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In vitro assessment of compatibility of PPFM with KCl for foliar spray in paddy under drought stress

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Abstract

Supplementation of potassium nutrient to paddy crop is highly essential under drought stress to support the crop for enhanced water uptake, water use efficiency and prevention of lodging. Farmers are spraying 1% KCl under such conditions since it has been recommended in 1990. Once the role of PPFM in enhancing drought tolerance by breaking the formation of ethylene was established, the farmers were advised to go for foliar spray of PPFM @ 200ml in 200 litres of water per acre of paddy since 2013. Even though this KCl and PPFM applications are benefiting the crop in different ways, both have to be foliar sprayed. It will reduce the labour cost and water usage for the farmers if these two are combined together and given as a single spray. Hence an attempt was made to study the survival of PPFM when it is mixed with 1% KCl or any other alternate K supplement in a sprayer tank. Invitro studies on growth of PPFM colonies were done by combining with 1% KCl solution and 1% KH₂ PO₄. After 24 hours of incubation, 4 x 10¹³ CFU/ ml was observed in PPFM alone whereas it was 1x 10¹⁰ and 3 x10¹¹ for PPFM+1% KCl solution and PPFM+1% KH₂ PO₄ solution respectively. When plated after 48 hours the colony count was 20 x 10¹⁴, 22 x 10¹³, 5 x 10¹³ CFU/ml in PPFM alone, PPFM+1% KCl, PPFM+1% KH₂ PO₄ in Glycerol peptone broth respectively. The observation at 48 hours showed that even though there was numerical variation in these 3 treatments but they were statistically on par. This indicated that it is safe to combine PPFM with 1% KCl while spraying for paddy under drought condition which could ease the job of farmers.

Keywords: Paddy, drought, PPFM spray, KCl spray

Introduction

Pink Pigmented Facultative Methyloph is a Gram negative, rod shaped, obligate aerobe which is ubiquitously present everywhere especially on the phyllosphere utilising the plant released one carbon compounds such as methanol, formaldehyde, formate and also organic acids, alcohols etc. These are well studied for their importance as phyto symbiont (Trotsenko *et al.*, 2001) ^[1] in seed germination, seedling vigor, induced systemic resistance against fungal pathogens (Poorniammal *et al.*, 2010; Savitha *et al.*, 2015) ^[2, 3] and drought stress tolerance (Madhaiyan *et al.*, 2006; Chinnadurai *et al.*, 2009) ^[4, 5]

It was previously proposed that much of the ACC ((1-aminocyclopropane-1-carboxylate) formed are exuded from seeds or plant roots along with other small molecules normally present and may be taken up by the PGP bacteria and subsequently hydrolyzed by the ACCD enzyme. This in turn, would lead to more ACC exudation from inside the plant to maintain the equilibrium thus reducing ACC and the amount of ethylene evolved by the plant (Glick *et al.* 1998) ^[6]. The ability of PPFM to synthesise IAA and ACCD (1-aminocyclopropane-1-carboxylate deaminase) is much utilised in enhancing drought tolerance in paddy. Under drought conditions, ethylene level is more which leads to the expression of drought symptoms in crops. But ACCD produced by PPFM breaks down ACC, an immediate precursor of ethylene, to ammonia and α -ketobutyrate, which can be further metabolized by bacteria for their growth. ACC deaminase is an inducible enzyme whose synthesis is induced in the presence of its substrate ACC. Hence PPFM inhibits the formation of ethylene thereby delays the symptoms of drought in plants. It has been proved that IAA secreted by a bacterium may promote root growth directly by stimulating plant cell elongation or cell division or indirectly by influencing bacterial ACCD activity (Patten and Glick 2002). Methylobacterium inoculation increased the IAA concentrations of the plants resulting in increased ACC activity. However, the ACC and ethylene concentrations inside the tissues were reduced due to the activity of bacterial ACCD (Madhaiyan *et al.*, 2006). Foliar spray of ACCD positive methylobacterial isolates have lowered the ethylene level (60–80%) in the rice seedlings under gnotobiotic condition (Chinnadurai *et al.*, 2009) ^[5].

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Regarding the nutrients potassium is considered as an essential element under drought stress as it helps to prevent lodging. It is a major osmotically active cation of plant cell (Mehdi *et al.*, 2007) [7] where it enhances water uptake and root permeability and acts as a guard cell controller, beside its role in increasing water use efficiency (Zekri and Obreza, 2009) [8]. Foliar applied K can be beneficial when K uptake via the root zone is limited under certain conditions like drought which limits the transport of K to roots.

Various sources of K salts are used such as potassium chloride (KCl), potassium sulphate (K_2SO_4), potassium nitrate (KNO_3) and mono-potassium phosphate (KH_2PO_4) (Magen, 2004) [9]. Normally farmers adopt foliar spraying of 1% KCl since foliar fertilization supplies small amounts of nutrients directed to the leaves at lower cost and also at ease of application. So it is essential to spray both PPFM and 1% KCl to paddy facing drought stress for better results. Then there raises the question, whether they can be sprayed together so that the number of spraying can be minimised and also that it eases the work of farmers. Hence an attempt has been made to check for the survival of PPFM when combined with different K sources up to 48 hours.

