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Effects of Biopriming on Germination Characteristics of PMK (R) 4 under Salinity Conditions

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ABSTRACT

Keywords

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Study was conducted to determine the effect of seed bio-priming on germination and vigour of rice under salinity stress condition. Seeds of rice var. PMK (R) 4 were primed with water, liquid formulation of azospirillum, phosphobacteria, silicate soluble bacteria and their combinations at 20% concentration for 18 h. at 25⁰C and evaluated under salt stress conditions created by 0.25, 0.50, 0.75 and 1.0% NaCl solutions for its germination, vigour index, seedling length, dry matter production, total phenol, chlorophyll and malondialdehyde content were studied. The untreated dry seeds served as control. Results revealed that seeds bio-primed with combinations of azospirillum + phosphobacteria @ 20% solution for 18 h performed better in germination and vigour and also maximum phenol content and lower MDA content at 8dSm⁻¹ NaCl concentration. The next best bio-inoculant was 20% of azospirillum alone. Hence, suggested azospirillum + phosphobacteria @ 20% solution as a best seed enhancement technique for improving seed germination of rice under salinity stress condition.

Introduction

Poor soil health and polluted environment is the major cause for lower productivity in Indian Agriculture. Soil Salinity is one of the important factor which limits plant growth at all developmental stages by creating an osmotic pressure that prevent germination of seeds and decrease water uptake of plant roots, due to ionic toxicity of Na⁺ and Cl⁻ (Almansouri *et al.*, 2001) which ultimately leads to lower productivity (Epstein *et al.*, 1980; Lutts *et al.*, 2004; Yagmur and Kaydan, 2008). Though high quality seeds are used for sowing in the field, it undergoes many stresses during the emergence and

establishment leading to poor survival and reduced plant stands on the main field.

Salinity, as an abiotic hazard, induces numerous disorders in seeds and propagules during germination. Salinity either completely inhibits germination at higher levels or induces a state of dormancy at lower levels (Khan and Ungar, 1997).

Salinity affects germination by facilitating the intake of toxic ions, which can cause change of certain enzymatic or hormonal activities of the seed. Salinity has been reported to cause

significant reductions in the rate and final percentage of germination and emergence of radish, which in turn may lead to uneven stand establishment and ultimately reduced crop yields.

Seed priming a pre sowing seed enhancement technique, enhances the speed, vigour and uniformity of seedling formation (Demir and Van De Venter, 1999). Seed priming with bio-agents not only improves the germination but also increases stress tolerance during early seedling growth (Krishna *et al.*, 2008; Fallahi *et al.*, 2011). In order to overcome the salt stress during seed germination, several authors have recommended different seed priming techniques such as hydropriming, halopriming, osmopriming and solid matrix priming.

Bio priming is also a sustainable tool to improve the stress tolerance capacity of seeds during early seedling growth (Fallahi *et al.*, 2011). Hence, this present study was formulated to investigate the effect of bio-priming of rice seeds under salinity conditions upon their germination and vigour during the early growth stages.

Materials and Methods

Seeds of rice var. PMK(R) 4 with an initial germination of 90% and 10.2% moisture were used for these studies. Experiments were carried out in the Department of Seed Science and Technology, Agricultural College and Research Institute, Madurai (TNAU). Total nine treatments were followed with three replications, along with control.

The nine treatments follows as untreated seeds as a control (T₀), Seeds soaked in water (Hydro priming) (T₁); and seeds treated with 20 % solutions of azospirillum (T₂); phosphobacteria (T₃); silicate soluble bacteria (T₄); azospirillum + phosphobacteria (T₅);

azospirillum + silicate soluble bacteria (T₆); phosphobacteria + silicate soluble bacteria (T₇) and azospirillum + phosphobacteria + silicate soluble bacteria (T₈) with a soaking duration of 18 h at 25⁰C respectively for all the bio-inoculants. Then the bio-inoculants treated seeds were dried to its original moisture content and evaluated for its germination potential under induced salinity stress conditions created by using NaCl at different concentration of 0.25%, 0.50%, 0.75% and 1.0% expressing the electrical conductivity (EC) values of 2, 4, 6 and 8 dSm⁻¹ respectively. The germination media was moistened with NaCl solution for the entire period of germination as per the treatment and germination test was conducted.

