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RESEARCH PAPER

Standardization of *Azospirillum* concentration and duration of biopriming for rice seed vigour improvement

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Abstract : *Azospirillum lipoferum* is nitrogen fixing biofertilizer which can be used in seed treatment for improving seedling vigour. In order to standardize the optimum concentration of liquid *Azospirillum* for rice biopriming seed treatment and duration of biopriming, a laboratory experiment was conducted in Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore in Completely Randomized Design with four replicates. Rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R seeds were bioprimed separately with 10, 15 and 20 per cent of *Azospirillum* for 6, 12, 18 and 24h of durations. Unprimed and hydroprimed seeds were used as control. The result revealed that rice seeds bioprimed with 20 per cent *Azospirillum* for 12 h was found to be the best seed biopriming treatment for both CORH 4 rice hybrid and its parental lines to enhance the germination rate, total germination percentage, seedling growth and vigour. In rice hybrid CORH 4, biopriming with 20 per cent liquid *Azospirillum* for 12h expressed an increase of 14 per cent of vigour index over the non-primed control seeds.

Key Words : *Azospirillum lipoferum*, Biopriming, Seed germination, Seedling vigour

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INTRODUCTION

Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops (Taylor and Harman, 1990). In priming, seeds are exposed to restricted water availability under controlled conditions which allows some of the physiological processes of germination to occur and before germination is completed, the seeds are usually re-dried for short term storage before sowing. Inoculation of seeds with biological agents in combination with priming has, in several cases, been reported to enhance and stabilize the efficacy of biological agents (Callan *et al.*, 1991).

Biopriming is the process of biological seed treatment that refers to combination of seed hydration and inoculation with beneficial organism to enhance the seed germination and other character. Liquid bio-fertilizers has always more user friendly and eco-friendly effect than the inorganic fertilizers (Gomathy *et al.*, 2009). Many studies are available on the beneficial effects of inoculating biofertilizerson crop productivity. But, the studies on the effect of seed treatment with liquid biofertilizers on the germination and seedling vigour are

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very scarce. The biofertilizer *Azospirillum lipoferum* was 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 (Fayez *et al.*, 1985). Hence, the study was undertaken to standardize an optimum concentration and duration of soaking for seed biopriming using liquid *Azospirillum* in rice hybrid CORH 4 and its parental lines (COMS 23A, CB 174R).

MATERIAL AND METHODS

Genetically pure seeds of rice hybrid CORH 4 and its parental lines (COMS 23A, CB 174R) were obtained from Department of Rice, Tamil Nadu Agricultural University, Coimbatore were bioprimed with liquid biofertilizer *Azospirillum lipoferum* obtained from the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore.

The laboratory experiment was conducted to standardize an optimum concentration of liquid biofertilizer and priming duration was conducted and analyzed with four replications in factorial completely randomized design (FCRD) at the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore.

For this study, the seeds of paddy hybrid CORH 4 and its parental lines were soaked in double the volume of water and 10, 15 and 20 per cent of liquid *Azospirillum* (*A. lipoferum*) solutions for 6h, 12h, 18h and 24h, separately. Unprimed seed served as control. Treated seeds along with control were assessed for the physiological seed quality parameters as follows.

The laboratory germination test was carried out in quadruplicate using 100 seeds each with 4 sub replicates of 25 seeds in the paper medium (ISTA, 2009). The test conditions of 25 ± 2°C temperature and 95 ± 3 per cent relative humidity were maintained in a germination room. Germination was counted at 24 hour intervals and continued until no further germination occurred. At the end of 14 days, the number of normal seedlings was counted and the mean was expressed as a percentage. Speed of germination was calculated based on the following formula of Maguire (1962) :

$$\text{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots + \frac{X_n - X_{n-1}}{Y_n}$$

where; X_1, X_2 and X_n are number of seeds germinated on first, second and n^{th} day, respectively and Y_1, Y_2 and Y_n are number of days from sowing to first, second and n^{th} count, respectively.

Root length and shoot length were measured at the time of germination count from ten normal seedlings selected at random from each replication and the mean was expressed in centimeter and those seedlings used for growth measurement were placed in a paper cover and dried in shade for 24h and then they were kept in an oven maintained at 85±2°C for 48h. The dried seedlings were weighed to estimate the dry matter production and the mean values were expressed in g per 10 seedlings. The vigour index was computed as described by Abdul-Baki and Anderson (1973) as follows and expressed in whole numbers.

$$\mathbf{VI = GP \times (RL + SL)}$$

where, VI is vigour index, GP is germination percentage, RL is root length (cm), SL is shoot length (cm).

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.

