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RESEARCH ARTICLE

# ApoplastAssociated *Bacillus methylotrophicus* RABA6 Induced Growth and Yield Attributes Leading to Drought Tolerance in Rice

**Punitha, S.[1*], Kalarani M.K.[2] and Sivakumar Uthandi[3]**

[1] Department of Crop Physiology, Tamil Nadu Agricultural University,Coimbatore– 641003
[2]Tapioca and Castor Research Station, Yethapur, Salem – 636119
[3] Biocatalysts lab, Department of Agricultural Microbiology,Tamil Nadu Agricultural University, Coimbatore – 641003

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## ABSTRACT

The present investigation was conducted with anobjective to evaluate the efficiency of anapoplastic bacterial isolate,*Bacillus methylotrophicus* RABA6 to alleviate moisture stress in rice variety CO51.The seeds were biotized with *B. methylotrophicus* RABA6, @$10^6$CFU $ml^{-1}$ with an $OD_{660}$ of 1.0. Irrigation was withheld for ten days during panicle initiation to impose moisture stress and biometric observations were recorded. The results showed significant differences both in vegetative growth parameters as well as yield attributes compared with the uninoculatedcontrol under moisture stress. During panicle initiation stage (85th DAS), the plant height (105.26, 104.36 cm), root length (29.57, 28.23 cm), number of productive tillers $plant^{-1}$(12.34, 10.75), leaf area (1113.75, 796.52$cm^2$ $plant^{-1}$), total number of panicles/ plant (15.63, 13.69) and1000 grain weight (23.65, 22.19 g) registered more in *B. methylotrophicus* RABA6 inoculated plants compared touninoculated control under both irrigated and moisture stressed conditions respectively. The present study envisages that the inoculation of rice with the apoplastic bacterium *B. methylotrophicus* RABA6 could promote general plant health and productivityunder moisture stress. Hence,the apoplastic bacterium *B. methylotrophicus* RABA6 can be recommended as a bio-inoculant for alleviating drought stress in rice and reduce the dependency on chemical fertilizers thereby, providing a step forward towards sustainable agriculture.

**Keywords***: Apoplast, Bacillus methylotrophicus, rice, growth, yield, drought*

## INTRODUCTION

The contemporary climate and increasing demand for limited fresh water threaten agriculture. Rice (*Oryzasativa*),being a C3 plant, is very sensitiveto even milder water stress both at vegetative and reproductive stages (Ray *et al*., 2015). In Asia alone, water stress affected nearly 23 million hectares of rice growing area (Serraj*et al*., 2011).As an important food crop thatfeeds more than one-half of the world's population, unstable rice production due to recurring drought can have a potential global socioeconomic impact. In the face of these challenges, enhanced rice yield under normal, as well as stress conditions, is an ideal trait that will have a huge impact on rice productivity.Plant scientists are in the urge to develop strategies to cope up with these challenges. Since the breeding and biotechnological approaches are time-consuming, use of bioinoculants can be an eco-friendly alternative to mitigate the abiotic stress.

The apoplast is a distinct extracellular compartment in plant tissues that lies outside the plasma membrane and includes the cell wall. It is a dynamic compartment because it continuously changes during growth and development. Microorganisms have the ability to multiply to high densities in the apoplast, which they access through natural openings such as stomata or through wounds (Rico *et al*., 2009). Bacteria that inhabit the plant tissues as endophytes are not harmful toplant growth. These endophytes can display potentials to improve plant growth by augmenting a symbiotic environment.The beneficial effects of the apoplasticmicrobiome arepoorly understood when compared with general endophytes in the root system and phyllosphere. Unlike biotic stress tolerance, abiotic stress tolerance mechanisms are not yet elucidated. Such endophytes harbouringapoplast region mediates communication with the pathogen and plant plasma membrane as well, towards maintaining healthy crop plants. In order to understand the role of apoplast associated bacteria on rice crop, the present investigation was aimed at studying the effect ofapoplastic strain,*Bacillus*

*Corresponding author's e-mail: usivakumartnau@gmail.com

*methylotrophicus* on growth and yield improvement in rice under moisture stressed conditions.

## MATERIALS AND METHODS

### *Leaf sample collection*

Leaf samples of three drought tolerant rice cultivars (CO 51, Anna (R) 4, IR 64 DRT) and one indigenous landrace (Nootripathu) used in this study were obtained from the core germplasm collection of Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

### *Plant materials, microbes and stress conditions*

Apoplast associated bacteria, *B. methylotrophicus* RABA6 isolated and identified as plant growth promoting and moisture stress protecting bacteria is used in this study to evaluate its effect on rice. Seeds of rice variety CO51 were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore. Healthy seeds were surface sterilized with sodium hypochlorite for 10 min and washed with sterile distilled water. The seeds were soaked in sterile distilled water over-night for sprouting. The bio-inoculant *B.methylotrophicus*RABA6was grown in Nutrient broth at 28°C for 16 h. The sprouted paddy seeds were biotizedwith the cell pellet of *B.methylotrophicus*RABA6 and allowed to shade dry. Seeds were grown in a container filled with organic soil and irrigated regularly with water under a 12 h photoperiod at temperatures ranging from 25 to 29°C and the relative humidity of 70–75% in the glasshouse, Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore.

