proteins from stress (Yoshiba *et al.*, 1997).

2.3.5.11 Gibberellic Acid

Anurajan (2003), for the first time reported the production of gibberellic acid by *Methylobacterium* sp. which acted as plant growth regulator by modifying plant morphology.

2.4.5.12 Super Oxide Dismutase

Beltrano *et al.* (2003) reported that SOD catalyzes the dis mutation of superoxide into molecular oxygen (O^{2-}) and H_2O_2 that will be subsequently dis muted into H_2O and oxygen by catalase.

Sharma and Dubey (2005) studied the effect of mild and high drought stress on superoxide dismutase (SOD) activity and they observed that total SOD activity increased significantly in roots as well as shoots of both the rice cultivars (Malviya-36 and Pant12). The level of total SOD activity was higher in shoots than in roots. Twenty-day-old mild drought stressed ((PEG-6000 of 17%) seedlings showed about 71 to 78% increase in total SOD activity in roots and 56 to 90% increased activity in shoots compared to control seedlings. High drought stress (PEG-6000 of 41.2%) led to an increase between 15 and 105% in Cu/Zn-SOD, 56 to 93% in Fe-SOD and 53 to 63% in Mn-SOD activity in 20 days old seedlings.

2.3.5.13 Catalase

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).

Shukla *et al.* (2012), Sandhya *et al.* (2011) and Gusain *et al.* (2015) reported that under conditions of environmental stress, when ROS such as H_2O_2 are produced, catalase enzyme triggered by the bacteria act as scavenging enzymes and play a central role in protecting the cell from oxidative damage.

Kumar *et al.* (2017) reported that *B. altitudinis* FD48 and *Methylobacterium* spp. (PPFM) treated rice plants showed more catalase activity than control under drought condition.

Chandrasekaran *et al.* (2017) reported that the PPFM (2%) and brassinolide (1 ppm) treatments were found to superior in improving germination associated traits, stress tolerant index and anti-oxidant enzyme catalase activity which have the ability to protect the plant under abiotic stress condition.

Gawad *et al.* (2015) found that the antioxidant enzymes like catalase and SOD activity were increased by the PPFM in snap bean.

Chandrasekaran *et al.* (2017) noticed that PPFM (2%) recorded highest catalase activity of 2.96 μg H_2O_2 g^{-1} min^{-1} under stress condition in tomato.

The catalase activity increased under drought condition with *B. altitudinis* FD48 treated plants with significantly higher activity followed by *Methylobacterium* spp. (PPFM) treated plants. The least catalase activity was observed in control (Kumar *et al.*, 2017).

2.3.5.14 Peroxidase

Peroxidases and catalases also play an important role in the fine regulation of reactive oxygen species in the cell through activation and deactivation of several apoplastic enzymes may also generate reactive oxygen species under normal and stressful conditions (Sairam *et al.,* 2005).

Increased activity of peroxidase in stressed seedlings can be correlated to oxidative reactions corresponding to accumulation of peroxides and free radicals in the plant cells (Radotic *et al.*, 2000).

Accumulation of excess H_2O_2 in cells was prevented by Ascorbate peroxidase (APX) through ascorbate-glutathione pathway (Foyer and Halliwell, 1976).

Stressful conditions induces enhanced expression of APX in cytosol as well as in cellular organelles (Yoshimura, 2000).

In drought stressed seedlings an increased cytosolic APX activity led to decrease in H_2O_2 concentration (Madhusudhan *et al.*, 2002).

2.3.6 Effect of Water Stress on Yield Attributes and Yield

Being a drought sensitive crop, rice exhibits deleterious effects when exposed to drought at critical growth stages such as panicle initiation, anthesis and grain filling (Weisburg *et al.*, 1991).

Sarkarung, *et al.* (1995) reported that yield losses are more severe when drought occurs during the reproductive phase by slow growth during development of panicle, which reduces number of grains and size of grain.

Wang *et al.* (2003) reported that drought is a serious environmental stress which affects agriculture productivity and yield more than 50 per cent.

Sah and Zamora (2005) observed that water deficit at vegetative as well as reproductive stages significantly reduced the grain yield per plant in maize as compared to well-watered plant. The reduction was 19.5% and 48.5% due to water deficit in vegetative and reproductive stages, respectively, as compared to well-watered plants.

Lower CGR recorded under stress induced at PI and flowering stage along with control, might have resulted in lower recovery of the crop and thereby causing reduction in the grain yield (Thangamani, 2005).

Jaleel *et al.* (2008b) observed that drought is one of the serious environmental factors affecting yield and quality. Rice is sensitive to drought stress particularly during flowering stage, resulting in severe yield losses. The physiological processes during the sensitive flowering stage, negatively affects spikelet fertility under water stress.

Nysanth (2018) reported that the application of PPFM isolates significantly influenced the yield and yield attributes of paddy. The per cent increase in yield due to application with PPFM 11 was 37.59 against uninoculated control and 20.57 against the reference strain.

Sivakumar *et al.* (2018) studied that the foliar spray of 2% PPFM documented significantly superior fruit yield of 552.90 g which is closely followed by brassinolide (509.40 g) and salicylic acid (472.60 g) in tomato under drought conditions. Yield showed positive response to PGRs and PPFM under water deficit conditions. In the present study, fruit yield increased up to 35.00 per cent by PPFM (2%) followed by brassinolide (24.50%).

Materials and Methods

3. MATERIALS AND METHODS

The experiment on "Screening of Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy" was carried out during the period from 2017-2019 in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram. The details of the materials used and methods followed in the present study are included in this chapter.

3.1 Purification of Pink Pigmented Facultative Methylotrophs (PPFMs)

Based on the preliminary study conducted in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, during 2015-2017, twenty isolates of PPFM of paddy were selected on the basis of carotenoid pigment production, IAA production, proline content, seedling vigour index and yield (Nysanth, 2018). They were purified by the streak plate method. After preparing the streak plates, all the plates were incubated at 25 °C for a 10 days and allowed to develop its characteristic pink pigment. After incubation, well isolated colonies on the plates were preserved in peptone glycerol (enrichment medium) slants and were kept at 4 °C in a refrigerator for further use.

3.2 *IN VITRO* SCREENING OF PPFM ISOLATES FOR WATER STRESS TOLERANCE

3.2.1 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 °C for 10 days (Plate 1).

3.2.2 Soaking of Paddy Seed

Rice seeds (variety Harsha) were soaked overnight in 1 per cent liquid culture of 10 days old PPFM isolates (Plate 2).

Plate 1. Liquid culture of PPFM isolates

Plate 2. Seeds soaked in PPFM cultures

3.2.3 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 paddy seeds were selected randomly and given the different treatments T_1 to T_{21} and controls. From these treated seeds, eight seeds were randomly selected and placed between a pair of moist paper towels. The paper towels were rolled and the ends were closed by threads and covered by polyethylene paper to prevent drying (Plate 3). The rolled paper towel containing T_1 to T_{21} were dipped in different water stress levels induced by 1% mannitol, 2% mannitol, 3% mannitol and control (water). After 14 days of incubation, observations were recorded.

3.2.4 Details of *In vitro* Screening of PPFM Isolates

Design	: Completely Randomized Design
Treatments	: 84 + (4 x 3) (Control)
T_1-T_{20}	: KAU isolates of PPFM
T_{21}	: TNAU isolate
Water stress levels	: 4
WS_1	: 1% mannitol
WS_2	: 2% mannitol
WS_3	: 3% mannitol
WS_4	: Control (water)
Variety	: Harsha
Replication	: 2

Note:

Control 1: Application of 0.5% methanol

Control 2: Application of AMS liquid medium supplemented with 0.5% methanol

Control 3: Absolute control

3.2.5 Observations

3.2.5.1 Germination Percentage

Germination percentage was calculated after 14 days. Germination percentage was calculated by using the equation:

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

3.2.5.2 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 cm.

3.2.5.3 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 cm.

3.2.5.4 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.

3.2.5.5 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.

3.2.5.6 Seedling Vigour Index

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

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

3.2.5.7. Weighted Average Ranking

Weighted average ranking was done for finding the best PPFM isolate imparting the water stress tolerance. For the ranking, germination percentage, shoot length, root length, shoot dry weight and seedling vigour index were considered. The treatment of PPFM showing highest values in each parameter was given 1st rank and the next lower was given 2nd rank and so on and was ranked upto 20. For each PPFM treatment the ranks of different parameters were added to obtain weighted average rank. The PPFM treatment having the lowest value in the weighted rank was assigned 1st rank, the second lowest value was given 2nd rank and thus the 20 PPFM isolates were ranked accordingly.

3.3 POT CULTURE EXPERIMENT TO STUDY THE EFFECT OF PINK PIGMENTED FACULTATIVE METHYLOTROPH (PPFM) ISOLATES ON GROWTH AND YIELD OF PADDY UNDER WATER STRESS CONDITIONS.

Five isolates selected from the *in vitro* study (3.1) were used for the pot culture experiment and its effect on growth and yield of paddy under varying moisture levels were assessed (Plate 4). The isolate obtained from the commercial product of TNAU was used as the reference culture.

3.3.1 Crop Variety

Harsha (PTB 55), a short duration (105-110 days) variety having straw coloured grains with red kernel released from Regional Agricultural Research Station, Pattambi was used for the experiment. This photo insensitive variety shows moderate resistance to blue beetle and moisture stress. It exhibits low susceptibility to blast and sheath blight. Besides, it is a non-lodging and non-shattering variety with excellent milling and cooking qualities.

3.3.2 Source of Seed

Seeds for the experiment were procured from Regional Agricultural Research Station, Pattambi, Kerala, India.

3.3.3 Pot Culture Experiment

Location	: College of Agriculture, Vellayani
Crop	: Rice
Variety	: Harsha (PTB 55)
Design	: Completely Randomized Design
Treatments	: 18 + 3 (Control)
Replication	: 3
Season	: Summer (Fig. 1)

Treatment details:

A : PPFM isolates (I)

i_1, i_2, i_3, i_4, i_5 :KAU isolates

i_6 : TNAU isolate

B : Moisture levels (M)

m_1 : at field capacity

m_2 : 75% available water

m_3 : 50% available water

Note:

Control 1: Application of 0.5% methanol

Control 2: Application of AMS liquid medium supplemented with 0.5% methanol

Control 3: Absolute control

3.3.4 Preparation of Pots

The pots were filled with potting mixture. Potting mixture was prepared by mixing soil, cowdung and sand in 3:2:1 ratio.

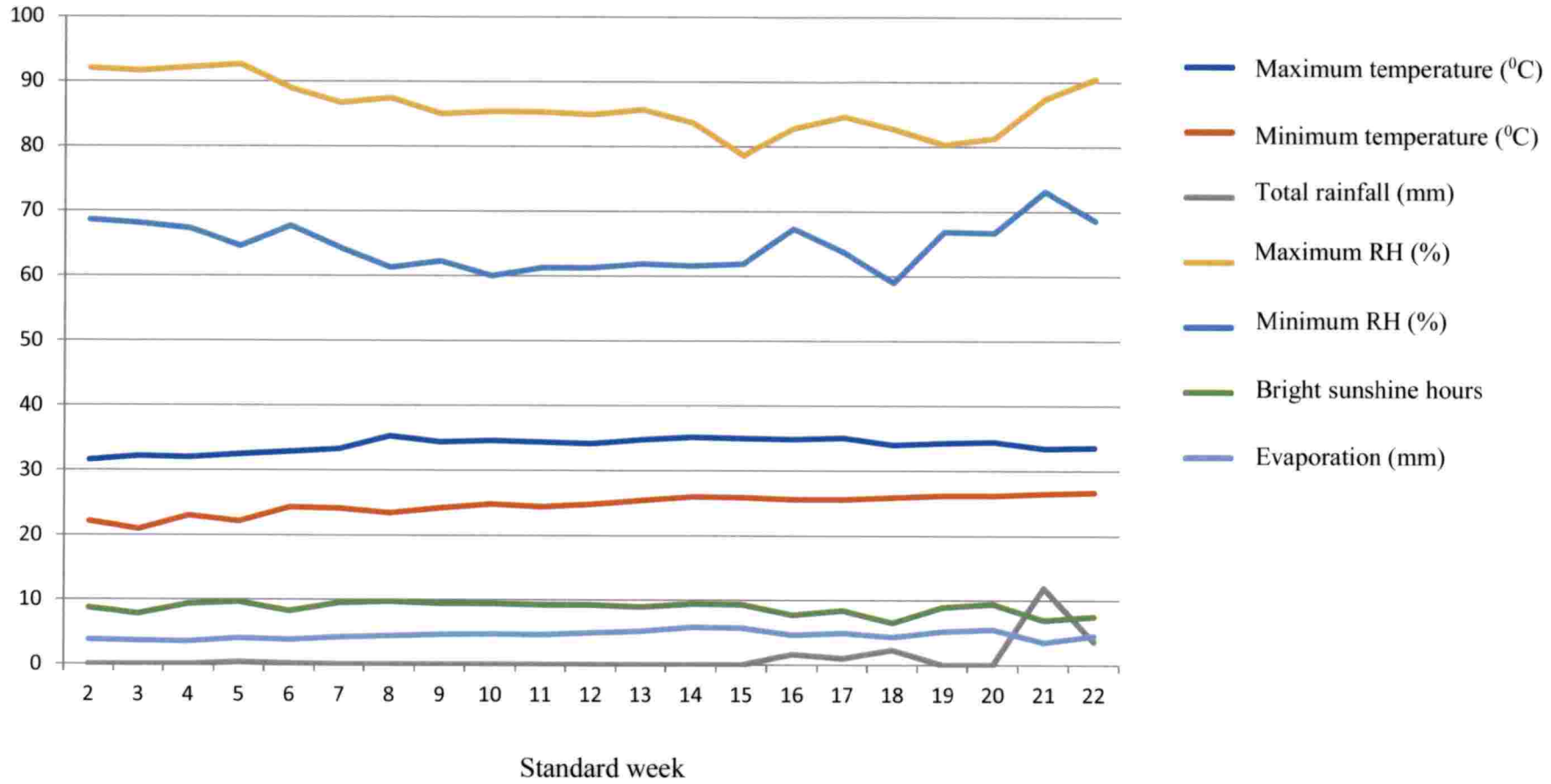

Figure 1. Weather data during the cropping period (January- June 2019)

Plate 3. General view of *in vitro* experiment

Plate 4. General view of pot culture experiment

3.3.5 Fertilizer Application

Fertilizers were applied as per the recommended dose of 70:35:35 kg NPK per hectare (KAU, 2016). N, P, K were applied in the form of urea, rajphos and muriate of potash.

3.3.6 Preparation of PPFM Inoculum

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

3.3.7 Seed Treatment

Rice seeds (variety Harsha) were soaked overnight in 1 per cent (10^5 cfu/ ml) liquid culture of 10 days old PPFM isolates and sown in pots for raising seedlings.

3.3.8 Seedling Dip

Roots of 18 days old seedlings were dipped in 2 per cent (10^5 cfu/ ml) solution of the respective isolates of PPFM for 30 minutes before transplanting. After seedling dip, the seedlings were transplanted in the pots.

3.3.9 Foliar Application

The PPFM cultures were grown for 14 days and one per cent (10^5 cfu/ ml) solution for foliar spray was prepared. It was applied at 15 and 30 days after transplanting.

3.3.10 Observations

3.3.10.1 Biometric Parameters of the Plant

3.3.10.1.1 Height of the Plant

The height of the plant was measured from the base to the growing tip of the top most leaf at 30, 60 DAT and at harvest. It was expressed in cm. At harvest, the height was recorded from the base to the tip of the longest panicle.

3.3.10.1.2 Leaf Area Index

The length and breadth of the fourth leaf from top were measured at 30 DAT and 60 DAT (Palanisamy and Gomez, 1974).

Leaf area = K (LxB)

K = 0.75 (Yoshida *et al.*, 1976)

L = Leaf length (cm)

B = Maximum breadth of the leaf (cm)

LAI was calculated as follows

$$\text{Leaf Area Index} = \frac{\text{Total leaf area per tiller x Number of tillers m}^{-2}}{\text{Area occupied by tillers m}^{-2}}$$

3.3.10.1.3 Number of Tillers per Hill

Total number of tillers were recorded after 30 and 60 DAT.

3.3.10.2 Physiological Parameters

3.3.10.2.1 Leaf Rolling Score

The plants were scored for leaf rolling at 30 days and 60 days after stress. Drought reactions were scored at 30 days and 60 days after stress using 0-9 scale of standard evaluation system for rice (IRRI, 1996).

Table 1: Description of leaf rolling score

Scale	Description
0	Leaves healthy
1	Leaves starts to fold
3	Leaves folding (deep V- shaped)
5	Leaves fully cupped (U- shaped)
7	Leaves margins touching (O-shaped)
9	Leaves tightly rolled

3.3.10.2.2 Leaf Drying Score

The plants were scored for leaf drying at 30 days and 60 days after stress. Drought reactions were scored at 30 days and 60 days after stress using 0-9 scale of standard evaluation system for rice (IRRI, 1996).

Table 2: Description of leaf drying score

Scale	Description	Rate
0	No symptoms	Highly resistant
1	Slight tip drying	Resistant
3	Tip drying extended to ¼ length in most leaves	Moderately resistant
5	¼ to ½ of the leaves fully dried	Moderately susceptible
7	More than 2/3 of all leaves fully dried	Susceptible
9	All plants apparently dead	Highly susceptible

3.3.10.2.3 Leaf Temperature

Leaf temperature was measured in the morning between 9 a.m. and 11 a.m. using infrared thermometer and expressed in °C.

3.3.10.2.4 Cell Membrane Integrity

Cell membrane integrity was calculated as per the procedure described by Blum and Ebercon (1981). Samples collected from all the treatments were washed three times in deionized water to remove electrolytes adhered on the surface. Samples were kept in capped vial (20 mL) containing 10 mL of deionised water and incubated in the dark for 24 hours at room temperature. The conductance was measured with a conductivity meter. Then these vials were autoclaved for 15 minutes to kill the leaf tissue and release electrolytes. After cooling, the second conductivity reading was taken. These two measurements were made individually for all the treatments. Cell membrane integrity was calculated by using following formula and expressed as per cent.

$$CMI\ (\%) = [1-(T_1/T_2)/1-(C_1/C_2)] \times 100$$

Where, T and C refer to the stress and control samples respectively. The subscripts 1 and 2 refer to the initial and final conductance readings, respectively.

3.3.10.2.5 Relative Water Content

The relative water content was measured based on the method described by Turner (1981). The relative leaf water content was determined in the fully expanded leaf. The fresh weights of the sample leaves were recorded, and the leaves were immersed in distilled water in a petri dish. After 2 h, the leaves were removed, the surface water was blotted off and the turgid weight was recorded. The samples were then dried in an oven at 70°C for 48 h. Then the dry weight was recorded. The relative leaf water content was calculated using the following formula and expressed as per cent.

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

Where, FW is the fresh weight; DW is the dry weight; and TW is the turgid weight.

3.3.10.2.6 Chlorophyll Stability Index

Chrorophyll content of leaf samples were estimated as per the procedure by Hiscox and Israelstam (1979). One hundred mg leaf sample was taken from fully expanded third leaf and were chopped into pieces. 5 mL of DMSO (Dimethyl sulfoxide): Acetone (80%) (1:1) mixture was added to samples and incubated overnight. The supernatant was collected and absorbance was measured at 645 and 663 nm. Total chlorophyll content and chlorophyll stability index was calculated using the formula given below and expressed in mg g^{-1} of fresh leaf weight.

Total chlorophyll = {[20.2(OD at 645) + 8.01(OD at 663)] x V} / (Wx1000)

Where V = volume of the solution made up and W = fresh weight of leaves

$$\text{Chlorophyll stability index} = \frac{\text{Total chlorophyll in stress}}{\text{Total chlorophyll in control}} \times 100$$

3.3.10.2.7 Rooting Depth

The rooting depth was taken at 30 DAT and 60 DAT. The plants were uprooted and the roots were washed under tap water to remove clods and soil particles. The rooting depth was expressed in centimeter.

3.3.10.2.8 Root Weight

The weight of root was taken in an electronic single pan balance immediately after uprooting the plants and expressed in 'g'.

3.3.10.2.9 Root Volume

The root volume was estimated by water displacement method. Individual plants were uprooted and roots were immersed in known volume of water. The amount of water displaced was measured and expressed in cubic centimeter.

3.3.10.2.10 Shoot Dry Weight

The shoot dry weight was taken at 30 DAT and 60 DAT. Shoots were dried at 60°C in a hot air oven and the weight was expressed in g.

3.3.10.2.11 Root Dry Weight

The root dry weight was taken at 30 DAT and 60 DAT. Roots were dried at 60°C in a hot air oven and the weight was expressed in g.

3.3.10.2.12 Root Shoot Ratio

After taking the dry weight of shoot and root (g) at 30 DAT and 60 DAT, root shoot ratio was calculated using the equation,

$$\text{Root shoot ratio} = \frac{\text{Dry weight of root (g)}}{\text{Dry weight of shoot (g)}}$$

3.3.10.2.13 Soil Moisture Percentage

Soil moisture was determined by gravimetric method. This method involves weighing a moist sample, oven drying it at 105 °C for 48h, reweighing, and calculating the mass of water lost as a percentage of the mass of the dried soil.

$$\text{SMP (\%)} = \frac{\text{Weight of the moist soil} - \text{Weight of the dry soil}}{\text{Weight of the dry soil}} \times 100$$

3.3.10.2.14 Drought Susceptibility Index

The drought susceptibility index is an important criterion for selection for stress environment, which provides a measure of drought based on loss of yield under drought condition in comparison to moist condition. It is used for screening of tolerance genotypes to drought (Fischer and Maurer, 1978). Drought susceptibility index was calculated by the formula given below.

$$DSI = \frac{1 - (Ys / Yp)}{1 - (\bar{Y}s / \bar{Y}p)}$$

Where,

Ys = Seed yield of genotypes under moisture stress condition (g $hill^{-1}$)

Yp = Seed yield of genotypes under irrigated condition (g $hill^{-1}$)

$\bar{Y}s$ = Mean yield of all strains under moisture stress condition (g $hill^{-1}$)

$\bar{Y}p$ = Mean yield of all strains under irrigated condition (g $hill^{-1}$)

3.3.10.2.15 Proline Content

Proline was estimated as per the procedure described by Bates *et al.* (1973). A known quantity (0.5 g) of mid-leaf portion was homogenized with 10 mL of 3 per cent aqueous sulphosalicylic acid and centrifuged at 3000 rpm for 15 minutes. Two mL of the supernatant was taken and mixed with an equal quantity of glacial acetic acid and acid ninhydrin. The contents were allowed to react at 100 °C for one hour in water bath. The reaction was terminated by keeping it in ice bath for 10 min. The reaction mixture was mixed with 4 mL toluene using vortex mixture for 15-20 seconds. The chromophore containing toluene was aspirated from aqueous phase, warmed to room temperature and the optical density was read at 520 nm with toluene as blank. A standard curve was drawn using concentration verses absorbance.

3.3.10.2.16 Gibberellic Acid

Gibberelic acid extraction and estimation was modified from method suggested by Sunderbarg (1990) and Kojima (1995). Two hundred and fifty milligram plant sample homogenised with ice cold methanol was kept at 4 °C in dark for four hours. The homogenate was centrifuged and filtered and all the extracts was collected and concentrated to a water residue at 50 °C for one hour. The volume was adjusted with phosphate buffer to 10 ml and partitioned in a separating funnel with 10 mL diethyl. The aqueous phase was adjusted to pH 2.7 with 0.4 M HCl and the ether phase was discarded. The aqueous phase was again partitioned and the aqueous phase collected was further partitioned two times with 0.4 M $NaHCO_3$.

The final partitioned aqueous phase was collected and separated again with 10 ml ethyl acetate. The aqueous phase was transferred and 2 mL methanol was added and stored at 4 °C. The GA content was then estimated by adding Zinc acetate, Potassium ferrocyanide. The supernatant collected after centrifugation and was kept at 20 °C for 75 minutes after adding 30 per cent HCl. Then the absorbance was read at 254 nm using a UV-VIS spectrophotometer. The GA content was calculated and expressed in $\mu g\ g^{-1}$.

3.3.10.2.17 Super Oxide Dismutase

Super oxide dismutase activity was measured by the method described by Beauchamp and Fridovich (1971). Grind 1g of clean leaf tissue in 10 ml ice cold 50 mM potassium phosphate buffer, pH 7.8 in a pre-chilled pestle and mortar. Centrifuge the homogenate at 10000 rpm for 10 min at 4 °C and the supernatant was used for assay. Mix a 3 mL reaction mixture containing 50 mM potassium phosphate buffer, 13 mM methionine, 2 µM riboflavin, 0.1 mM EDTA, 75 µM NBT and 50 µL of crude enzyme extract, in duplicate. Made up the volume equal by adding double distilled water. Set a blank without enzyme and NBT to calibrate the spectrophotometer. Set another control having NBT but no enzyme as reference control. Expose all the tubes to 400 W bulb (4 x 100 W

bulbs) for 15 min. Read the absorbance immediately at 560 nm. Calculate the percentage inhibition. The 50 % inhibition of the reaction between riboflavin and NBT in the presence of methionine was taken as 1 unit of SOD activity.

3.3.10.2.18 Catalase

Catalase activity was assayed by the method suggested by Barber (1980). The fresh leaves (0.5 g) were ground in 20 mL of cold potassium phosphate buffer and centrifuged at 15,000 rpm for 15 min. The enzyme extract was brought up to 25 ml with potassium phosphate buffer. One mL (1 mL) of enzyme extract, 2 ml of 0.1M H_2O_2 and 3 ml of potassium phosphate buffer were placed in a test tube. After 5 min. the reaction in test tube was stopped by adding 1 mL of 0.7 N concentrated sulphuric acid. The test tube was incubated for 5 min. at 27 °C and the residual hydrogen peroxide in the test tube was titrated against 0.01 M $KMnO_4$ until a faint purple color persisted for at least 15 second. The amount of H_2O_2 destroyed by catalase was calculated by the formula given below.

$$\frac{25 \times 0.85}{2} \times \frac{V}{W}$$

Where,

W = weight of sample

V = volume of $KMnO_4$ utilized (Titer value)

3.3.10.2.19 Peroxidase

The peroxidase activity in plant was estimated following the method described by Reddy *et al.* (1995). Leaf sample of 200 mg was homogenized in 1 ml of 0.1 M phosphate buffer (pH 6.5) and centrifuged at 5000 rpm for 15 minute at 4 °C. To 3.0 ml of pyrogallol solution, 0.1 ml of the enzyme extract was added and adjusted to read zero at 430 nm. The enzyme reaction was started by adding 0.5 ml of one percent hydrogen peroxide (H_2O_2) into sample cuvettes

and change in absorbance was measured every 30 second up to 3 minute. One unit of peroxidase is defined as the change in absorbance per minute at 430 nm.

3.3.10.3 Yield and Yield Attributes

3.3.10.3.1 Number of Panicles per Hill

Total number of panicles from each hill were counted and expressed as number of panicles per hill.

