

Rice Seed Priming with Swine Manure Extract to Ameliorate Vigor and Nutrient Status of Seedlings

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ABSTRACT

Seed priming is one of the strategies for improving rice seedling development in an unfavorable environment. The effects of seed priming by concentrated swine manure extract (CSME) solution were evaluated on rice seedling vigor and nutrient status during the germination of aged and non-aged rice seed of the cultivar Pathum Thani 1 at the Suwanvajokkasikit Animal Research and Development Institute, Kasetsart University, Kamphaeng Saen Campus from March to August 2012. The CSME was diluted with water to ratios of 1:1, 1:2, 1:5, 1:25, 1:50, 1:100 and 1:200 volume to volume to make the tested CSME dilutions for the rice seed priming. The results showed that CSME seed priming significantly ($P < 0.05$) increased the vigor of rice seedlings, including the shoot and root length, fresh weight and dry weight at 21 d after sowing. Seed priming with a CSME dilution of 1:100 provided the optimal results for rice seedling improvement by significantly improving seedling vigor and the contents of P, Ca, Fe, Mn, B, Mo, Ni and Na in the rice seedlings when compared to the non-priming group. The results confirmed that CSME seed priming can enhance seedling vigor and the nutrient status of the rice seeds.

Keywords: swine manure extract, rice, seed priming, seedling vigor, nutrient uptake

INTRODUCTION

Traditional rice seed broadcasting cultivation can be confronted with poor crop results and weed infestation, like any crop. Seedling vigor is an indicator of performance to determine the overall rice growing efficiency and rice yield. Seed priming or a pre-sowing hydration technique are strategies to improve seed germination ability and seedling vigor which can result in a better crop and a higher yield of

rice as seed priming not only produces faster seed emergence and better seedling uniformity, but also helps to maintain vigorous crop growth under suboptimal field conditions as well as to improve earlier flowering which results in a higher grain yield than with non-primed seeds (Harris *et al.*, 2001). Techniques of seed priming include: hydropriming, osmoconditioning, osmohardening, hardening and hormonal priming (Farooq *et al.*, 2006a). These techniques are employed for earlier and better nursery establishment, resulting in an

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improvement over traditional rice production performance and increased reinvigoration of aged seed (Bailly *et al.*, 1998). International Seed Testing Association (2007) suggested that accelerated ageing of seed by exposing the seeds to high temperature and high relative humidity is a validated vigor test for many crops. After the accelerated ageing, the high vigor seed lots maintained a high germination rate, while the low vigor seed lots showed a reduced germination rate (International Seed Testing Association, 2004). There is always a relationship between the vigor test and seedling growth in the field (Milošević *et al.*, 2010).

Nutripriming by soaking seeds in a micronutrient solution of a specific concentration for a specific duration is well documented (Farooq *et al.*, 2012). Several studies have addressed the efficiency of nutripriming in promoting the seedling vigor of rice (Johnson *et al.*, 2005; Farooq *et al.*, 2006b; Prom-u-thai *et al.*, 2012). Slaton *et al.* (2001) demonstrated that seed priming with zinc sulfate solution increased the grain yield by 14.57 to 28.25% over the untreated control. Likewise, priming rice seed with 0.008 M boric acid for 36 hr improved the grain boron content by 700% over the untreated control (Johnson *et al.*, 2005). In addition, osmopriming of rice seeds by KCl and CaCl₂ improved the germination rate, kernel yield and the quality of the field sown rice (Farooq *et al.*, 2006a). The reinvigoration of aged seed by priming maize seed with gibberellin and KNO₃ was demonstrated by Siadat *et al.* (2012). However, the effects of seed priming on rice seedling development under accelerated ageing conditions by using biological waste containing plant micronutrients, is of interest, but poorly understood.

