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PHYSIOLOGICAL CHANGES DURING BIOPRIMING OF RICE PMK(R) 4

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ABSTRACT
Biopriming is a process of biological seed treatment that refers combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism to protect seed. In order to utilize the biopriming influence on seedling growth in rice, this experiment was conducted to study the physiological changes of bioprimed rice seeds PMK(R) 4 on germination. Seeds of rice were primed with various liquid biopriming agents *viz*., *Azospirillum, Phosphobacteria, Silicate Solublizing Bacteria* and *Pseudomonas fluorescens at* 20% for 18 hrs and result revealed that seed bioprimed with 20% *Pseudomonas fluorescens* for 18hrs recorded early activation of metabolic events which were maintained throughout the germination period resulting in the highest percentage of radicle emergence in a shortest duration. This treatment showed a higher imbibition rate (42.5%) at 48h, radical emergence (82.0%), longest radicle length (2.1cm) at 48h, mean emergence time of (3.9) at 60h and seed metabolic efficiency (0.33).

KEYWORDS: Imbibition rate, Mean Emergence Time, Seed Metabolic Efficiency, *Pseudomonas fluorescens.*

INTRODUCTION

Rice (*Oryza sativa* L.) is the second largest produced cereal in the World contributing world's staple food particularly in South Eastern countries. The demand for rice production is still rising, because of the continuous increase in World population. The World population is predicted to reach approximately 8 billion by 2030 and there is a need to further increase rice production by 40% in the next 20 years (Bernier *et al.,* 2008). One possible way to enhance rice production is to improve yield and tolerance to stresses by means of rhizosphere microbial manipulation. Rice, being a high delta crop, is a key target for water saving because, it is the most widely grown of all crops under irrigation. There is also much evidence that water scarcity already prevails in rice-growing areas, where rice farmers need technologies to cope with water shortage and ways must be sought to grow rice with lesser amount of available water. The challenge is to develop novel technologies and production systems that would allow rice production to be maintained or increased at the face of declining water availability. Direct seeding in aerobic culture is being taken as an attractive alternative to traditional rice production system throughout the World. Nevertheless, poor stand establishment and high weed infestation are major constraints in its mass scale adoption. In this regard, seed priming techniques are pragmatic approaches to achieve proper stand establishment in this new rice culture (Farooq *et al.,* 2009). Bio-priming is a process of biological seed treatment that refers combination of seed hydration (Physiological aspect of disease control) and inoculation (Biological aspect of disease control) of seed with beneficial organism to protect seed. It is an ecological approach using selected fungal antagonists against the soil and seed-borne pathogens. Biological seed treatments may provide an alternative to chemical control. Seed may be planted moist or dried for storage. Excessive and continuous use of chemical fertilizers coupled with pesticides and fungicides have damaged the soil health which causes deleterious effects on crop cultivation and productivity. Now-a-days, the chemical fertilizers are replaced by environment friendly biofertilizers. Most of the biofertilizers manufactured in India are solid carrier based and generally suffer from shorter shelf life, poor quality, high contamination and low field performance (Hedge, 2002). At present, the carrier based biofertilizers are replaced by liquid formulations which are easy to use as it spreads well, mixed uniformly and does not require sticker agent. Rice and Olsen (1992) suggested that liquid formulations were an effective method for seed inoculation of biofertilizer than carrier based inoculant application. Research information on the use of liquid biofertilizers as seed treatment, especially seed biopriming is very scanty and need further investigations about its efficacy in different crop seeds. In view of the above facts, a study of pre-germinative and post-germinative physiological changes during germination of seed biopriming undertake by using liquid biocontrol agent *Pseudomonas fluorescens* and liquid biofertilizers such as *Azospirillum,* Phosphobacteria and Silicate Solublizing Bacteria in rice cv. PMK 4.