RESULTS AND DISCUSSION

Biofertilizers have several beneficial roles in seed germination and seedling establishment. Beneficial bioagents contained the properties of plant growth promotion. Generally, the simplest method of beneficial microbial inoculation in crop production programme is the application of liquid suspension either directly to the soil or to the seeds. Carrier based biofertilizers had the problem of short shelf life, poor quality, high contamination and unpredictable field performances. However, liquid inoculant formulations showed good field performance with the use of low cost materials and easily attainable by small producers could overcome many problems associated with solid carriers (Singleton *et al.*, 2002).

In the present investigation, the rice seeds bioprimed with 20 per cent *Azospirillum* for 12 h quickened the germination with high percentage (Table 1 and 2) and recorded longest root and shoot (Table 3 and 4), maximum dry matter production (Table 5) and vigour index (Fig.1).

Table 1 : Standardization of seed biopriming with *Azospirillum* on speed of germination of rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R

Biopriming treatment (T) \ Soaking duration (D)	COMS 23A					CB 174R					CORH 4				
	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean
Hydropriming	5.9	6.8	6.3	6.1	6.3	5.9	6.3	6.3	6.1	6.2	5.7	7.3	7.1	6.1	6.6
Azospirillum 10 %	6.5	7.1	6.8	6.3	6.7	5.9	6.0	6.1	6.3	6.1	5.6	7.4	7.2	6.3	6.6
*Azospirillum*15 %	5.9	7.1	7.1	6.5	6.7	6.1	6.6	6.8	6.3	6.5	6.3	7.5	7.3	6.4	6.9
Azospirillum 20%	6.4	7.6	7.3	6.8	7.0	6.1	7.8	7.5.	6.8	6.9	6.3	7.6	7.5	7.2	7.2
Mean	6.2	7.2	6.9	6.4	6.7	6.0	6.7	6.4	6.4	6.4	6.0	7.4	7.3	6.5	6.8
Nonprimed seed (C)			5.8					5.6					5.8		
	T	D	T×D	C vs Rest		T	D	T×D	C vs Rest		T	D	T ×D	C vs Rest	
S.E. ±	0.052	0.052	0.104	0.076		0.056	0.056	0.113	0.082		0.181	0.181	0.362	0.264	
C.D. (P=0.05)	0.104	0.104	0.208	0.152		0.113	0.113	0.227	0.165		0.364	0.364	0.728	0.531	

Table 2 : Standardization of seed biopriming with *Azospirillum* on seed germination (%) of rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R

Biopriming treatment (T) \ Soaking duration (D)	COMS 23A					CB 174R					CORH 4				
	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean
Hydropriming	89 (70.63)	91 (72.54)	91 (72.54)	90 (71.57)	90 (71.57)	90 (71.57)	92 (73.57)	92 (73.57)	91 (72.54)	91 (72.54)	88 (69.73)	90 (71.57)	89 (70.63)	89 (70.63)	89 (70.63)
Azospirillum 10 %	89 (70.63)	91 (72.54)	92 (73.57)	90 (71.57)	91 (72.54)	90 (71.57)	92 (73.57)	92 (73.57)	91 (72.54)	91 (72.54)	88 (69.73)	90 (71.57)	89 (70.63)	89 (70.63)	89 (70.63)
Azospirillum 15 %	90 (71.57)	93 (74.66)	92 (73.57)	91 (72.54)	92 (73.57)	91 (72.54)	94 (75.82)	94 (75.82)	92 (73.57)	93 (74.66)	88 (69.73)	90 (71.57)	90 (71.57)	89 (70.63)	89 (70.63)
Azospirillum 20 %	91 (72.54)	94 (75.82)	93 (74.66)	92 (73.57)	92 (73.57)	93 (74.66)	95 (77.08)	94 (75.82)	93 (74.66)	94 (75.82)	89 (70.63)	93 (74.66)	92 (73.57)	89 (70.63)	91 (72.54)
Mean	90 (71.57)	92 (73.57)	92 (73.57)	91 (72.54)	91 (72.54)	91 (72.54)	93 (74.66)	93 (74.66)	92 (73.57)	92 (73.57)	88 (69.73)	91 (72.54)	90 (71.57)	89 (70.63)	90 (71.57)
Nonprimed seed (C)			89 (70.63)					90 (71.57)					88 (69.73)		
	T	D	T × D	C vs Rest		T	D	T × D	C vs Rest		T	D	T × D	C vs Rest	
S.E. ±	0.643	0.643	1.286	0.937		0.678	0.678	1.356	0.989		0.427	0.427	0.854	0.623	
C.D. (P=0.05)	1.292	1.292	NS	1.883		1.362	1.362	NS	1.986		0.858	0.858	NS	1.251	

