

Mitigation of Water Scarcity with Sustained Growth of Rice by Pink Pigmented Facultative Methylophiles (PPFMs) Bacteria

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ABSTRACT

Pink pigmented facultative methylophiles (PPFMs), ubiquitous in nature, one such an organism of paramount importance of PPFMs which is capable of mitigating the effects of drought on crops and thereby improving its growth and productivity. PPFMs are bacteria capable of growing on C1 compounds like methanol, formaldehyde and methylamine; commonly found in soils, leaf surface, seeds and in the rhizosphere of a wide variety of plants, with highest numbers present on actively growing meristematic tissue. It produces plant growth regulators such zeatin, cytokinins and auxins which have an impact on seed germination and seedling development. PPFMs are known to improve plant growth by adopting various mechanisms viz., nitrogen fixation, nodule formation, phosphate solubilisation, production of urease enzyme and vitamin B12, plant growth regulators and

synthesis of siderophores. PPFMs promote plant growth by producing an enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase is responsible for drought management during the beneficial interaction with plants. PPFMs is responsible for a variety of beneficial effects on plants, including accelerating vegetative growth by producing phytohormones, increasing leaf area index, chlorophyll content, advancing flowering and fruit maturation by a few days, enhancing fruit quality, colour and seed weight, 10% yield enhancement and also mitigating the drought. Methylo-trophic bacteria generate 1-aminocyclopropane-1-carboxylate (ACC) deaminase and encourage plants to create antioxidant enzymes and osmolytes, which aid in reducing the stress caused by drought.

INTRODUCTION

Pink Pigmented Facultative Methylo-trophs, commonly abbreviated to PPFMs, are bacteria that are members of the genus *Methylobacterium* and are commonly found in soil, dust, various fresh water supplies and on plant surfaces. PPFMs are specially selected beneficial microbes that establish a natural, permanent partnership with plants. They were first isolated as covert contaminants from the tissue cultures of liverwort, *Scapania nemorosa*, but later identified as *Methylobacterium*. The *Methylobacterium* are known to metabolize methanol but also a limited number of alternative carbon substrates, such as organic acids and alcohols.

There is evidence that methanol is consumed by *Methylobacterium* and contributes to the epiphytic fitness of the organism. However, in addition to the peak of methanol emission in the morning, *Methylobacterium* should adapt its metabolism to use additional carbon sources during the rest of the day when methanol emission is low or during the night when stomata are closed and methanol is consequently no longer available. PPFMs are especially abundant on leaves of field grown crops averaged about 10⁶ cfu of PPFMs per leaflet and typically >80% of the viable bacteria recovered from leaves. The occurrence of soil methylo-trophs is probably related to the abundance of plant lignin and pectin in soils; these polymers are major potential sources of methanol.

Although gram negative, *Methylobacteria* often stain gram variable and are easily isolated using methanol-based mineral medium. Their pigmentation, which is

frequently pink but may also be yellow or orange, is thought to provide protection from solar UV radiation which damages the DNA of bacteria at low doses because of their small cell size (Anandakumar Selvaraj, 2021). This colour is present due to the carotenoid pigments within the cell. *Methylobacterium* is composed variety of Pink Pigmented Facultative Methylo-trophic (PPFMs) bacteria, which can be grow on one carbon compounds such as formate, formaldehyde and methanol as sole source of carbon and energy as well as on a wide range of multi-carbon growth substrate. Aerobic, gram-negative bacteria known as methylo-trophs PPFMs can promote the growth and development of plants by synthesising hormones such cytokinin, zeatin and IAA. PPFMs could be potential bio-inoculants to increase plant growth and drought stress tolerance in sustainable agricultural ecosystem.

Production of Hormones and ACC Deaminase

The presence of ethylene in plants disrupts a variety of biotic and abiotic processes that are essential for plant growth and production. High ethylene levels in plants have negative impacts. Ethylene interacts with other plant hormones including auxin and abscisic acid in addition to affecting photosynthesis and stomata conductance when plants are treated with PPFMs that produce the ACC deaminase enzyme, the ACC is broken down into ammonia and alpha ketobutyrate (Madhaiyan et al., 2004). Under stressful conditions, plants produce less ethylene due to the bacterial ACC deaminase enzyme. Moreover, it is capable of producing phytohormones including auxins,

cytokinins and gibberellic acid that encourage cell division in roots and shoots (Chinnadurai et al., 2009).