Swine manure extract (SME) is a liquid form of plant nutrient derived from steeping dry swine manure in water for 72 hr and contains a full profile of both the macronutrients and micronutrients required by plants (Kanto *et al.*,

2012). This procedure is a beneficial practice for swine waste utilization where there is limited availability of swine effluent or swine waste in crop growing areas. Monthly foliar and soil applications of SME not only elevated the chlorophyll content and starch content in fresh tubers of cassava, but also improved the dry matter content, the nutrient uptake of the cassava tuber and tuber yields (Kanto *et al.*, 2011, 2012). The extract has also been shown to increase the yields of rice, vegetables and ornamental plants under practical farm conditions in Thailand (Jattupornpong *et al.*, 2012). Therefore, SME is a potential organic plant nutrient source for seed priming. The mechanism of SME in promoting the seed growth of plants needs to be fully elucidated. The hypothesis underlying this study focused on whether seed priming with SME in the form of concentrated swine manure extract (CSME) could increase the vigor of the rice seedling cultivar Pathum Thani 1. Thus, the aims of the study were to provide the first evidence that rice seed priming with CSME promoted the vigor and nutrient status of rice seedlings. The specific objective was to investigate the optimum concentration of CSME seed priming regimes for practical field conditions.

MATERIAL AND METHODS

Production of swine manure extract and determination of nutrient content

Concentrated swine manure extract (CSME) was produced as a stock solution for making SME seed priming by steeping three sets of the dried manure consecutively in the same water at a ratio of manure to water of 1:10 (weight to volume; w:v) for 24 hr with each set (the tea-bag method), making a final ratio of total manure to water of 3:10 (w:v) after 72 hr of steeping. Solids were filtered out after the completion of steeping for each set. The CSME was kept standing unstirred in a plastic drum for 14 d to allow for further fermentation and ageing (Kanto

et al., 2013). The CSME was analyzed for pH, electrical conductivity (EC) and macro-, micro- and trace-mineral contents using a potentiometer, an EC meter and an inductive coupled plasma spectrophotometer (Quantima ICP-OES; GBC Scientific Equipment; Braeside VIC, Australia), respectively. The results indicated that the CSME was alkaline with a pH of 8.7 and EC of 15.5 $\text{mS}\cdot\text{cm}^{-1}$. However, the EC value decreased when the CSME was further diluted with water by the ratio employed in the study. CSME employed in this study was analyzed for the plant nutrient content. The result indicated a full profile of plant nutrients in the CSME with average concentrations of 0.12% N, 11.73 $\text{mg}\cdot\text{kg}^{-1}$ P, 0.56% K, 11.53 $\text{mg}\cdot\text{kg}^{-1}$ Ca, 99.27 $\text{mg}\cdot\text{kg}^{-1}$ Mg, 24.47 $\text{mg}\cdot\text{kg}^{-1}$ Fe, 67.86 $\text{mg}\cdot\text{kg}^{-1}$ Cu, 8.92 $\text{mg}\cdot\text{kg}^{-1}$ Zn, 2.03 $\text{mg}\cdot\text{kg}^{-1}$ Ni, 0.23 $\text{mg}\cdot\text{kg}^{-1}$ B, 20.17 $\text{mg}\cdot\text{kg}^{-1}$ Se and 640 $\text{mg}\cdot\text{kg}^{-1}$ Na.

Seed materials

Seeds of 'Pathum Thani 1' paddy rice with an initial moisture content of 11.95% (dry weight basis), all from the same seed lot, were obtained from the Rice Research Center, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. A sample of 7,200 seeds of the same size was carefully selected and weighed. The seeds were divided into two groups of 3,600 seeds each and one group (Group 1) was randomly subjected to accelerated ageing treatment where the seeds were exposed to 42°C and 100% relative humidity (RH) for 84 hr (Association of Official Seed Analysts, 1983) while the other 3,600 seeds (Group 2) underwent no accelerated ageing treatment as they were not subjected to any treatment. Each group of seeds was further divided into 9 subgroups of 400 seeds each. The seed in each sub-group was further divided into 4 sub-subgroups of 100 seeds to provide 4 replications.

Seed priming treatments and seedling germination

Randomly assigned rice seed in each group was selected to receive seed priming treatments: a) non-priming, b) hydropriming by soaking seeds in aerated distilled water for 24 hr and c) nutripriming by soaking seed in a series of CSME dilutions (CSME:water) of 1:200, 1:100, 1:50, 1:25, 1:5, 1:2 and 1:1 (volume to volume; v:v) for 24 hr. The primed seeds were redried to their initial moisture content under shade with forced air. The ratio of seed weight to solution volume was 1:5 ($\text{g}\cdot\text{mL}^{-1}$) according to Farooq *et al.* (2007). The experiment was conducted as a factorial using a completely randomized design with ageing and seed priming factors with four replications (100 seeds per replication).