3.3.10.3.2 Number of Grains per Panicle

The entire spikelets including filled and unfilled grains were counted and the mean number of grains per panicle was worked out.

3.3.10.3.3 1000 Grain Weight

One thousand bold grains were counted from cleaned and dried produce in the observational plants and the weight of the grains was recorded in 'g'.

3.3.10.3.4 Grain Yield

Plants were harvested from the pot, threshed, cleaned, dried to 14 per cent moisture, weighed and the grain yield expressed in g $hill^{-1}$.

3.3.10.3.5 Percentage Relative Yield Reduction

Relative yield reduction (RYR) under stress was computed as:

$$RYR = 1 - (\text{grain yield stress}/\text{grain yield control}) \times 100$$

3.3.10.3.6 Straw Yield

The straw obtained from each hill was dried under sun, weighed and straw yield expressed in g $hill^{-1}$.

3.3.10.4 Incidence of Pest and Diseases

The incidence of pest and disease was monitored throughout the crop period.

3.3.10.5 Weighted Average Ranking

Weighted average ranking of PPFM isolates was done separately for physiological parameters and yield and yield attributes. Physiological parameters like leaf rolling score, leaf drying score, rooting depth, drought susceptibility index, proline content, super oxide dismutase, catalase, peroxidase and yield and yield attributes such as number of panicles per hill, number of grains per panicle, 1000 grain weight, grain yield and percentage relative yield reduction were considered for ranking.

The treatment of PPFM showing highest value was given 1st rank and the next lower was given 2nd rank and so on upto rank 5. In the case of leaf rolling score, leaf drying score, drought susceptibility index and percentage relative yield reduction, the lowest value was ranked 1st and the next higher value ranked 2nd and so on upto rank 5. For each treatment, the ranks were added to obtain weighted average rank separately for physiological parameters and yield attributes and yield. The lowest value in the weighted rank was assigned 1st rank, the second lowest value was given 2nd rank and thus the 5 PPFM isolates were ranked accordingly.

3.4 Statistical Analysis

The experiment was laid out in Factorial Completely Randomized Design (FCRD) and data analyzed using analysis of variance technique (Gomez and Gomez, 1984). One way ANOVA for 2 factors (A-treatments, B-water stress levels) was carried out and critical difference (CD) was calculated based on their significance.

Results

4. RESULTS

The present study on "Screening of Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy" was conducted during 2017-2019, in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala. The results based on statistically analyzed data pertaining to the experiment conducted during the course of investigation are presented below:

4.1. EFFECT OF PPFM ISOLATES ON PADDY SEED GERMINATION AND SEEDLING GROWTH UNDER *IN VITRO* CONDITIONS

4.1.1 Germination Percentage

The data on the effect of different PPFM isolates and water stress levels on germination percentage of paddy seeds are presented in Table 3.

The results revealed that in 1% mannitol, the germination percentage was maximum in seeds treated with PPFM 6 (Plate 5) and water treated control (87.50 %). These treatments were on par with PPFM 11 (81.25 %), PPFM 16 (68.75 %), PPFM 17 (75.00 %), PPFM 19 (68.75 %), PPFM 26 (68.75 %), PPFM 32 (75.00 %), PPFM 37 (81.25 %), PPFM 47 (75.00 %), 0.5% methanol (68.75%) and AMS media (68.75 %). The lowest germination percentage of 43.75 % was recorded with PPFM 2, PPFM 4, PPFM 34 and PPFM 38.

However, in 2% mannitol, the germination percentage was maximum in seeds treated with PPFM 42 (Plate 6) and PPFM 46 (93.75 %). These isolates were on par with PPFM 2 (87.50 %), PPFM 3 (68.75 %), PPFM 4 (75.00 %), PPFM 9 (81.25 %), PPFM 11 (81.25 %), PPFM 15 (68.75 %), PPFM 22 (87.50 %), PPFM 24 (87.50 %), PPFM 32 (81.25 %), PPFM 34 (68.75 %), PPFM 38 (68.75 %) and 0.5% methanol (68.75%). The lowest germination percentage of 43.75 % was recorded with PPFM 16, PPFM 17 and PPFM 37.

The results indicated that in 3% mannitol, the germination percentage was maximum in seeds treated with PPFM 26 (87.50 %) (Plate 7). It was also on par with PPFM 2 (68.75 %), PPFM 4 (81.25 %), PPFM 11 (81.25 %), PPFM 15 (75.00 %), PPFM 16 (68.75 %), PPFM 24 (68.75 %), PPFM 34 (75.00 %), PPFM 35 (75.00 %), PPFM 37 (81.25 %), PPFM 38 (68.75 %), PPFM 47 (68.75 %) and 0.5% methanol (68.75%). The lowest germination percentage of 37.50 % was recorded with PPFM 9 and PPFM 46.

The results revealed that in water alone, *i.e.,* without mannitol, maximum germination percentage was recorded with PPFM 2 (93.75 %) and PPFM 11 (93.75 %). These isolates were also on par with PPFM 3 (68.75 %), PPFM 6 (75.00 %), PPFM 16 (81.25 %), PPFM 17 (68.75 %), PPFM 32 (68.75 %), PPFM 34 (81.25 %), PPFM 35 (68.75 %), PPFM 47 (75.00 %), 0.5% methanol (68.75%) and AMS media (81.25 %). The lowest germination percentage was recorded with PPFM 42 (43.75 %).

4.1.2 Shoot Length

The data on the effect of different PPFM isolates and water stress levels on shoot length of paddy seedlings are presented in Table 4.

Perusal of the results revealed that in 1% mannitol, the shoot length was maximum in seeds treated with PPFM 2 (10.21 cm). It was also on par with PPFM 3 (8.45 cm), PPFM 4 (9.10 cm), PPFM 6 (9.17 cm), PPFM 9 (8.95 cm), PPFM 11 (9.20 cm), PPFM 15 (9.32 cm), PPFM 16 (9.84 cm), PPFM 19 (9.11 cm), PPFM 22 (9.51 cm), PPFM 24 (8.82 cm), PPFM 26 (9.11 cm), PPFM 35 (9.68 cm), PPFM 42 (8.97 cm), PPFM 47 (9.72 cm), 0.5% methanol (9.20 cm) and water (8.80 cm). The lowest shoot length was recorded with PPFM 38 (5.38 cm).

The results revealed that in 2% mannitol, the shoot length was maximum in seeds treated with PPFM 34 (10.88 cm). It was also on par with PPFM 2 (8.61 cm), PPFM 3 (9.48 cm), PPFM 4 (9.05 cm), PPFM 11 (8.99 cm), PPFM 16

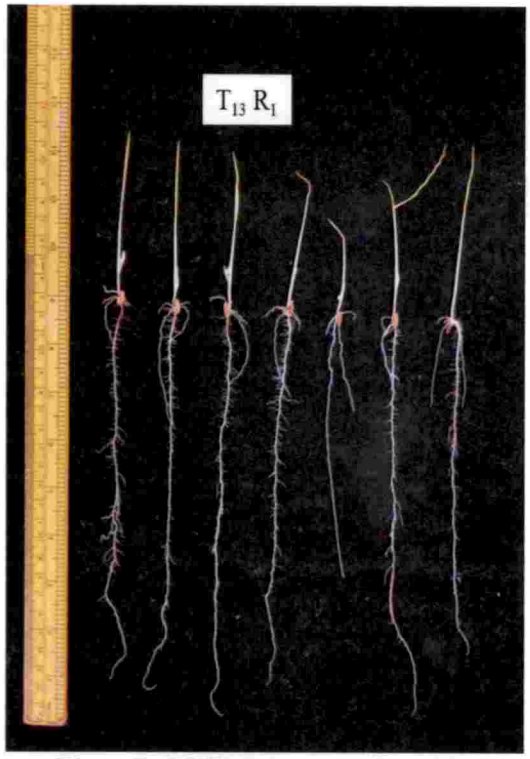

Plate 5. PPFM 6 treated paddy seedlings in 1% mannitol

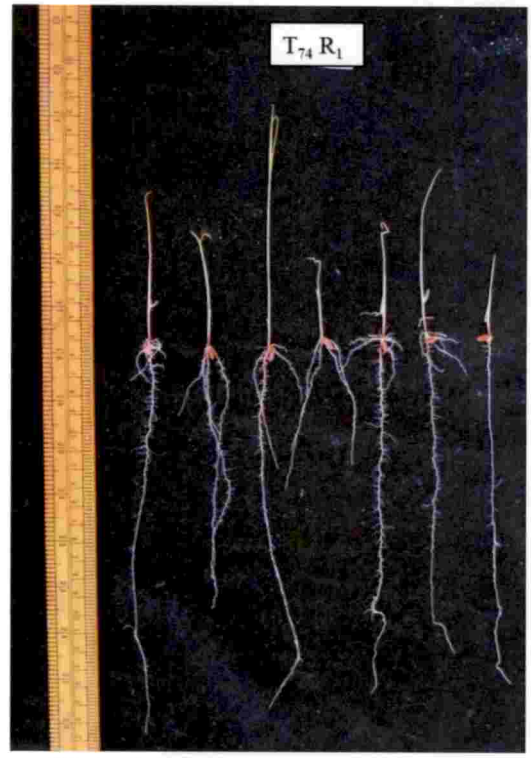

Plate 6. PPFM 42 treated paddy seedlings in 2% mannitol

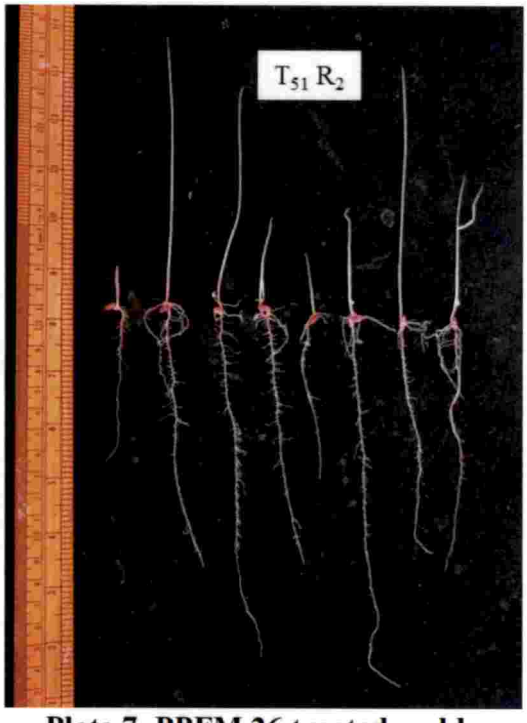

Plate 7. PPFM 26 treated paddy seedlings in 3% mannitol

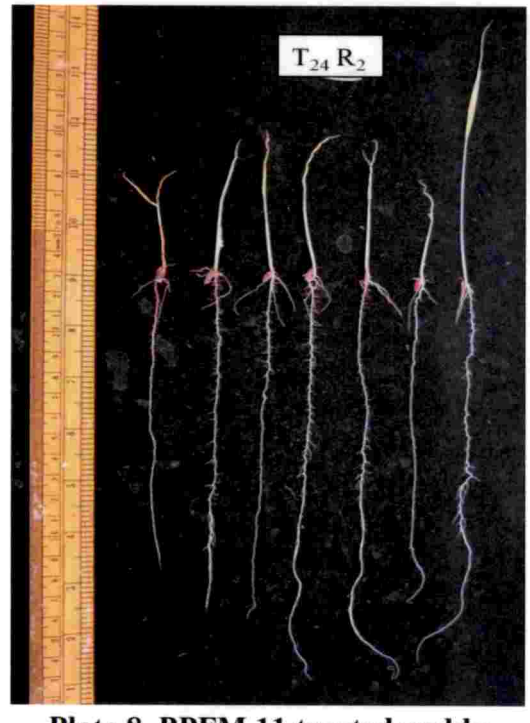

Plate 8. PPFM 11 treated paddy seedlings in water

(8.84 cm), PPFM 17 (8.65 cm) and PPFM 35 (10.51 cm). The lowest shoot length was recorded with PPFM 32 (5.62 cm).

It was also observed that in 3% mannitol, the shoot length was maximum in seeds treated with PPFM 26 (9.47 cm). It was also on par with PPFM 3 (8.24 cm), PPFM 4 (7.11 cm), PPFM 6 (7.85 cm), PPFM 11 (8.88 cm), PPFM 15 (9.39 cm), PPFM 16 (8.36 cm), PPFM 22 (8.06 cm), PPFM 24 (7.10 cm), PPFM 38 (8.65 cm), 0.5% methanol (7.49 cm) and AMS media (7.78 cm). The lowest shoot length was recorded with PPFM 19 (5.25 cm).

The results revealed that in water alone, *i.e.,* without mannitol, maximum shoot length was recorded with PPFM 37 (12.37 cm). It was also on par with PPFM 16 (10.63 cm), PPFM 24 (11.15 cm), PPFM 26 (10.51 cm) and PPFM 46 (11.90 cm). The lowest shoot length was recorded with PPFM 34 (7.13 cm).

4.1.3 Root Length

The results of effect of different PPFM isolates and water stress levels on root length of paddy seedlings are presented in Table 5.

The results indicated that in 1% mannitol, the root length was maximum in seeds treated with PPFM 3 (23.26 cm). It was also on par with PPFM 2 (20.28 cm), PPFM 9 (20.82 cm), PPFM 34 (21.33 cm) and PPFM 37 (19.97 cm). The lowest root length was recorded with PPFM 38 (10.90 cm).

It was also observed that in 2% mannitol, the root length was maximum in seeds treated with PPFM 4 (22.91 cm). It was also on par with PPFM 2 (17.44 cm), PPFM 3 (20.82 cm), PPFM 6 (19.38 cm), PPFM 9 (18.17 cm), PPFM 11 (20.91 cm), PPFM 15 (18.48 cm), PPFM 16 (21.95 cm), PPFM 17 (16.85 cm), PPFM 19 (20.37 cm), PPFM 22 (18.65 cm), PPFM 24 (21.38 cm), PPFM 34 (19.86 cm), PPFM 38 (18.96 cm) and AMS media (17.43 cm). The lowest root length was recorded with PPFM 47 (9.13 cm).

Table 3. 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 4. Effect of PPFM isolates on shoot length of paddy seedlings, cm

Treatments	Shoot length			
	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 5. Effect of PPFM isolates on root length of paddy seedlings, cm

Treatments	Root length			
	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

The results pointed out that in 3% mannitol, the root length was maximum in seeds treated with PPFM 15 (18.38 cm). It was also on par with PPFM 2 (14.38 cm), PPFM 3 (16.79 cm), PPFM 4 (15.53 cm), PPFM 6 (16.33 cm), PPFM 9 (15.14 cm), PPFM 11 (14.93 cm), PPFM 16 (16.11 cm), PPFM 17 (14.40 cm), PPFM 19 (15.35 cm), PPFM 22 (14.28 cm), PPFM 24 (15.75 cm), PPFM 26 (15.38 cm), PPFM 34 (14.61 cm), PPFM 35 (15.36 cm), PPFM 37 (18.07 cm), PPFM 38 (15.28 cm), PPFM 46 (15.19 cm), PPFM 47 (17.26 cm), 0.5% methanol (16.76 cm), AMS media (14.33 cm) and water (17.13 cm). The lowest root length was recorded with PPFM 42 (7.08 cm).

The results also revealed that in water alone, *i.e.,* without mannitol, maximum root length was recorded with PPFM 4 (24.17 cm). It was also on par with PPFM 2 (23.46 cm), PPFM 3 (20.96 cm), PPFM 6 (20.35 cm), PPFM 15 (20.25 cm), PPFM 16 (21.00 cm), PPFM 17 (22.46 cm), PPFM 22 (22.68 cm), PPFM 24 (22.08 cm), PPFM 26 (21.51 cm), PPFM 35 (22.89 cm), PPFM 37 (23.45 cm), PPFM 38 (20.68 cm) and PPFM 46 (21.80 cm). The lowest root length was recorded with PPFM 19 (16.62 cm).

4.1.4 Shoot Dry Weight

The data on the effect of different PPFM isolates and water stress levels on shoot dry weight of paddy seedlings are presented in Table 6.

The results pointed out that in 1% mannitol, the shoot dry weight was maximum in seeds treated with PPFM 22 (7.65 mg). It was also on par with PPFM 2 (6.15 mg), PPFM 3 (7.05 mg), PPFM 4 (7.55 mg), PPFM 6 (6.00 mg), PPFM 9 (7.50 mg), PPFM 11 (6.45 mg), PPFM 15 (6.20 mg), PPFM 17 (6.45 mg), PPFM 19 (6.10 mg), PPFM 24 (7.20 mg), PPFM 34 (6.10 mg), PPFM 35 (6.80 mg), PPFM 37 (7.00 mg), PPFM 47 (6.05 mg), 0.5% methanol (6.25 mg) and water (6.00 mg). The lowest shoot dry weight was recorded with PPFM 32 (4.55 mg).

The results indicated that in 2% mannitol, the shoot dry weight was maximum in seeds treated with PPFM 16 (8.25 mg). It was also on par with PPFM 2 (6.60 mg), PPFM 3 (8.05 mg), PPFM 4 (7.45 mg), PPFM 6 (6.55 mg), PPFM 9 (7.60 mg), PPFM 11 (7.15 mg), PPFM 19 (6.75 mg), PPFM 24 (7.00 mg), PPFM 26 (7.30 mg), PPFM 34 (7.20 mg) and PPFM 35 (7.05 mg). The lowest shoot dry weight was recorded with PPFM 37 (4.35 mg).

However, in 3% mannitol, the shoot dry weight was maximum in seeds treated with PPFM 15 (7.40 mg). It was also on par with PPFM 3 (6.40 mg), PPFM 4 (6.05 mg), PPFM 6 (6.30 mg), PPFM 9 (5.95 mg), PPFM 11 (6.25 mg), PPFM 16 (6.35 mg) and PPFM 26 (7.00 mg). The lowest shoot dry weight was recorded with PPFM 42 (4.00 mg).

The results revealed that in water alone, *i.e.,* without mannitol, maximum shoot dry weight was recorded with PPFM 22 (8.85 mg). It was also on par with PPFM 17 (8.05 mg). The lowest shoot dry weight was recorded with PPFM 42 (4.25 mg).

4.1.5. Root Dry Weight

The results of effect of different PPFM isolates and water stress levels on root dry weight of paddy seedlings are presented in Table 7.

The results indicated that in 1% mannitol, the root dry weight was maximum in seeds treated with PPFM 9 (3.70 mg). It was also on par with PPFM 2 (3.35 mg), PPFM 3 (3.30 mg), PPFM 4 (3.65 mg), PPFM 6 (2.75 mg), PPFM 11 (2.80 mg), PPFM 15 (2.95 mg), PPFM 17 (3.35 mg), PPFM 19 (3.15 mg), PPFM 22 (2.95 mg), PPFM 24 (3.35 mg), PPFM 26 (2.85 mg), PPFM 34 (2.85 mg), PPFM 35 (2.85 mg) and PPFM 37 (2.80 mg). The lowest root dry weight was recorded with PPFM 42 (1.90 mg).

The results also revealed that in 2% mannitol, the root dry weight was maximum in seeds treated with PPFM 4 (5.35 mg). It was also on par with

PPFM 16 (4.05 mg), PPFM 24 (3.85 mg), PPFM 26 (4.35 mg), PPFM 35 (3.95 mg) and PPFM 38 (5.05 mg). The lowest root dry weight was recorded with PPFM 47 (1.90 mg).

Perusal of the data indicated that in 3% mannitol, the root dry weight was maximum in seeds treated with PPFM 9 (4.50 mg). It was also on par with PPFM 3 (3.20 mg), PPFM 17 (4.25 mg), PPFM 19 (4.20 mg), PPFM 22 (3.60 mg), PPFM 26 (3.75 mg), PPFM 35 (3.90 mg), PPFM 37 (3.20 mg), PPFM 38 (3.80 mg), PPFM 46 (3.85 mg) and PPFM 47 (3.25 mg). The lowest root dry weight was recorded with PPFM 4 (2.05 mg).

The results also revealed that in water alone, *i.e.,* without mannitol, maximum root dry weight was recorded with PPFM 22 (4.60 mg). It was also on par with PPFM 4 (3.75 mg) and PPFM 6 (4.35 mg). The lowest root dry weight of 2.30 mg was recorded with PPFM 32 and PPFM 47.

4.1.6 Seedling Vigour Index

The results of effect of different PPFM isolates and water stress levels on seedling vigour index (SVI) of paddy seedlings are presented in Table 8.

A critical analysis of the results revealed that in 1% mannitol, the SVI was maximum in seeds treated with water treatment (2256.63). It was also on par with PPFM 3 (1982.82), PPFM 6 (2538.38), PPFM 9 (1860.32), PPFM 11 (2097.00), PPFM 16 (1932.00), PPFM 17 (2054.63), PPFM 19 (1822.25), PPFM 26 (1756.25), PPFM 35 (1806.13), PPFM 37 (2256.00), and PPFM 47 (1733.63). The lowest SVI was recorded with PPFM 38 (718.57).

The results further revealed that in 2% mannitol, the SVI was maximum in seeds treated with PPFM 24 (2588.26). It was also on par with PPFM 2 (2293.75), PPFM 3 (2085.44), PPFM 4 (2396.63), PPFM 9 (2069.88), PPFM 11 (2435.82), PPFM 22 (2162.57), PPFM 34 (2101.69), PPFM 38 (1851.25), PPFM 42

Table 6. Effect of PPFM isolates on shoot dry weight of paddy seedlings, mg

Treatments	Shoot dry weight			
	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 7. Effect of PPFM isolates on root dry weight of paddy seedlings, mg

Treatments	Root dry weight			
	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 8. 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 9. 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

(2079.44) and PPFM 46 (1903.94). The lowest SVI was recorded with PPFM 37 (921.63).

It was also pointed out that in 3% mannitol, the SVI was maximum in seeds treated with PPFM 26 (2143.25). It was also on par with PPFM 4 (1832.63), PPFM 11 (1939.25), PPFM 15 (2070.82), PPFM 16 (1671.00), PPFM 35 (1675.13), PPFM 37 (2038.32), PPFM 38 (1638.50), PPFM 47 (1633.63) and 0.5% methanol (1653.63). The lowest SVI was recorded with PPFM 32 (865.00).

However, in water alone, *i.e.,* without mannitol, maximum SVI was recorded with PPFM 2 (3080.63) (Plate 8). It was also on par with PPFM 11 (2702.07) and PPFM 16 (2559.19). The lowest SVI was recorded with PPFM 42 (1275.07).

Weighted Average Ranks

Maximum stress level of 3% mannitol was selected for calculating the weighted average of PPFM isolates, which was presented in Table 9.

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 having top weighted average ranks were PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 (Plate 7, 9, 10, 11 and 12). These isolates which secured ranks from 1 to 5 were selected for the subsequent pot culture experiment.

4.2 EFFECT OF PPFM ISOLATES ON GROWTH AND YIELD OF PADDY

4.2.1 Biometric Parameters of the Plant

4.2.1.1 Height of the Plant

The data on height of the plant as influenced by PPFM isolates and moisture levels at 30 DAT, 60 DAT and 90 DAT are presented in Table 10, Table 11 and 12 respectively.

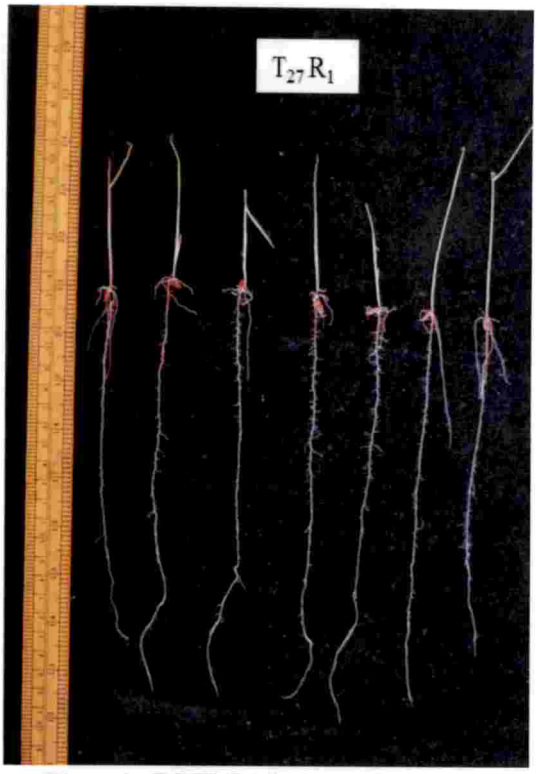

Plate 9. PPFM 15 treated paddy seedlings in 3% mannitol

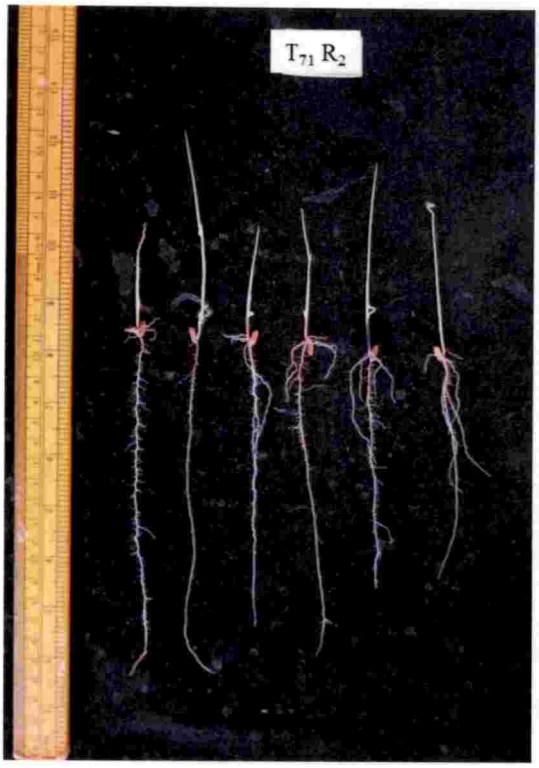

Plate 10. PPFM 38 treated paddy seedlings in 3% mannitol

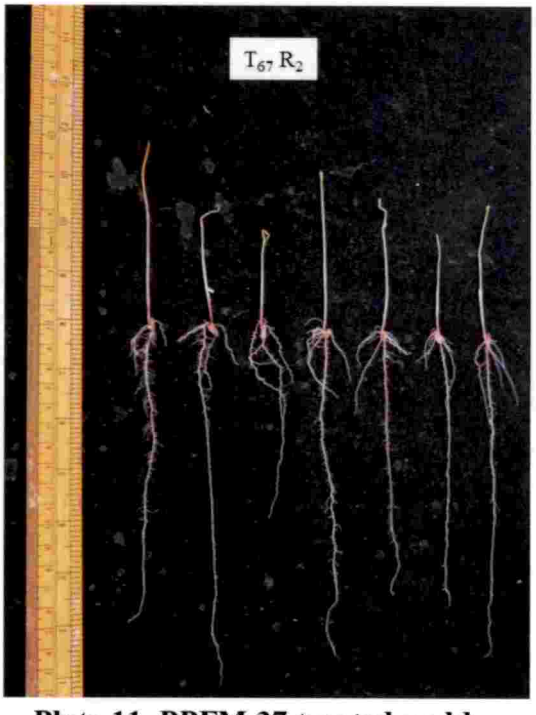

Plate 11. PPFM 37 treated paddy seedlings in 3% mannitol

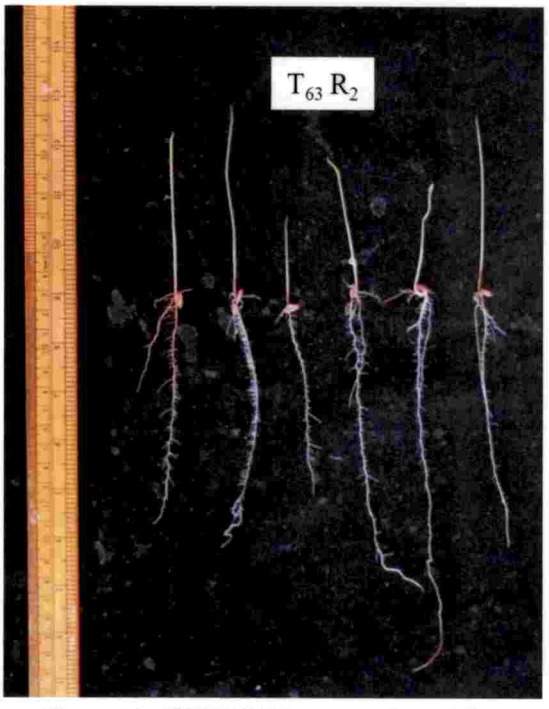

Plate 12. PPFM 35 treated paddy seedlings in 3% mannitol

Among the different PPFM isolates tested, the highest mean plant height of 42.55 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (39.00 cm) at 30 DAT. Mean plant height was the least with isolate PPFM 15 (22.01 cm).