Seeds were considered as germinated when radicals were 2 mm long. Speed of germination was recorded every 24 h for 14 days (Maguire, 1962) and percentage of germination was calculated (ISTA, 2009). Root length, shoot length were also measured at the end of 14th days. Vigour index was calculated using the formula of Abdul-Baki and Anderson (1973).The end of 14 days of germination, total chlorophyll content (Yoshida *et al.*, 1971) and total phenol content (Thimmaiah, 1999), Malondialdehyde (MDA) content (Heath and Packer, 1968) were also estimated using the seedlings raised under salt stress conditions against hydropriming.

For the comparison amongst treatments, salt tolerance index was calculated where, a salt sensitive treatment was chosen as susceptible standard and the performance of other treatments were compared (Zeng *et al.*, 2003).

The untreated seeds served as susceptible standard as it had highest mean for MDA content and lower mean for other characters.

Salt tolerance index =

Mean of a treatment for a trait over
salt stress treatments

Mean of susceptible standard for the trait over
salt stress treatments

The data obtained from different experiments were analyzed by the 'F' test of significance (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 per cent probability level.

Results and Discussion

Seed bio-priming with 20% solutions of all the organisms had a significant impact on speed of germination and germination percentage under saline conditions compare to control. Under zero level of salinity, the highest speed of germination was register with seeds bio-primed with azospirillum + phosphobacteria (T₅) (4.6) compared to control (3.6) and all other treatments (Figure 1).

Bio-priming might have improved the stress tolerance capacity during early seedling growth in which crop (Fallahi *et al.*, 2011).

The maximum germination (96%) was achieved in rice seeds bio-primed with azospirillum and phosphobacteria (T₅) when compare with normal conditions (0%).

The same trend was observed in T₅ (azospirillum + phosphobacteria) under saline conditions also, it registered maximum germination (93%) with salt tolerance index of 0.61 than all other bio-priming treatments. Minimum germination (84%) was in unprimed seeds (control) at all stress levels with salt tolerance index of 1.00 in each (Figure 2).

Poor germination under salt stress conditions was due to an osmotic effect and ion toxicity. Salt stress causes reactive oxygen species stress, leading to gradual peroxidation of lipid and antioxidant enzyme inactivation and finally reduces germination and plant growth (Song *et al.*, 2008; Zheng *et al.*, 2009). In the present study, seedling length was also much affected and it decreased as salt stress increased. The shoot and root length reduced to almost 25% from the normal growth of 8.6 cm and 15.0 cm, 6.0 cm and 13.4 cm, respectively at the stress level of 1% in untreated plants (control).

However, it could be noted that seedlings obtained from T₅ (azospirillum + phosphobacteria) had significantly higher shoot and root length than control (8.7 cm and 15.0 cm, respectively at 1% NaCl). Seed bio-priming with azospirillum has a positive effect on the production of plant growth hormones increased the number of root hairs, seedling length and dry matter concentration of wheat, sesame, canola and bean (Mirshekari and Baser, 2010).

In the present study also next to treatment (T₅-azospirillum + phosphobacteria) azospirillum treated seeds (T₂) expressed its vigour in term of root and shoot length (13.8cm and 7.5cm) under stress conditions (Table 1).

When salinity was increased to 1% level, fresh and dry weight of seedlings decreased irrespective of the treatments. It is probably due to decrease in remobilization of reservoirs from cotyledons to embryo axis (Akita and Cabuslay, 1990). Drastic reduction in plumule length of wheat, barley, pea and cabbage seeds when salinity stress was increased (Mer *et al.*, 2000). They pointed out that decreasing growth was because of reduced water absorption by radicle, and subsequently by accumulation of soluble salts in cells and water potential of root cells decreases.

Table.1 Effect of seed bioprimer on root length, shoot length and dry matter production in rice var. PMK 4–under salt stress condition