Two hundred seeds in replicates of 100 of the same size and strength were carefully selected, and then placed on paper towels saturated with distilled water having pH 6.2 and conductivity 0.1 $\mu\text{S}\cdot\text{cm}^{-1}$ in covered transparent polystyrene germination boxes (33 × 20 × 10 cm). The seeds were germinated in a germination cabinet with an average temperature of 25°C for 14 d. The experiment was carried out using four replicates. Seeds with a primary root, shoot axis, cotyledon and coleoptile were counted as germinating. The first and final counts were carried out on the 4th and 14th days after sowing, respectively (International Seed Testing Association, 2007). The final germination percentage was calculated according to the equations of Ellis and Roberts (1981). After the first day of germination, rice seeds were determined using an injury index. The injury index was estimated from measurement of the electrolyte leakage as described by the method of González-Aguilar *et al.* (2004). After pretreatments, 1 g samples of rice seeds were washed with distilled water to remove the solution from the injured cells and each sample was immersed in 20 mL of mannitol of 0.3 M solution in a 50 mL plastic tube at room temperature. The

tubes were gently shaken for 3 hr and the electrical conductivity of the solution was measured using a conductrometer (ATI Orion model 162; Thermo Scientific Corporation; Beverly, MA, USA) at 25°C. The initial electrical conductivity was measured and then each tube was immersed in boiling water for 30 min and set aside until reaching room temperature. The final electrical conductivity was measured again in the resultant solution. Cell membrane stability was estimated from the formula as described by Kocheva *et al.* (2004).

After the seed priming, the experimental seed in each group (100 seed in each) was germinated by placing the rice seed in a sand box (33 × 20 × 10 cm) containing approximately 2,500 g of cleaned sand for 21 d until the emergence of the rice seedlings. The sand-growing medium was prepared by ensuring 90% of the sand particles passed through a 0.8 mm sieve. The sand was rinsed with tap water until the rinsed water was clean, followed by a single final rinsing with distilled water. The sand-growing medium contained 8.08 mg.kg⁻¹ P, 66.4 mg.kg⁻¹ K, 21.9 mg.kg⁻¹ Fe, 0.0306 mg.kg⁻¹ Cu, 0.349 mg.kg⁻¹ Zn, 0.476 mg.kg⁻¹ Mn, 0.0775 mg.kg⁻¹ Mo, 0.561 mg.kg⁻¹ B, 0.543 mg.kg⁻¹ Ni and 36.2 mg.kg⁻¹ Na. To minimize the effect of nutrient contamination from other sources, and to confirm the promotional effect on rice seedling growth by the seed priming treatment, only de-ionized water was added to maintain the field capacity of the sand-growing medium. The sand boxes were placed under ambient conditions where the average day/night conditions were: temperature, 32.40/26.66°C; RH, 59.71/77.04%; and average daily photosynthetic photon flux density (PPFD) ranged from 651.96 to 1,080.8 μmol PPF.m⁻².s⁻¹.

Seedling vigor measurement, photosynthetic pigment content and nutrient status determination

Root and shoot length, and the fresh

and dried weights of the seedlings were recorded at 21 d after sowing. Ten rice seedlings from one replication were sampled and determined for the chlorophyll a, b, total chlorophyll and carotenoid contents at 14 d after sowing by the 80% acetone extraction method as described by Inskeep and Bloom (1985).

A sample of 50 rice seedlings from one replication (200 plants per treatment) was determined for the nutrient status of the rice seedlings by determination of the contents of K, P, Ca, Mg, Fe, Cu, Mn, Zn, Ni, Mo, B and Na in the whole rice seedling plants at 14 d after sowing. The procedure was carried out by wet digestion of the sample with a digestive acid (HNO₃/H₂O₂) and the mineral concentrations were determined by inductive coupled plasma spectrophotometry (Quantima ICP-OES; GBC Scientific Equipment; Braeside, VIC, Australia). The separated samples were determined for total N by the micro-Kjeldahl procedure (Bremner and Tabatabai, 1972).

Data analysis

The experimental data were subjected to analysis of variance, and the differences among means were determined by Duncan's multiple range test (SAS Institute, 2003). The correlation coefficient (r) between plasma membrane integrity and nutrient concentration was studied. Means that differed at $P < 0.05$ were considered significant.

Place and date of the study

The study was conducted at the Suwanvajokkasikit Animal Research and Development Institute (SARDI), Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand during March to August 2012.