Reactive Oxygen Species Inhibition

During stress, plants release reactive oxygen species (ROS), which interact with proteins, lipids and DNA to cause oxidative damage and impair normal plant processes. To overcome the negative effect of ROS, plants develop antioxidant defense systems, comprising both enzymatic and non-enzymatic components that serve to prevent ROS accumulation and alleviate the oxidative damage occurring during drought stress (Anandakumar Selvaraj, 2021). Superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase are really a few of the enzymes and non-enzymatic components that PPFMs spray causes plants to produce to combat ROS, relieving the plant of its drought stress (Sivakumar et al., 2017). 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.

Application methods of PPFMs

- Seed treatment: Imbibe the seeds in 1.0% volume for 5-10 mins.
- Foliar spray of 1% PPFMs
- Spray during morning or evening
- Recommended for all crops
- Spray at critical stage of crop growth (or) 30 days interval

Precaution: Do not mix with pesticide / fungicide

Drought mitigation techniques in rice with PPFMs spray

The PPFMs is widely used in rice crops by recommended spraying of bacteria to mitigate drought and save crops. Application of PPFMs helps the rice crops remain green for 15 to 20

days and it's helpful only during the terminal stages of the crops such as boot leaf stage and flowering stage after which panicle initiation would take place (Anandakumar Selvaraj, 2021). During drought condition, the exogenous methanol of plant stimulated the growth of *Methylobacterium* spp, which provided plants with cytokinins and auxin which inturn enhanced plant development and ultimately increased the rice yield. This might be due to the supply of cytokinin by PPFMs. Foliar application of PPFMs prevents the chlorophyll breakdown under drought thus leading to retention of chlorophyll and delay of senescence in rice. Chlorophyll stability index of PPFMs was significantly higher that it can be a good choice for the organic rice growers under drought situation. Rice seeds treated with inoculated *Methylobacterium* spp., strain PPFMs-Os-07 expressed increased plant height, number of tillers, plant biomass and grain yield by over 24% than the control (Madhaiyan et al., 2004). Application of 0.1% PPFM as foliar spray in transplanted rice record higher growth attributes, improved root growth and activity, which ultimately enhanced the yield attributes and grain yield. Apart from that wetland rice contributes upto 20% of the global CH₄ emissions. CH₄ emissions will need to be reduced by microbial CH₄ oxidation (as happens with *Methylobacterium*).

Effects of PPFMs and PGRs on Proline

Proline, an amino acid plays a highly beneficial role in plants exposed to various stress conditions. Besides acting as an excellent osmolyte, proline plays three major roles during stress, as a metal chelator, an antioxidative defence molecule and a signalling molecule. Water stressful environment results in an overproduction of proline in plants which in turn imparts stress tolerance by maintaining cell turgor or osmotic balance, stabilizing membranes thereby preventing electrolyte leakage and bringing concentrations of reactive oxygen species (ROS) within normal ranges, thus preventing oxidative burst in plants. Proline accumulation is a significant response of plant under drought stress. 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. Among the PGRs, the foliar application of PPFMs (2%) increased the proline content by 11.3% followed by brassinolide (8.3%) and salicylic acid (7.8%) to absolute control. This might be due to the fact that PGRs and PPFMs reduced the impact of stress leading to high level of proline accumulation (Sivakumar et al., 2017). Proline acts as a compatible solute and a protective agent for cytoplasmic enzymes and structures. 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.

CONCLUSION

Methylobacterium, known as pink pigmented facultative methylophilic bacteria play multifunctional positive role ranging from moisture stress mitigation by regulation of ethylene level in crop plants, seed germination to plant growth and development. This bio-inoculant can be exclusively recommended for rainfed and dryland farming systems. Agricultural production has been declined year by year due to many abiotic stresses especially drought. Of the various management practices available, mitigation drought in rice through PPFMs spraying are the promising techniques to enhance water status of the plant, photosynthetic rate, compatible osmolytes like proline and antioxidant enzyme like catalase activity which protects the plant under any abiotic stress condition. Similarly, methylophilic bacteria also offer drought-tolerant mechanisms by nitrogen fixation, phytohormones production, ACC deaminase production and phosphate solubilization. Therefore, 10% increase in production is achieved by foliar spraying of 1% PPFMs in rice to protect the plant from drought. PPFMs

could be potential bio-inoculants to increase plant growth and drought stress tolerance in sustainable agricultural systems.

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