The effect of different soil moisture levels on plant height showed that 50% AW resulted in significantly higher values for mean plant height (33.51 cm) than 75% AW (32.33 cm) and FC (26.52 cm).

The interaction effect between moisture levels and PPFM isolates revealed that plants were significantly taller with PPFM 38 at all the three moisture levels. The results also revealed that at FC, the maximum plant height of 40.51 cm was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (34.92 cm) and PPFM 47 (33.55 cm). The lowest plant height was recorded with water (17.51 cm) at FC. At 75% AW, the maximum plant height of 43.11 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (39.43 cm) and PPFM 47 (34.48 cm). The lowest plant height was recorded with PPFM 15 (19.80 cm) at 75% AW. At 50% AW, the maximum plant height of 44.01 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (42.64 cm). The lowest plant height was recorded with PPFM 15 (26.73 cm) at 50% AW.

Among the different PPFM isolates tested, highest mean plant height of 59.41 cm was recorded with PPFM 38 at 60 DAT. Mean plant height was least with isolate PPFM 15 (46.49 cm).

The effect of different soil moisture levels on plant height showed that 50% AW resulted in significantly higher values for mean plant height (53.44 cm) compared to the height at 75% AW (50.60 cm) and at FC (48.82 cm).

The interaction effect between moisture levels and the PPFM isolates revealed that plants were significantly taller with PPFM 38 at all the three moisture levels. The results also revealed that at FC, the maximum plant height of

57.03 cm was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (52.20 cm), PPFM 35 (51.23 cm), PPFM 47 (50.90 cm) and PPFM 26 (48.77 cm). The lowest plant height was recorded with 0.5% methanol (43.43 cm) at FC. At 75% AW, the maximum plant height of 60.03 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (54.17 cm) and PPFM 35 (52.03 cm) (Plate 13). The lowest plant height was recorded with water (44.67 cm) at 75% AW. At 50% AW, the maximum plant height of 61.17 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (56.10 cm), PPFM 35 (54.60 cm) and PPFM 47 (53.07 cm) (Plate 14). The lowest plant height was recorded with water (51.07 cm) at 50% AW.

Among the different PPFM isolates tested, highest mean plant height of 80.79 cm was recorded with PPFM 37 which was statistically on par with PPFM 38 (80.63 cm) and PPFM 35 (75.96 cm) at 90 DAT. Mean plant height was least with water treated control (68.09 cm).

The effect of different soil moisture levels on plant height showed that at 50% AW resulted in significantly higher values for mean plant height (76.24 cm) and it was statistically on par with the height at 75% AW (73.26 cm) and at FC (72.67 cm).

Interaction effect between moisture levels and the PPFM isolates was significant with respect to plant height. The results revealed that at FC, the maximum plant height of 79.53 cm was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (77.90 cm), PPFM 35 (75.07 cm), PPFM 26 (74.13 cm), PPFM 47 (73.63 cm), PPFM 15 (71.40 cm) and AMS media (69.23 cm). The lowest plant height was recorded with water (65.70 cm) at FC. At 75% AW, the maximum plant height of 80.87 cm was recorded with PPFM 38 which was statistically on par with PPFM 37 (79.30 cm), PPFM 35 (73.73 cm), PPFM 26 (71.47 cm), PPFM 15 (71.63 cm), PPFM 47 (71.73 cm) and AMS media (72.93 cm). The lowest plant height was recorded with water (68.30 cm) at 75% AW. At 50% AW, the maximum plant height of

85.17 cm was recorded with PPFM 37 which was statistically on par with PPFM 38 (81.50 cm), PPFM 35 (79.07 cm), PPFM 26 (77.43 cm) and PPFM 47 (76.40 cm) (Plate 15). The lowest plant height was recorded with water (70.27 cm) at 50% AW.

4.2.1.2 Leaf Area Index

The data on leaf area index as influenced by PPFM and moisture levels at 30 DAT and 60 DAT are presented in Table 13 and 14 respectively.

Among the different PPFM isolates tested, the highest mean leaf area index of 3.86 was recorded with PPFM 38 which was statistically on par with PPFM 37 (3.68) at 30 DAT. Mean leaf area index was the least with water treated control (1.79).

The effect of different soil moisture levels on leaf area index showed that 50% AW resulted in significantly higher values for mean leaf area index (3.16) compared to the leaf area index at 75% AW (2.95) and at FC (2.61).

Interaction effect between moisture levels and the PPFM isolates on leaf area index was significant at 30 DAT. The results revealed that at FC, the maximum leaf area index of 3.68 was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (3.41). The lowest leaf area index was recorded with water (1.40) at FC. At 75% AW, the maximum leaf area index of 3.96 was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest leaf area index was recorded with water (1.93) at 75% AW. At 50% AW, the maximum leaf area index of 4.01 was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.95) and PPFM 26 (3.81). The lowest leaf area index was recorded with water (2.03) at 50% AW.

At 60 DAT, among the different PPFM isolates tested, the highest mean leaf area index of 4.78 was recorded with PPFM 38 which was statistically on par

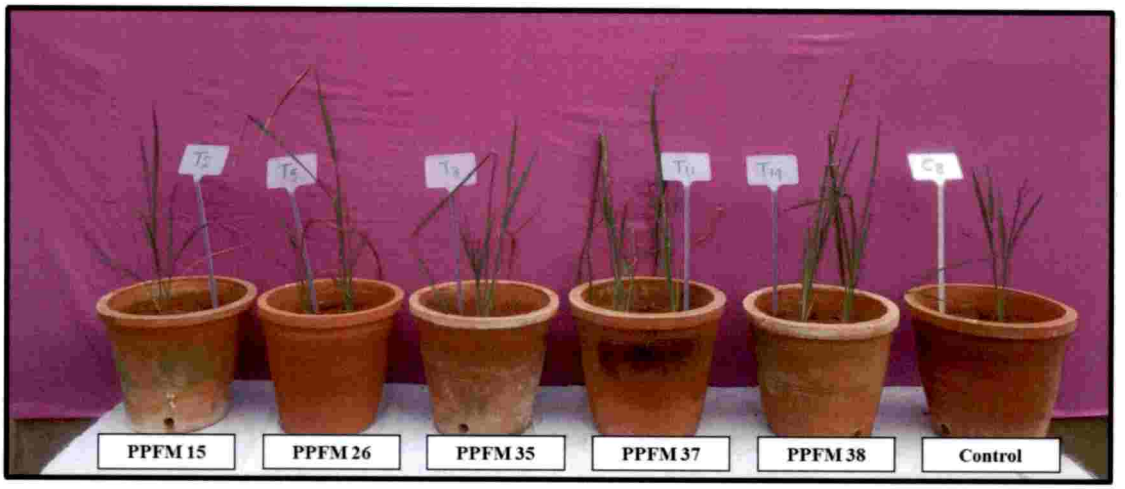

Plate 13. PPFM treated plants (75% AW) at 60 DAT compared to water control

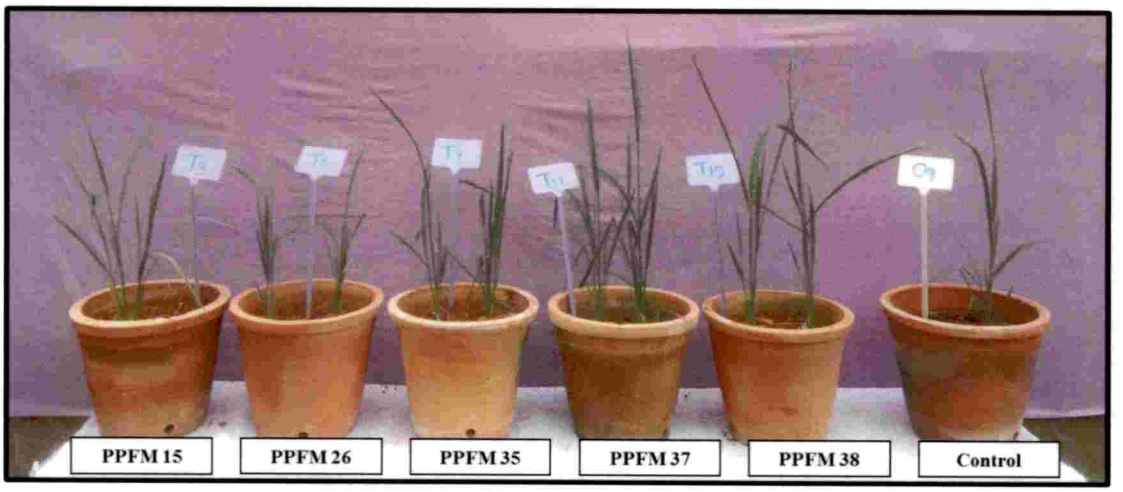

Plate 14. PPFM treated plants (50% AW) at 60 DAT compared to water control

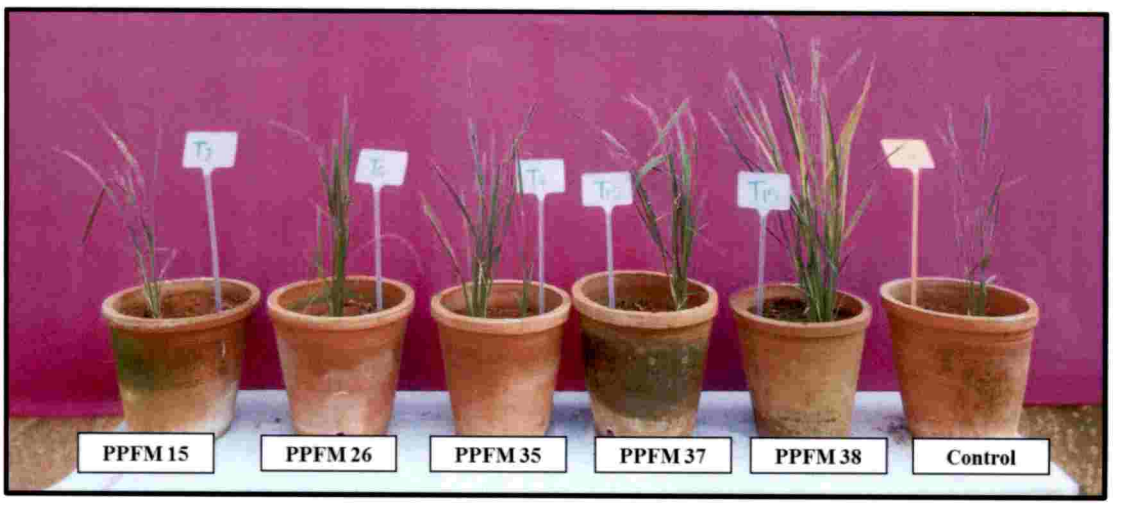

Plate 15. PPFM treated plants (50% AW) at 90 DAT compared to water control

with PPFM 37 (4.76). Mean leaf area index was the least with water treated control (2.97).

The effect of different soil moisture levels on leaf area index showed that at 50% AW significantly higher values for mean leaf area index (3.83) was observed and it was statistically on par with the leaf area index at 75% AW (3.79) and the lowest leaf area index was noticed at FC (3.58).

The interaction effect between moisture levels and the PPFM isolates on leaf area index at 60 DAT was significant. The results revealed that at FC, the maximum leaf area index of 4.79 was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (4.59). The lowest leaf area index was recorded with water (2.92) at FC. At 75% AW, the maximum leaf area index of 4.91 was recorded with PPFM 38 which was statistically on par with PPFM 37 (4.67). The lowest leaf area index was recorded with water (2.95) at 75% AW. At 50% AW, the maximum leaf area index of 5.02 was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest leaf area index was recorded with water (3.04) at 50% AW.

4.2.1.3 Number of Tillers per Hill

Effect of PPFM isolates on number of tillers per hill at 30 DAT was found to be the same (1 tiller per hill) for all the treatments.

The data on number of tillers per hill at 60 DAT are presented in Table 15. Among the different PPFM isolates tested, the highest mean number of tillers per hill (5.56) was recorded with PPFM 37 which was statistically on par with PPFM 38 (5.33). Mean number of tillers per hill was the least with PPFM 15 (3.44).

Soil moisture levels failed to have significant effect on the number of tillers per hill at 60 DAT.

Table 10. Effect of PPFM isolates on height of the plant at 30 DAT, cm

Treatments (A)	Height of the plant			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	19.50	19.80	26.73	22.01
PPFM 26	28.44	33.91	27.20	29.85
PPFM 35	22.75	31.22	32.03	28.67
PPFM 37	34.92	39.43	42.64	39.00
PPFM 38	40.51	43.11	44.01	42.55
PPFM 47 (TNAU)	33.55	34.48	34.57	34.20
0.5% Methanol	19.68	31.78	31.77	27.74
AMS	21.79	33.09	34.75	29.88
Water	17.51	24.13	27.87	23.17
Mean B	26.52	32.33	33.51	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	5.381	1.89		
B	3.107	1.09		
AxB	9.172	3.28		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 11. Effect of PPFM isolates on height of the plant at 60 DAT, cm

Treatments (A)	Height of the plant			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	44.07	45.33	50.07	46.49
PPFM 26	48.77	49.27	50.23	49.42
PPFM 35	51.23	52.03	54.60	52.62
PPFM 37	52.20	54.17	56.10	54.16
PPFM 38	57.03	60.03	61.17	59.41
PPFM 47 (TNAU)	50.90	51.57	53.07	51.84
0.5% Methanol	43.43	47.50	52.20	47.71
AMS	46.03	50.83	52.43	49.77
Water	45.67	44.67	51.07	47.13
Mean B	48.82	50.60	53.44	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	5.100	1.79		
B	2.945	1.04		
AxB	8.453	3.11		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 12. Effect of PPFM isolates on height of the plant at 90 DAT, cm

Treatments (A)	Height of the plant			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	71.40	71.63	71.80	71.61
PPFM 26	74.13	71.47	77.43	74.34
PPFM 35	75.07	73.73	79.07	75.96
PPFM 37	77.90	79.30	85.17	80.79
PPFM 38	79.53	80.87	81.50	80.63
PPFM 47 (TNAU)	73.63	71.73	76.40	73.92
0.5% Methanol	67.40	69.40	71.83	69.54
AMS	69.23	72.93	72.73	71.63
Water	65.70	68.30	70.27	68.09
Mean B	72.67	73.26	76.24	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	6.308	2.22		
B	3.678	1.28		
AxB	11.033	3.84		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 13. Effect of PPFM isolates on leaf area index at 30 DAT

Treatments (A)	Leaf area index			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	1.90	2.53	2.65	2.36
PPFM 26	2.34	3.23	3.81	3.13
PPFM 35	3.17	3.01	3.41	3.20
PPFM 37	3.41	3.62	4.01	3.68
PPFM 38	3.68	3.96	3.95	3.86
PPFM 47 (TNAU)	2.38	2.91	3.26	2.85
0.5% Methanol	2.03	2.52	2.36	2.30
AMS	3.13	2.86	2.95	2.98
Water	1.40	1.93	2.03	1.79
Mean B	2.61	2.95	3.16	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.192	0.07		
B	0.111	0.04		
AxB	0.333	0.12		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 14. Effect of PPFM isolates on leaf area index at 60 DAT

Treatments (A)	Leaf area index			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	2.98	3.17	3.26	3.14
PPFM 26	3.08	4.19	4.12	3.80
PPFM 35	3.94	3.99	3.60	3.84
PPFM 37	4.59	4.67	5.02	4.76
PPFM 38	4.79	4.91	4.64	4.78
PPFM 47 (TNAU)	3.52	3.81	4.17	3.83
0.5% Methanol	3.04	3.01	3.10	3.05
AMS	3.36	3.37	3.50	3.41
Water	2.92	2.95	3.04	2.97
Mean B	3.58	3.79	3.83	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.206	0.07		
B	0.119	0.04		
AxB	0.357	0.13		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 15. Effect of PPFM isolates on number of tillers per hill at 60 DAT

Treatments (A)	Number of tillers per hill			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	3.67	3.00	3.67	3.44
PPFM 26	4.00	5.00	4.00	4.33
PPFM 35	4.00	4.00	5.00	4.33
PPFM 37	5.00	6.33	5.33	5.56
PPFM 38	4.67	5.00	6.33	5.33
PPFM 47 (TNAU)	3.67	4.67	4.67	4.33
0.5% Methanol	4.33	4.00	4.00	4.11
AMS	4.00	4.00	4.67	4.22
Water	3.33	3.67	3.67	3.56
Mean B	4.07	4.41	4.59	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	1.191	0.42		
B	NS	0.24		
AxB	NS	0.73		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Interaction effect between moisture levels and PPFM isolates was also not significant with respect to number of tillers per hill at 60 DAT.

4.2.2 Physiological Parameters

4.2.2.1 Leaf Rolling Score

The data on leaf rolling score as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 16 and 17 respectively.

At 30 DAT, among the different PPFM isolates tested, the least leaf rolling score of 3.33 was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.36), PPFM 35 (3.63) and PPFM 47 (3.63). Mean leaf rolling score was maximum with water treated control (4.28).

The effect of different soil moisture levels on leaf rolling score showed that at FC resulted in least mean leaf rolling score (1.23) compared to the leaf rolling score at 75% AW (3.53) and at 50% AW (6.56).

At 60 DAT, among the different PPFM isolates tested, the least leaf rolling score of 4.26 was recorded with PPFM 37 which was statistically on par with PPFM 38 (4.30), PPFM 35 (4.44) and PPFM 47 (4.53). Mean leaf rolling score was maximum with water treated control (5.21).

The effect of different soil moisture levels on leaf rolling score showed that at FC least mean leaf rolling score (1.94) was observed compared to the leaf rolling score at 75% AW (4.82) and at 50% AW (7.26).

No interaction effect was observed between moisture levels and the PPFM isolates in leaf rolling score at 30 and 60 DAT.

4.2.2.2 Leaf Drying Score

The data on leaf drying score as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 18 and Table 19 respectively.

At 30 DAT, among the different PPFM isolates tested, the least leaf drying score of 3.48 was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.49), PPFM 35 (3.83), PPFM 47 (3.76) and PPFM 26 (3.77). Mean leaf drying score was maximum with water treated control (4.40).

The effect of different soil moisture levels on leaf drying score showed that mean leaf drying score of 3.24 was least at FC compared to the leaf drying score at 75% AW (3.59) and at 50% AW (4.82).

At 60 DAT, among the different PPFM isolates tested, the least leaf drying score of 2.80 was recorded with PPFM 37 and PPFM 38 which was statistically on par with PPFM 35 (3.08) and PPFM 47 (3.10). Mean leaf drying score was maximum with water treated control (3.62).

The effect of different soil moisture levels on leaf drying score showed that mean leaf drying score of 1.89 was least at FC compared to the leaf drying score at 75% AW (3.25) and at 50% AW (4.39).

No interaction effect could be observed between moisture levels and the PPFM isolates in leaf drying score at 30 and 60 DAT.

4.2.2.3 Leaf Temperature

The data on leaf temperature as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 20 and Table 21 respectively.

At 30 DAT among the different PPFM isolates tested, the least leaf temperature of 27.33 °C was recorded with PPFM 38 which was statistically on

Table 16. Effect of PPFM isolates on Leaf rolling score at 30 DAT

Treatments (A)	Leaf rolling score			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	1.40	3.67	6.60	3.89
PPFM 26	1.33	3.47	6.53	3.78
PPFM 35	1.20	3.30	6.40	3.63
PPFM 37	0.83	2.97	6.20	3.33
PPFM 38	0.93	3.13	6.00	3.36
PPFM 47 (TNAU)	1.03	3.40	6.47	3.63
0.5% Methanol	1.50	3.93	6.93	4.12
AMS	1.17	3.80	6.80	3.92
Water	1.63	4.13	7.07	4.28
Mean B	1.23	3.53	6.56	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.332	0.12		
B	0.192	0.07		
AxB	NS	0.20		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 17. Effect of PPFM isolates on Leaf rolling score at 60 DAT

Treatments (A)	Leaf rolling score			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	2.03	5.07	7.40	4.83
PPFM 26	1.93	4.80	7.20	4.64
PPFM 35	1.80	4.60	6.93	4.44
PPFM 37	1.63	4.33	6.80	4.26
PPFM 38	1.77	4.47	6.67	4.30
PPFM 47 (TNAU)	1.87	4.67	7.07	4.53
0.5% Methanol	2.17	5.20	7.73	5.03
AMS	1.97	4.87	7.60	4.81
Water	2.30	5.40	7.93	5.21
Mean B	1.94	4.82	7.26	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.366	0.13		
B	0.212	0.07		
AxB	NS	0.22		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 18. Effect of PPFM isolates on leaf drying score at 30 DAT

Treatments (A)	Leaf drying score			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	3.43	3.67	4.93	4.01
PPFM 26	3.17	3.53	4.60	3.77
PPFM 35	3.30	3.47	4.73	3.83
PPFM 37	2.83	3.27	4.33	3.48
PPFM 38	2.60	3.33	4.53	3.49
PPFM 47 (TNAU)	3.13	3.47	4.67	3.76
0.5% Methanol	3.53	3.80	5.20	4.18
AMS	3.47	3.73	4.87	4.02
Water	3.67	4.07	5.47	4.40
Mean B	3.24	3.59	4.82	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.369	0.13		
B	0.213	0.08		
AxB	NS	0.23		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 19. Effect of PPFM isolates on leaf drying score at 60 DAT

Treatments (A)	Leaf drying score			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	1.93	3.33	4.53	3.27
PPFM 26	1.83	3.20	4.40	3.14
PPFM 35	1.77	3.27	4.20	3.08
PPFM 37	1.67	3.07	3.67	2.80
PPFM 38	1.60	2.93	3.87	2.80
PPFM 47 (TNAU)	1.83	3.13	4.33	3.10
0.5% Methanol	2.13	3.40	4.80	3.44
AMS	2.00	3.37	4.67	3.34
Water	2.27	3.53	5.07	3.62
Mean B	1.89	3.25	4.39	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.308	0.11		
B	0.178	0.06		
AxB	NS	0.19		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 20. Effect of PPFM isolates on leaf temperature at 30 DAT, °C

Treatments (A)	Leaf temperature			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	27.63	28.03	29.80	28.49
PPFM 26	27.37	28.07	29.40	28.28
PPFM 35	27.17	27.43	28.63	27.74
PPFM 37	27.03	27.37	28.53	27.64
PPFM 38	26.73	27.07	28.20	27.33
PPFM 47 (TNAU)	27.30	27.73	28.90	27.98
0.5% Methanol	27.93	28.37	30.23	28.84
AMS	27.77	28.27	29.77	28.60
Water	28.07	28.83	30.50	29.13
Mean B	27.44	27.91	29.33	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.640	0.23		
B	0.370	0.13		
AxB	NS	0.39		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 21. Effect of PPFM isolates on Leaf temperature at 60 DAT, °C

Treatments (A)	Leaf temperature			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	31.80	32.27	32.57	32.21
PPFM 26	31.57	31.80	31.93	31.77
PPFM 35	31.47	31.77	31.83	31.69
PPFM 37	30.37	31.03	31.23	30.88
PPFM 38	30.20	30.80	30.93	30.64
PPFM 47 (TNAU)	31.60	31.90	32.13	31.88
0.5% Methanol	32.57	32.63	32.93	32.71
AMS	31.73	32.10	32.47	32.10
Water	32.40	32.53	32.67	32.53
Mean B	31.52	31.87	32.08	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	1.170	0.41		
B	0.680	0.24		
AxB	NS	0.71		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

par with PPFM 37 (27.64 °C) and PPFM 35 (27.74 °C). Mean leaf temperature was maximum with water treated control (29.13 °C).

The effect of different soil moisture levels on leaf temperature showed that mean leaf temperature of 27.44 °C was least at FC compared to that at 75% AW (27.91 °C) and at 50% AW (29.33 °C).

At 60 DAT, among the different PPFM isolates tested, the least leaf temperature of 30.64 °C was recorded with PPFM 38 which was statistically on par with PPFM 37 (30.88 °C) PPFM 35 (31.69 °C) and PPFM 26 (31.77 °C). Mean leaf temperature was maximum with 0.5% methanol treated control (32.71 °C).

The effect of different soil moisture levels on leaf temperature showed that mean leaf temperature of 31.52 °C was least at FC and it was statistically on par with leaf temperature at 75% AW (31.87 °C) and at 50% AW (32.08 °C).

No interaction effect was noticed between moisture levels and the PPFM isolates for leaf temperature at 30 and 60 DAT.

4.2.2.4 Cell Membrane Integrity

The data on cell membrane integrity (CMI) as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 22 and 23 respectively.

Among the different PPFM isolates tested, the highest mean CMI of 89.10 % was recorded with PPFM 38, which was statistically on par with PPFM 37 (88.46 %), PPFM 35 (88.04 %), PPFM 26 (86.50 %), PPFM 15 (86.15 %), PPFM 47 (87.49 %) and AMS media (86.18 %) at 30 DAT. Mean CMI was the least with water treated control (82.37 %).

The effect of different soil moisture levels on CMI showed significantly higher values for mean CMI (91.19 %) at 75% AW compared to the CMI at 50% AW (81.79 %).