RESULTS

Effect of accelerated ageing on seed germination, seedling vigor, photosynthetic pigment content and nutrient concentration in rice seedlings

There were no significant differences

in the effects of accelerated and non-accelerated seed ageing on the final germination percentage, seedling shoot length, root length and the seedling dry weight. However, non-accelerated ageing significantly increased rice seedling fresh weight compared to the accelerated ageing group (Tables 1 and 2). Accelerated ageing of the seeds caused a significant increase in the contents of chlorophyll b, total chlorophyll, carotenoids and in the carotenoid:chlorophyll (Car:Chl) ratio of the rice seedling by 144.52, 21.09, 29.96 and 16.68%, respectively, but there were no significant differences in the chlorophyll a content between the rice seedlings in the accelerated and non-

accelerated ageing groups (Table 3). Accelerated ageing of the seeds significantly decreased the plasma membrane integrity which was 18.97% lower than for the seeds in the non-accelerated ageing group (Table 1).

The rice seedlings in the non-accelerated ageing group had a significantly higher accumulation than those of the accelerated ageing group of P (2.63 versus 2.49 g.kg⁻¹), Mg (2.25 versus 2.06 g.kg⁻¹), Fe (1.15 versus 0.90 g.kg⁻¹) and Mo (8.57 versus 7.19 g.kg⁻¹), but had significantly lower accumulation of N (9.46 versus 9.91 g.kg⁻¹), Mn (55.13 versus 60.52 g.kg⁻¹), Ni (48.56 versus 54.25 g.kg⁻¹) and Na (3.36 versus

Table 1 Effect of seed priming with concentrated swine manure extract (CSME) on final germination percentage and plasma membrane integrity of accelerated ageing of rice seedlings and non-accelerated ageing of seedling during germination.

Factor	Final germination percentage (%)	Plasma membrane integrity (%)
Ageing (A)		
Non-ageing	84.79	51.49 ^a
Ageing	82.67	45.32 ^b
Treatment (T)		
Non-priming	72.50 ^b	40.04 ^c
Hydropriming	83.88 ^a	50.18 ^{ab}
CSME solution (v:v)		
1:200	90.00 ^a	52.76 ^a
1:100	89.13 ^a	50.23 ^{ab}
1:50	88.63 ^a	52.48 ^a
1:25	89.50 ^a	51.21 ^{ab}
1:5	90.75 ^a	50.63 ^{ab}
1:2	88.13 ^a	40.10 ^b
1:1	58.00 ^c	40.78 ^c
F-test		
A	ns	**
T	**	**
A × T	ns	**

v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at $P = 0.05$.

ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

3.79 g.kg⁻¹) concentrations (Table 4-6). There were no significant differences in the K, Ca, Cu, Zn and B concentrations between the non-accelerated ageing seeds and the accelerated ageing seeds (Tables 4-6).

Effects of seed priming on seedling vigor, photosynthetic pigment contents, and nutrient concentration in rice seedlings

The effects of seed priming which comprised non-priming, hydropriming and CSME dilutions of 1:200, 1:100, 1:50, 1:25, 1:5, 1:2

and 1:1 (v:v) priming on the final germination percentage, seedling vigor, photosynthetic pigment contents and nutrient concentration in rice seedling are shown in Tables 1-6. These results showed that both hydropriming and CSME priming produced a significantly higher final germination percentage, shoot length, root length, seedling fresh weight and seedling dry weight at 21 d after sowing than those in the non-priming group (Tables 1 and 2). Seed priming with CSME at a low concentration significantly improved the plasma membrane integrity which was higher than in the non-priming

Table 2 Effect of seed priming with concentrated swine manure extract (CSME) on shoot length, root length, seedling fresh weight and seedling dry weight of accelerated ageing of rice seedlings and non-accelerated ageing of seedlings 21 d after sowing.