MATERIALS & METHODS

Fresh seeds of rice cv. PMK (R) 4, a short duration variety sourced from Department of Farm Management and liquid biofertilizers viz. *Azospirillum,* Phosphobacteria and Silicate Solublizing Bacteria from the Department of Agricultural Microbiology, AC&RI, Madurai, along with liquid biocontrol

agent *Pseudomonas fluorescens* obtained from the Department of Plant Pathology, TNAU, Coimbatore were used for this study. Six treatments *viz.,* non primed seed (control)(T_1), hypropriming (T_2), biopriming with *Azospirillum*(T_3), Phosphobacteria(T_4), Silicate Solublizing Bacteria(T_5) and *Pseudomonas fluorescens*(T_6) were taken up in this study. The experiment was carried out with four replications in Completely Randomized Block Design (CRD). In order to standardize the optimum concentration of biopriming agent and duration, liquid bioagents were prepared in three different concentration *viz.,* 10, 15 and 20 percent and three different duration viz.,6,12 and 18hrs. Seeds were soaked in equal volume of solution in different concentrations in each of the biopriming agents. For hydropriming, simple water is used for soaking. The nonprimed seeds formed the control. After soaking, the seeds were removed from the solutions and shade dried at room temperature for assessing the seed quality parameters. The number of seeds germinated on each day was recorded daily upto 14th day. End of fourteen days germination their performance were evaluated for germination (%), dry matter production (g seedlings^{-10}) and vigour index.

The best treatments one each from biocontrol agent, liquid biofertilizers along with hydropriming and unprimed seeds were subjected to germination and the germinating seeds were collected at 12, 18, 24, and 30 h. During different stages of germination the bioprimed and unprimed seeds were evaluated for the following physiological characterization. The study was conducted adopting factorial completely randomised design with four replications.

Rate of imbibition

Four replicates of 25 seeds were weighed and placed between premoistened germination paper at 25±1°C for 0 to 48 h. At 12 h interval seeds were taken out and dried superficially with absorbent paper and weighed with an analytical balance. Later, the difference between initial and final wet weight was calculated to determine the percent of water imbibed (Takahashi, 1961).

Radicle emergence

Germination test was conducted using 4 × 25 seeds between two layers of premoistened germination paper kept in petri dishes at 25 ± 2°C temperature and 90 ± 3% RH. A seed was considered germinated when the radicle pierced the seed coat up to 2 mm length. Radicle emergence was observed for every 12 h till 48 h and the number of seeds with radicles emerged were counted and expressed in percentage.

Radicle length

Seeds observed for radicle emergence was again utilized for measuring the length of the radicle and length was expressed in centimeter.

Mean Emergence Time (MET)

Seeds observed for 60h were recorded as germinated when the coleoptyle and coleorhiza were visible. Mean Emergence Time (MET) was calculated using the formula of Ellis and Roberts (1981).

$$MET = \frac{\Sigma Dn}{\Sigma n}$$

Where,

n - Number of seeds that germinated on the day (*D*)

D - number of days counted from the beginning of germination.

Endosperm and embryo degradation (Seed metabolic efficiency)

Seed metabolic efficiency (SME) may be defined as the amount of shoot and root drymatter (g) produced from 1 unit (g) of dry seed weight that was respired. Thus higher the value of seed metabolic efficiency, the higher is the efficiency of seed as more seed reserves would be used for producing roots and shoots. Amount of seed metabolic rate (SMR) was calculated as:

SMR = SDW-(SHW+RTW+RSW)

Where,

SDW - Seed dry weight before germination
SHW - Shoot dry weight
RTW - Root dry weight
RSW - Remaining seed dry weight

Seed Metabolic Efficiency (SME) was calculated using the following formula (Rao an Sinha, 1993)

$$SME = \frac{SHW + RTW}{SMR}$$

Statistical Analysis

The data obtained from different experiments were analysed for the 'F' test of significance following the methods described by Panse and Sukhatme (1985). Wherever necessary, the per cent values were transformed to angular (Arc-sine) values before analysis. The critical differences (CD) were calculated at 5 percent probability level. The data were tested for statistical significance. If the F test is non-significant, it was indicated by the letters NS.

