Standardization of Suitable Concentration and duration of Seed Biopriming with Liquid Biofertilizers for Seedling Vigour Improvement in Rice

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Abstract**—** *An investigation was carried out with PMK (R) 4 riceseed in order to standardize suitable concentration and duration of seed biopriming with liquid biofertilizers (Azospirillum, Phosphobacteria and Silicate Solubilizing Bacteria) and liquid biocontrol agent (Pseudomonas fluorescens) a laboratory experiment was conducted in Department of Seed Science and Technology, Madurai, Tamil Nadu Agricultural University, in Completely Randomized Design with four replicates along with control. The results of theexperiments revealed that seeds bioprimed with20% concentration Pseudomonas fluorescens and 20% Azospirillum for 18h duration germinated earlier and expressed its vigour in terms of increased speed ofgermination, germination percentage, shoot and root length and vigour index compared to other treatments.*

Keywords— Biopriming, Germination, Pseudomonas fluorescens and Vigour.

I. INTRODUCTION

Rice (Oryza saiva L.) is the world's most important staple food-grain grown in over 100 countries, consumed regularly by over two billion people and the primary source of protein for millions. India is the leading rice producing country in terms of area and is the second largest producer next to China. Rice plays an important role in food as well as livelihood security for almost every household, more so in India. To feed this estimated 1.6 billion population of India by 2050 calls for stepping up the current production of 106 mt of milled rice to 140 mt. Higher production and productivity of crop is possible only through use of good quality seeds and proper management practices (Punithavathi et al.,2020). Good quality seeds imply vigour, uniformity and structural soundness besides its genetic and physical purity. Seed priming with living bacterial inoculum is termed as biopriming that involves the application of plant growth promoting rhizobacteria. This type of priming increases germination rate, seedling vigour and also protects seeds against various soil and seed borne pathogens. Bio priming also enhances the ability of plants to withstand against extreme environmental conditions. The bacteria used in biopriming are able to colonize the rhizosphere and help plant through direct or indirect mechanism. Biopriming has emerged as an effective approach for increasing seed vigour. The biofertilizers were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis (Kokila and Baskaran,2016). There is no standard procedure in biopriming as the treatment duration, concentration depends on species, cultivars and seed types. The optimum such variability is a major limitation of the priming method since numerous trials are required to identify the most appropriate strategy for each situation. There is no general rule concerning modalities of seed priming and there is no clear trend of priming response according to the taxonomic position of the species. This undoubtedly limits the commercial implementation of priming treatments. 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 (Nethery, 1991). Rice and Olsen, 1992 suggested that liquid formulations were an effective method for seed inoculation of biofertilizer than carrier based inoculants application. Many studies are available on the beneficial effects of inoculating biofertilizers on crop productivity.

Research information on the use of liquid biofertilizers as seed treatment is very scanty and the concentrations or duration of soaking are to be in different crops. With this background, the present investigation was carried out to standardize the appropriate concentration and priming duration for rice var. PMK(R) 4.

II. MATERIALS AND METHODS

Genetically pure, fresh seeds of PMK(R) 4 rice formed the base seeds for this study. Standardization and evaluation of priming treatments were carried out at the Department of Seed Science and Technology, Agricultural Collage & Research Institute, Tamil Nadu Agricultural University, Madurai. In order to standardize the optimum concentration of biopriming agents and duration of priming, fresh seeds of PMK-4 rice having 92% germination were imposed with water and liquid biopriming agents *viz*., *Azospirillum, Phosphobacteria, Silicate Solubilizing Bacteria* and *Pseudomonas fluorescens*. Priming solutions were prepared in three different concentrations viz., 10, 15 and 20 per cent and the seeds were soaked in double the volume of solutions for three different durations viz., 6, 12 and 18 hours. After priming, the seeds were removed from the solutions, shade dried for 12hrs at room temperature and subjected to germination test (ISTA, 1999). The duration of hydropriming was also standardized by priming the seeds in water with nonprimed seed serving as control.