The interaction effect between moisture levels and the PPFM isolates revealed that PPFM 38 showed significantly higher CMI at all the moisture levels. The results pointed out that at 75% AW, the highest CMI of 93.12 % was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (92.69 %), PPFM 35 (92.25 %), PPFM 26 (91.85 %), PPFM 15 (91.43 %), PPFM 47 (91.91 %), 0.5% methanol (89.12 %) and AMS media (91.01 %). The lowest CMI was recorded with water (87.34 %) at 75% AW. The results also indicated that at 50% AW, the maximum CMI of 85.07 % was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (84.23 %), PPFM 35 (83.83 %), PPFM 26 (81.14 %), PPFM 15 (80.87 %), PPFM 47 (83.06 %) and AMS media (81.35 %). The lowest CMI was registered with water (77.40 %) at 50% AW.

At 60 DAT, among the different PPFM isolates tested, the highest mean CMI of 84.52 % was recorded with PPFM 38 which was statistically on par with PPFM 37 (82.82 %). Mean CMI was the least with water treated control (71.55 %).

The effect of different soil moisture levels on CMI showed significantly higher values for mean CMI (81.84 %) at 75% AW compared to the CMI at 50% AW (74.59 %).

The interaction effect between moisture levels and the PPFM isolates revealed that PPFM 38 showed significantly higher CMI at all the moisture levels. The results showed that at 75% AW, the maximum CMI of 87.97 % was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (86.44 %) and PPFM 35 (84.40 %). The lowest CMI was recorded with water treated control (74.97 %) at 75% AW. The results revealed that at 50% AW, the maximum CMI of 81.07 % was recorded with isolate PPFM 38 which was statistically on par with

PPFM 37 (79.21 %) and PPFM 35 (78.27 %). The lowest CMI was recorded with water treated control (68.12 %) at 50% AW.

4.2.2.5 Relative Water Content

The data on relative water content (RWC) as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 24 and 25 respectively.

Among the different PPFM isolates tested, the highest mean RWC of 76.84 % was recorded with PPFM 38 which was statistically on par with PPFM 37 (75.91 %), PPFM 35 (75.57 %), PPFM 47 (74.67 %) and PPFM 26 (74.34 %) at 30 DAT. Mean RWC was the least with water treated control (71.56 %).

The effect of different soil moisture levels on RWC showed significantly higher values for mean RWC of 86.80 % at FC compared to at 75% AW (72.26 %) and at 50% AW (63.09 %).

At 60 DAT, among the different PPFM isolates tested, highest mean RWC of 67.26 % was recorded with PPFM 38 which was statistically on par with PPFM.37 (66.33 %), PPFM 35 (65.57 %) and PPFM 47 (64.84 %). Mean RWC was the least with water treated control (61.18 %).

The effect of different soil moisture levels on RWC showed that FC resulted in significantly higher values for mean RWC (76.37 %) compared to that at 75% AW (63.95 %) and at 50% AW (52.25 %).

No interaction effect was observed between moisture levels and the PPFM isolates for RWC at 30 and 60 DAT.

4.2.2.6 Chlorophyll Stability Index

The data on chlorophyll stability index (CSI) as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 26 and 27 respectively.

Among the different PPFM isolates tested, the highest mean CSI of 88.54 % was recorded with PPFM 38 which was statistically on par with PPFM 37 (87.37 %) and PPFM 35 (85.70 %) at 30 DAT. Mean CSI was the least with water treated control (75.89 %).

The effect of different soil moisture levels on CSI showed significantly higher values for mean CSI of 86.72 % was recorded at 75% AW compared to the CSI at 50% AW (78.31 %).

At 60 DAT, among the different PPFM isolates tested, the highest mean CSI of 84.54 % was recorded with PPFM 38 which was statistically on par with PPFM 37 (83.32 %), PPFM 35 (81.80 %), PPFM 47 (80.65 %) and AMS media (81.82 %). Mean CSI was the least with water treated control (74.62 %).

The effect of different soil moisture levels on CSI showed significantly higher values for mean CSI of 82.73 % was recorded at 75% AW compared to the CSI at 50% AW (77.59 %).

No interaction effect was noticed between moisture levels and the PPFM isolates for CSI at 30 and 60 DAT.

4.2.2.7 Rooting Depth

The data on rooting depth as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 28 and 29 respectively.

Among the different PPFM isolates tested, the highest mean rooting depth of 10.74 cm was recorded with PPFM 38 which was statistically on par with

Table 22. Effect of PPFM isolates on cell membrane integrity at 30 DAT, %

Treatments (A)	Cell membrane integrity		
	Moisture levels (B)		Mean A
	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	91.43	80.87	86.15
PPFM 26	91.85	81.14	86.50
PPFM 35	92.25	83.83	88.04
PPFM 37	92.69	84.23	88.46
PPFM 38	93.12	85.07	89.10
PPFM 47 (TNAU)	91.91	83.06	87.49
0.5% Methanol	89.12	79.17	84.15
AMS	91.01	81.35	86.18
Water	87.34	77.40	82.37
Mean B	91.19	81.79	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	3.211	1.12	
B	1.514	0.53	
AxB	4.610	1.58	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 23. Effect of PPFM isolates on cell membrane integrity at 60 DAT, %

Treatments (A)	Cell membrane integrity		
	Moisture levels (B)		Mean A
	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	80.66	72.52	76.59
PPFM 26	82.76	75.59	79.18
PPFM 35	84.40	78.27	81.34
PPFM 37	86.44	79.21	82.82
PPFM 38	87.97	81.07	84.52
PPFM 47 (TNAU)	82.05	74.80	78.43
0.5% Methanol	77.41	70.07	73.74
AMS	79.90	71.68	75.79
Water	74.97	68.12	71.55
Mean B	81.84	74.59	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	3.043	1.06	
B	1.435	0.50	
AxB	4.251	1.50	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 24. Effect of PPFM isolates on relative water content at 30 DAT, %

Treatments (A)	Relative water content			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	86.24	70.75	61.65	72.88
PPFM 26	86.77	72.59	63.65	74.34
PPFM 35	87.59	74.83	64.28	75.57
PPFM 37	87.84	75.07	64.83	75.91
PPFM 38	88.76	75.51	66.26	76.84
PPFM 47 (TNAU)	86.97	73.02	64.02	74.67
0.5% Methanol	85.86	69.66	61.08	72.20
AMS	86.04	70.11	61.32	72.49
Water	85.16	68.80	60.72	71.56
Mean B	86.80	72.26	63.09	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	3.090	1.09		
B	1.790	0.63		
AxB	NS	1.88		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 25. Effect of PPFM isolates on relative water content at 60 DAT, %

Treatments (A)	Relative water content			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	74.85	64.14	51.47	63.49
PPFM 26	75.39	64.29	51.89	63.86
PPFM 35	78.22	65.43	53.06	65.57
PPFM 37	78.48	66.85	53.67	66.33
PPFM 38	79.96	66.50	55.32	67.26
PPFM 47 (TNAU)	77.54	64.72	52.27	64.84
0.5% Methanol	74.55	61.21	51.15	62.30
AMS	74.67	62.19	51.73	62.86
Water	73.67	60.20	49.65	61.18
Mean B	76.37	63.95	52.25	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	3.290	1.16		
B	1.900	0.67		
AxB	NS	2.00		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 26. Effect of PPFM isolates on chlorophyll stability index at 30 DAT, %

Treatments (A)	Chlorophyll stability index		
	Moisture levels (B)		Mean A
	At 75% AW (B1)	At 50% AW (B2)	
PPFM 15	85.05	76.69	80.87
PPFM 26	86.17	77.18	81.67
PPFM 35	90.58	80.82	85.70
PPFM 37	91.03	83.71	87.37
PPFM 38	92.69	84.38	88.54
PPFM 47 (TNAU)	88.68	78.23	83.46
0.5% Methanol	82.00	74.15	78.08
AMS	85.10	77.06	81.08
Water	79.18	72.59	75.89
Mean B	86.72	78.31	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	4.440	1.54	
B	2.093	0.73	
AxB	NS	2.18	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 27. Effect of PPFM isolates on chlorophyll stability index at 60 DAT, %

Treatments (A)	Chlorophyll stability index		
	Moisture levels (B)		Mean A
	At 75% AW (B1)	At 50% AW (B2)	
PPFM 15	81.09	75.67	78.38
PPFM 26	81.25	77.46	79.36
PPFM 35	84.43	79.17	81.80
PPFM 37	85.95	80.69	83.32
PPFM 38	86.88	82.20	84.54
PPFM 47 (TNAU)	82.57	78.73	80.65
0.5% Methanol	80.04	73.89	76.96
AMS	84.81	78.83	81.82
Water	77.53	71.70	74.62
Mean B	82.73	77.59	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	4.301	1.49	
B	2.027	0.70	
AxB	NS	2.11	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 28. Effect of PPFM isolates on rooting depth at 30 DAT, cm

Treatments (A)	Rooting depth			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	4.57	6.53	6.47	5.86
PPFM 26	6.23	5.03	4.93	5.40
PPFM 35	5.27	7.07	8.43	6.92
PPFM 37	10.67	6.93	13.17	10.26
PPFM 38	9.13	10.67	12.43	10.74
PPFM 47 (TNAU)	6.63	6.17	9.10	7.30
0.5% Methanol	3.67	5.80	5.70	5.06
AMS	10.40	6.43	8.23	8.36
Water	6.23	4.33	4.83	5.13
Mean B	6.98	6.55	8.14	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	1.704	0.60		
B	0.984	0.35		
AxB	2.951	1.04		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 29. Effect of PPFM isolates on rooting depth at 60 DAT, cm

Treatments (A)	Rooting depth			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	13.03	16.37	16.47	15.29
PPFM 26	13.57	17.97	19.07	16.87
PPFM 35	14.47	19.07	20.03	17.86
PPFM 37	18.23	22.27	22.10	20.87
PPFM 38	17.73	17.53	24.60	19.96
PPFM 47 (TNAU)	13.93	17.80	18.53	16.76
0.5% Methanol	14.53	13.90	14.30	14.24
AMS	14.40	17.20	16.50	16.03
Water	12.63	12.03	12.43	12.37
Mean B	14.73	17.13	18.23	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	3.334	0.82		
B	1.348	0.47		
AxB	3.391	1.42		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

PPFM 37 (10.26 cm) at 30 DAT. Mean rooting depth was the lowest with 0.5% methanol treated control (5.06 cm).

The effect of different soil moisture levels on rooting depth showed that at 50% AW significantly higher values for mean rooting depth (8.14 cm) was obtained compared to that at 75% AW (6.55 cm) and at FC (6.98 cm).

The interaction effect between moisture levels and the PPFM isolates on rooting depth was significant. At FC, maximum rooting depth of 10.67 cm was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (9.13 cm) and AMS media (10.40 cm). The lowest rooting depth was recorded with 0.5% methanol treated control (3.67 cm). At 75% AW, the maximum rooting depth of 10.67 cm was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest rooting depth was recorded with water treated control (4.33 cm). At 50% AW, the maximum rooting depth of 13.17 cm was recorded with PPFM 37 which was statistically on par with PPFM 38 (12.43 cm). The lowest rooting depth was recorded with water treated control (4.83 cm).

At 60 DAT, among the different PPFM isolates tested, the highest mean rooting depth of 20.87 cm was recorded with PPFM 37 which was statistically on par with PPFM 38 (19.96 cm) and PPFM 35 (17.86 cm). Mean rooting depth was the lowest with water treated control (12.37 cm).

The effect of different soil moisture levels on rooting depth showed that mean rooting depth significantly higher (18.23 cm) at 50% AW was statistically on par 75% AW (17.13 cm) and lowest at FC (14.73 cm).

The interaction effect between moisture levels and the PPFM isolates on rooting depth was significant. At FC, maximum rooting depth of 18.23 cm was recorded with isolate PPFM 37 (Plate 16) which was statistically on par with PPFM 38 (17.73 cm). The lowest rooting depth was recorded with water treated control (12.63 cm). At 75% AW, the maximum rooting depth of 22.27 cm was

Plate 16. Root of PPFM 37 treated plants (at FC) at 60 DAT compared to water control

Plate 17. Root of PPFM 37 treated plants (75% AW) at 60 DAT compared to water control

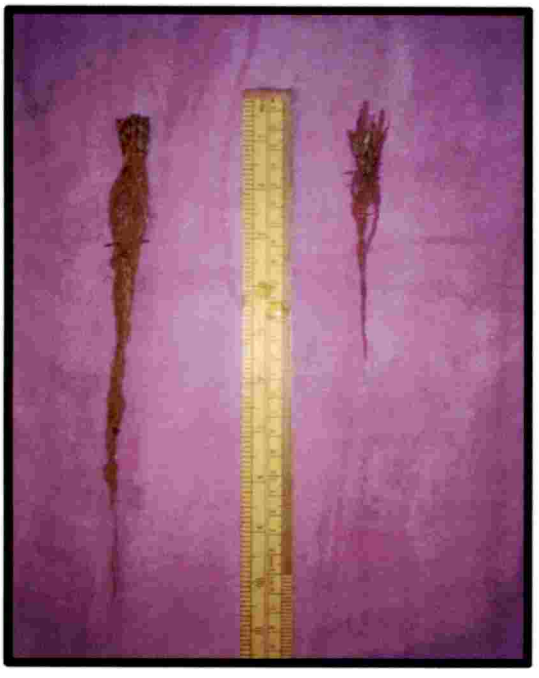

Plate 18. Root of PPFM 38 treated plants (50% AW) at 60 DAT compared to water control

Plate 19. Root of PPFM 37 treated plants (50% AW) at 60 DAT compared to water control

recorded with PPFM 37 (Plate 17) which was statistically on par with PPFM 35 (19.07 cm). The lowest rooting depth was recorded with water treated control (12.03 cm). At 50% AW, the maximum rooting depth of 24.60 cm was recorded with PPFM 38 (Plate 18) which was statistically on par with PPFM 37 (22.10 cm) (Plate 19). The lowest rooting depth was recorded with water treated control (12.43 cm).

4.2.2.8 Root Weight

The data on root weight as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 30 and 31 respectively.

Among the different PPFM isolates tested, the highest mean root weight of 0.223 g was recorded with PPFM 38. Mean root weight was the least with 0.5% methanol (0.066 g).

The effect of different soil moisture levels on root weight showed that at 50% AW significantly higher values for mean root weight (0.133 g) was recorded compared to that at 75% AW (0.102 g) and at FC (0.109 g).

The interaction effect between moisture levels and the PPFM isolates revealed that significantly higher root weight was observed in PPFM 38 treated plants at all the three moisture levels. It was also pointed out that at FC, maximum root weight of 0.189 g was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (0.175 g). The lowest root weight was recorded with 0.5% methanol (0.054 g). At 75% AW, the maximum root weight of 0.221 g was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest root weight was recorded with water treated control (0.055 g). At 50% AW, the maximum root weight of 0.273 g was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest root weight was recorded with 0.5% methanol treated control (0.065 g).

At 60 DAT, among the different PPFM isolates tested, the highest mean root weight of 4.03 g was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.68 g). Mean root weight was the lowest with 0.5 % methanol (1.35 g).

No significant effect was observed for different soil moisture levels on root weight at 60 DAT.

The interaction effect between moisture levels and the PPFM isolates on root weight was significant. The results revealed that at FC, maximum root weight of 3.54 g was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (2.92 g). The lowest root weight was recorded with water treated control (1.46 g). At 75% AW, the maximum root weight of 5.03 g was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest root weight was recorded with 0.5% methanol (1.20 g). At 50% AW, the maximum root weight of 4.20 g was recorded with PPFM 38 which was statistically on par with PPFM 37 (4.15 g). The lowest root weight was recorded with 0.5% methanol treated control (1.32 g).

4.2.2.9 Root Volume

The data on root volume as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 32 and 33 respectively.

At 30 DAT, among the different PPFM isolates tested, the highest mean root volume of 0.811 cm^3 was recorded with PPFM 38. Mean root volume was the least with water treated control (0.133 cm^3).

The effect of different soil moisture levels on root volume showed that at 50% AW significantly higher values were observed for mean root volume (0.441 cm^3) compared to the root volume at 75% AW (0.341 cm^3) and at FC (0.263 cm^3).

The interaction effect between moisture levels and PPFM isolates on root volume at 30 DAT was significant. The results revealed that at FC, the maximum root volume of 0.533 cm^3 was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (0.433 cm^3). The lowest root volume was recorded with water treated control (0.133 cm^3). At 75% AW, the maximum root volume of 0.933 cm^3 was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest root volume was recorded with water treated control (0.133 cm^3). At 50% AW, the maximum root volume of 1.067 cm^3 was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest root volume was recorded with water (0.133 cm^3).

At 60 DAT, among the different PPFM isolates tested, highest mean root volume of 5.31 cm^3 was recorded with PPFM 37 which was significantly higher compared to all other treatments. Mean root volume was the lowest with water treated control (2.04 cm^3).

No significant effect was observed for the different soil moisture levels on root volume at 60 DAT.

The interaction effect between moisture levels and PPFM isolates was non-significant for root volume at 60 DAT.

4.2.2.10 Shoot Dry Weight

The data on shoot dry weight as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 34 and 35 respectively.

Among the different PPFM isolates tested, the highest mean shoot dry weight of 0.669 g was recorded with PPFM 38 which was statistically on par with PPFM 37 (0.628 g) at 30 DAT. Mean shoot dry weight was the lowest with water treated control (0.340 g).

The effect of different soil moisture levels on shoot dry weight showed that at 50% AW, significantly higher values for mean shoot dry weight (0.507 g)

was observed compared to the shoot dry weight at 75% AW (0.464 g) and at FC (0.425 g).

The interaction effect between moisture levels and the PPFM isolates on shoot dry weight was found to be significant. The results revealed that at FC, the maximum shoot dry weight of 0.610 g was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (0.563 g) and PPFM 26 (0.567 g). The lowest shoot dry weight was recorded with 0.5% methanol treated control (0.247 g). At 75% AW, the maximum shoot dry weight of 0.640 g was recorded with PPFM 38 which was statistically on par with PPFM 37 (0.523 g). The lowest root volume was recorded with water (0.327 g). At 50% AW, the maximum shoot dry weight of 0.797 g was recorded with PPFM 37 which was statistically on par with PPFM 38 (0.757 g). The lowest shoot dry weight was recorded with PPFM 15 (0.320 g).

At 60 DAT, among the different PPFM isolates tested, highest mean shoot dry weight of 1.58 g was recorded with PPFM 37 and PPFM 38 which was significantly higher as compared to all other treatments. Mean shoot dry weight was the lowest with water (1.22 g).

No significant effect of different soil moisture levels was observed on shoot dry weight at 60 DAT.

No interaction effect was noticed between moisture levels and the PPFM isolates for shoot dry weight at 60 DAT.

4.2.2.11 Root Dry Weight

The data on root dry weight as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 36 and 37 respectively.

Among the different PPFM isolates tested, the highest mean root dry weight of 0.029 g was recorded with PPFM 38 which was statistically on par with

Table 30. Effect of PPFM isolates on root weight at 30 DAT, g

Treatments (A)	Root weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.079	0.095	0.097	0.091
PPFM 26	0.113	0.075	0.084	0.091
PPFM 35	0.093	0.115	0.164	0.124
PPFM 37	0.189	0.117	0.221	0.176
PPFM 38	0.175	0.221	0.273	0.223
PPFM 47 (TNAU)	0.095	0.081	0.114	0.097
0.5% Methanol	0.054	0.081	0.065	0.066
AMS	0.104	0.078	0.091	0.091
Water	0.080	0.055	0.090	0.075
Mean B	0.109	0.102	0.133	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.023	0.01		
B	0.013	0.01		
AxB	0.040	0.01		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 31. Effect of PPFM isolates on root weight at 60 DAT, g

Treatments (A)	Root weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	2.18	2.54	1.96	2.23
PPFM 26	1.69	1.55	1.34	1.53
PPFM 35	2.10	2.52	2.04	2.22
PPFM 37	2.92	5.03	4.15	4.03
PPFM 38	3.54	3.31	4.20	3.68
PPFM 47 (TNAU)	1.75	1.70	1.64	1.70
0.5% Methanol	1.54	1.20	1.32	1.35
AMS	2.05	1.92	1.56	1.84
Water	1.46	1.47	1.42	1.45
Mean B	2.14	2.36	2.18	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.381	0.13		
B	NS	0.08		
AxB	0.661	0.23		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 32. Effect of PPFM isolates on root volume at 30 DAT, cm^3

Treatments (A)	Root volume			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.167	0.267	0.267	0.233
PPFM 26	0.267	0.200	0.233	0.233
PPFM 35	0.233	0.367	0.500	0.367
PPFM 37	0.533	0.400	0.767	0.567
PPFM 38	0.433	0.933	1.067	0.811
PPFM 47 (TNAU)	0.267	0.333	0.333	0.311
0.5% Methanol	0.100	0.233	0.233	0.189
AMS	0.233	0.200	0.433	0.289
Water	0.133	0.133	0.133	0.133
Mean B	0.263	0.341	0.441	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.142	0.05		
B	0.082	0.03		
AxB	0.245	0.09		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 33. Effect of PPFM isolates on root volume at 60 DAT, cm^3

Treatments (A)	Root volume			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	2.73	3.07	2.87	2.89
PPFM 26	2.43	2.97	3.03	2.81
PPFM 35	3.13	3.77	3.57	3.49
PPFM 37	4.17	6.23	5.53	5.31
PPFM 38	4.37	4.07	4.93	4.46
PPFM 47 (TNAU)	2.83	3.13	3.10	3.02
0.5% Methanol	2.20	2.20	2.43	2.28
AMS	2.57	3.03	2.77	2.79
Water	1.97	1.90	2.27	2.04
Mean B	2.93	3.37	3.39	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.784	0.28		
B	NS	0.16		
AxB	NS	0.48		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 34. Effect of PPFM isolates on shoot dry weight at 30 DAT, g

Treatments (A)	Shoot dry weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.343	0.480	0.320	0.381
PPFM 26	0.567	0.383	0.330	0.427
PPFM 35	0.367	0.513	0.570	0.483
PPFM 37	0.563	0.523	0.797	0.628
PPFM 38	0.610	0.640	0.757	0.669
PPFM 47 (TNAU)	0.390	0.480	0.670	0.513
0.5% Methanol	0.247	0.450	0.340	0.346
AMS	0.410	0.377	0.410	0.399
Water	0.327	0.327	0.367	0.340
Mean B	0.425	0.464	0.507	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.070	0.03		
B	0.040	0.01		
AxB	0.121	0.04		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 35. Effect of PPFM isolates on shoot dry weight at 60 DAT, g

Treatments (A)	Shoot dry weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	1.44	1.36	1.31	1.37
PPFM 26	1.50	1.34	1.42	1.42
PPFM 35	1.40	1.48	1.40	1.43
PPFM 37	1.47	1.57	1.69	1.58
PPFM 38	1.53	1.58	1.64	1.58
PPFM 47 (TNAU)	1.50	1.45	1.47	1.47
0.5% Methanol	1.35	1.24	1.26	1.28
AMS	1.31	1.36	1.35	1.34
Water	1.14	1.20	1.32	1.22
Mean B	1.41	1.40	1.43	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.088	0.03		
B	NS	0.02		
AxB	NS	0.05		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 36. Effect of PPFM isolates on root dry weight at 30 DAT, g

Treatments (A)	Root dry weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.012	0.017	0.012	0.013
PPFM 26	0.021	0.015	0.014	0.017
PPFM 35	0.015	0.024	0.028	0.023
PPFM 37	0.023	0.023	0.036	0.027
PPFM 38	0.022	0.028	0.037	0.029
PPFM 47 (TNAU)	0.014	0.020	0.033	0.022
0.5% Methanol	0.007	0.015	0.012	0.011
AMS	0.014	0.013	0.016	0.015
Water	0.011	0.012	0.013	0.012
Mean B	0.016	0.019	0.022	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.004	0.001		
B	0.002	0.001		
AxB	0.007	0.002		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 37. Effect of PPFM isolates on root dry weight at 60 DAT, g

Treatments (A)	Root dry weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.523	0.52	0.515	0.519
PPFM 26	0.443	0.409	0.452	0.435
PPFM 35	0.513	0.561	0.568	0.547
PPFM 37	0.611	0.67	0.772	0.684
PPFM 38	0.74	0.796	0.859	0.798
PPFM 47 (TNAU)	0.475	0.48	0.522	0.492
0.5% Methanol	0.402	0.399	0.422	0.408
AMS	0.437	0.479	0.498	0.471
Water	0.374	0.409	0.457	0.413
Mean B	0.502	0.525	0.563	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.046	0.02		
B	0.026	0.01		
AxB	NS	0.03		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

PPFM 37 (0.027 g) at 30 DAT. Mean root dry weight was the lowest with 0.5% methanol treated control (0.011 g).

The effect of different soil moisture levels on root dry weight showed that at 50% AW significantly higher values for mean root dry weight (0.022 g) was observed compared to the root dry weight at 75% AW (0.019 g) and at FC (0.016 g).

Statistically significant interaction effect between moisture levels and the PPFM isolates on root dry weight was noticed. At FC, maximum root dry weight of 0.023 g was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (0.022 g) and PPFM 26 (0.021 g). The lowest root dry weight was recorded with 0.5% methanol treated control (0.007 g). At 75% AW, the maximum root dry weight of 0.028 g was recorded with PPFM 38 which was statistically on par with PPFM 37 (0.023 g) and PPFM 35 (0.024 g). The lowest root dry weight was recorded with water (0.012 g). At 50% AW, the maximum root dry weight of 0.037 g was recorded with PPFM 38 which was statistically on par with PPFM 37 (0.036 g). The lowest root dry weight was recorded with 0.5% methanol treated control (0.012 g).

At 60 DAT, among the different PPFM isolates tested, the highest mean root dry weight of 0.798 g was recorded with PPFM 38 which was significantly higher as compared to all other treatments. Mean root dry weight was the lowest with 0.5% methanol treated control (0.408 g).

The effect of different soil moisture levels on root dry weight showed significantly higher values for mean root dry weight (0.563 g) at 50% AW compared to the root dry weight at 75% AW (0.525 g) and at FC (0.502 g).

No interaction effect could be noticed between moisture levels and the PPFM isolates for root dry weight at 60 DAT.

4.2.2.12 Root Shoot Ratio

The data on root shoot ratio as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 38 and 39 respectively.

At 30 DAT, no significant variation in root shoot ratio was observed due to PPFM isolates and moisture levels.

At 60 DAT, among the different PPFM isolates tested, the highest mean root shoot ratio of 0.506 was recorded with PPFM 38 and it was significantly higher compared to all other treatments. Mean root shoot ratio was the least with PPFM 26 (0.307).

The effect of different soil moisture levels on root shoot ratio showed that at 50% AW significantly higher value was recorded for mean root shoot ratio (0.390) compared to the root shoot ratio at 75% AW (0.372) and at FC (0.357).

The interaction effect between moisture levels and the PPFM isolates for root shoot ratio at 30 and 60 DAT was non-significant.

4.2.2.13 Soil Moisture Percentage

The data on soil moisture percentage as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 40 and 41 respectively.

No significant effect was observed on the mean soil moisture percentage at 30 DAT and 60 DAT, due to the PPFM isolates.

The effect of different soil moisture levels at 30 DAT on soil moisture percentage showed, significantly higher values for mean soil moisture percentage (33.33 %) at FC compared to that at 75% AW (27.36 %) and at 50% AW (21.15 %).