The pot culture experiment withtwo treatments in four replicationswere maintained in both irrigated and moisture stressed conditions.Morphological observations were recordedduringa drought period. Yield and yield components were recorded during harvest.Moisture stress was imposed at the panicle initiation stage, by withholding water for 10 days. The control plants under irrigated conditionswere watered daily to 100 % field capacity (FC). The treatment details are as follow:

$T_1$ : Control

$T_2$ : Seed biotization with *Bacillus methylotrophicus* RABA6

### *Morphological and plant growth traits*

### *Plant height*

Plant height was measured from the ground level to the tip of the growing point and expressed as cm.

### *Root length*

Rice plants were carefully uprooted from the pot with minimum root damage. The roots were washed carefully and the distance from the base of the shoot to the tip of the longest root was measured and the average was calculated for three plants in each replication and expressed in cm.

### *Number of productive tillers plant$^{-1}$*

In each treatment, three hills were selected at the time of panicle initiation, labeled and a number of panicle producing tillers hill$^{-1}$ was counted and the average value was recorded.

### *Leaf area*

Leaf area per plant was measured using a Leaf Area Meter (LICOR, Model LI 3000) and expressed as cm$^2$ plant$^{-1}$.

### *Days to 50 % flowering*

Days to flowering (the number of days from seedling emergence to 50% flower opening) was recorded for 3 plants per pot and represented as DAS.

### *Total Number of Panicles/ Plant*

A number of panicles per plant was counted in three plants and average values areexpressed.

### *Thousand-grain weight*

One thousand filled grains were sampled from each plant and weighed at 14 per cent moisture content and expressed in g.

### *Statistical analysis*

Factorial Completely Randomized Design (FCRD) analysis was carried out on various parametersfrom pot experiments(Gomez and Gomez, 1984) in AGRES software package.Wherever the treatment differences are found significant, critical differences were worked out at 5% probability level and the values with respective standard errors of means are furnished.

## RESULTS AND DISCUSSION

Apoplast associated bacterial strain,*B. methylotrophicus*RABA6 isolated fromtheapoplastic fluid of rice variety Anna (R) 4 leaves wereused in this pot culture experiment to evaluate its effect on drought protection and growth promotion in rice crop.The results obtained from the present study revealed that there was a significant difference between the *B.methylotrophicus*RABA6 inoculated and uninoculated control plants. *B.methylotrophicus*RABA6 registered higher plant height (105.2, 104.3cm) and root length (29.5, 28.23 cm) when compared with uninoculated control under both irrigated and moisture stress conditions respectively (Fig.1). The positive response in morphological attributes in *B.methylotrophicus*inoculated plants,might bedue to the additional effect of IAA from microbial origin, which is responsible for cell proliferation

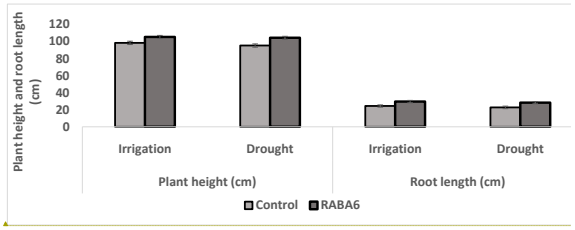


**Fig.1. Effect of *Bacillus methylotrophicus*RABA6 on plant height and root length of rice variety CO51**

and elongation. Similar results were reported by Timmusk*et al.* (2014)where, wheat plants treated with *Bacillus thuringiensis*AZP2 had three times longer root hairs and longer, denser lateral roots. Also,Vardharajula*et al.,* (2011) observed improved shoot growthin corn plants inoculated withplant growth promoting *Bacillus* spp.

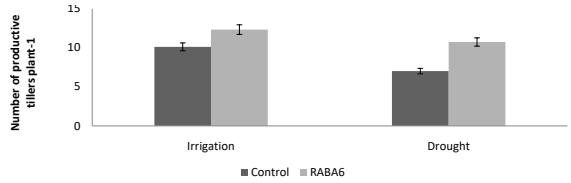


**Fig. 2. Effect of *Bacillus methylotrophicus*RABA6on Number of productive tillers plant$^{-1}$ of rice variety CO51**

The findings of the present study also agree with Ashrafuzzaman*et al*. (2009) and Isahak*et al*. (2012), that biofertilizers significantly improved rice plants growth by increasing the soil nutrients such as nitrogen and phosphorus.The increased nutrient levelsinfluence rice seedlings growth and significantly enhancing plant height which leads to high photosynthesis rate. Similarly, Chi*et al*. (2005) observed an increase in plant height up to 23.63% over un-inoculated control in rice and argued indole acetic acid and gibberellins production as the key mechanism for growth promotion.