The number of seeds germinated on each day was recorded daily up to fourteen days. After fourteen days of germination their performance were evaluated for germination (ISTA, 1999), shoot length (cm), root length (cm) dry matter production per 10 seedlings (g), speed of germination (Maguire, 1962) and vigour index values (Abdul-Baki and Anderson, 1973). The experiment was carried out with four replications in Factorial Completely Randomized Block Design. The data obtained were analysed by the 'F' test of significance following the methods described by Rangaswamy (2002). The per cent values were transformed to arc-sine values and used for analysis. The critical differences (CD) were calculated at 1 and 5 per cent probability level. The data were tested for statistical significance by two way ANOVA.

III. RESULTS AND DISCUSSION

Statistically significant variations were observed for all the parameters tested including speed of germination and germination due to priming treatments and its concentration, duration of biopriming and its interaction effect. Among the biopriming agents the performance of *Pseudomonas fluorescens* was better compared to others at all concentrations. This might be due to the plant growth hormones and secondary metabolites produced by *Pseudomonas fluorescens* in alleviating the effect of disease causing organism. The vigour of the seeds registered as germination, root and shoot length and vigour index values were all the highest with the seeds primed with 20% concentration with a soaking duration of 18h irrespective of the priming agents. The effect of priming was much pronounced when concentration of the priming solution was increased with advancement in soaking duration.

3.1 Standardization Biopriming of *Azospirillum:*

Biopriming with *Azospirillum* at 10, 15 and 20% concentrations, the duration of 6, 12 and 18h revealed that seed biopriming at 20% for 18h was the best which recorded the highest speed of germination of 6.1. Early germination was observed in seeds bioprimed at 20% concentration (5.8) against nonprimed seeds (2.8). Among the priming durations, 18h priming recorded the maximum values (5.5). An increase in speed of germination was noticed with an increase in the soaking duration and increase was from 4.2 (12h) to 4.5 (18h) duration. Similar trend was noticed with germination percentage also where maximum germination (97%) was observed at 20% concentration with a priming duration of 18h against 85 per cent in nonprimed seed (Figure.1). Vigour parameters expressed as root and shoot length (19.7 and 10.2cm), drymatter production (0.121g 10seedlings^{-1}). Among the biopriming treatments, *Azospirillum* 20% recorded the greatest vigour index values (2959) and lowest was with nonprimed seeds (1590). Regarding the durations of biopriming, 18h had the greatest vigour index value of 2424 and the least was recorded with 6h (2142). The interaction effect indicated that vigour index values (3130) were also maximum with same combination of azospirillum 20% with 18h soaking duration. Punithavathi *et al*.,2020 reported that that it enhanced seedling vigour encompassing speed of germination, seedling length and dry weight of high vigour as well as low vigour seedlots. Similar results were demonstrated in rice seeds where *Azospirillum liporferum* registered increased germination rate (Kavitha, 2011), tomato and snackgouard (Gowthamy *et al*,2017).

In this study, the colonization of *Azospirillum* was the highest in 20% concentration ($11.0x10^6$CFU ml^{-1}) than 15% ($9.0x10^6$CFU ml^{-1}) and 10% ($9.3x10^5$CFU ml^{-1}) concentrations and adequate population might be the reason for the best performance of the seedlings at 20% concentration.