Treatments/ Different concentration of NaCl (%)	Root Length (cm)						Shoot Length (cm)						Dry matter production (g/10 seedlings)					
	0	0.25%	0.50%	0.75%	1%	mean	0	0.25%	0.50%	0.75%	1%	mean	0	0.25%	0.50%	0.75%	1%	mean
T0	15	14.8	14.5	13.4	10	13.5	8.6	8.3	7.7	7.1	6	7.5	1.000	0.099	0.093	0.086	0.082	0.272
T1	17.2	16.2	16	15	11.3	15.1	10	8.6	7.8	7.4	6.4	8.0	1.004	1.002	1.000	0.096	0.091	0.639
T2	19.5	18	17.3	16.8	13.8	17.1	11	10	9	8.4	7.5	9.0	1.110	1.108	1.005	1.000	0.096	0.864
T3	17.6	16.9	16.3	15.5	13.5	16.0	10.2	9.8	8.5	7.8	6.6	8.6	1.100	1.006	1.003	0.097	0.093	0.660
T4	17	16	15.8	14.8	12	15.1	9.8	8.3	8	7.3	6.3	7.9	1.000	1.000	0.098	0.094	0.091	0.457
T5	20	19.2	18.6	18.2	15	18.2	11.8	11	10.3	9.4	8.7	10.2	1.120	1.116	1.111	1.107	1.100	1.111
T6	17.7	16.8	16.3	15.5	12	15.7	10.4	9	8.4	7.6	7	8.5	1.007	1.004	1.000	0.095	0.094	0.640
T7	15.6	15.4	14.6	14	11.3	14.2	8.8	8.2	8	7.2	6.1	7.7	1.002	0.099	0.094	0.09	0.089	0.275
T8	18.6	17.8	17	16	13	16.5	10.7	9.6	8.8	8.1	6.8	8.9	1.008	1.006	1.001	0.096	0.092	0.641
Mean	17.6	16.8	16.3	15.5	12.4	15.7	10.1	9.2	8.5	7.8	6.8	8.5	1.039	0.827	0.712	0.307	0.203	0.617
SE(d)	T		C		TXC		T		C		TXC		T		C		TXC	
	0.187		0.139		0.418		0.081		0.060		0.181		0.009		0.007		0.021	
CD (0.05%)	0.370**		0.275**		0.827**		0.160**		0.119**		0.359**		0.019**		0.014**		0.043**	

Table.2 Effect of seed bioprimer on total chlorophyll, total phenol content and MDA in rice var. PMK 4 - NaCl salt stress condition

	Chlorophyll content ($\mu\text{g g}^{-1}$)						Total phenol content (mg/100 g)						MDA ($\mu\text{mol/g fr.wt}$)					
	0	0.25%	0.50%	0.75%	1%	mean	0	0.25%	0.50%	0.75%	1%	mean	0	0.25%	0.50%	0.75%	1%	mean
T0	0.020	0.016	0.011	0.006	0.005	0.012	1.50	1.63	1.94	2.22	2.81	2.02	1.952	2.720	4.600	6.000	6.521	4.359
T1	0.024	0.022	0.020	0.014	0.011	0.018	1.78	1.86	2.12	2.38	3.46	2.32	1.620	1.850	2.700	3.650	4.100	2.784
T2	0.031	0.026	0.023	0.018	0.014	0.022	1.85	1.98	2.20	2.66	3.78	2.49	1.330	1.735	2.360	2.863	3.540	2.366
T3	0.028	0.026	0.023	0.019	0.016	0.022	1.80	1.95	2.18	2.45	3.53	2.38	1.430	1.780	2.664	3.563	4.006	2.689
T4	0.023	0.021	0.018	0.014	0.010	0.017	1.76	1.84	2.11	2.38	3.40	2.30	1.548	1.905	2.925	3.828	4.560	2.953
T5	0.035	0.031	0.027	0.023	0.017	0.027	1.88	2.03	2.44	3.27	4.00	2.72	1.200	1.510	2.110	2.854	3.252	2.185
T6	0.026	0.021	0.019	0.015	0.012	0.019	1.81	1.87	2.08	2.32	3.30	2.28	1.275	1.628	2.350	2.720	3.400	2.275
T7	0.022	0.019	0.013	0.010	0.008	0.014	1.60	1.78	2.04	2.28	3.15	2.17	1.720	2.336	3.755	5.600	6.250	3.932
T8	0.029	0.024	0.020	0.016	0.013	0.020	1.82	1.90	2.14	2.40	3.35	2.32	1.486	1.854	2.520	2.880	3.860	2.520
mean	0.026	0.023	0.019	0.015	0.012	0.019	1.76	1.87	2.14	2.48	3.42	2.33	1.507	1.924	2.887	3.773	4.388	2.896
SE(d)	T		C		TXC		T		C		TXC		T		C		TXC	
	0.0002		0.0001		0.0005		0.02		0.01		0.05		0.03		0.02		0.07	
CD (0.05%)	0.0005**		0.0003**		0.0011**		0.04**		0.03**		0.09**		0.06**		0.04**		0.14**	