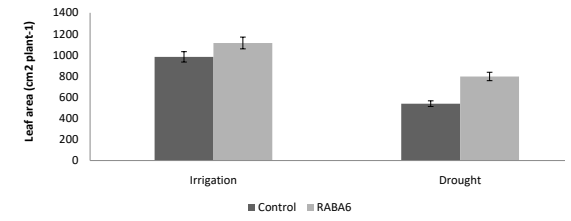


**Fig.3. Effect of *Bacillus methylotrophicus*RABA6on leaf areaof rice variety CO51**

Furthermore, in general,moisture stress reduced the number of productive tillers (10.75, 7.02) and leaf area (796.52, 538.78cm$^2$ plant$^{-1}$) in both *B.m ethylotrophicus*RABA6inoculated and uninoculated plants. However, withinthe treatment under both irrigated and moisture stressed condition,*B. methylotrophicus*RABA6 inoculation registered an increase in the number of productive tillers and leaf area when compared to uninoculated control (Fig.2&3).Such improved response might be due to phytohormone production and enhanced nutrient uptake facilitated by *B.methylotrophicus*RABA6. Dar and Bali (2007) also stated that application of biofertilizers on rice under low land conditions proved significantly beneficial in increasing the number of leaves and improving leaf area index (LAI) and all yield attributing aspects. According to Kim (2013),higher tiller numberin pepper plants treated with *Bacilluslicheniformis* K11, when exposed to drought stress was attributed due to the growth promoting the ability of *B. licheniformis* K11. The similar findings observed in the present investigation might be due to the plant growth promoting attributes of the apoplastic bacterium *B. methylotrophicus* RABA6, more specifically phytohormone production.

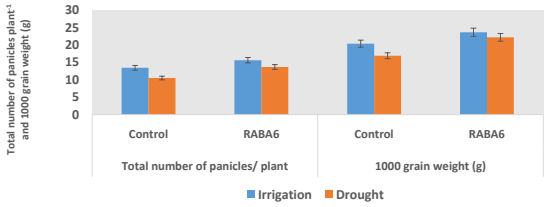


**Fig.4. Effect of *Bacillus methylotrophicus*RABA6on total number of panicles and thousand grain weightof rice variety CO51**

The drought stress at vegetative growth especially booting stage (Pantuwan*et al.*, 2002), flowering and terminal periods can interrupt floret initiation, causing spikelet sterility and slow grain filling, resulting in lower grain weight and ultimately poor yield (Kamoshita*et al*., 2004). In general, moisture stress reduced the total number of panicles and 1000 grain weight in both *B.methylotrophicus*RABA6 inoculated (13.69, 22.19g) and control plants (10.52, 16.91 g) respectively. Among the treatments,*B.methylotrophicus*RABA6 inoculated plants showed more panicle number and grain weight than uninoculatedcontrol (Fig. 4). Under the irrigated situation, both uninoculatedcontrol and *B.methylotrophicus*RABA6 inoculated plants,attained 50% flowering on 85 to 86DAS.On the other hand, *B.methylotrophicus*RABA6 inoculated plants showed early flowering when compared with uninoculated control plants under moisture stressed condition (Fig. 5).*B. methylotrophicus*RABA6 inoculated plants registered 13.9 and 23.7% yield improvement in terms of thousand-grain weight under irrigated and drought conditions respectively.This shows that*B. methylotrophicus*RABA6 played a predominat role in advancing the days to 50% flowering by the active role of phytohormones, enhanced P uptake and accumulation of photosynthetic assimilates. The results corroborate with the findings of Peng*et al.* (2002) where, biofertilizer inoculation registered 27.11% increase ina number of panicles per plant

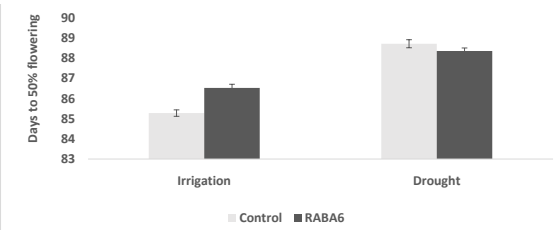


**Fig.5. Effect of RABA6 *Bacillus methylotrophicus*on days to 50%of rice variety C051**

over the uninoculated control in rice and improved the yield under drought. The overall performance in the yield attributes of *B.methylotrophicus*RABA6 treated plants is the resultant effect of nutrient and water uptake, root growth, hormonal regulation and well-balanced partitioning of photosynthetic assimilates.

## CONCLUSION

The apoplastis the primary sites of endophyte invasion and harbors several beneficial microbes. The apoplastic bacteria *B.methylotrophicus* RABA6 isolated in the present investigation displayed a pronouncedeffect in plant performance under drought condition *viz*., increased biometric and yield attributes. The PGP attribute of *B.methylotrophicus*RABA6 might be due to phytohormone production and nutrient uptake. Further studies under field condition may lead to the development of *B.methylotrophicus*RABA6 as a mitigation measure for prevailing drought stress and increased rice productivity.

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