The effect of different soil moisture levels at 60 DAT on soil moisture percentage showed that at FC, significantly higher values for mean soil moisture percentage (32.57 %) was noticed compared to that at 75% AW (26.64 %) and at 50% AW (20.62 %).

No interaction effect between moisture levels and the PPFM isolates for soil moisture percentage at 30 DAT and 60 DAT was noticed.

4.2.2.14 Drought Susceptibility Index

The data on drought susceptibility index (DSI) as influenced by PPFM isolates and moisture levels are presented in Table 42.

Among the different PPFM isolates tested, the least mean DSI of 0.78 was recorded with PPFM 37 which was statistically on par with PPFM 38 (0.82), PPFM 35 (0.87), PPFM 26 (0.98), PPFM 15 (1.08) and PPFM 47 (0.94). Mean DSI was the highest with water treated control (1.34).

The effect of different soil moisture levels on DSI showed that at 75% AW significantly lower values for mean DSI (1.00) was obtained compared to the DSI at 50% AW (1.02).

No interaction effect was observed between moisture levels and the PPFM isolates for DSI.

4.2.2.15 Proline Content

The data on proline content as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 43 and 44 respectively.

Among the different PPFM isolates tested, the highest mean proline content of 88.16 µg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other treatments at 30 DAT. Mean proline content was the least with AMS media treated control (17.63 µg g^{-1} tissue).

Table 38. Effect of PPFM isolates on root shoot ratio at 30 DAT

Treatments (A)	Root shoot ratio			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.034	0.036	0.037	0.035
PPFM 26	0.038	0.041	0.042	0.04
PPFM 35	0.041	0.048	0.050	0.046
PPFM 37	0.042	0.043	0.045	0.044
PPFM 38	0.038	0.044	0.049	0.044
PPFM 47 (TNAU)	0.036	0.042	0.049	0.042
0.5% Methanol	0.033	0.035	0.036	0.034
AMS	0.035	0.036	0.040	0.037
Water	0.036	0.037	0.038	0.037
Mean B	0.037	0.040	0.043	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.003		
B	NS	0.002		
AxB	NS	0.006		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 39. Effect of PPFM isolates on root shoot ratio at 60 DAT

Treatments (A)	Root shoot ratio			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.363	0.384	0.393	0.380
PPFM 26	0.296	0.307	0.319	0.307
PPFM 35	0.369	0.382	0.405	0.385
PPFM 37	0.415	0.428	0.457	0.434
PPFM 38	0.487	0.506	0.525	0.506
PPFM 47 (TNAU)	0.319	0.332	0.356	0.336
0.5% Methanol	0.301	0.321	0.336	0.319
AMS	0.335	0.352	0.368	0.352
Water	0.327	0.338	0.347	0.337
Mean B	0.357	0.372	0.390	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.041	0.01		
B	0.024	0.01		
AxB	NS	0.03		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 40. Effect of PPFM isolates on soil moisture percentage at 30 DAT, %

Treatments (A)	Soil moisture percentage			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	33.42	27.50	21.08	27.33
PPFM 26	32.98	27.71	21.23	27.31
PPFM 35	33.27	27.02	21.22	27.17
PPFM 37	33.13	27.25	21.04	27.14
PPFM 38	32.83	26.84	20.94	26.87
PPFM 47 (TNAU)	33.49	27.51	21.05	27.35
0.5% Methanol	33.62	27.62	21.57	27.61
AMS	33.56	26.98	21.16	27.24
Water	33.64	27.78	21.08	27.50
Mean B	33.33	27.36	21.15	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.18		
B	0.290	0.10		
AxB	NS	0.30		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 41. Effect of PPFM isolates on soil moisture percentage at 60 DAT, %

Treatments (A)	Soil moisture percentage			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	32.72	26.60	20.65	26.65
PPFM 26	32.42	26.74	20.49	26.55
PPFM 35	32.59	26.68	20.41	26.56
PPFM 37	32.40	26.54	20.58	26.51
PPFM 38	32.44	26.31	20.63	26.46
PPFM 47 (TNAU)	32.65	26.83	20.79	26.76
0.5% Methanol	32.52	26.46	20.57	26.52
AMS	32.76	26.85	20.76	26.79
Water	32.61	26.79	20.70	26.70
Mean B	32.57	26.64	20.62	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.13		
B	0.210	0.07		
AxB	NS	0.22		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 42. Effect of PPFM isolates on drought susceptibility index

Treatments (A)	Drought susceptibility index		
	Moisture levels (B)		Mean A
	At 75% AW (B1)	At 50% AW (B2)	
PPFM 15	1.04	1.11	1.08
PPFM 26	1.01	0.95	0.98
PPFM 35	0.89	0.84	0.87
PPFM 37	0.79	0.77	0.78
PPFM 38	0.83	0.82	0.82
PPFM 47 (TNAU)	0.96	0.91	0.94
0.5% Methanol	1.10	1.22	1.16
AMS	1.09	1.18	1.13
Water	1.27	1.40	1.34
Mean B	1.00	1.02	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	0.336	0.12	
B	0.179	0.06	
AxB	NS	0.18	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 43. Effect of PPFM isolates on proline content at 30 DAT, µg g^{-1} tissues

Treatments (A)	Proline content			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	70.88	73.90	78.75	74.51
PPFM 26	43.05	47.90	51.60	47.52
PPFM 35	35.86	38.18	43.06	39.03
PPFM 37	83.19	88.57	92.74	88.16
PPFM 38	48.51	49.65	58.70	52.29
PPFM 47 (TNAU)	38.07	42.35	47.29	42.57
0.5% Methanol	19.04	19.71	21.86	20.20
AMS	15.81	17.66	19.44	17.63
Water	30.67	31.25	33.87	31.93
Mean B	42.79	45.46	49.70	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	3.736	1.31		
B	2.157	0.76		
AxB	6.480	2.28		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 44. Effect of PPFM isolates on proline content at 60 DAT, µg g^{-1} tissues

Treatments (A)	Proline content			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	74.68	81.84	91.60	82.70
PPFM 26	45.88	52.88	63.07	53.94
PPFM 35	41.14	45.44	52.48	46.35
PPFM 37	90.18	104.88	113.06	102.71
PPFM 38	53.18	69.83	78.24	67.08
PPFM 47 (TNAU)	42.08	48.94	54.66	48.56
0.5% Methanol	20.92	28.25	29.97	26.38
AMS	17.69	21.79	23.95	21.14
Water	31.48	35.82	36.70	34.67
Mean B	46.36	54.41	60.41	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	4.917	1.73		
B	2.837	0.99		
AxB	8.602	2.99		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

The effect of different soil moisture levels on proline content showed that at 50% AW significantly higher values was observed for mean proline content (49.70 µg g^{-1} tissue) compared to the proline content at 75% AW (45.46 µg g^{-1} tissue) and at FC (42.79 µg g^{-1} tissue).

The interaction effect between moisture levels and the PPFM isolates revealed that significantly higher proline content was recorded in plants treated with PPFM 37 at all the three moisture levels. The results also revealed that at FC, the maximum proline content of 83.19 µg g^{-1} tissue was recorded with isolate PPFM 37 which was significantly higher compared to all other treatments. The lowest proline content was recorded with AMS media (15.81 µg g^{-1} tissue). At 75% AW, the maximum proline content of 88.57 µg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest proline content was recorded with AMS media (17.66 µg g^{-1} tissue). At 50% AW, the maximum proline content of 92.74 µg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest proline content was recorded with AMS media (19.44 µg g^{-1} tissue).

At 60 DAT, among the different PPFM isolates tested, the highest mean proline content of 102.71 µg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other isolates. Mean proline content was the least with AMS media treated control (21.14 µg g^{-1} tissue).

The effect of different soil moisture levels on proline content showed that 50% AW recorded significantly higher values for mean proline content (60.41 µg g^{-1} tissue) compared to that at 75% AW (54.41 µg g^{-1} tissue) and at FC (46.36 µg g^{-1} tissue).

The interaction effect between moisture levels and the PPFM isolates indicated that significantly higher proline content of plants was recorded with PPFM 37 at all the three moisture levels. The results also revealed that at FC, the maximum proline content of 90.18 µg g^{-1} tissue was recorded with isolate PPFM 37 which was significantly higher compared to all other treatments. The

lowest proline content was recorded with AMS media (17.69 μg g^{-1} tissue). At 75% AW, the maximum proline content of 104.88 μg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest proline content was recorded with AMS media (21.79 μg g^{-1} tissue). At 50% AW, the maximum proline content of 113.06 μg g^{-1} tissue was recorded with PPFM 37 which was significantly higher compared to all other treatments. The lowest proline content was recorded with AMS media (23.95 μg g^{-1} tissue).

4.2.2.16 Gibberellic Acid

The data on gibberellic acid content as influenced by PPFM and moisture levels at 30 DAT and 60 DAT are presented in Table 45 and 46 respectively.

No significant effect of PPFM isolates was observed on the mean gibberellic acid at 30 DAT and 60 DAT.

The effect of different soil moisture levels at 30 DAT on gibberellic acid showed that 50% AW resulted in significantly higher values for mean gibberellic acid (3.54 μg g^{-1}) compared to the gibberellic acid content at 75% AW (3.32 μg g^{-1}) and at FC (3.06 μg g^{-1}).

The effect of different soil moisture levels at 60 DAT on gibberellic acid content showed that 50% AW resulted in significantly higher values (3.52 μg g^{-1}) compared to that at 75% AW (3.39 μg g^{-1}) and at FC (3.19 μg g^{-1}).

No interaction effect was observed between moisture levels and the PPFM isolates for gibberellic acid content at 30 and 60 DAT.

4.2.2.17 Super oxide Dismutase

The data on super oxide dismutase (SOD) as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 47 and 48 respectively.

Among the different PPFM isolates tested, the highest mean SOD of 0.302 activity g^{-1} min^{-1} was recorded with PPFM 37 which was statistically on par with PPFM 38 (0.295 activity g^{-1} min^{-1}) and PPFM 35 (0.300 activity g^{-1} min^{-1}) at 30 DAT. Mean SOD was the least with 0.5% methanol treated control (0.220 activity g^{-1} min^{-1}).

The effect of different soil moisture levels on SOD showed that 50% AW registered significantly higher values for mean SOD (0.281 activity g^{-1} min^{-1}) compared to that at 75% AW (0.265 activity g^{-1} min^{-1}) and at FC (0.248 activity g^{-1} min^{-1}).

At 60 DAT, among the different PPFM isolates tested, the highest mean SOD of 0.312 activity g^{-1} min^{-1} was recorded with PPFM 37 which was statistically on par with PPFM 38 (0.306 activity g^{-1} min^{-1}) and PPFM 35 (0.308 activity g^{-1} min^{-1}). Mean SOD was least with 0.5% methanol treated control (0.245 activity g^{-1} min^{-1}).

The effect of different soil moisture levels on SOD showed that at 50% AW significantly higher values for mean SOD (0.299 activity g^{-1} min^{-1}) was observed compared to that at 75% AW (0.281 activity g^{-1} min^{-1}) and at FC (0.264 activity g^{-1} min^{-1}).

No interaction effect was observed between moisture levels and the PPFM isolates for SOD at 30 and 60 DAT.

4.2.2.18 Catalase

The data on catalase activity as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are furnished in Table 49 and 50 respectively.

Among the different PPFM isolates tested, the highest mean catalase activity of 11.34 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 37 which was statistically on par with PPFM 38 (10.86 µg H_2O_2 g^{-1} min^{-1}), PPFM 35

(9.21 µg H_2O_2 g^{-1} min^{-1}) and PPFM 26 (9.45 µg H_2O_2 g^{-1} min^{-1}) at 30 DAT. Mean catalase was the lowest with water treated control (6.38 µg H_2O_2 g^{-1} min^{-1}).

The effect of different soil moisture levels on catalase activity showed that at 50% AW, significantly higher value was recorded for mean catalase activity (10.23 µg H_2O_2 g^{-1} min^{-1}) compared to that at 75% AW (8.90 µg H_2O_2 g^{-1} min^{-1}) and at FC (6.61 µg H_2O_2 g^{-1} min^{-1}).

No interaction effect was observed between moisture levels and the PPFM isolates for catalase activity at 30 DAT.

Among the different PPFM isolates tested, the highest mean catalase activity of 15.35 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 37 which was statistically on par with PPFM 38 (15.11 µg H_2O_2 g^{-1} min^{-1}) at 60 DAT. Mean catalase activity was the lowest with water treated control (6.38 µg H_2O_2 g^{-1} min^{-1}).

The effect of different soil moisture levels on catalase activity showed that at 50% AW significantly higher values for mean catalase activity (13.38 µg H_2O_2 g^{-1} min^{-1}) was recorded compared to that at 75% AW (11.65 µg H_2O_2 g^{-1} min^{-1}) and at FC (7.79 µg H_2O_2 g^{-1} min^{-1}).

The interaction effect between moisture levels and the PPFM isolates on catalase activity was significant. The results revealed that at FC, the maximum catalase activity of 9.92 µg H_2O_2 g^{-1} min^{-1} was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (9.21 µg H_2O_2 g^{-1} min^{-1}), PPFM 35 (8.50 µg H_2O_2 g^{-1} min^{-1}), PPFM 26 (7.79 µg H_2O_2 g^{-1} min^{-1}), PPFM 15 (7.79 µg H_2O_2 g^{-1} min^{-1}), PPFM 47 (8.50 µg H_2O_2 g^{-1} min^{-1}), 0.5% methanol (6.38 µg H_2O_2 g^{-1} min^{-1}) and AMS media (7.09 µg H_2O_2 g^{-1} min^{-1}). The lowest catalase activity was recorded with water treated control (4.96 µg H_2O_2 g^{-1} min^{-1}). At 75% AW, the maximum catalase activity of 17.71 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 38 which was statistically on par with PPFM 37 (16.29 µg H_2O_2 g^{-1} min^{-1}). The lowest catalase activity was recorded with water

(6.38 µg H_2O_2 g^{-1} min^{-1}). At 50% AW, the maximum catalase activity of 19.84 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 37 which was statistically on par with PPFM 38 (18.42 µg H_2O_2 g^{-1} min^{-1}).The lowest catalase activity was recorded with water (7.79 µg H_2O_2 g^{-1} min^{-1}).

4.2.2.19 Peroxidase

The data on peroxidase activity as influenced by PPFM isolates and moisture levels at 30 DAT and 60 DAT are presented in Table 51 and 52 respectively.

Among the different PPFM isolates tested, the highest mean peroxidase activity of 38.96 activity g^{-1} min^{-1} was recorded with PPFM 38 which was significantly higher compared to all other isolates, at 30 DAT. Mean peroxidase was the least with water treated control (11.83 activity g^{-1} min^{-1}).

The effect of different soil moisture levels on peroxidase activity showed that at 50% AW significantly higher values for mean peroxidase activity (28.34 activity g^{-1} min^{-1}) was recorded compared to that at 75% AW (25.75 activity g^{-1} min^{-1}) and at FC (23.91 activity g^{-1} min^{-1}).

The interaction effect between moisture levels and the PPFM isolates revealed that significantly higher peroxidase activity of plants was recorded with PPFM 38 at all the three moisture levels. The results also revealed that at FC, the maximum peroxidase activity (35.33 activity g^{-1} min^{-1}) was recorded with isolate PPFM 38 which was statistically on par with PPFM 35 (31.17 activity g^{-1} min^{-1}). The lowest peroxidase activity was recorded with water (9.57 activity g^{-1} min^{-1}). At 75% AW, the maximum peroxidase of 38.23 activity g^{-1} min^{-1} was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest peroxidase was recorded with water (12.57 activity g^{-1} min^{-1}). At 50% AW, the maximum peroxidase activity of 43.30 activity g^{-1} min^{-1} was recorded with PPFM 38 which was significantly higher as compared to all other

Table 45. Effect of PPFM isolates on gibberellic acid at 30 DAT, µg g^{-1}

Treatments (A)	Gibberellic acid			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	3.02	3.23	3.53	3.26
PPFM 26	3.07	3.30	3.58	3.32
PPFM 35	3.05	3.32	3.55	3.31
PPFM 37	3.22	3.50	3.80	3.51
PPFM 38	3.23	3.58	3.85	3.56
PPFM 47 (TNAU)	3.07	3.35	3.52	3.31
0.5% Methanol	3.02	3.32	3.48	3.27
AMS	2.97	3.17	3.28	3.14
Water	2.93	3.08	3.23	3.08
Mean B	3.06	3.32	3.54	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.13		
B	0.215	0.08		
AxB	NS	0.23		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 46. Effect of PPFM isolates on gibberellic acid at 60 DAT, µg g^{-1}

Treatments (A)	Gibberellic acid			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	3.15	3.28	3.50	3.31
PPFM 26	3.25	3.50	3.53	3.43
PPFM 35	3.18	3.47	3.57	3.41
PPFM 37	3.32	3.55	3.77	3.54
PPFM 38	3.37	3.65	3.72	3.58
PPFM 47 (TNAU)	3.28	3.45	3.62	3.45
0.5% Methanol	3.10	3.25	3.43	3.26
AMS	3.13	3.30	3.37	3.27
Water	2.93	3.05	3.15	3.04
Mean B	3.19	3.39	3.52	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.13		
B	0.214	0.08		
AxB	NS	0.23		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 47. Effect of PPFM isolates on super oxide dismutase at 30 DAT, activity g^{-1} min^{-1}

Treatments (A)	Super oxide dismutase			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.223	0.258	0.282	0.254
PPFM 26	0.239	0.269	0.294	0.267
PPFM 35	0.286	0.303	0.311	0.300
PPFM 37	0.278	0.308	0.320	0.302
PPFM 38	0.280	0.291	0.313	0.295
PPFM 47 (TNAU)	0.256	0.270	0.298	0.275
0.5% Methanol	0.215	0.219	0.225	0.220
AMS	0.231	0.236	0.243	0.237
Water	0.225	0.230	0.240	0.232
Mean B	0.248	0.265	0.281	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.015	0.005		
B	0.009	0.003		
AxB	NS	0.009		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 48. Effect of PPFM isolates on super oxide dismutase at 60 DAT, activity g^{-1} min^{-1}

Treatments (A)	Super oxide dismutase			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	0.257	0.279	0.306	0.281
PPFM 26	0.261	0.282	0.317	0.287
PPFM 35	0.291	0.311	0.322	0.308
PPFM 37	0.281	0.318	0.336	0.312
PPFM 38	0.287	0.308	0.324	0.306
PPFM 47 (TNAU)	0.274	0.291	0.313	0.293
0.5% Methanol	0.234	0.244	0.256	0.245
AMS	0.249	0.252	0.260	0.254
Water	0.241	0.248	0.252	0.247
Mean B	0.264	0.281	0.299	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.013	0.009		
B	0.007	0.003		
AxB	NS	0.008		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 49. Effect of PPFM isolates on catalase at 30 DAT, µg H_2O_2 g^{-1} min^{-1}

Treatments (A)	Catalase			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	5.67	7.09	9.21	7.32
PPFM 26	6.38	10.63	11.34	9.45
PPFM 35	7.09	9.92	10.63	9.21
PPFM 37	7.79	12.05	14.17	11.34
PPFM 38	8.50	11.34	12.75	10.86
PPFM 47 (TNAU)	6.38	8.50	10.63	8.50
0.5% Methanol	5.67	7.09	7.80	6.85
AMS	6.38	7.09	8.50	7.32
Water	5.67	6.38	7.09	6.38
Mean B	6.61	8.90	10.23	

Treatment effects	CD (0.05)	SEm (±)
A	2.336	0.82
B	1.349	0.47
AxB	3.793	1.42

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 50. Effect of PPFM isolates on catalase at 60 DAT, µg H_2O_2 g^{-1} min^{-1}

Treatments (A)	Catalase			
	Moisture levels (B)			
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	Mean A
PPFM 15	7.79	11.33	12.75	10.63
PPFM 26	7.79	12.75	14.17	11.57
PPFM 35	8.50	12.04	13.46	11.34
PPFM 37	9.92	16.29	19.84	15.35
PPFM 38	9.21	17.71	18.42	15.11
PPFM 47 (TNAU)	8.50	12.05	14.88	11.81
0.5% Methanol	6.38	7.80	9.21	7.79
AMS	7.09	8.50	9.92	8.50
Water	4.96	6.38	7.79	6.38
Mean B	7.79	11.65	13.38	

Treatment effects	CD (0.05)	SEm (±)
A	2.271	0.80
B	1.311	0.46
AxB	3.934	1.38

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 51. Effect of PPFM isolates on peroxidase at 30 DAT, activity g^{-1} min^{-1}

Treatments (A)	Peroxidase			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	23.43	24.70	25.93	24.69
PPFM 26	29.27	30.63	33.67	31.19
PPFM 35	31.17	32.97	37.43	33.86
PPFM 37	27.63	29.57	32.83	30.01
PPFM 38	35.33	38.23	43.30	38.96
PPFM 47 (TNAU)	24.73	25.90	28.63	26.42
0.5% Methanol	19.33	20.67	21.43	20.48
AMS	14.73	16.50	18.47	16.57
Water	9.57	12.57	13.37	11.83
Mean B	23.91	25.75	28.34	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	2.945	1.04		
B	1.700	0.60		
AxB	5.117	1.79		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 52. Effect of PPFM isolates on peroxidase at 60 DAT, activity g^{-1} min^{-1}

Treatments (A)	Peroxidase			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	25.27	27.83	30.20	27.77
PPFM 26	31.63	35.57	41.67	36.29
PPFM 35	35.60	41.27	48.80	41.89
PPFM 37	29.10	32.23	38.63	33.32
PPFM 38	39.77	46.20	54.67	46.88
PPFM 47 (TNAU)	27.13	29.57	33.03	29.91
0.5% Methanol	21.03	21.73	23.03	21.93
AMS	15.80	17.00	19.13	17.31
Water	12.13	12.63	15.17	13.31
Mean B	26.39	29.34	33.82	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	2.802	0.99		
B	1.618	0.57		
AxB	4.854	1.71		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

treatments. The lowest peroxidase activity was recorded with water (13.37 activity g^{-1} min^{-1}).

At 60 DAT, among the different PPFM isolates tested, the highest mean peroxidase activity (46.88 activity g^{-1} min^{-1}) was recorded with PPFM 38 which was significantly higher compared to all other treatments. Mean peroxidase activity was the least with water treated control (13.31 activity g^{-1} min^{-1}).

The effect of different soil moisture levels on peroxidase activity showed that at 50% AW significantly higher values for mean peroxidase activity (33.82 activity g^{-1} min^{-1}) was observed compared that at 75% AW (29.34 activity g^{-1} min^{-1}) and at FC (26.39 activity g^{-1} min^{-1}).

The interaction effect between moisture levels and the PPFM isolates was significant and significantly higher peroxidase activity of plants was recorded with PPFM 38 at all the three moisture levels. The results also revealed that at FC, the maximum peroxidase activity (39.77 activity g^{-1} min^{-1}) was recorded with isolate PPFM 38 which was statistically on par with PPFM 35 (35.60 activity g^{-1} min^{-1}). The lowest peroxidase activity was recorded with water (12.13 activity g^{-1} min^{-1}). At 75% AW, the maximum peroxidase activity (46.20 activity g^{-1} min^{-1}) was recorded with PPFM 38 which was significantly higher compared to all other treatments. The lowest peroxidase was recorded with water (12.63 activity g^{-1} min^{-1}). At 50% AW, the maximum peroxidase of 54.67 activity g^{-1} min^{-1} was recorded with PPFM 38 which was significantly higher as compared to all other treatments. The lowest peroxidase was recorded with water (15.17 activity g^{-1} min^{-1}).

4.2.3 Yield and Yield Attributes

4.2.3.1 Number of Panicles per Hill

The data on number of panicles per hill as influenced by PPFM isolates and moisture levels are presented in Table 53.

Among the different PPFM isolates tested, the highest mean number of panicles per hill (3.89) was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.56) and PPFM 35 (3.22). Mean number of panicles per hill was the lowest with water treated control (2.11).

The effect of different soil moisture levels on number of panicles per hill showed significantly higher mean number of panicles per hill (3.11) at 50% AW compared to 75% AW (3.07) and FC (2.44).

The interaction effect between moisture levels and the PPFM isolates on number of panicles per hill was significant and at FC, the highest number of panicles per hill of 3.33 was recorded with isolate PPFM 37 which was statistically on par with PPFM 38 (2.67), PPFM 35 (3.00), PPFM 26 (2.33), PPFM 15 (2.33), PPFM 47 (2.00), 0.5% methanol (2.00), AMS media (2.33) and water (2.00). At 75% AW, the highest number of panicles per hill of 4.33 was recorded with PPFM 37 which was statistically on par with PPFM 38 (3.67), PPFM 35 (3.33), PPFM 47 (3.00) and 0.5% methanol (3.00). The lowest number of panicles per hill was recorded with water (2.33). At 50% AW, the highest number of panicles per hill of 4.33 was recorded with PPFM 38 which was statistically on par with PPFM 37 (4.00), PPFM 35 (3.33), PPFM 26 (3.00), PPFM 15 (3.33) and PPFM 47 (3.33).The lowest number of panicles per hill was recorded with water (2.00).

4.2.3.2 Number of Grains per Panicle

The data on number of grains per panicle as influenced by PPFM isolates and moisture levels are presented in Table 54.

Among the different PPFM isolates tested, the highest mean number of grains per panicle of 64.11 was recorded with PPFM 37 which was significantly higher compared to all other isolates. Mean number of grains per panicle was the lowest with 0.5% methanol treated control (31.56).

The effect of different soil moisture levels on number of grains per panicle showed that at FC, significantly higher values was recorded for mean number of grains per panicle (46.59) compared to that at 75% AW (41.59) and at 50% AW (38.37).

The interaction effect between moisture levels and the PPFM isolates was also significant the highest value was registered with PPFM 38 at all the three moisture levels. At FC, the highest number of grains per panicle of 76.33 was recorded with isolate PPFM 37 which was significantly higher compared to all other isolates. The lowest number of grains per panicle was recorded with water (34.00). At 75% AW, the highest number of grains per panicle of 59.67 was recorded with PPFM 37 which was statistically on par with PPFM 38 (52.33) and PPFM 35 (47.67). The lowest number of grains per panicle was recorded with 0.5% methanol (30.67). At 50% AW, the maximum number of grains per panicle of 56.33 was recorded with PPFM 37 which was statistically on par with PPFM 38 (47.67), PPFM 35 (44.33) and PPFM 47 (41.67).The lowest number of grains per panicle was recorded with AMS media treated control (27.33).

4.2.3.3 Thousand Grain Weight

The data on thousand grain weight as influenced by PPFM isolates and moisture levels are presented in Table 55.

The different PPFM isolates tested had no significant effect on the mean thousand grain weight.

No significant effect of different soil moisture levels was also observed on thousand grain weight.

The interaction effect observed between moisture levels and the PPFM isolates for thousand grain weight was also non-significant.

4.2.3.4 Grain Yield

The data on grain yield as influenced by PPFM isolates and moisture levels are presented in Table 56.

Among the different PPFM isolates tested, the highest mean grain yield of 6.06 g hill^{-1} was recorded with PPFM 37 which was significantly higher compared to all other isolates. Mean grain yield was the lowest with water treated control (3.81 g hill^{-1}).