Factor	Plant shoot length (cm)	Plant root length (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)
Ageing (A)				
Non-ageing	14.42	6.28	149.24 ^a	35.63
Ageing	14.40	5.99	143.14 ^b	35.81
Treatment (T)				
Non-priming	13.80 ^d	5.19 ^b	142.41 ^{bc}	31.80 ^d
Hydropriming	14.75 ^{abc}	5.67 ^b	149.70 ^{ab}	37.89 ^{ab}
CSME solution (v:v)				
1:200	14.18 ^{bcd}	6.01 ^b	144.38 ^{abc}	33.78 ^{cd}
1:100	14.87 ^{ab}	6.58 ^{ab}	150.34 ^{ab}	38.32 ^a
1:50	14.60 ^{bc}	6.22 ^b	151.97 ^{ab}	36.62 ^{abc}
1:25	13.97 ^{cd}	7.88 ^a	137.84 ^c	34.30 ^{cd}
1:5	13.70 ^d	5.97 ^b	137.72 ^c	38.30 ^a
1:2	14.34 ^{bcd}	5.89 ^b	146.56 ^{abc}	34.96 ^{bcd}
1:1	15.43 ^a	5.83 ^b	154.80 ^a	35.41 ^{abc}
F-test				
A	ns	ns	*	ns
T	**	*	*	**
A × T	**	*	ns	**

v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at $P = 0.05$.

ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

group (Table 1). However, there were no significant differences in the contents of chlorophyll a, b, a+b, carotenoids and the Car:Chl ratio in rice seedlings among the seed priming groups (Table 3). There was variation in the rice seedling vigor response to different dilution levels of CSME seed priming. The highest values of the shoot length, root length, seedling fresh weight and seedling dry weight at 21 d after sowing of the rice seedlings were obtained from seed primed with CSME dilutions of 1:1, 1:25, 1:1 and 1:100, respectively.

Hydropriming produced significantly

higher contents of P, K, Ca and Mg, but significantly lower contents of Na in the rice seedlings than those of the non-priming group. However, there were no significant differences in the N, Cu, Mn, Zn, B, Ni and Mo contents of rice seedlings between the hydropriming and non-priming groups (Tables 4-6). There was also variation in the rice seedling nutrient content as a result of the different dilution levels of CSME seed priming. The CSME dilution 1:100 seed priming produced the highest contents of P, K, Ca, Fe, Mn, Zn, B, Ni and Mo in rice seedlings, while the CSME dilutions 1:2,

Table 3 Effect of seed priming with concentrated swine manure extract (CSME) on chlorophyll and carotenoid contents of accelerated ageing of rice seedlings and non- accelerated ageing of seedlings 14 d after sowing.

Factor	Chl a (mg .g ⁻¹ FW)	Chl b (mg.g ⁻¹ FW)	Chl (a+b) (mg.g ⁻¹ FW)	Carotenoid (mg.g ⁻¹ FW)	Car:Chl
Ageing (A)					
Non-ageing	0.416 ^a	0.146 ^b	0.569 ^b	0.514 ^b	0.929 ^b
Ageing	0.336 ^b	0.357 ^a	0.689 ^a	0.668 ^a	1.084 ^a
Treatment (T)					
Non-priming	0.317	0.182	0.499	0.531	1.062
Hydropriming	0.312	0.240	0.552	0.613	1.162
CSME solution (v:v)					
1:200	0.420	0.172	0.592	0.635	1.106
1:100	0.360	0.175	0.556	0.558	1.077
1:50	0.377	0.291	0.668	0.602	0.915
1:25	0.352	0.242	0.594	0.628	1.126
1:5	0.408	0.408	0.817	0.682	0.971
1:2	0.422	0.354	0.776	0.614	0.819
1:1	0.355	0.232	0.587	0.487	0.913
F-test					
A	*	**	*	*	*
T	ns	ns	ns	ns	ns
A × T	ns	**	ns	ns	*

FW = Fresh weight; Chl a = Chlorophyll a; Chl b = Chlorophyll b; Car:Chl = Carotenoid:Chlorophyll;
v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at $P = 0.05$.

ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

1:1 and 1:200 seed priming produced the highest contents of Mg, Cu and Na in the seedlings, respectively. The CSME dilution 1:100 seed priming produced significantly higher contents of Fe, Cu, Mn, B, Ni and Na in the rice seedlings than those of the hydropriming. There were no significant differences in the contents of N, P, K, Ca, Mg, Zn and Mo in rice seedlings derived from seed primed with the CSME dilution of 1:100 and hydropriming (Tables 4-6). The results also indicated that the appropriate CSME dilution for rice seed priming is 1:100.