Figures in parentheses indicate arcsine values NS=Non-significant

Table 3 : Standardization of seed biopriming with *Azospirillum* on root length (cm) of rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R

Biopriming treatment (T) \ Soaking duration (D)	COMS 23A					CB 174R					CORH 4				
	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean
Hydropriming	21.2	23.5	22.2	22.0	22.2	18.8	19.3	19.2	19.0	19.1	21.1	23.0	22.6	22.2	22.2
Azospirillum 10 %	21.4	23.6	23.0	22.0	22.5	19.0	19.5	19.4	19.4	19.3	21.2	23.0	22.8	22.2	22.3
Azospirillum 15 %	22.0	24.0	23.1	22.8	23.0	19.5	21.0	21.0	19.8	20.3	21.3	23.0	23.0	22.9	22.5
Azospirillum 20 %	22.8	25.0	24.8	23.8	24.1	20.0	22.0	21.6	21.2	21.2	21.3	23.8	23.1	23.1	22.8
Mean	22.0	24.0	23.3	22.7	23.0	19.3	20.5	20.3	19.9	20.0	21.2	23.2	22.8	22.6	22.5
Nonprimed seed (C)			21.0					18.5					20.5		
	T	D	T×D	C vs Rest		T	D	T×D	C vs Rest		T	D	T×D	C vs Rest	
S.E. ±	0.141	0.141	0.283	0.206		0.113	0.113	0.226	0.165		0.172	0.172	0.344	0.251	
C.D. (P=0.05)	0.284	0.284	0.568	0.414		0.227	0.227	0.454	0.331		0.345	0.345	0.691	0.504	

Table 4 : Standardization of seed biopriming with *Azospirillum* on shoot length (cm) of rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R

Biopriming treatment (T) \ Soaking duration (D)	COMS 23A					CB 174R					CORH 4				
	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean
Hydropriming	11.2	12.0	11.8	11.8	11.7	10.3	11.0	10.5	10.5	10.6	11.5	12.4	12.3	12.2	12.1
Azospirillum 10 %	11.4	12.0	12.1	12.0	11.9	10.5	11.3	11.2	10.8	11.0	11.7	12.4	12.3	12.3	12.2
Azospirillum 15 %	11.6	12.3	12.3	12.2	12.1	11.0	11.6	11.5	11.2	11.3	11.8	12.6	12.4	12.3	12.3
Azospirillum 20 %	11.8	12.7	12.5	12.3	12.3	11.2	12.2	11.8	11.6	11.7	12.3	12.8	12.6	12.5	12.6
Mean	11.6	12.3	12.2	12.1	12.0	10.8	11.5	11.3	11.0	11.1	11.8	12.6	12.4	12.3	12.3
Nonprimed seed (C)			11.1					10.2					11.3		
	T	D	T×D	C vs Rest		T	D	T×D	C vs Rest		T	D	T×D	C vs Rest	
S.E. ±	0.083	0.083	0.167	0.122		0.059	0.059	0.119	0.087		0.103	0.103	0.207	0.151	
C.D. (P=0.05)	0.168	0.168	0.335	0.244		0.119	0.119	0.239	0.174		0.208	0.208	0.415	0.303	

Table 5 : Standardization of seed biopriming with *Azospirillum* on drymatter production (g / 10 seedlings) of rice hybrid CORH 4 and its parental lines COMS 23A and CB 174R

Biopriming treatment (T) \ Soaking duration (D)	COMS 23A					CB 174R					CORH 4				
	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean	6h	12h	18h	24h	Mean
Hydropriming	0.108	0.125	0.123	0.118	0.118	0.085	0.090	0.090	0.087	0.088	0.117	0.123	0.120	0.115	0.119
Azospirillum 10 %	0.110	0.125	0.123	0.120	0.120	0.088	0.092	0.093	0.089	0.091	0.118	0.126	0.127	0.120	0.123
Azospirillum 15 %	0.110	0.126	0.126	0.120	0.121	0.09	0.096	0.094	0.092	0.093	0.119	0.125	0.125	0.124	0.123
Azospirillum 20 %	0.115	0.128	0.128	0.125	0.124	0.090	0.110	0.098	0.094	0.098	0.120	0.134	0.130	0.125	0.127
Mean	0.111	0.126	0.125	0.122	0.121	0.088	0.097	0.094	0.090	0.092	0.118	0.127	0.125	0.121	0.123
Nonprimed seed (C)			0.105					0.083					0.111		
	T	D	T×D	C vs Rest		T	D	T×D	C vs Rest		T	D	T×D	C vs Rest	
S.E. ±	0.001	0.001	0.002	0.001		0.001	0.001	0.001	0.001		0.001	0.001	0.003	0.002	
C.D. (P=0.05)	0.002	0.002	0.004	0.003		0.001	0.001	0.003	0.002		0.003	0.003	0.005	0.004	