RESULTS & DISCUSSION

The imbibition rate was very low for the non primed seeds and even hydroprimed seeds. Among the priming treatments, the seeds bioprimed with *Pseudomonas fluorescens* at 20% for 18h recorded the highest imbibition rate of 27.5% at 12h of germination. The imbibition rate increased to 42.5% at 48h of germination against 12 and 9% with non primed and hydroprimed seeds. Next to *Pseudomonas fluorescens, Azospirillum* at 20% for 18h has showed the advancement in imbibition rate (36%) than other microorganisms (Table 1). McDonald (1999) and Ghassemi-Golezanik *et al.* (2008) observation of early and faster radicle emergence due to priming water soaking was is also confirmed in the present investigation. Over the germination period from 12 to 48h, all the biopriming treatments and hydropriming recorded early radicle emergence from 36h of germination. Kokila (2014) who showed that highest imbibition rate was measured in rice and Gowthamy (2016) in snake gourd seeds bioprimed with *Pseudomonas fluorescens* (4%) for 12h, due to which the primed seeds germinate faster when compared to nonprimed seeds.

TABLE 1: Effect of biopriming on rate of imbibition (%) during germination in rice cv. PMK (R) 4

Treatments (T)	Time course of germination (H)				
	12	24	36	48	Mean
T_1	18.0	20.0	24.0	30.0	23.0
T_2	19.0	22.5	25.0	33.0	24.9
T_3	22.5	25.5	28.0	36.0	28.0
T_4	21.5	23.0	27.0	34.0	26.4
T_5	20.0	21.5	26.0	31.5	24.8
T_6	27.5	32.5	35.0	42.5	34.4
Mean	21.4	24.2	27.5	34.5	26.9
	T		H		TXH
SEd	0.177		0.145		0.355
CD (P=0.05)	0.354**		0.289**		0.709**

TABLE 2: Effect of biopriming on radicle emergence (%) and radicle length (cm) during germination in rice cv. PMK (R) 4

Treatments (T)	Radicle emergence					Radicle length				
	Time course of germination (H)					Time course of germination (H)				
	12	24	36	48	Mean	12	24	36	48	Mean
T_1	0.0	0.0	0.0	34.0	8.5	0.0	0.0	0.0	0.4	0.1
T_2	0.0	0.0	5.0	50.0	13.8	0.0	0.0	0.4	1.6	0.5
T_3	0.0	0.0	10.0	74.0	21.0	0.0	0.0	0.3	1.5	0.5
T_4	0.0	0.0	8.0	69.0	19.3	0.0	0.0	0.2	1.1	0.3
T_5	0.0	0.0	7.0	59.0	16.5	0.0	0.0	0.3	1.5	0.6
T_6	0.0	0.0	15.0	82.0	24.3	0.0	0.0	0.4	2.1	0.4
Mean	0.0	0.0	7.5	61.3		0.0	0.0	0.3	1.0	0.4
	T		H		TXH	T		H		TXH
SEd	0.98		0.80		1.96	0.01		0.01		0.02
CD (P=0.05)	1.95**		1.59**		3.91**	0.02**		0.02**		0.04**

TABLE 3: Effect of biopriming on mean emergence time (MET) (days) and seed metabolic efficiency during germination in rice cv. PMK (R) 4

Treatments (T)	Mean Emergence Time Time course of germination (H)					Seed Metabolic Efficiency
	24	36	48	60	Mean	
T_1	0.0	0.0	0.0	2.0	0.5	0.05
T_2	0.0	0.0	1.3	3.1	1.1	0.14
T_3	0.0	0.0	2.1	3.6	1.5	0.27
T_4	0.0	0.0	1.9	3.5	1.4	0.23
T_5	0.0	0.0	1.7	3.3	1.3	0.20
T_6	0.0	0.0	2.5	3.9	1.6	0.33
Mean	0.0	0.0	1.6	3.3		0.20
	T		H		TXH	
SEd	0.08		0.06		0.16	0.008
CD (P=0.05)	0.16**		0.13**		0.32**	0.016**