The effect of different soil moisture levels on grain yield showed that at FC, significantly higher values was recorded for mean grain yield (5.17 g hill^{-1}) compared to that at 75% AW (4.78 g hill^{-1}) and at 50 % AW (4.53 g hill^{-1}).

The interaction effect between moisture levels and the PPFM isolates revealed that the grain yield was maximum with PPFM 37 at all the three moisture levels. At FC, the maximum grain yield of 6.40 g hill^{-1} was recorded with isolate PPFM 37 (Plate 20) which was statistically on par with PPFM 38 (5.96 g hill^{-1}). The lowest grain yield was recorded with water (4.19 g hill^{-1}). At 75% AW, the maximum grain yield of 5.99 g hill^{-1} was recorded with PPFM 37 (Plate 21) which was statistically on par with PPFM 38 (5.56 g hill^{-1}). The lowest grain yield was recorded with water (3.77 g hill^{-1}). At 50% AW, the maximum grain yield of 5.78 g hill^{-1} was recorded with PPFM 37 (Plate 22) which was statistically on par with PPFM 38 (5.35 g hill^{-1}) (Plate 23). The lowest grain yield was recorded with water treated control (3.46 g hill^{-1}).

4.2.3.5 Percentage Relative Yield Reduction

The data on percentage relative yield reduction as influenced by PPFM isolates and moisture levels are presented in Table 57.

Among the different PPFM isolates tested, the least percentage relative yield reduction of 7.80 was recorded with PPFM 37 which was statistically on par with PPFM 38 (8.31), PPFM 35 (8.60), PPFM 26 (9.79) and PPFM 47 (9.31).

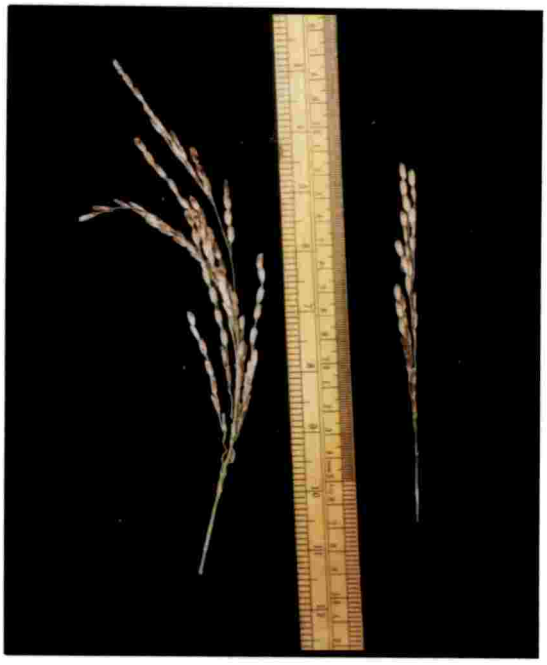

Plate 20. Panicle of PPFM 37 treated plants at FC compared to water treated control

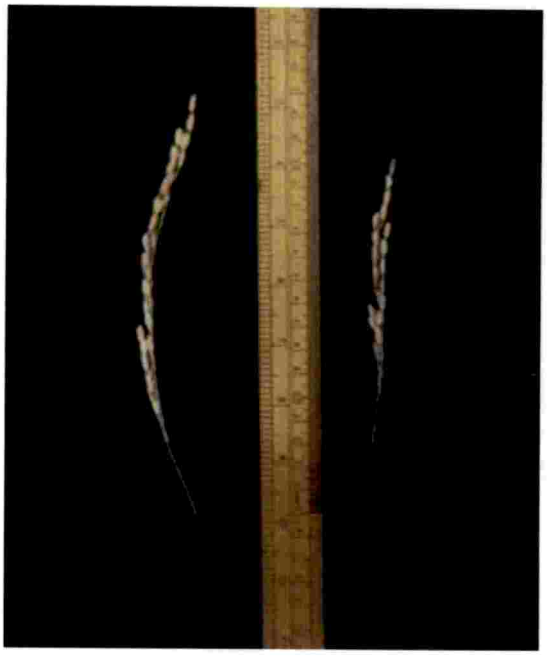

Plate 21. Panicle of PPFM 37 treated plants at 75% AW compared to water treated control

Plate 22. Panicle of PPFM 37 treated plants at 50% AW compared to water treated control

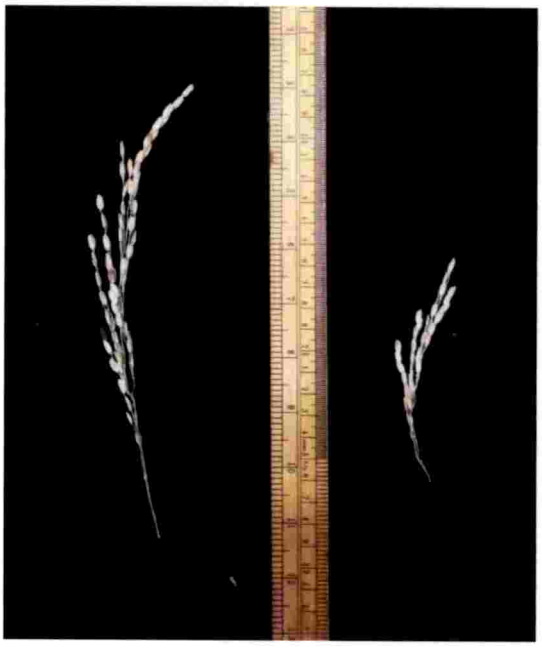

Plate 23. Panicle of PPFM 38 treated plants at 50% AW compared to water treated control

Table 53. Effect of PPFM isolates on number of panicles per hill

Treatments (A)	Number of panicles per hill			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	2.33	2.67	3.33	2.78
PPFM 26	2.33	2.67	3.00	2.67
PPFM 35	3.00	3.33	3.33	3.22
PPFM 37	3.33	4.33	4.00	3.89
PPFM 38	2.67	3.67	4.33	3.56
PPFM 47 (TNAU)	2.00	3.00	3.33	2.78
0.5% Methanol	2.00	3.00	2.33	2.44
AMS	2.33	2.67	2.33	2.44
Water	2.00	2.33	2.00	2.11
Mean B	2.44	3.07	3.11	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.795	0.28		
B	0.459	0.16		
AxB	2.941	0.48		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 54. Effect of PPFM isolates on number of grains per panicle

Treatments (A)	Number of grains per panicle			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	40.00	35.33	33.33	36.22
PPFM 26	43.67	40.67	34.00	39.44
PPFM 35	49.33	47.67	44.33	47.11
PPFM 37	76.33	59.67	56.33	64.11
PPFM 38	57.33	52.33	47.67	52.44
PPFM 47 (TNAU)	47.33	43.67	41.67	44.22
0.5% Methanol	34.67	30.67	29.33	31.56
AMS	36.67	31.67	27.33	31.89
Water	34.00	32.67	31.33	32.67
Mean B	46.59	41.59	38.37	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	9.468	3.33		
B	5.467	1.92		
AxB	16.64	5.77		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 55. Effect of PPFM isolates on thousand grain weight, g

Treatments (A)	Thousand grain weight			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	23.68	23.50	23.33	23.50
PPFM 26	23.72	23.58	23.27	23.52
PPFM 35	23.67	23.53	23.31	23.50
PPFM 37	23.72	23.52	23.35	23.53
PPFM 38	23.76	23.61	23.39	23.59
PPFM 47 (TNAU)	23.70	23.51	23.30	23.50
0.5% Methanol	23.64	23.47	23.25	23.46
AMS	23.61	23.51	23.28	23.46
Water	23.58	23.46	23.24	23.43
Mean B	23.68	23.52	23.30	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	NS	0.04		
B	0.064	0.02		
AxB	NS	0.07		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 56. Effect of PPFM isolates on grain yield, g hill^{-1}

Treatments (A)	Grain yield			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	4.89	4.50	4.22	4.54
PPFM 26	5.01	4.62	4.42	4.68
PPFM 35	5.72	5.34	5.12	5.40
PPFM 37	6.40	5.99	5.78	6.06
PPFM 38	5.96	5.56	5.35	5.62
PPFM 47 (TNAU)	5.60	5.18	4.96	5.25
0.5% Methanol	4.34	3.97	3.67	3.99
AMS	4.47	4.10	3.82	4.13
Water	4.19	3.77	3.46	3.81
Mean B	5.17	4.78	4.53	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.295	0.10		
B	0.170	0.06		
AxB	0.507	0.18		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 57. Effect of PPFM isolates on percentage relative yield reduction, %

Treatments (A)	Percentage relative yield reduction		
	Moisture levels (B)		Mean A
	At 75% AW (B1)	At 50% AW (B2)	
PPFM 15	8.02	13.78	10.90
PPFM 26	7.76	11.81	9.79
PPFM 35	6.76	10.44	8.60
PPFM 37	6.15	9.46	7.80
PPFM 38	6.47	10.15	8.31
PPFM 47 (TNAU)	7.41	11.21	9.31
0.5% Methanol	8.47	15.16	11.81
AMS	8.32	14.65	11.49
Water	9.78	17.36	13.57
Mean B	7.68	12.67	
Treatment effects	**CD (0.05)**	**SEm (±)**	
A	3.291	1.14	
B	1.551	0.54	
AxB	NS	1.62	

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 58. Effect of PPFM isolates on straw yield, g hill^{-1}

Treatments (A)	Straw yield			
	Moisture levels (B)			Mean A
	At FC (B1)	At 75% AW (B2)	At 50% AW (B3)	
PPFM 15	2.08	2.51	2.91	2.50
PPFM 26	2.31	3.30	2.93	2.85
PPFM 35	2.95	3.40	3.03	3.12
PPFM 37	3.70	4.61	4.11	4.14
PPFM 38	4.09	4.98	4.69	4.59
PPFM 47 (TNAU)	3.59	3.50	3.75	3.61
0.5% Methanol	2.76	2.91	2.92	2.86
AMS	2.92	3.03	3.29	3.08
Water	2.57	2.72	2.84	2.71
Mean B	3.00	3.44	3.39	
Treatment effects	**CD (0.05)**	**SEm (±)**		
A	0.418	0.15		
B	0.241	0.09		
AxB	0.683	0.26		

FC=Field capacity, AW= Available water, AMS= Ammonium mineral salt media

Table 59. Ranking of PPFM isolates based on physiological parameters, yield attributes and yield of paddy

Isolates	Rank (Physiological parameters)	Rank (Yield attributes & yield)
PPFM 15	5	4
PPFM 26	4	3
PPFM 35	3	2
PPFM 37	2	1
PPFM 38	1	1

Mean percentage relative yield reduction was the highest with water treated control (13.57).

The effect of different soil moisture levels on percentage relative yield reduction showed that 75% AW resulted in least mean percentage relative yield reduction (7.68) compared to that at 50% AW (12.67).

No significant interaction effect was noticed between moisture levels and the PPFM isolates on percentage relative yield reduction.

4.2.3.6 Straw Yield

The data on straw yield as influenced by PPFM isolates and moisture levels are presented in Table 58.

Among the different PPFM isolates tested, the highest mean straw yield of 4.59 g $hill^{-1}$ was recorded with PPFM 38 which was significantly higher compared to all other treatments. Mean straw yield was the lowest with PPFM 15 (2.50 g $hill^{-1}$).

The effect of different soil moisture levels on straw yield showed that at 75% AW significantly higher values were recorded for mean straw yield (3.44 g $hill^{-1}$) compared to that at 50% AW (3.39 g $hill^{-1}$) and at FC (3.00 g $hill^{-1}$).

The interaction effect between moisture levels and the PPFM isolates revealed that the straw yield was the highest with PPFM 38 at all the three moisture levels. At FC, the highest straw yield of 4.09 g $hill^{-1}$ was recorded with isolate PPFM 38 which was statistically on par with PPFM 37 (3.70 g $hill^{-1}$) and PPFM 47 (3.59 g $hill^{-1}$). The lowest straw yield was recorded with PPFM 15 (2.08 g $hill^{-1}$). At 75% AW, the maximum straw yield of 4.98 g $hill^{-1}$ was recorded with PPFM 38 which was statistically on par with PPFM 37 (4.61 g $hill^{-1}$). The lowest straw yield was recorded with PPFM 15 (2.51 g $hill^{-1}$). At 50% AW, the maximum straw yield of 4.69 g $hill^{-1}$ was recorded with PPFM 38 which was

statistically on par with PPFM 37 (4.11 g hill^{-1}). The lowest straw yield was recorded with water treated control (2.84 g hill^{-1}).

Weighted Average Ranks

The weighted average rank of PPFM isolates calculated based on physiological parameters, yield attributes and yield of paddy are presented in Table 59. Considering the major drought tolerance parameters such as leaf rolling score, leaf drying score, rooting depth, drought susceptibility index, proline content, super oxide dismutase, catalase and peroxidase, PPFM 38 was ranked first among the PPFM isolates tested in the pot culture experiment. With respect to the yield attributes and yield of rice under water stress, the effect of PPFM 37 and PPFM 38 were observed to be at par.

4.2.4 Incidence of Pest and Diseases

Less incidence of pest and diseases were observed in the experimental field and hence there was no economical loss due to pest and diseases. Since the pest and disease incidence did not reach the economic threshold level, uniform score was given to all plants.

Discussion

5. DISCUSSION

During the course of the experiment entitled, 'Screening of Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy', many significant responses were noted due to the treatments constituting the experiment. In this chapter, efforts have been made to assign reasons responsible for such responses that occurred due to different treatments.

The present programme comprised two experiments. In the first experiment, *in vitro* screening of PPFM isolates for water stress tolerance was done by using mannitol as an osmotic stress inducer. Observations on seed germination, growth of seedlings and seedling vigour index were recorded. The results showed high variation in performance of PPFM isolates with respect to seed germination and seedling growth at different water stress levels (1%, 2%, 3% mannitol and water control). This may be due to the genetic inherent character of the PPFM isolates.

In the present study it was observed that maximum osmotic stress was contributed by 3 per cent mannitol and it induced water stress to the paddy seeds and seedlings. Zgallai *et al.* (2005) reported that polyethylene glycol and mannitol could be used to stimulate osmotic stress and these neutral polymers are being widely used to impose water stress in plants. Polyethylene glycol and mannitol have a significant effect on germination percentage. 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. 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 many 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

(1%, 2%, 3% mannitol) along with water control. Since maximum stress was induced by 3% mannitol treatment, results of the same has been discussed in this chapter.

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, 9.47 cm and 2143.25 respectively were recorded in PPFM 26 treated seeds. Seeds treated with PPFM 15 recoded the maximum root length (18.38 cm) and shoot dry weight (7.40 mg). 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. 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).

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 induced 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). 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 rice seeds under different PEG concentration (Kumar *et al.*, 2017). It has been suggested 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 were done taking into consideration germination percentage (Fig. 2), shoot length root length, shoot dry weight, root dry weight (Fig. 3) and seedling vigour index of paddy seedlings. The isolates PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 having top weighted

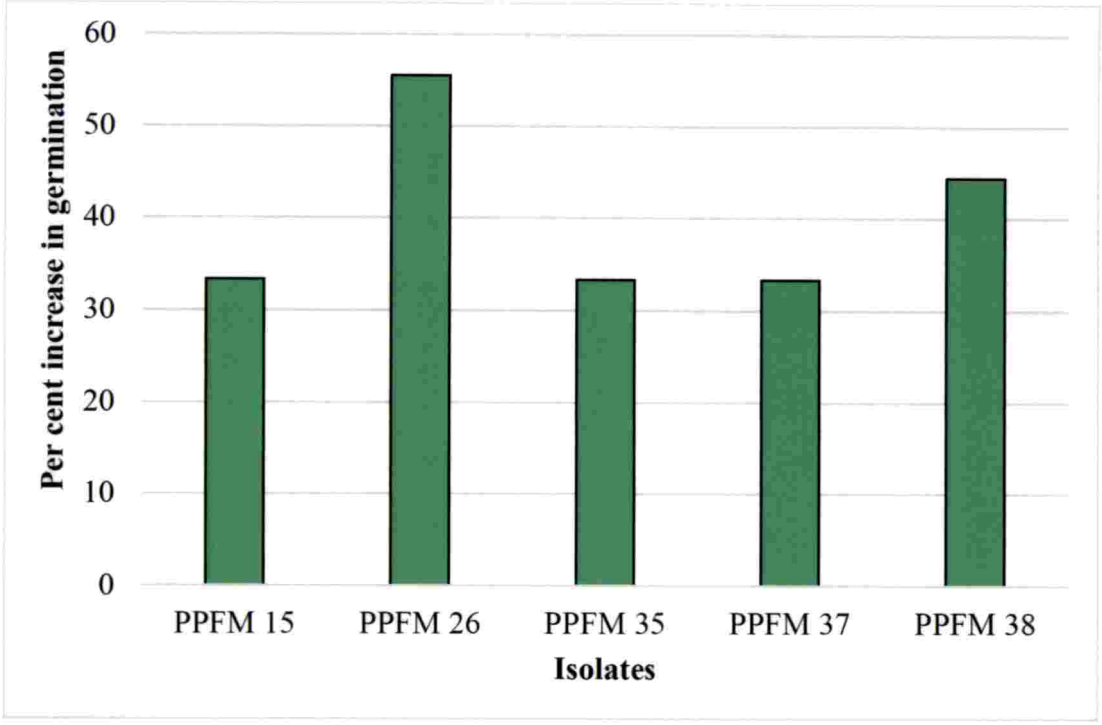

Figure 2. Per cent increase in germination of paddy seeds by selected PPFM isolates in 3% mannitol over water treated control

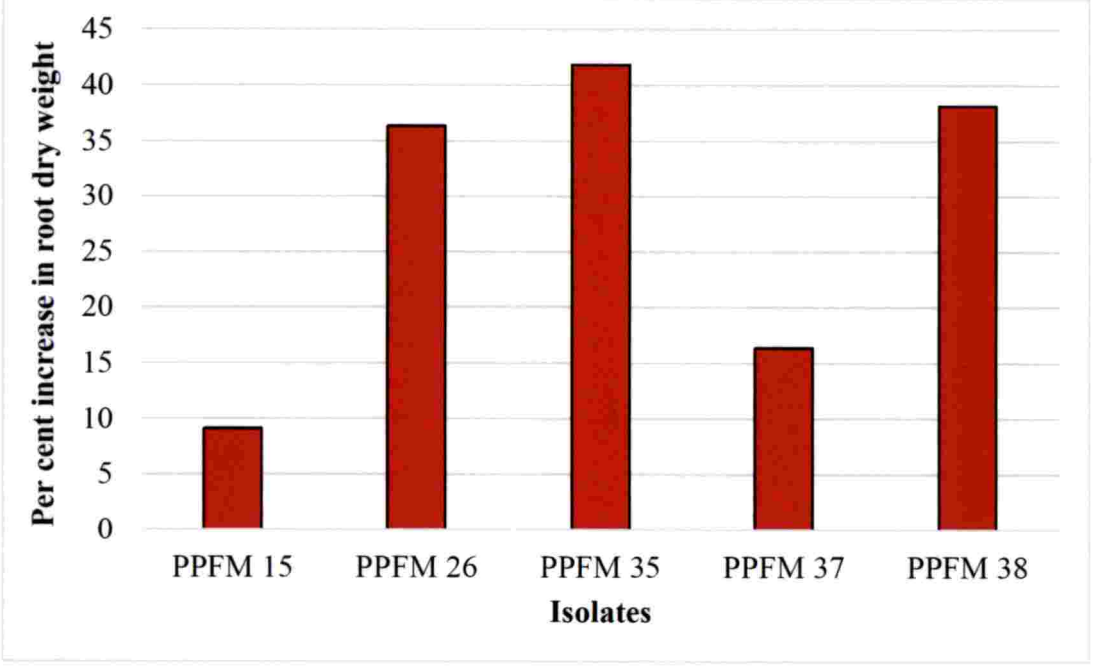

Figure 3. Per cent increase in root dry weight of paddy seedlings by selected PPFM isolates in 3% mannitol over water treated control

average ranks which secured ranks from 1 to 5 were selected for the subsequent pot culture experiment.

The second experiment was a pot culture experiment to study the effect of PPFM isolates on growth and yield of paddy under water stress conditions. The experiment was laid out in CRD with 21 treatments and three replications, during summer 2019. The treatments comprised six PPFM isolates (5 KAU isolates of PPFM and one TNAU isolate) and three moisture levels (at field capacity, 75 per cent available water and 50 per cent AW) and three control treatments (0.5% methanol, AMS liquid medium supplemented with 0.5% methanol and absolute control). The treatments were given as seed treatment, seedling root dip and foliar application at 15 and 30 DAT. Observations on biometric and physiological parameters, yield attributes and yield were recorded. The study revealed that PPFM isolates had significant effect on biometric parameters, physiological parameters, yield attributes and yield of paddy under water stress condition. 50 per cent AW resulted maximum water stress to the plants.

Drought stress suppressed the plant height, leaf number, size and tillers which finally lowered the dry matter production (Khan and Abdullah, 2003). The present investigation conclusively proved that, under water stress PPFM inoculation in paddy had significant positive effect on growth parameters like plant height, leaf area index and tiller production.

In the present study, maximum plant height of 44.01 cm and 61.17 cm were recorded with PPFM 38 at 30 and 60 DAT whereas PPFM 37 recorded maximum plant height at 90 DAT (85.17 cm) under highest level of water stress condition. Leaf area index of 4.01 and 5.02 were recorded with PPFM 37 at 30 and 60 DAT under maximum water stress condition. Among the different PPFM isolates tested, the highest mean number of tillers per hill (5.56) was recorded with PPFM 37 at 60 DAT.

These results clearly indicated that the production and release of important growth promoting substances by non-pathogenic *Methylobacteria* might have

been involved in the regulation of plant growth and highly correlated with drought tolerance (Sivakumar *et al.*, 2017). Ajaykumar and Krishnasamy (2018) also observed that PPFM (1%) application at panicle initiation to flowering stages of rice increases the leaf area index and crop growth rate than uninoculated control. The PPFM mediated hormonal activity might be attributed to the increase in leaf area, crop growth rate and other growth parameters (Ajaykumar and Krishnasamy, 2018). Growth promotion by the plant growth promoting bacteria (PGPB) may be attributed to mechanisms such as production of plant growth promoting hormones and other plant growth promoting activities (Glick, 1995). One of the main internal factors controlling the growth and development of plant is plant hormones (Kelen *et al.*, 2004). GA_3 can be taken into account as vegetative growth promoter commonly used in the crop. In the present investigation PPFM inoculated plants showed more gibberellic acid over water treated control.

Leaf rolling character and leaf drying character of leaves are good criteria for assessing drought tolerance levels in large scale screening (Chang, *et al.,* 1974). Dingkuhn *et al.* (1991) reported that leaf rolling is an adaptive mechanism found in rice plants to escape drought. Blum (1989) reported that delayed leaf rolling is associated with better osmotic adjustment and avoidance of dehydration under water stress in rice. In the present investigation, among the different PPFM isolates tested, the least mean leaf rolling score of 3.33 and 4.26 were recorded with PPFM 37 at 30 and 60 DAT. Among the different PPFM isolates tested, the least leaf mean drying score of 3.48 was recorded with PPFM 37 at 30 DAT whereas least drying score of 2.80 was recorded with PPFM 37 and PPFM 38 at 60 DAT. The present study revealed that the PPFM isolates treated plants maintained better relative water content under water stress condition and showed lesser leaf rolling and leaf drying symptoms. Hence, it could be suggested that leaf rolling and leaf drying are adaptive mechanism in rice to escape drought.

Leaf temperature is considered as an index to measure water stress in crop plants. High temperature causes membrane collapse, which leads to chlorophyll

degradation in the plant. As soil water diminishes, leaf temperature increases because transpiration is reduced (Blum, 1988). In the present study, among the different PPFM isolates tested, the least mean leaf temperature of 27.33°C and 30.64°C were recorded with PPFM 38 at 30 and 60 DAT whereas higher mean leaf temperature was recorded in uninoculated control. Plants with deeper root system would maintain cooler canopy temperature and ultimately higher yield under drought. Canopy temperature was found to have a positive correlation with leaf rolling and leaf drying and negative correlation with root thickness in rice (Babu *et al.*, 2003). A substantial decrease in relative water content, leaf water potential and transpiration rate and a simultaneous increase in leaf temperature were observed when rice plants were exposed to drought stress (Akram *et al.*, 2013). The present study revealed that the PPFM isolates treated plants maintained better relative water content under water stress and showed lesser leaf temperature. Hence, it could be suggested that leaf temperature is associated with leaf water potential.