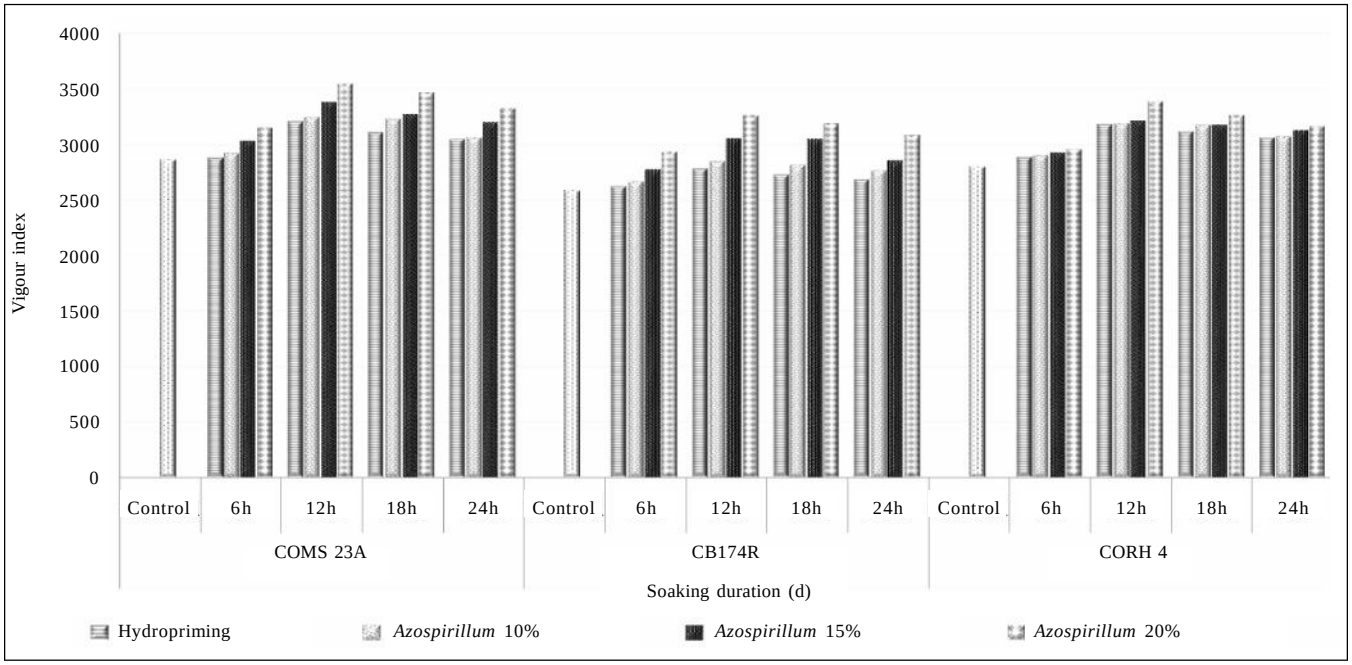

Fig. 1 : Seed biopriming with *Azospirillum* on vigour index of rice hybrid CORH 4 and itsparental lines COMS 23A and CB 174R

The increase of vigour index in seeds bioprimed with 20 per cent *Azospirillum* was 10, 15 and 4 per cent over hydroprimed seeds and 18, 21 and 14 per cent over nonprimed seeds of COMS 23A, CB 174R and CORH 4, respectively. The increase in physiological attributes of *Azospirillum* bioprimed seeds might be due to the production of biologically active substance such as auxins, gibberellins, cytokinins, amino acids and vitamins (Afifi *et al.*, 2003). The phytohormones released by *Azospirillum lipoferum* could stimulate plant growth, absorption of nutrients and photosynthesis (Fayez *et al.*, 1985). Biofortification of rice seeds with *Azospirillum* enhanced seedling vigour, speed of germination, seedling length and dry weight of both high and low vigour seed lots (Ramamoorthy *et al.*, 2000).

Azospirillum sp. are non-specific plant growth-promoting rhizobacterias (PGPRs) providing many contributions to the enhancement of growth in many agricultural crop species (Bharathi *et al.*, 2004). Similar result was observed by Kapulnik *et al.* (1981) in rice; Raju *et al.* (1999) in sorghum; Niranjan *et al.* (2004) in pearl millet.

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