The rapid imbibition rate stimulated the metabolic activities and increased the extensibility of radicle cell wall, which results in faster radicle emergence in primed seeds. The radicle started emerging out 36h onwards in primed seeds. In nonprimed seed it took 48h to emerge out. At 36h of imbibition, 15% of the seeds had a radicle emergence length of 4mm when seeds were primed with *Pseudomonas fluorescens*, while it was 10% when treated with *Azospirillum.* But hydropriming had registered only 5% seeds with 4mm radicle. This 5% increased to 50% at 48h in hydroprimed seeds whereas primed seed recorded 82% at 48h. The radicle length was also 2.1cm against 1.6cm with hydroprimed seeds. The nonprimed seeds registered only 0.4cm radicle at 48h of germination. Priming accelerated the imbibition which stimulates the metabolic activities leading to mobilization of food reserves to initiated germination. The metabolic events like cell cycle related events (De Castro *et al.,* 2000), endosperm weakening by hydrolase activities due to activated imbibition rate results in quicker emergence of radicle (Bradford *et al.*, 2000).

Among the biopriming treatments, seed bioprimed with *Pseudomonas fluorescens* (20%) for 18h registered highest radicle emergence which was 48 per cent increase over nonprimed seed. However, in unprimed seed, radicle emergence was noticed only at 48h of germination (Table 2). Faster radicle emergence reflected on the length of the radicle and seeds bioprimed with 20% *Pseudomonas fluorescens* for 18h produced longest radicle at 48h of germination. The significant and faster rate of radicle emergence and radicle length noticed in the bioprimed seed in the present study might be attributed to the quicker uptake of water coupled with early initiation of high metabolic changes. This fact is also supported by Ghassemi-Golezanik *et al.* (2008) in lentil, Pegah *et al.* (2008) in maize, Afzal *et al.* (2009) in tomato, Kokila (2014) in rice and Gowthamy and Selvaraju (2016) in snakegourd who observed early radicle protrusion due to priming.

The results on Mean Emergence Time recorded at 36 and 48h of germination revealed the superiority of seeds bioprimed with *Pseudomonas fluorescens* (20%) for 18h by recording a Mean Emergence Time of 3.9 at 60h.This value was 95% and 25% increase over non primed and hydroprimed seed respectively. Similar results with respect to Mean Emergence Time in primed and nonprimed seed were reported by Afzal *et al.* (2009) in tomato and Kavitha (2011) and Kokila (2014) in rice. Different priming agents lower the mean emergence time due to shortening of lag phase, the stimulatory impacts of priming on the earlier process of germination by mediation of cell division in germinating seed.

The highest metabolic efficiency is closely related to higher mobilization of food reserves that decide the germination and seedling growth. The metabolic efficiency was more in the bioprimed seed with *Pseudomonas fluorescens* (20%) for 18h (1.6). The metabolic efficiency of *Pseudomonas fluorescens* (20%) for 18h (68%) increased over the nonprimed seed (Table 3). The relative increase in the breakdown of food reserves might be due to early onset of metabolic events in the bioprimed seeds. The results of this study are in close agreement with the reports of Job *et al.*,(2000) who recorded high mobilization rate of storage protein in the primed seed of sugar beet.

CONCLUSION

The documentation of physiological events of bioprimed seed during germination (0 to 48h) revealed that the *Pseudomonas fluorescens* (20%) bioprimed seed were found to imbibe faster and initiate quick and early metabolic activities compared to nonprimed seed. Finally, increasing metabolic events helped the bioprimed seed for efficient degradation of endosperm and mobilization of food materials to the growing embryo which resulted in early radicle emergence, increasing radicle length and ultimately highest germination.

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