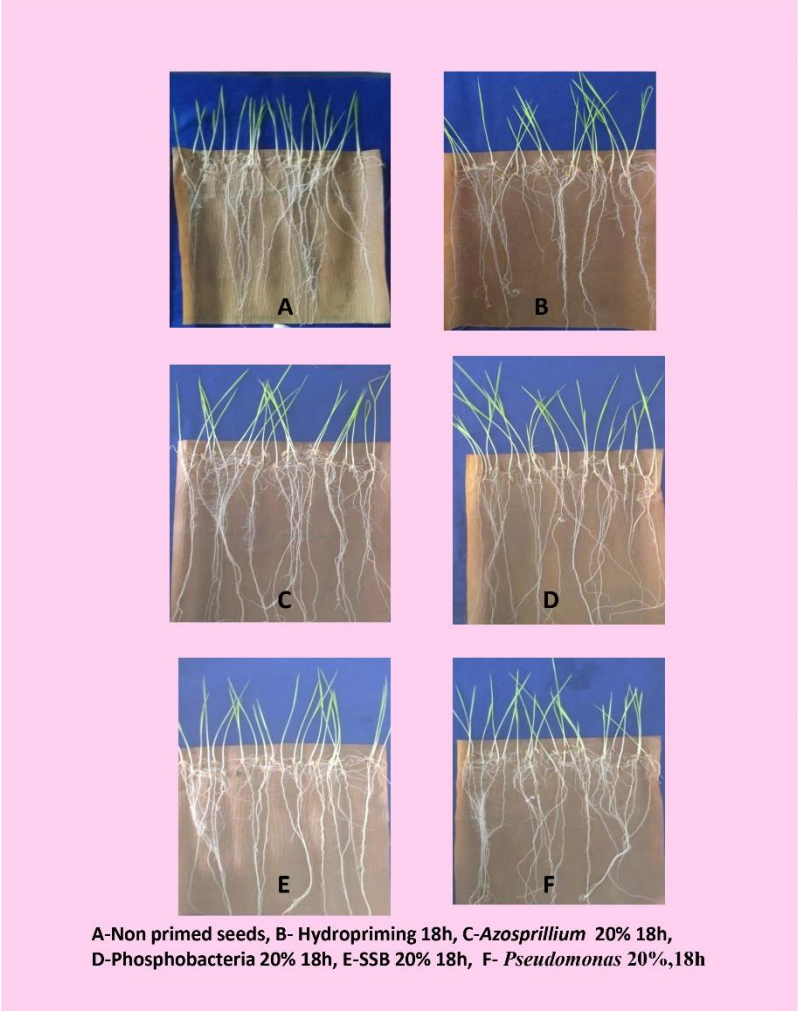

FIGURE 1: Influence of Seedling vigour on 14th day of Germination of rice var. PMK(R) 4