Drought stress caused a disturbance in membrane permeability expressed by an increase in solute leakage (Premchandra *et al.*, 1990; Deshmukh *et al.*, 1991). PPFM treatment exerted significant influence on plant cell membrane integrity under water stress condition. In the present study significantly higher cell membrane integrity of 85.07 per cent and 81.07 per cent was noticed with PPFM 38 under maximum water stress condition at 30 DAT and 60 DAT respectively (Fig. 4 and 5)). Similar results had already been reported by Kumar *et al.* (2017) observed that inoculation of *Bacillus altitudinis* FD48 and *Methylobacterium* sp. reduced severe membrane damage whereas higher leakage of solutes and severe membrane damage was observed in untreated plants. The plasma membrane is generally protected from desiccation induced damage by the presence of membrane compatible solutes, such as sugars and amino acids. Therefore, a link may exist between the capacity for osmotic adjustment and the degree of membrane protection from the effect of dehydration. Accumulation of antioxidant enzymes may also result in protecting membrane stability (Sese and

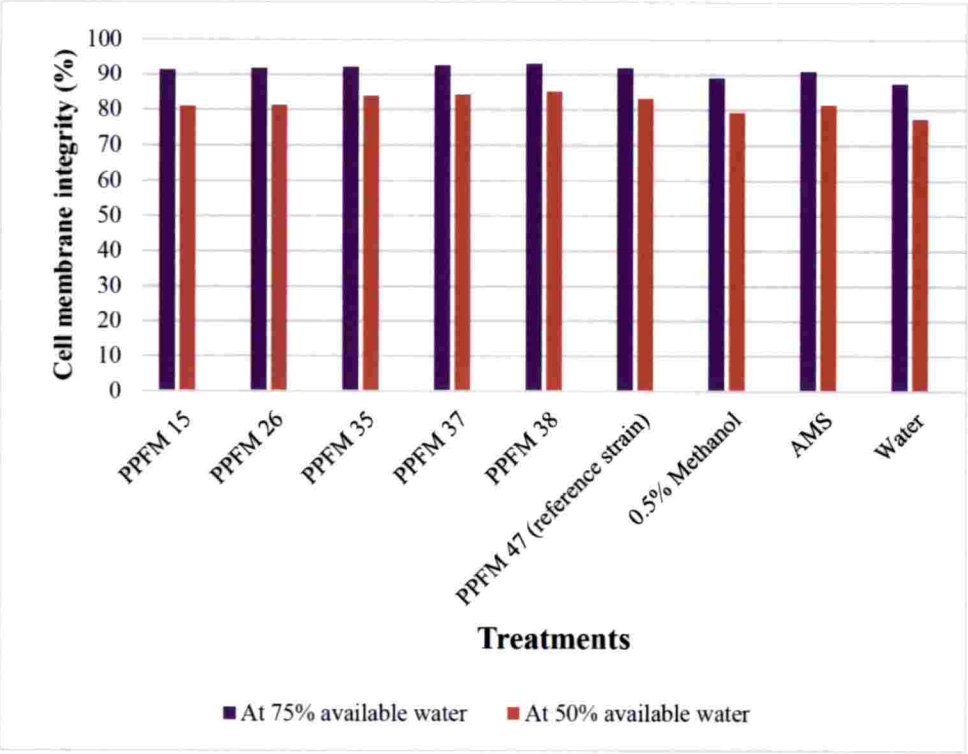

Figure 4. Effect of PPFM isolates on cell membrane integrity at 30 DAT

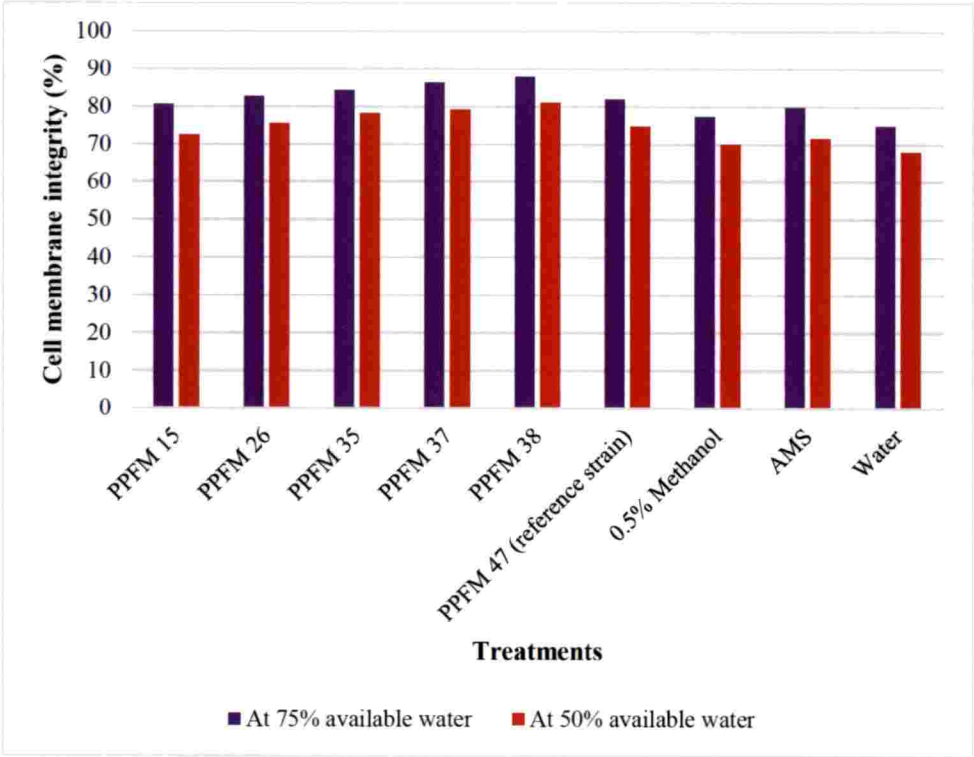

Figure 5. Effect of PPFM isolates on cell membrane integrity at 60 DAT

Tobita, 1998). Treatment with PPFM 38 resulted in an increase of 9.90 per cent and 19.01 per cent cell membrane integrity over water treated control at 30 and 60 DAT.

It has been suggested that the variation in drought tolerance among the rice cultivars is mostly the reflection of the variation in plant water status during stress periods. In the present study, among the different PPFM isolates tested, the highest mean relative water content (RWC) of 76.84 per cent and 67.26 per cent were recorded with PPFM 38 at 30 DAT and 60 DAT. Under drought stress, relative water content (RWC) declined in inoculated and uninoculated seedlings. However, bacterial inoculation did help seedlings to maintain their RWC during drought periods. Similar report was made on the use of *Pseudomonas* spp. inoculation which helps the maize plants to maintain their RWC under drought conditions (Sandhya *et al.*, 2010). *Bacillus altitudinis* FD48 and *Methylobacterium* spp. (PPFM) treated plants had more RWC compared to control under induced drought conditions (Kumar *et al.*, 2017). However, the mechanism behind the increased RWC when treated with PGPB is yet to be elucidated. Some studies predict that this may be the result of bacterial absicisic acid which results in closure of stomata (Casanovas *et al.*, 2002).

Chlorophyll stability index (CSI) is a function of temperature, the property of chlorophyll pigments which can be correlated with drought tolerance/susceptibility of the crop plants. Prolonged drought stress reduces the chlorophyll stability index. In the present study, among the different PPFM isolates tested, the highest mean CSI of 88.54 per cent and 84.54 per cent were recorded with PPFM 38 at 30 and 60 DAT. Sathyan *et al.* (2018) studied the effect of PPFM bacteria and synthetic materials on small cardamom (*Elettaria cardamomum* Maton.) under drought and reported a significant increase in the CSI in the PPFM treated plants. *B. altitudinis* FD48 and *Methylobacterium* spp. (PPFM) treated plants showed more CSI compared to control under drought conditions (Kumar *et al.*, 2017). Sivakumar *et al.* (2017) also reported that foliar application of PPFM prevented the chlorophyll breakdown under drought leading

to retention of chlorophyll and delay of senescence. Treatment with PPFM 38 resulted in an increase of 16.67 per cent and 13.29 per cent CSI over water treated control at 30 and 60 DAT.

In the present study, effect of PPFM isolates on root traits were tested and the results revealed that the treated plants under water stress condition showed significant increase in root length, root weight, root volume and root dry weight. Chang *et al.* (1986) also found that deep rooted rice cultivars tolerate drought better than shallow rooted cultivars because of their ability to extract moisture from the deeper layers of soil. Maximum root length of 13.17 cm was recorded with PPFM 37 at 30 DAT (Fig. 6) whereas PPFM 38 recorded 24.60 cm at 60 DAT (Fig. 7) under maximum water stress condition of 50% AW. This treatment with PPFM 37 resulted in an increase of 172.67 per cent at 30 DAT over water treated control whereas 97.91 per cent increase in rooting depth over control with PPFM 38 at 60 DAT. Root length was increased up to 26.34 per cent by PPFM (2%) than control followed by brassinolide (22.93%) in tomato under drought condition (Sivakumar *et al.*, 2018). Similar observations were also reported in tomato by Chandrasekaran *et al.* (2017).

Maximum root weight of 0.273 g and 4.20 g were recorded with PPFM 38 at 30 and 60 DAT under maximum water stress condition. Maximum root volume of 1.067 cm^3 was recorded with PPFM 38 at 30 DAT under maximum water stress condition, whereas highest mean root volume of 5.31 cm^3 was recorded with PPFM 37 at 60 DAT. This is in conformition with the findings of Sivakumar *et al.* (2018) who reported that the foliar spray of PGRs and PPFM helped to alleviate drought by improving the lateral root growth which increased the root volume. This increase in root volume can be attributed to their ability to increase root biomass in order to extract moisture from deeper layers of soil and hence the ability to tolerate drought condition. Maximum root dry weight of 0.037 g was recorded with PPFM 38 at 30 DAT under maximum water stress condition, whereas highest mean root dry weight of 0.798 g was recorded with PPFM 38 at 60 DAT. The increase in root biomass under water stress condition is a function

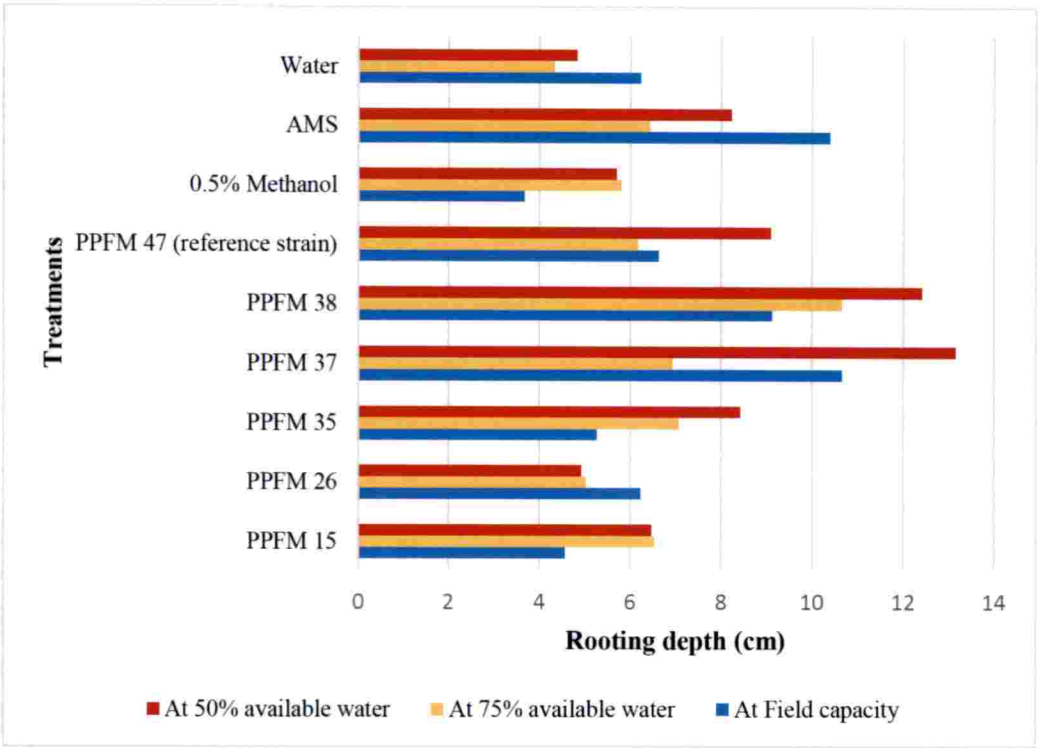

Figure 6. Effect of PPFM isolates on rooting depth at 30 DAT

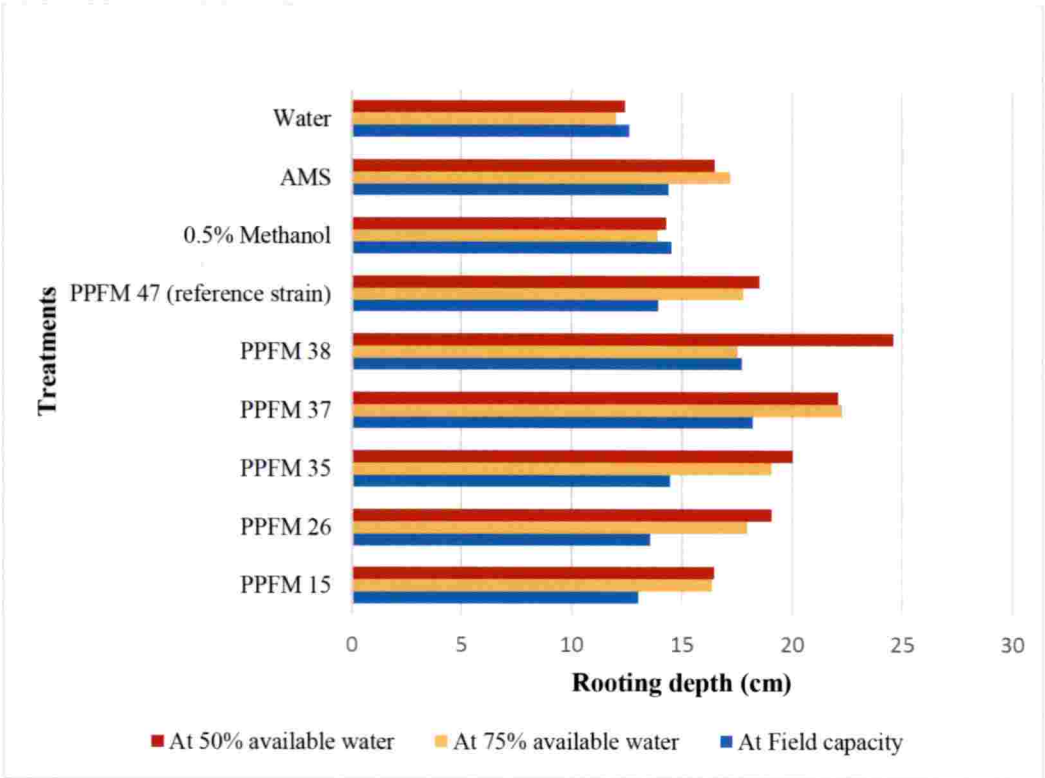

Figure 7. Effect of PPFM isolates on rooting depth at 60 DAT

of the ability to tolerate drought in rice (Cruz *et al.*, 1986). This increment by PPFM might be due to the fact that, *Methylobacterium* are capable of generating plant growth regulators such as auxins and cytokinins (Ivanova *et al.*, 2000) which induce cell division and cell elongation. Drought stress increases the concentrations of ABA in the root, which in turn maintain root growth and increase root hydraulic conductivity, which can postpone development of water stress by increase in water uptake (Gowda *et al.*, 2011). In addition to general plant growth, indole acetic acid (IAA) stimulates stress tolerance because of physical and chemical changes in plant caused by these Plant Growth Promoting Bacteria (Mayak *et al.*, 2004). IAA can improve root proliferation and help plants to accumulate water from the surrounding environment, thereby improving the response to drought stress.

Root shoot ratio can be considered as an important parameter in determining drought tolerance in rice. In the present study, the highest mean root shoot ratio of 0.506 was recorded with PPFM 38 at 60 DAT. Such an increase in root shoot ratio can be linked with maintenance of leaf water status under drying soil. Similar results have already been reported by Boyer (1985) who observed an increase in root shoot ratio under soil moisture deficit. Nysanth (2018) also reported that root shoot ratio of rice seedlings showed significant increase when seeds were treated with PPFM isolates.

In the present study, shoot dry weight of 0.797 g was recorded with PPFM 37 at 30 DAT under maximum water stress condition. But at 60 DAT, the maximum mean shoot dry weight of 1.58 g was recorded with PPFM 37 and PPFM 38. Wahid (2007) reported that high temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane. Kumar *et al.* (2017) reported that *B. altitudinis* FD48 (5.11 mg) showed the highest shoot dry weight followed by *Methylobacterium* spp. (PPFM) (4.43 mg) compared to unicoculated control.

Drought susceptibility index (DSI) is a very important criterion under selection for stress environment, which provides a measure of drought based loss of yield in comparison to moist condition which has been used for screening of drought tolerance genotypes (Brukner and Frohberg, 1987). In the present study, the least mean DSI of 0.78 was recorded with PPFM 37 and the highest in water treated control (Fig. 8). Chandrasekaran *et al.* (2017) also reported that stress tolerance index was more in PPFM and PGRs treated seeds than uniculated control. PPFM bacteria are predominant and explored largely for their ability to release plant-growth regulation molecules (Dourado *et al.,* 2015) and thereby increase the tolerance capacity of plants under drought conditions.

Proline is one of the most important osmolytes that accumulate in plants during severe drought stress (Yoshiba *et al.*, 1997). It not only acts as an osmolyte for osmotic adjustment but also helps to stabilize sub-cellular structures (eg. proteins and membranes). It is also involved in scavenging free radicals and buffering cellular redox potential. In the present investigation, proline content of plants inoculated with PPFM isolates was higher compared to uninoculated control. Proline content of 92.74 µg g^{-1} tissue and 113.06 µg g^{-1} tissue were recorded with PPFM 37 at 30 and 60 DAT under maximum water stress condition (Fig. 9 and 10). Treatment with PPFM 38 resulted in an increase of 173.81 % and 208.81 % over water treated control and 96.10 % and 106.84 % increase over reference strain (PPFM 47) at 30 and 60 DAT respectively.

The positive effect of PPFM might be due to the increment of osmolytes like proline and enhanced water uptake which helped to maintain water status of the plant (Sivakumar *et al.,* 2017). These osmolytes might increase the osmotic pressure of cytoplasm and enhance water flow into the different plant organs and tissues. These researchers reported that foliar application of PPFM (2%) increased the proline content by 11.34 per cent followed by brassinolide (8.34%) and salicylic acid (7.89%) compared to absolute control. *Azospirillum* and arbuscular mycorrhizal inoculation increased the shoot proline content in rice under drought conditions compared to control (Sanchez *et al.*, 2011). The results obtained herein

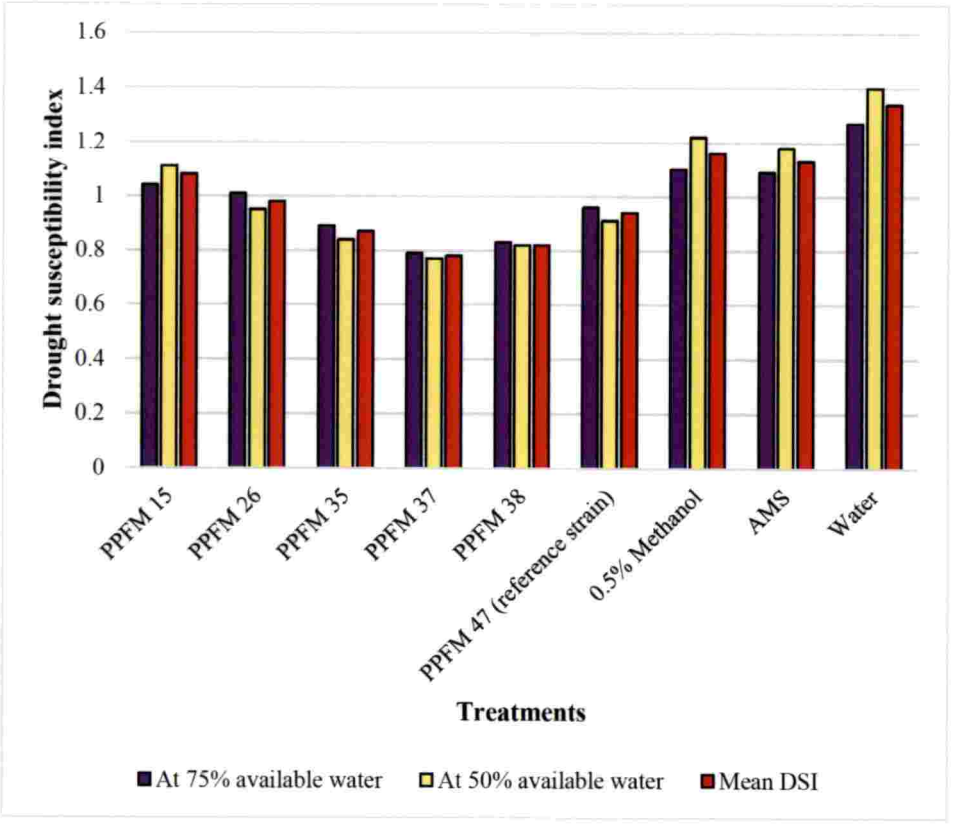

Figure 8. Effect of PPFM isolates on drought susceptibility index

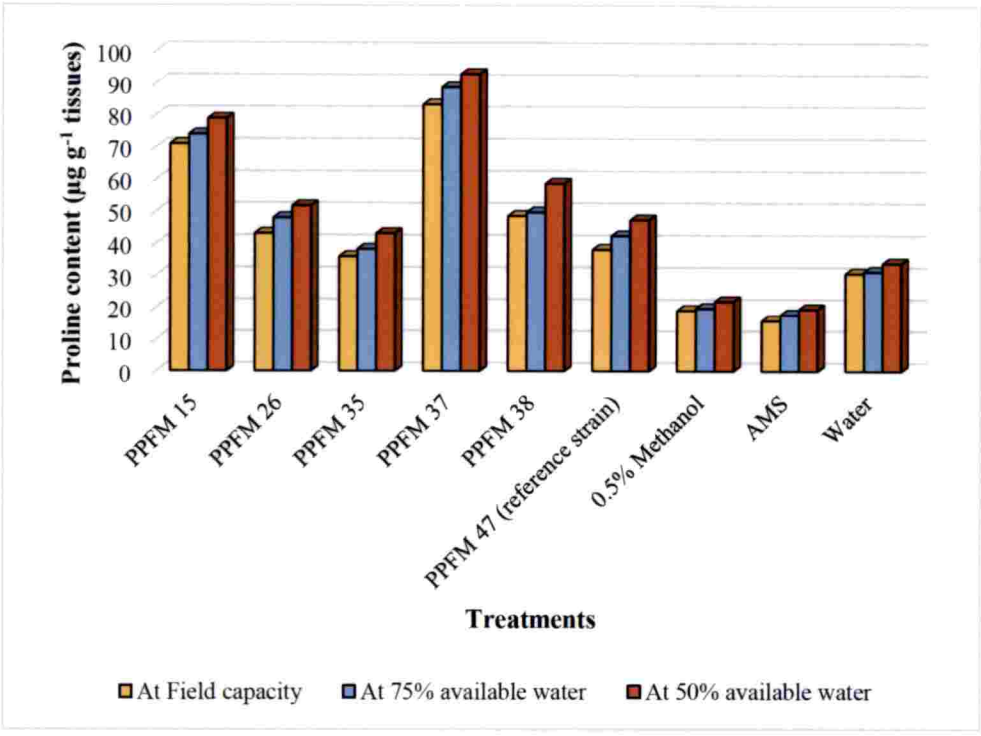

Figure 9. Effect of PPFM isolates on proline content at 30 DAT

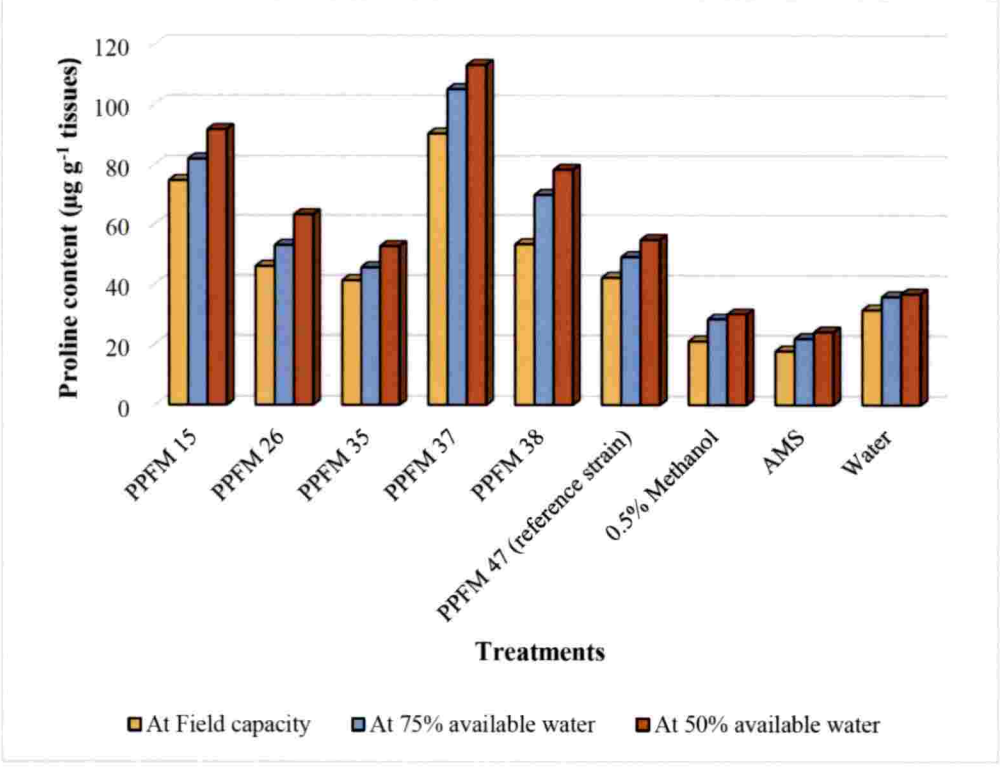

Figure 10. Effect of PPFM isolates on proline content at 60 DAT

are in conformation with the findings of Sivakumar *et al.* (2017) who reported that treatment of plants with *Methylobacterium* spp. lead to an increase in proline content. The inoculation also increased proline content under drought stress compared to control which may be due to up regulation of proline biosynthesis pathway to keep proline in high levels, which helps in maintaining cell water status, protects membranes and proteins from stress (Yoshiba *et al.*, 1997).

Cao *et al.* (2009) explained that high activity of antioxidants in plants might be one of the physiological mechanisms for stress tolerance in rice. Antioxidant enzymes including polyphenol oxidase (PPO), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD) are most important in this respect. In the present study super oxide dismutase, catalase and peroxidase activity were increased under drought conditions. Peroxidases and catalases also play an important role in the fine regulation of reactive oxygen species in the cell through activation and deactivation of several apoplastic enzymes which may also generate reactive oxygen species under normal and stressful conditions (Sairam *et al.,* 2005). In the present investigation, the highest mean SOD of 0.302 activity g^{-1} min^{-1} and 0.312 activity g^{-1} min^{-1} were recorded with PPFM 37 at 30 and 60 DAT. These treatments showed an increase of 25.83 per cent and 26.32 per cent over water treated control at 30 and 60 DAT respectively. Gawad *et al.* (2015) observed that the antioxidant enzymes like catalase and SOD activity were increased by PPFM inoculation in snap bean. In the present investigation, the highest mean catalase of 11.34 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 37 at 30 DAT. Whereas at 60 DAT maximum catalase of 19.84 µg H_2O_2 g^{-1} min^{-1} was recorded with PPFM 37 under maximum water stress condition of 50% AW. These treatments showed an increase of 77.74 % and 154.69 % in catalase over water treated control at 30 and 60 DAT respectively (Fig. 11). Kumar *et al.* (2017) reported that *B. altitudinis* FD48 and *Methylobacterium* spp. (PPFM) treated rice plants showed more catalase activity than control under drought conditions. Infact the control treatment recorded least catalase activity. Chandrasekaran *et al.* (2017) noticed that PPFM (2%) gave

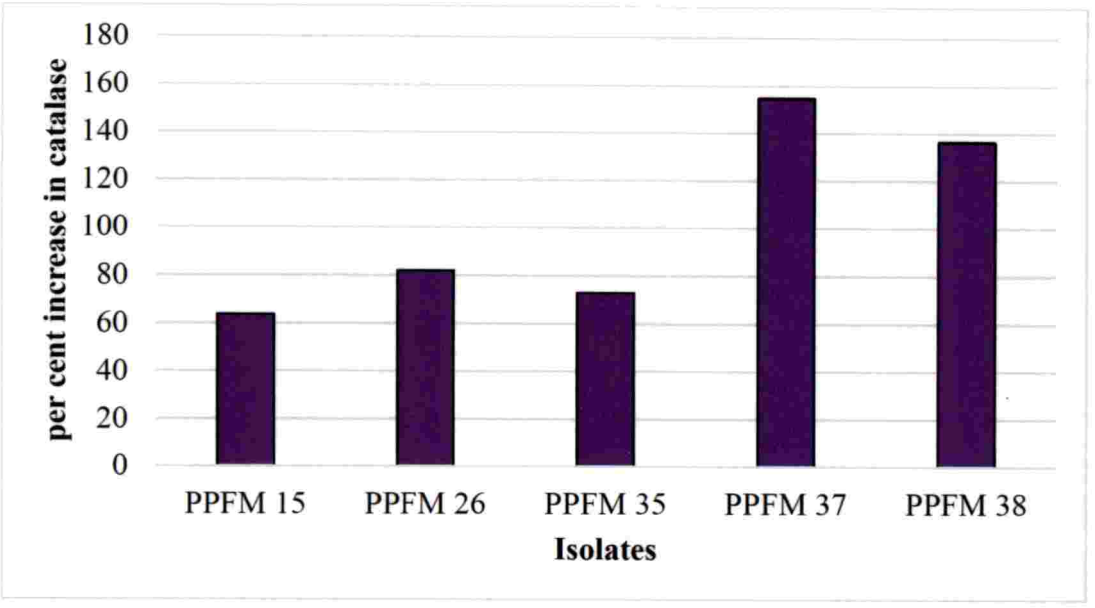

Figure 11. Per cent increase in catalase activity by PPFM isolates at 60 DAT (50% AW) over water treated control

highest catalase activity of 2.96 µg H_2O_2 g^{-1} min^{-1} under stress conditions in tomato.

