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Methylobacterium: A Foliar Bioinoculant for Barnyard Millet

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Abstract

India is the biggest producer of barnyard millet, both in terms of area (0.146 m/ha) and production (0.147 mt) with average productivity of 1034 kg/ha during the last 3 years. Barnyard millet is primarily cultivated for human consumption, though it is also used as a livestock feed. Among many cultivated and wild species of barnyard millet, two of the most popular species are *Echinochloa frumentacea* (Indian barnyard millet) and *Echinochloa esculenta* (Japanese barnyard millet). Barnyard millet is a short duration crop that can grow in adverse environmental conditions with almost no input and can withstand various biotic and abiotic stresses. *Methylobacterium* popularly called as PPFM (pink pigmented facultative methylotroph) found to colonize the plant surface ubiquitously. The foliar application of PPFMS and seed imbibitions improves the germination percentage, plant growth and yield characters under dryland conditions.

Introduction

Barnyard millet (*Echinochloa* species) has become one of the most important minor millet crops in Asia, showing a firm upsurge in world production. The genus *Echinochloa* comprises of two major species, *Echinochloa esculenta* and *Echinochloa frumentacea*, which are predominantly cultivated for human consumption and livestock feed. They are less susceptible to biotic and abiotic stresses. Barnyard millet grain is a good source of protein, carbohydrate, fiber, and, most notably, contains more micronutrients (iron and zinc) than other major cereals. Despite its nutritional and agronomic benefits, barnyard millet has remained an underutilized crop. Over the past decades, very limited attempts have been made to study the features of this crop.

Barnyard millet is gaining recognition as a health food due to its high protein, minerals and fibre content. This makes it suitable for the preparation of commercial foods, especially for diabetic patients, patients with gluten intolerance, infants and pregnant women. In India, barnyard millet is grown up to 2300 m from mean sea level (MSL) predominantly in kharif season in the hilly tracts of Tamil Nadu states. Like other small millets, barnyard millet also is gaining attention as a health food. Traditionally it was used as a substitute for rice in Northern India. Traditional foods prepared from barnyard millet such as *idli*, *dosa* and *muruku* are very popular in Southern India. Recently, it is being used for developing various value-added products such as biscuits, sweets, vermicelli, ready mixes, multi-grain *atta* (flour), *etc*. As a health food, it is highly suitable and recommended for infants, pregnant women and people with diabetes. In addition, it has important use as cattle feed due to the high palatability of its fodder, which can be used for making hay or silage. Barnyard millet

(*Echinochloa frumentacea*) has the least maturation time of 45-70 days among millets, which is half to the rice maturation (120-140 days) time. Millets fall under the group of C4 cereals. C4 cereals take more carbon dioxide from the atmosphere and convert it to oxygen, have high efficiency of water use, require low input and hence are more environment friendly (Gomashe, 2017).

Biotic stresses such as insect pests and diseases are a cause for substantial yield losses to diverse types of millets. However, abiotic stresses are the biggest contributor to losses every year. Although, in general, millets perform better than cereals such as wheat and rice in semi-arid environments, these challenging climatic and soil conditions are by no means an optimum environment for millet cultivation. In semi-arid and arid environments where millets are the dominant crop, drought or inadequate moisture is the major abiotic stress affecting productivity.

Small millets are genetically diverse and grown where major cereals fail to produce satisfactory. Among small millets group, barnyard millet has emerged as very important feed as well as fodder crop. Barnyard millet grains are nutritious as similar to other millets. However, productivity of the crop is reduced due to the number of factors *viz.,* non-availability of high yielding variety, quality seeds, diseases (*Helminthosporium)* leaf spot, grain smut, head smut, sheath blight) and insect pest attack. Among these factors, diseases alone cause 63.5% reduction of grain yield (Renganathan *et al.,* 2020).

Responses to Biotic and Abiotic Stresses

The *Echinochloa* species generally has potential resistance against various biotic and abiotic stresses. However, cultivated species such as *E. esculenta* and *E. frumentacea* are widely threatened by pest and diseases (*i.e.*, shoot fly, stem borer, grain smut, and loose smut) at different growth stages of the crop. Aphid's infection at the vegetative stage causes considerable yield reduction to *E. frumentacea*. So far, DHBM 996 and TNEF-204 were found to be resistant genotypes for shoot fly and stem borer.

Echinochloa species are also the preferable choice of farmers for cultivation in various adverse environments such as those prone to drought or flooding. These features showed that the *Echinochloa* species might have some specialized rhizosphere organizations that can facilitate the uptake and release of oxygen (O_2) from their roots at stressful conditions. Barnyard millet sustained and increased the water use efficiency, leaf area index, and dry matter production in both drought and flooding conditions. Therefore, it is also worth investigating the *Echinochloa* species mechanism behind the tolerance to drought and flooding stress.