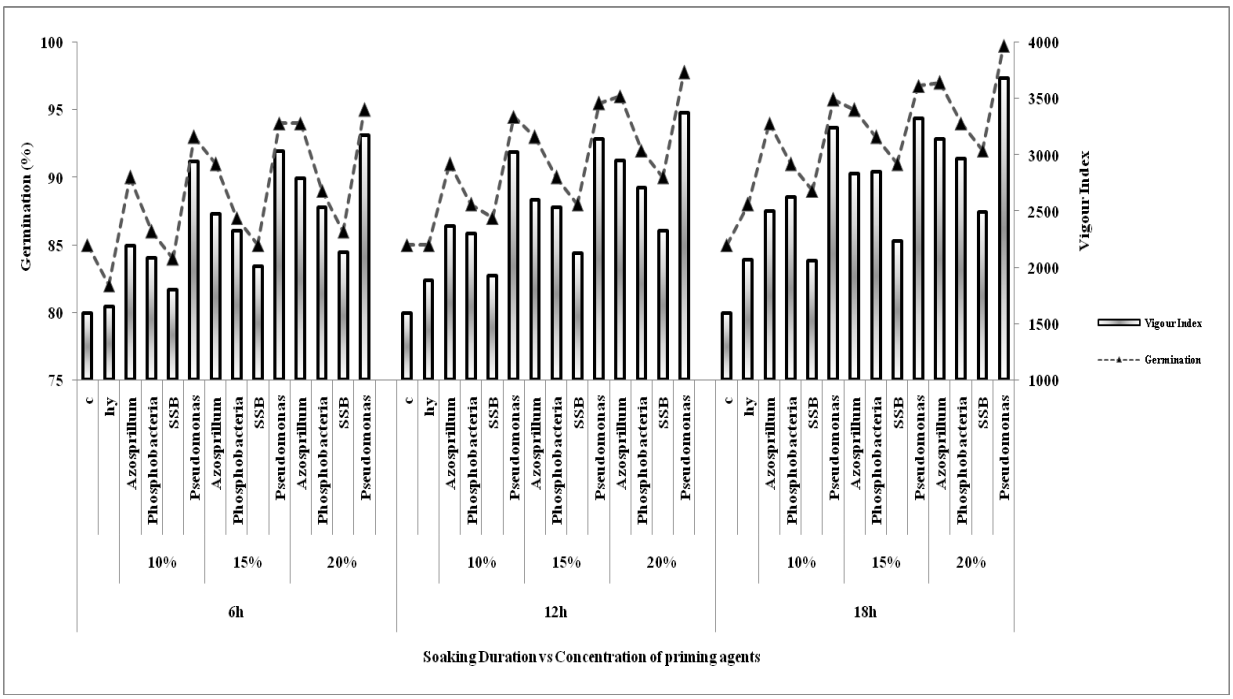

FIGURE 2: Effect of different biopriming agents and duration of priming on germination (%) and vigour index of rice var.PMK(R) 4

TABLE 1

EFFECT OF DIFFERENT BIOPRIMING AGENTS AND DURATION OF PRIMING ON SPEED OF GERMINATION, GERMINATION, SHOOT AND ROOT LENGTH AND DRY MATTER PRODUCTION OF RICE VAR.PMK(R) 4

Biopriming T+A1:Q24reatments / Biopriming agents	Speed of Germination				Shoot Length(cm)				Root Length(cm)				Dry Matter Production(g/10seedlings)			
	6	12	18	Mean	6	12	18	Mean	6	12	18	Mean	6	12	18	Mean
Azosprillium sp 10%	4.5	4.6	4.7	4.6	9.5	9.9	10.1	9.8	14.8	17.4	20.2	17.5	0.106	0.108	0.114	0.109
Azosprillium sp 15%	5.3	5.4	5.6	5.4	9.7	10	10.3	10	16	18	21	18.3	0.111	0.113	0.117	0.114
Azosprillium sp 20%	5.6	5.7	6.1	5.8	9.8	10.2	10.6	10.2	17	20	22	19.7	0.115	0.116	0.121	0.117
Mean	5.1	5.2	5.5	5.3	9.7	10	10.3	10	15.9	18.5	21.1	18.5	0.111	0.112	0.117	0.113
Phosphobacteria 10%	4.8	4.9	5.1	4.9	9.4	9.8	10.2	9.8	15	16.8	17.8	16.5	0.104	0.106	0.107	0.106
Phosphobacteria 15%	5.1	5.2	5.5	5.3	9.6	10	10.3	10	17	18.3	19	18.1	0.105	0.109	0.11	0.108
Phosphobacteria 20%	5	5.4	5.7	5.4	9.7	10.1	10.4	10.1	18	18	20.6	18.9	0.108	0.111	0.115	0.111
Mean	5	5.2	5.4	5.2	9.6	10	10.3	9.9	16.7	17.7	19.1	17.8	0.106	0.109	0.111	0.108
SSB 10%	4	4.2	4.3	4.2	9.2	9.6	9.8	9.5	12.3	13.6	14.3	13.4	0.102	0.106	0.108	0.105
SSB 15%	4.6	4.7	5.1	4.8	9.4	10	10.1	9.7	13.4	14.6	15.6	14.5	0.104	0.107	0.11	0.107
SSB 20%	4.6	4.8	4.8	4.7	9.5	9.9	10.2	9.9	14.5	15	17	15.5	0.105	0.109	0.111	0.108
Mean	4.4	4.6	4.7	4.6	9.4	9.7	10	9.7	13.4	14.4	15.6	14.5	0.104	0.107	0.11	0.107
Pseudomonas 10%	5.4	5.6	6	5.7	11	11.4	11.6	11.3	20.6	20.6	21.8	21	0.113	0.115	0.116	0.115
Pseudomonas 15%	6.6	7.3	7.5	7.1	11.1	11.5	11.8	11.5	21	21.3	23	21.8	0.116	0.116	0.118	0.117
Pseudomonas 20%	7.2	7.4	7.7	7.4	11.3	11.7	12.3	11.8	22.4	22.6	24.5	23.2	0.118	0.12	0.122	0.12
Mean	6.4	6.8	7.1	6.7	11.1	11.5	11.9	11.5	21.3	21.5	23.1	22	0.116	0.117	0.119	0.117
Hydro priming	2.8	3	3.4	3.1	9	9.2	9.3	9.2	11.2	13	14.2	12.8	0.099	0.1	0.105	0.101
Control	2.8				7.5				11.2				0.086			
Grand Mean	3.4	3.5	3.6	3.5	7.5	7.7	7.9	7.7	10.8	12.5	13.5	12.5	0.084	0.086	0.088	0.086
T		**D**	**TXD**		**T**	**D**		**TXD**	**T**	**D**		**TXD**	**T**	**D**		**TXD**
SE(D)	0.06	0.02	0.11		0.13	0.06		0.23	0.24	0.11		0.41	0.001	0.0004		0.001
CD (P=0.05%)	0.12**	0.05**	0.21**		0.27**	0.12**		0.46**	0.47**	0.22**		0.82**	0.002**	0.0009**		0.003**