Table.3 Salt tolerance index (STI) amongst treatments at mean value of salt stress condition

	Speed of germination	Germination (%)	Shoot Length(cm)	Root Length(cm)	Phenol content (mg/100 g)	Chlorophyll content ($\mu\text{g g}^{-1}$)	MDA ($\mu\text{mol/g fr.wt}$)
T0	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T1	1.05	1.03	1.07	1.12	1.15	1.57	0.64
T2	1.26	1.09	1.20	1.26	1.23	1.93	0.54
T3	1.09	1.04	1.14	1.18	1.18	1.93	0.62
T4	1.04	1.02	1.05	1.12	1.14	1.48	0.68
T5	1.43	1.12	1.36	1.34	1.35	2.29	0.50
T6	1.16	1.04	1.12	1.16	1.13	1.60	0.52
T7	1.04	1.01	1.02	1.05	1.07	1.24	0.90
T8	1.12	1.07	1.19	1.22	1.15	1.76	0.58
SE(d)	0.02	0.02	0.02	0.02	0.026	0.038	0.015
CD(0.05)	0.05**	0.04**	0.05**	0.05**	0.053**	0.078**	0.032**

Fig.1 Effect of seed bio-priming on speed of germination in rice var. PMK 4 - NaCl salt stress condition

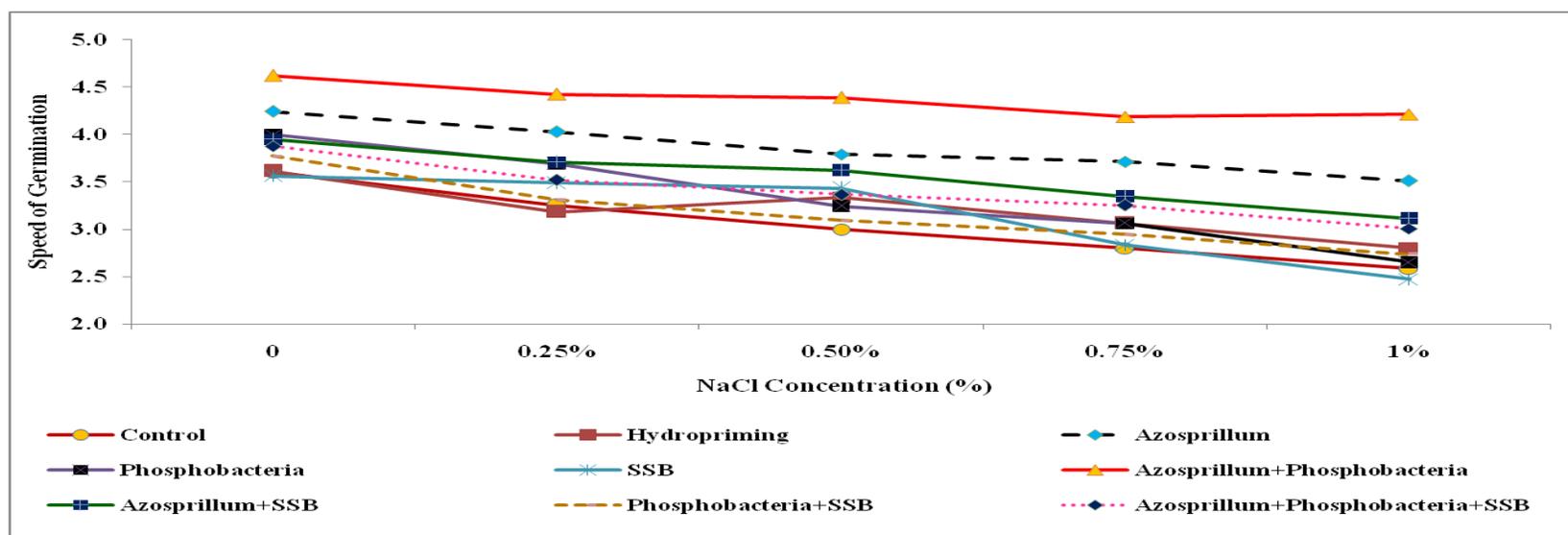


Fig.2 Effect of seed bio-priming on germination (%) in rice var. PMK 4 - NaCl salt stress condition