In the present study, the highest peroxidase of 43.30 activity g^{-1} min^{-1} and 54.67 activity g^{-1} min^{-1} were recorded with PPFM 38 at 30 and 60 DAT under maximum water stress condition of 50% AW (Fig. 12 and 13). These treatments showed 223.86 per cent and 260.38 per cent increase in peroxidase over water treated control and 51.23 per cent and 65.51 per cent increase over reference strain (PPFM 47) at 30 and 60 DAT respectively. Increased activity of peroxidase in stressed seedlings could be correlated to oxidative reactions corresponding to accumulation of peroxides and free radicals in the plant cells (Radotic *et al.*, 2000). PGPRs, *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81 and *Arthrobacter nitroguajacolicus* strain YB3 and strain YB5 used as consortia enhanced plant growth and induction of stress related enzymes (SOD, CAT, peroxidase (POD), APX and lower level of H_2O_2, malondialdehyde (MDA)) in variety Sahbhagi (drought tolerance) and IR-64 (drought sensitive) cultivars of rice (*O. sativa* L.) under drought stress compared to control (Gusain *et al.*, 2015). These studies provide evidence for the beneficial effect of PGPRs application in enhancing drought tolerance of plants by altering the antioxidants activity under water deficit conditions (Gusain *et al.*, 2015). The results of the present study are in agreement with the findings of Shukla *et al.* (2012), Sandhya *et al.* (2011) and Gusain *et al.* (2015) who reported that under conditions of environmental stress, when ROS such as H_2O_2 are produced, catalase enzyme triggered by the bacteria act as scavenging enzymes and play a central role in protecting the cell from oxidative damage.

Water stress at flowering stage is a serious problem that affects yield and yield related traits because it adversely affects pollination, flower and grain development and causes increase in percentage of unfilled grains (Hsiao *et al.*, 1976). Dey and Upadhyaya, (1996) suggested three different critical stages of growth – seedling, vegetative and anthesis, which are highly affected by water stress and reduces the estimates of component characters and finally grain

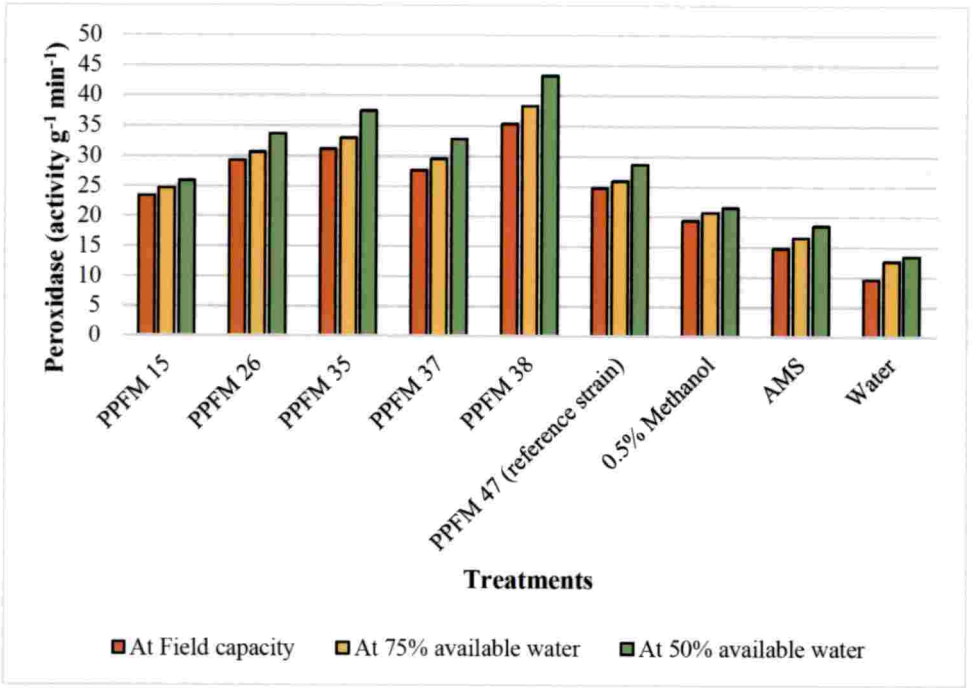

Figure 12. Effect of PPFM isolates on peroxidase at 30 DAT

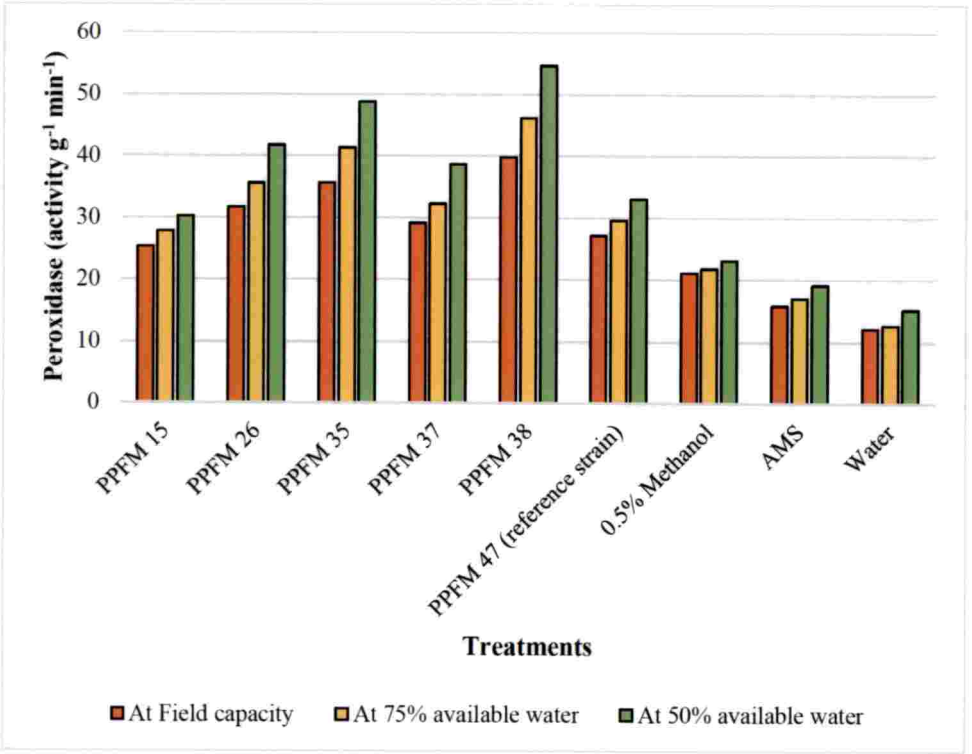

Figure 13. Effect of PPFM isolates on peroxidase at 60 DAT

yield. Lower crop growth rate (CGR) recorded under stress induced at panicle initiation and flowering stage along with control, which might have resulted in lower recovery of the crop and thereby causing reduction in the grain yield (Thangamani, 2005).

In the present investigation, maximum number of panicles per hill of 4.33 was recorded with PPFM 38 under maximum water stress condition of 50% AW. This treatment showed 116.5 per cent increase in number of panicles per hill over water treated control. However, maximum number of grains per panicle of 56.33 was recorded with PPFM 37 under maximum water stress condition. These treatments showed 79.79 per cent increase in number of grains per panicle over water treated control. Maximum grain yield of 5.78 g $hill^{-1}$ was recorded with PPFM 37 under maximum water stress condition (Fig. 14). These treatments showed 67.05 per cent increase in grain yield over water treated control and 16.53 per cent against reference strain (PPFM 47). Nysanth (2018) reported that the application of PPFM isolates significantly influenced the yield and yield attributes of paddy. Senthilkumar *et al.* (2003) also obtained increased yield in paddy due to PPFM inoculation.

In the present study, the lowest mean relative yield reduction of 7.80 per cent was recorded with PPFM 37 (Fig. 15). The yield reduction was 19.5 per cent and 48.5 per cent due to water deficit in vegetative and reproductive stages, respectively, as compared to well-watered plants in maize (Sah and Zamora, 2005). However, maximum straw yield of 4.69 g $hill^{-1}$ was recorded with PPFM 38 under maximum water stress condition of 50% AW. Sivakumar *et al.* (2018) observed that foliar spray of 2% PPFM documented significantly superior fruit yield compared to control under water deficit condition. The reduction in yield components might be due to decrease in translocation of assimilates towards reproductive organs under drought conditions (Rahman *et al.*, 2002).

Considering the major drought tolerance parameters, PPFM 38 was ranked first among the PPFM isolates tested in the pot culture experiment. With respect

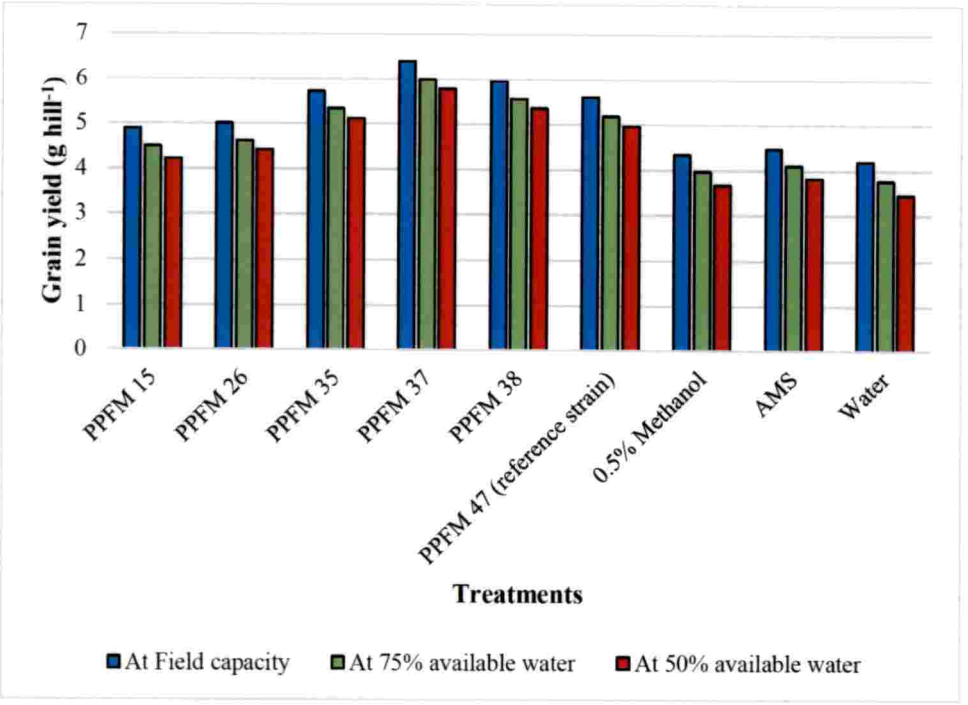

Figure 14. Effect of PPFM isolates on grain yield of paddy

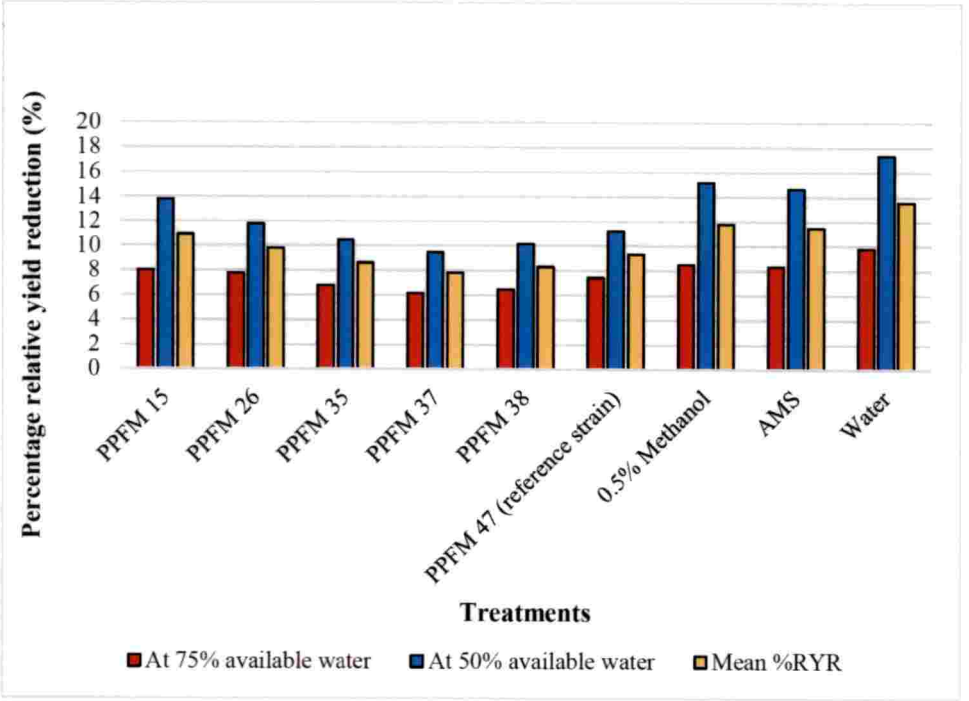

Figure 15. Effect of PPFM isolates on percentage relative yield reduction

to the yield attributes and yield of rice under water stress the effect of PPFM 37 and PPFM 38 were observed to be at par.

Hence, the present study revealed that the isolates PPFM 37 and PPFM 38 (seed treatment 1% PPFM broth culture + seedling dip 2% PPFM broth culture + foliar spray 1% PPFM broth culture at 15 and 30 DAT) were effective in improving the growth, yield and drought tolerance characters of rice.

Summary

6. SUMMARY

The investigation entitled "Screening of Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy" was undertaken in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram during 2017-2019. The main objective of the study was to screen the Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy. The salient findings are summarized below.

In the *in vitro* screening experiment, effect of selected isolates of PPFM on paddy seed germination and seedling growth was tested under maximum water stress condition. Osmotic stress was higher in 3 per cent mannitol treatment. Maximum germination percentage, shoot length and seedling vigour index of 87.50 %, 9.47 cm and 2143.25 respectively were recorded in PPFM 26 treated seeds. Seeds treated with PPFM 15 recorded the maximum root length (18.38 cm) and shoot dry weight (7.40 mg). Maximum root dry weight of 4.50 mg was recorded in seeds treated with PPFM 9. The isolates were assigned top weighted average ranks and PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 which secured ranks from 1to 5 were selected for the subsequent pot culture experiment.

The pot culture experiment was undertaken to study the effect of PPFM isolates on growth and yield of paddy under water stress. The treatments were given as seed treatment, seedling root dip and foliar application at 15 and 30 DAT. The results revealed that PPFM isolates had significant effect on biometric parameters, physiological parameters, yield and yield attributes of paddy under water stress.

Maximum plant height of 44.01 cm and 61.17 cm were recorded with PPFM 38 at 30 and 60 DAT respectively whereas PPFM 37 recorded maximum plant height (85.17 cm) at 90 DAT under maximum water stress condition. Leaf area index of 4.01 and 5.02 were recorded with PPFM 37 at 30 and 60 DAT respectively under maximum water stress condition. Among the different PPFM

isolates tested, the highest mean number of tillers per hill of 5.56 was recorded with PPFM 37 at 60 DAT.

The effect of PPFM isolates on mean leaf rolling score and leaf drying score were found to be the lowest with PPFM 37 at 30 and 60 DAT. Significantly lower leaf temperature was recorded with PPFM 38 at 30 DAT (27.33 ^{0}C) and 60 DAT (30.64 ^{0}C). Significantly higher cell membrane integrity of 85.07 % and 81.07 % observed with PPFM 38 under maximum water stress condition at 30 and 60 DAT respectively. Mean relative water content and mean chlorophyll stability index were the highest with PPFM 38 at 30 and 60 DAT.

Maximum rooting depth of 13.17 cm was recorded with PPFM 37 at 30 DAT whereas PPFM 38 recorded 24.60 cm at 60 DAT under maximum water stress condition. Maximum root weight of 0.273 g and 4.20 g were recorded with PPFM 38 at 30 and 60 DAT respectively under maximum water stress condition. Maximum root volume of 1.067 cm^3 was recorded with PPFM 38 at 30 DAT under maximum water stress condition, whereas highest mean root volume of 5.31 cm^3 was recorded with PPFM 37 at 60 DAT. Maximum root dry weight of 0.037 g was recorded with PPFM 38 at 30 DAT under maximum water stress condition, whereas highest mean root dry weight of 0.798 g was recorded with PPFM 38 at 60 DAT. Shoot dry weight of 0.797 g was recorded with PPFM 37 at 30 DAT under maximum water stress condition. But at 60 DAT, the maximum mean shoot dry weight of 1.58 g was recorded with PPFM 37 and PPFM 38. The highest mean root shoot ratio of 0.506 was recorded with PPFM 38 at 60 DAT.

The lowest mean drought susceptibility index of 0.78 was recorded with PPFM 37. Maximum proline content was recorded maximum with PPFM 37 at 30 and 60 DAT at all the three moisture levels. Mean super oxide dismutase and catalase activity were significantly higher with PPFM 37 at 30 and 60 DAT. Peroxidase activity was significantly higher with PPFM 38 at all moisture levels at 30 and 60 DAT.

PPFM isolate treatments exerted significant effect on yield and yield attributes of paddy under maximum water stress condition. Maximum number of panicles per hill and straw yield were recorded with PPFM 38 under maximum water stress condition, while number of grains per panicle and grain yield were recorded maximum with PPFM 37. The percentage relative yield reduction was lowest with PPFM 37.

Considering the major drought tolerance parameters, PPFM 38 was ranked first among the PPFM isolates tested in the pot culture experiment. With respect to the yield attributes and yield of rice under water stress, the effect of PPFM 37 and PPFM 38 were observed to be at par.

Hence, the present study revealed that the isolates PPFM 37 and PPFM 38 (seed treatment 1% PPFM broth culture + seedling dip 2% PPFM broth culture + foliar spray 1% PPFM broth culture at 15 and 30 DAT) were effective in improving the growth, yield and drought tolerance characters of rice.

In the present investigation, two PPFM isolates were selected based on superior performance of growth and yield of paddy under water stress condition. Further studies on the effect of these isolates on plants are required before developing commercial formulations. Hence the future studies may be focused on the following:

1. The selected isolates will have to be tested under field conditions in different agro ecological zones.
2. Molecular level identification of the selected isolates.
3. Evaluation of the effect of the selected isolates in imparting drought tolerance in other crops.
4. Detailed investigations on mechanism of drought tolerance.

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Appendices

APPENDIX – I

COMPOSITION OF MEDIA USED

1. Ammonium Mineral Salt Media

$(NH_4)_2SO_4$	-	0.5 g
K_2HPO_4	-	0.7 g
KH_2PO_4	-	0.54 g
$MgSO_4.7H_2O$	-	1.0 g
$CaCl_2.2H_2O$	-	0.2 g
$FeSO_4.7H_2O$	-	4 mg
$ZnSO_4.7H_2O$	-	100 µg
$MnCl_2.4H_2O$	-	30 µg
H_3BO_3	-	300 µg
$CoCl_2.6H_2O$	-	200 µg
$CuCl_2.2H_2O$	-	10 µg
$NiCl_2.6H_2O$	-	20 µg
$Na_2MoO_4.2H_2O$	-	60 µg
Distilled water	-	1000 mL

$(NH_4)2SO_4$, K_2HPO_4, KH_2PO_4, $MgSO_4.7H_2O$ and $CaCl_2.2H_2O$ were dissolved in 500 mL distilled water and volume made up to 1000 mL. Then autoclaved at 15 lbs pressure and 121 °C for 15 min. After cooling, all other nutrients (sterilized by filtration through a 0.2 µm pore size membrane filter) were added aseptically, followed by 5 mL of methanol and 10 µg of cyclohexamide were added.

2. Peptone Glycerol Agar

Glycerol	-	10 mL
Peptone	-	10 g
Agar-agar	-	20 g
Distilled water	-	1000 mL

Glycerol and peptone were dissolved in 500 mL distilled water and volume made up 1000 mL. 20 g agar-agar was added into this mixture and autoclaved at 15 lbs pressure and 121 °C for 15 min.

APPENDIX II

Weather parameters during the cropping period (January to June 2019)

Standard week	Mean temperature (^{0}C)		Total rainfall (mm)	Mean RH (%)		Bright sunshine hours	Evaporation (mm)
	Max.	Min.		Max.	Min.		
2 (8 Jan. – 14 Jan.)	31.6	22.1	0.0	92.0	68.6	8.7	3.8
3 (15 Jan. – 21 Jan.)	32.2	20.9	0.0	91.6	68.1	7.8	3.6
4 (22 Jan. – 28 Jan.)	32.0	23.0	0.0	92.1	67.3	9.3	3.5
5 (29 Jan. – 4 Feb.)	32.5	22.1	0.3	92.6	64.6	9.6	4.0
6 (5 Feb. – 11 Feb.)	32.9	24.3	0.1	88.9	67.7	8.2	3.8
7 (12 Feb. – 18 Feb.)	33.3	24.1	0.0	86.7	64.3	9.5	4.2
8 (19 Feb. – 25 Feb.)	35.3	23.4	0.0	87.4	61.3	9.7	4.4
9 (26 Feb.- 4 Mar.)	34.4	24.2	0.0	85.0	62.3	9.4	4.6
10 (5 Mar. – 11 Mar.)	34.6	24.8	0.0	85.4	60.0	9.4	4.7
11 (12 Mar. – 18 Mar.)	34.4	24.4	0.0	85.3	61.3	9.2	4.6
12 (19 Mar. – 25 Mar.)	34.2	24.8	0.0	84.9	61.3	9.2	4.9
13 (26 Mar.- 1 April)	34.8	25.4	0.0	85.7	61.9	8.9	5.2
14 (2 April- 8 April)	35.2	26.0	0.0	83.7	61.6	9.4	5.8
15 (9 April – 15 April)	35.0	25.9	0.0	78.6	61.9	9.3	5.7
16 (16 April –22 April)	34.9	25.6	1.6	82.8	67.3	7.7	4.6
17 (23 April – 29 April)	35.1	25.6	1.0	84.6	63.7	8.4	4.9
18 (30 April – 6 May)	34.0	25.9	2.3	82.7	59.0	6.5	4.3
19 (7 May – 13 May)	34.3	26.2	0.0	80.3	66.9	8.9	5.2

20 (14 May –20 May)	34.5	26.2	0.0	81.3	66.7	9.4	5.5
21 (21 May –27 May)	33.5	26.5	11.9	87.4	73.1	6.9	3.5
22 (28 May –3 June)	33.6	26.7	3.6	90.4	68.6	7.5	4.5

SCREENING OF PINK PIGMENTED FACULTATIVE METHYLOTROPH (PPFM) ISOLATES FOR WATER STRESS TOLERANCE AND YIELD IN PADDY

by

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ABSTRACT

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ABSTRACT

The study entitled "Screening of Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy" was undertaken during 2017-2019, in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram, with the objective to screen the Pink Pigmented Facultative Methylotroph (PPFM) isolates for water stress tolerance and yield in paddy.

The study comprised an *in vitro* screening experiment and a pot culture experiment 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.

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 for the subsequent experiment. Consequently, PPFM 26, PPFM 15, PPFM 38, PPFM 37 and PPFM 35 which secured ranks from 1 to 5 were selected for the pot culture experiment.

The pot culture experiment was undertaken to study the effect of PPFM isolates on growth and yield of paddy under water stress. The experiment was laid out in CRD with 21 treatments and three replications, during summer 2019. The treatments comprised six PPFM isolates (5 KAU isolates of PPFM and one TNAU isolate) and three moisture levels (at field capacity, 75% available water and 50% AW) and three control treatments (0.5% methanol, AMS liquid medium supplemented with 0.5% methanol and absolute control). The treatments were given as seed treatment, seedling root dip and foliar application at 15

and 30 DAT. The study revealed that PPFM isolates had significant effect on biometric parameters, physiological parameters, yield and yield attributes of paddy under water stress.

Maximum plant height and leaf area index was recorded with PPFM 38 at 30 DAT and 60 DAT whereas PPFM 37 recorded maximum number of tillers per hill at 60 DAT. Leaf rolling score and leaf drying score were found to be the lowest with PPFM 37 at 30 and 60 DAT. Cell membrane integrity, relative water content, chlorophyll stability index and root dry weight were the highest with PPFM 38 at 30 and 60 DAT. Rooting depth was the highest with PPFM 38 at 30 DAT and PPFM 37 at 60 DAT. Proline content (at all the three moisture levels) and super oxide dismutase (SOD) were significantly higher with PPFM 37 at 30 and 60 DAT. While at 60 DAT, PPFM 37 recorded significantly higher catalase activity at FC and 50% AW, PPFM 38 was found to be superior at 75% AW. Both these isolates were comparable at the different moisture levels. Crop treated with PPFM 37 also recorded the lowest drought susceptibility index. However, peroxidase activity was significantly higher with PPFM 38 at all moisture levels at 30 and 60 DAT. All the PPFM isolates had significant effect on yield attributes and yield of paddy under water stress. Maximum number of panicles per hill, number of grains per panicle (at all moisture levels), grain yield and the lowest relative percentage yield reduction was recorded with PPFM 37. While, PPFM 37 recorded significantly higher number of panicles per hill at FC and 75% AW, PPFM 38 was found to be superior at 50% AW. Both these isolates were comparable at different moisture levels. Though PPFM 37 recorded higher grain yield at all the moisture levels it was on par with PPFM 38.

Considering the major drought tolerance parameters such as leaf rolling score, leaf drying score, rooting depth, proline content, SOD, catalase and peroxidase, PPFM 38 was ranked first among the PPFM isolates tested in the pot culture experiment. With respect to the yield attributes and yield of rice under water stress the effect of PPFM 37 and PPFM 38 were observed to be at par.

The present study revealed that the isolates PPFM 37 and PPFM 38 (seed treatment 1% PPFM broth culture + seedling dip 2% PPFM broth culture + foliar spray 1% PPFM broth culture at 15 and 30 DAT) were effective in improving the growth, yield and drought tolerance characters of rice.