3.2 Standization of Phosphobacteria Biopriming:

In the present study, with reference to seed priming with phosphobacteria among the different concentrations seeds bioprimed with 20% registered maximum speed of germination (5.4). interaction (Table 1). Among the treatments, maximum germination was observed (92%) in the seeds bioprimed with Phosphobacteria at 20% concentration and minimum was with nonprimed seed (85%). Among the durations, the highest germination was observed in the seeds bioprimed for 18h (90%) and lowest (86 %) was in 6h treated seeds. The interaction effect indicated that seeds bioprimed with Phosphobacteria at 20% for 18h registered the highest germination of 94 per cent and lowest was with nonprimed seed (85 %). Similar trend was observed with reference to longest root and shoot length (18.9 and 10.1cm) and dry matter production than other concentrations. The seed germination and vigour status of primed seeds was in the increasing trend when the concentration and soaking duration was increased. The relative enhancement of germination and seedling vigour might be attributed to the role of phosphorus solubilising bacteria known as phosphobacteria in enhancing the solubilisation of insoluble phosphorus and making it available to the germinating seed with consequent enhancement in the metabolic activity which resulted in higher germination (Cooper, 1979). Phosphobacteria biopriming at 20% for 18h was the best recording the highest vigour index value (2963) and nonprimed seed registered the lowest value (1590). Karthika and Vanangamudi,2013 reported that, seed biopriming with phosphobacteria 20% for 12 h was found to be the best biopriming treatment for improving the seed germination and seedling vigour of COH(M)5 maize hybrid. In this present investigation also, the colonization of Phosphobacteria at 20, 15 and 10% concentrations were 9.7 x 10^6, 8 x 10^6 and 9 x 10^5 $CFUml^{-1}$. This heavy colonization may be the reason for increase in germination and seedling vigour.

3.3 Standardization Biopriming of *Silicate Solubilizing Bacteria:*

The maximum speed of germination was recorded @ 20% concentrations (5.4). With reference to soaking duration, 12h was found to be the best (4.7) than 6h duration. The interaction effect of soaking duration and concentration of biopriming agent, seeds bioprimed with 20% silicate

soluble bacteria for 12hrs recorded maximum values (4.8). Same trend was followed in germination (92%), root and shoot length (17cm and 10.2cm), Dry matter production (0.111g $10seedlings^{-1}$) and vigour index (2492) values. Vijayapriya and Muthukkaruppan, 2010, reported that presents of silicon (Si) in the silicate solubilizing bacteria inoculated seeds might have reduce the incidence of fungal diseases and also induced the plant growth in rice. The colonization of Silicate Solubilizing Bacteria was highest in 20% concentration at 18h (9 x 10^6 $CFUml^{-1}$) than 10% (8.6 x 10^5 $CFUml^{-1}$) concentrations at 18h.