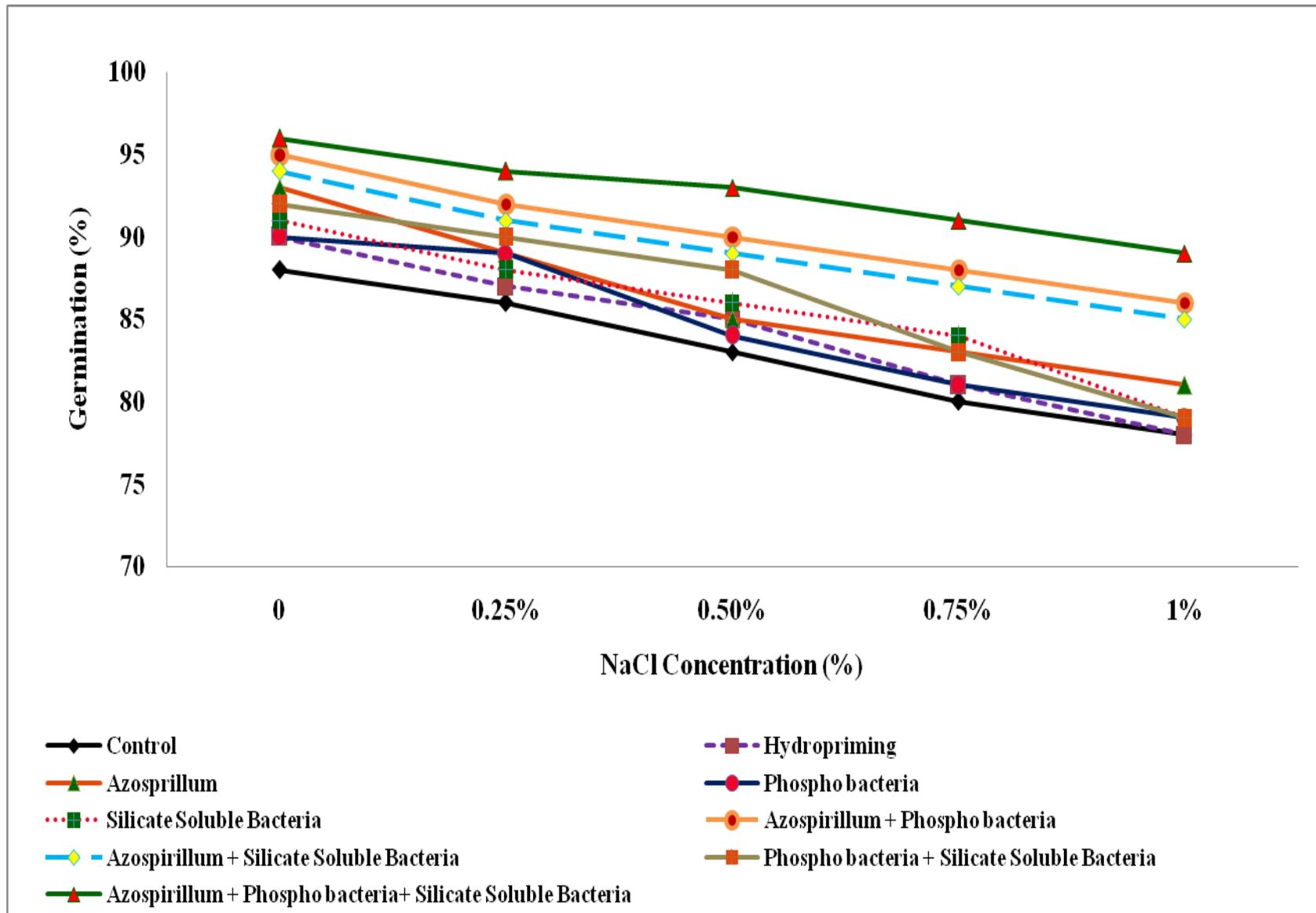


Fig.3 Effect of seed bio-priming on seedling growth of rice var. PMK 4 under i) normal and ii) salt stress conditions

i) Normal condition (0%)