Materials and Methods

The PPFM bacteria *Methylobacterium extorquens* AM1 was inoculated in Glycerol Peptone broth and incubated at 37 ± 1 °C for 24 hr with continuous agitation at 120 rpm. Bacterial suspensions were adjusted to approximately 10^8 CFU mL^{-1} by ultraviolet-visible spectrophotometry at 600 nm. Among the 3 set of 24 hr old culture one set was maintained as control without adding any K amendment (PPFM alone) and other 2 set were added with 1% KCl (T1) and 1% KH_2PO_4 (T2) separately. By using standard serial 10-fold dilution in buffered peptone water and eventually transferred 10 μ L for drop plating on AMS agar. The drops were absorbed to agar in less than 30 minutes. After the drops on the agar got absorbed, the plates were incubated at inverted positions. Enumeration of PPFM viable cells were done after 96-120 hrs at 35 ± 1 °C in aerobic incubation. Each dilution was plated in duplicate with four drops per plate. We averaged the total count of CFU over all at least 3 drops at the countable dilution and the viable cell counts were expressed as CFU mL^{-1} . The population count was taken by this drop plate technique at 0hr, 24 hrs and 48 hrs.

Results and Discussion

After allowing *Methylobacterium extorquens* AM1 to grow for 24 hours in glycerol peptone broth, the population count was taken at 0, 24 and 48 hrs for control and 1% KCl and 1% KH_2PO_4 amendments. Immediately after mixing with K supplements the PPFM count was taken and denoted as 0 hour reading. At 0 hour the population load were 2×10^8 , 3×10^8 and 4×10^8 CFU/ ml in control, T1 and T2 respectively. After 24 hours 4×10^{11} CFU/ ml was observed in PPFM alone solution whereas it was 1×10^{10} and 3×10^{11} for PPFM+1% KCl solution and PPFM+1% $KH_2 PO_4$ solution respectively. At 48 hours of incubation we could observe an appreciable amount of increase in the PPFM load in all 3 set of flasks. The colony count was 20×10^{14} in control which was almost greater by 10 folds than in PPFM+1% KCl, PPFM+1% $KH_2 PO_4$ solutions wherein it was 22×10^{13} and 5×10^{13} CFU/ml of the solutions respectively (Table 1). Though the values were numerically variable but were statistically on par with control. Moreover, addition of K supplements in the broth has not affected the growth of PPFM as the PPFM count was increasing gradually at 24 and 48 hours in both control and treatments.

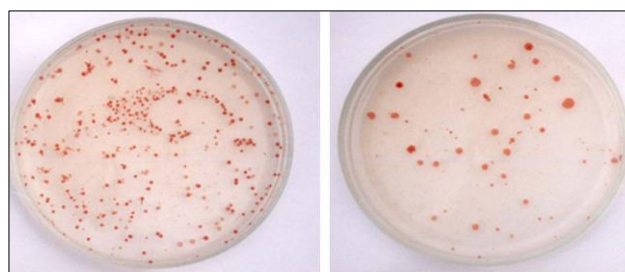


Fig 1a: *Methylobacterium extorquens* AM1 colonies in petriplates



Fig 1b: Liquid Bioinoculant of PPFM

Table 1: Effect of 1% K supplements on the growth of PPFM (*Methylobacterium extorquens* AM1)

Treatment	Microbial load of PPFM (CFU/ml)		
	0 hour	24 hours	48 hours
PPFM alone	2×10^8 (8.30) ^{ab}	4×10^{11} (11.60) ^a	20×10^{14} 15.30) ^a
PPFM+1% KCl	3×10^8 (8.48) ^a	1×10^{10} (10.00) ^{ab}	22×10^{13} 14.34) ^a
PPFM+1% $KH_2 PO_4$	4×10^8 (8.60) ^a	3×10^{11} (11.48) ^a	5×10^{13} (13.70) ^{ab}

Log values in paranthesis; Different letters in a single column show statistically significant differences for $P < 0.05$

We are providing 0.07% K_2HPO_4 and 0.05% KH_2PO_4 in AMS agar medium as K source whereas in GP broth K_2HPO_4 is supplied @ 0.02% for the growth of PPFM. Hence their K requirement is very much meagre and in this study it was observed that the presence of higher concentration of K supplement in the form of KCl or K_2HPO_4 is not affecting the growth of PPFM even upto an incubation period of 48 hours. Farmers have been recommended to spray 1% KCl for paddy under drought stress since 1990 and recommendation of PPFM spray is in practice since 2013. Normally it is the general

recommendation that bioinoculants should not be mixed with any kind of chemicals at the time of application to plants as it could kill the microbes. Hence this study was conducted to check whether the PPFM count is affected when it is combined with 1% KCl solution. This study has showed that there is no reduction in the PPFM load until 48 hours which would be sufficient for the KCl to be absorbed.

Conclusion

From this study it can be concluded that it is safe to combine bioinoculant spray and chemical spray i.e PPFM with 1% KCl as a single spray for paddy under drought condition which could reduce labour cost and ease the job of farmers.

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