It is also well known for its excellent nitrogen-use efficiency over cereal crops and has been recommended as a natural phyto-extractor in heavy metal (lead, cadmium, and chromium) contaminated soils and sodic soils due to hyper accumulation nature. Since heavy metals are currently of much environmental concern, phyto based soil reclamation is an alternative, cost-effective, and eco-friendly approach.

Biofertilizers Application

Microorganisms offering one or more of these mechanisms can directly stimulate plant growth. There are some reports on Rhizobia infecting and colonizing the roots of cereals and other nonleguminous crops and producing a large number of plant growth promoting molecules, such as auxins, cytokinins, abscisic acid, lumichrome, riboflavin, lipochito-oligosaccharides, and vitamins.

The use of chemicals to control diseases in crops like barnyard millet seems to be uneconomical. However, these problems can be overcome by using bio-agents like *Pseudomonas fluorescens*, *Trichoderma harzarium*, *Aspergillusniger*, *Fusarium moniliforme,* because bio-agents having bio-control and plant growth promoting (PGP) activities may be a viable alternative to minimize use of synthetic chemicals and their hazardous effects, to provide protection to the plants against resident pathogen populations.

Bio-fertilizer can be an important component of integrated nutrients management. Microorganisms that are commonly used as bio-fertilizer components include; nitrogen fixers (N-fixer), potassium and phosphorus solubilizers, growth promoting rhizobacteria (PGPRs), endo and ecto mycorrhizal fungi, cyanobacteria and other useful microscopic organisms. The use of bio-fertilizers leads to improved nutrients and water uptake, plant growth and plant tolerance to abiotic and biotic factors. These potential biological fertilizers would play a key role in productivity and sustainability of soil and also in protecting the environment as eco-friendly and cost effective inputs for the farmer.

Methylobacterium

Methylobacterium are ubiquitous in nature found in a variety of habitats. Bacteria of the genus Methylobacterium are also called as, pink-pigmented facultative methylotrophic bacteria (PPFMs). They are strict aerobic, gram-negative rods, able to grow on C1 compounds. Methylotrophs have been reported to influence seed germination and seedling growth by producing plant growth regulators like zeatin and related cytokinins, auxins and to alter agronomic traits like branching, seedling vigour, rooting and heat/ cold tolerance. These PPFMs are especially abundant on leaves of field-grown crops averaged about 10^6 cfu of PPFMs per leaflet, and typically >80% of the viable bacteria recovered from leaves were PPFMs. The occurrence of soil methylotrophs is probably related to the abundance of plant lignin and pectin in soils; these polymers are major potential sources of methanol.

The PGPMs contribute to mitigate the stress conditions by diverse mechanisms. The PGPMs directly enhancing the uptake of the micronutrients, through phytohormones production; fixing of atmospheric nitrogen; P, K, and Zn-solubilization or indirectly stimulating the immune system against various fungal pathogens by production of various compounds, enzymes, siderophores, antibiotics, osmolytes or improving either texture or structure of the soil.

Phyllosphere PPFM helps in augmenting the plant productivity. In the present study, increases in the number of PPFMs were positively correlated with the yield increases in kudiraivali. The beneficial role of PPFMs, when applied as seed inoculant in enhancing germinability, storability or vigour of the seeds was already attributed to their ability to produce the plant growth regulators like Indole acetic acid and ultimately to increased plant growth and yield and also having biocontrol ability. The foliar application of Methylobacterium had significantly improved the overall performance of the tomato plant growth and yield over uninoculated plants.

Methylotrophs are generally desiccation tolerant. Desiccation tolerance is desirable for production of microbial products where a rapidly soluble powder formulation is acceptable. Other Methylotrophs strains persist in a low water activity state and are therefore compatible with conventional emulsions and suspensions used for seed treatment. Many Methylotrophs contain hopanoid and trehalose biosynthesis genes which are both known to contribute to desiccation tolerance (Senthilkumar *et al.,* 2017). Experiments on Methylotrophs mixed with commonly used agricultural chemistries (insecticides, fungicides, herbicides) demonstrate very high tolerance and compatibility.

Conclusion

Exogenous application of PPFM produces some benefit in alleviating the adverse effects of drought stress and also improves germination, growth, development, quality and yield of crop plants. Foliar application of PPFM prevents the chlorophyll breakdown under drought leads to retention of chlorophyll and delay of senescence in kudiraivali. The barnyard millet seed Imbibition + 1% Foliar spray of *Methylobacterium* at 30 & 60 DAS recorded the highest plant growth and yield parameters under rain fed condition.

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