3.4 Standardization Biopriming of *Pseudomonas fluorescens:*

The result of seed priming with *Pseudomonas fluorescens* revealed that among the priming durations, 18h priming recorded the maximum speed of germination (5.5), germination (94%), longest root and shoot (23.1 and 11.9cm), seedling dry matter of 0.119 g 10 $seedlings^{-1}$ and highest vigour index value of 3416 irrespective of soaking durations. With respect to the interaction among the concentrations and priming durations 20% *Pseudomonas fluorescens* for 18hrs recorded maximum speed of germination (7.7). Pradhan *et al.,* (2022) found similar type findings in cowpea while Sharma *et al.,* (2018) reported similar in soybean seeds. Seeds primed with *Pseudomonas fluorescens* (20%) for 18h registered cent per cent germination against hydropriming (88%) and unprimed seed (85%) (Fig.1). The results are in confirmation with the findings of Kokila and Bhaskaran (2016) who reported that rice seeds bioprimed with *Pseudomonas fluorescens* for 12h expressed highest germination percentage and seedling vigour. Punithavathi et al.,2020, revealed that seed biopriming with Pseudomonas fluorescens produced desirable results, both promoting the 91% germination as well as increased the seedling growth and vigour of rice (2972). Similarly root and shoot length of the primed seeds were better than control. Advancement in metabolic activation during germination has registered 118 and 64% increased over control. But the increase was 72 and 32% over hydroprimining. Increased seedling length revealed more drymatter production (0.122 g $10seedlings^{-1}$) and vigour index (3680) which accounted for 41 and 131 per cent increased over the nonprimed seeds (Table 1). Nandakumar *et al*., 2001 found that bio-priming of rice seed with *Pseudomonas fluorescens* induce the production of plant growth regulators such as gibberellins, cytokinins and indole acetic acid, increased availability of minerals and other ions, extensive rooting which facilitates water and nutrient uptake thereby increased seed germination and seedling vigour over the unprimed seeds. Biopriming of sunflower seeds with *Pseudomonas fluorescens* UTPf76 and UTPf86 enhanced the ability of seeds to invigorate and seedlings to grow uniformly (Moeinzadeh *et al*. 2010). Mohan Raj and Sundareswaran (2016) reported that seed biopriming with liquid *Pseudomonas fluorescens* Pf 1 @ 8% concentration for 9 h was found to be the best biopriming treatment for improving the seed germination and production of vigourous diseases free seedling vigour of tomato. As that of other microorganisms, the number of $CFUml^{-1}$ of *Pseudomonas fluorescens* was significantly more (16 x 10^7 $CFUml^{-1}$) at 20% than 15% (13 x 10^7 $CFUml^{-1}$) and 10% (15 x 10^6 $CFUml^{-1}$).

Higher germination and enhanced seedling establishment was also obtained through seed priming with PGPR (Anitha *et al*., 2013) and PGPR inoculation increased the stress resistance and production of the crops; including wheat (Chakraborty *et al.*, 2013; Kumar *et al*. 2014), rice (Bal *et al.*, 2013; Lavakush *et al.*, 2014), soybean (Masciarelli *et al.*, 2014), groundnut (Paulucci *et al.*, 2015) and tomato (Mohan raj and Sundareswaran,2020).

IV. CONCLUSION

Overall it could be concluded that the increase in speed of germination, germination, shoot length, root length and dry matter production could be made seeds bioprimed with liquid *Pseudomonas fluorescens@* 20% for 18hrs for rice PMK(R) 4. Seed treatment with *Azospirillum* and *Phosphobacteria* can be done at the concentration of 20% for 18hrs to have better establishment in rice. Seed coating or priming techniques to be developed and standardized for all type of field, plantation and horticultural crops, which should be cost-effective, time-saving and affordable to all the level of farmers.

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