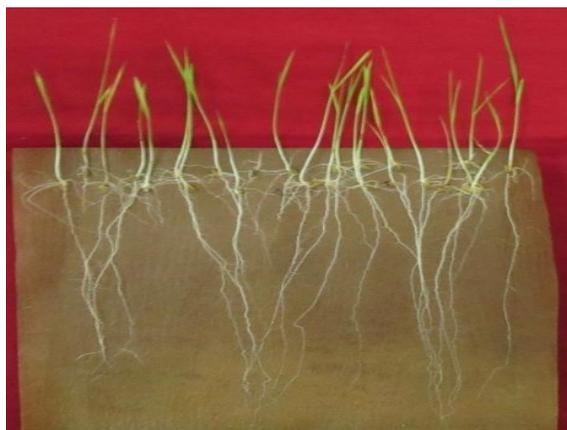


Unprimed Seeds of rice



Bio-primed seeds with 20% (Azospirillum + Phosphobacteria)

ii) Salt stress condition (1% NaCl)



Unprimed Seeds



Bio-primed seeds with 20% (Azospirillum + Phosphobacteria)

The reduction in vigour of the seedlings due to salinity was reversed by the bio-priming treatments in the present study, expressed as improved vigour index values in all treated seeds and T₅ (azospirillum + phosphobacteria) recorded maximum vigour index values both under salt free (3053) and salt stress conditions (2109) than other treatments.

This was expressed as the highest salt stress tolerance index for shoot and root length with seeds bio primed with (T₅) azospirillum+ phosphobacteria (index 1.36 and 1.34 respectively) compared to control (index 1.00 in each) and other treatments including hydropriming (1.07 and 1.12).

Plants exposed to saline environment generally have reduced chlorophyll content in all treatments. Sodium salt stress decreased total chlorophyll content of plants by increasing the activity of the chlorophyll degrading enzyme; chlorophyllase (Rao and Rao 1981), inducing the destruction of the chloroplast structure and the instability of pigment complexes. In the present study also maximum total chlorophyll content was recorded in T₅ (azospirillum + phosphobacteria) under zero level of salinity (0.035 µg/g) compared to control (0.020 µg/g) plants. The total chlorophyll content of leaves averaged over treatments indicated that it decreased from 0.027 µg/g at 0% to 0.012 µg/g at 1% NaCl indicating the salt stress over the growth of the seedlings. But bio-priming has induced salt resistance to the seedlings and it was expressed as the highest chlorophyll content in T₅ (azospirillum + phosphobacteria) treatment (0.017µg/g) compared to control (0.005mg/g) under 1% level of NaCl. However, the decrease in total chlorophyll content with increased salinity was observed in all the treatments.

Lipid peroxidation is the main index of the increase in active free radicals and MDA is the main by-product of the lipid peroxidation

process. Production of MDA, which is an indicative of oxidative stress, increases as salinity stress increases in plant, serves as an index of lipid peroxidation (Meloni *et al.*, 2003). Peroxidation damage of the plasma membrane leads to leakage of contents, rapid desiccation and cell death (Scandalios, 1993). In the present investigation also degree of accumulation of MDA under salt stress condition was higher in untreated plants compared to treated plants. As the salt stress was increased proposal increase in release of MDA was noticed in all treatments including control (4.359 µmol/g fr.wt). But lowest MDA content was recorded in seeds bio primed with (T₅) azospirillum + phosphobacteria (2.185 µmol/g fr.wt), which might be due to an increase in expression of stress related proteins such as, glutathione S-transferase (GST), glutathione dependent formaldehyde dehydrogenase (FALDH) and peroxidase.

The phenol content was also increased substantially with an increase of salt stress level. Treatments differed significantly both under normal and salt stress conditions.

A significantly higher level of phenol content was observed in T₅ as the salt stress increased from 0 dSm⁻¹ to 8 dSm⁻¹ in comparison to other bio-primed treatments. Highest phenol content under salt stress was observed in T₅ (azospirillum + phosphobacteria) treatment (2.72 mg/100 g) with salt tolerance indices of 1.35 respectively. Unprimed seeds (control) showed minimum phenol content (2.02 mg/100 g) with the lowest salt tolerance index of 1.00 (Table 2).

The PMK (R) 4 seeds bio-primed with azospirillum @ 20% (treatment number) registered better values for germination (95%) and vigour index (2898) parameters observed as next best treatment in all parameters under salt stress conditions (Table 3).

Hence, the present study, revealed that seeds bioprimered with 20% solution of azospirillum + phosphobacteria withstand salinity stress better compared to all other treatments. expressed in all observed parameters under salinity stress conditions rice seeds may be bioprimered with liquid biofertilizers and their combined treatments to have better performance, which might be due to the fact that azospirillum and phosphobacteria penetrates into the root tissues and initiates a series of morphological, physiological and biochemical changes in the plant.

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