DISCUSSION

The results clearly indicated that accelerated ageing of rice seeds significantly increased the contents of chlorophyll b, a+b, carotenoids and the Car:Chl ratio, but significantly decreased the content of chlorophyll a of the rice seedlings (Table 3). Smolikova *et al.* (2011) demonstrated that a biotic stress to the seeds always resulted in an elevated content of residual chlorophylls as a response to reduce the stress effects. Carotenoids were also found to accumulate in seeds during accelerated ageing and during seed

Table 4 Effect of seed priming with concentrated swine manure extract (CSME) on nitrogen, phosphorus, potassium, calcium and magnesium concentrations of accelerated ageing of rice seedlings and non-accelerated ageing of seedlings 14 d after sowing.

Factor	N (g.kg ⁻¹)	P (g.kg ⁻¹)	K (g.kg ⁻¹)	Ca (g.kg ⁻¹)	Mg (g.kg ⁻¹)
Ageing (A)					
Non-ageing	9.46 ^b	2.63 ^a	2.34	3.16	2.25 ^a
Ageing	9.91 ^a	2.49 ^b	2.26	3.19	2.06 ^b
Treatment (T)					
Non-priming	9.90	2.26 ^d	2.07 ^d	2.75 ^c	1.90 ^c
Hydropriming	9.86	2.53 ^{abc}	2.50 ^a	3.39 ^a	2.34 ^a
CSME solution (v:v)					
1:200	9.65	2.56 ^{abc}	2.30 ^{abc}	3.28 ^{ab}	2.14 ^{ab}
1:100	9.30	2.74 ^a	2.38 ^{ab}	3.39 ^a	2.15 ^{ab}
1:50	9.50	2.39 ^{ab}	2.14 ^{cd}	3.06 ^{bc}	2.01 ^{bc}
1:25	9.86	2.67 ^{ab}	2.27 ^{bcd}	3.22 ^{ab}	2.16 ^{ab}
1:5	9.68	2.70 ^a	2.33 ^{abc}	3.10 ^{ab}	2.24 ^{ab}
1:2	9.76	2.68 ^{ab}	2.36 ^{ab}	3.17 ^{ab}	2.25 ^a
1:1	9.69	2.47 ^{bcd}	2.37 ^{ab}	3.24 ^{ab}	2.23 ^{ab}
F-test					
A	**	**	ns	ns	**
T	ns	**	**	**	**
A × T	**	**	**	ns	**

v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at P = 0.05.

ns = Not significant; * = Significant at P < 0.05; ** = Significant at P < 0.01.

germination at high temperature. The ratio of the contents of carotenoid to chlorophyll (Car:Chl) is proposed as a measure of seed tolerance to stress factors. The results of the present study also revealed that the accelerated aged seeds had a significantly higher Car:Chl ratio than those of the non-accelerated ageing group (Table 3). It is noted that carotenoids might play an important role as antioxidants protecting the seeds against oxidative stress. Although no studies have been reported on the effects of accelerated rice seed ageing on the plant nutrient content of seedlings, the results of the current study found that accelerated ageing

significantly decreased the contents of P, Mg, Fe and Mo, but significantly increased the contents of N, Mn, Ni and Na of the rice seedlings compared to the non-accelerated ageing group, while there were no significant differences in the contents of K, Ca, Cu, Zn and B in the rice seedlings between the accelerated and non-accelerated groups (Tables 4–6). The reason is unknown for both the increases and decreases of the plant nutrients in the rice seedlings mentioned above. Since there is no loss or gain of indigenous mineral content in the rice seeds during the seedling germination, the differences in the plant nutrient content in the

Table 5 Effect of seed priming with concentrated swine manure extract (CSME) on iron, copper, manganese and zinc concentrations of accelerated ageing of rice seedlings and non-accelerated ageing of seedlings 14 d after sowing.

Factor	Fe (g.kg ⁻¹)	Cu (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Ageing (A)				
Non-ageing	1.15 ^a	10.89	55.13 ^b	33.95
Ageing	0.90 ^b	10.58	60.52 ^a	34.90
Treatment (T)				
Non-priming	1.01 ^{cd}	8.03 ^e	43.67 ^g	34.10
Hydropriming	0.83 ^e	8.06 ^e	45.55 ^{fg}	31.65
CSME solution (v:v)				
1:200	0.81 ^e	10.35 ^c	63.68 ^{bc}	37.38
1:100	1.30 ^a	9.71 ^{cd}	73.61 ^a	39.66
1:50	0.92 ^{de}	10.17 ^{cde}	55.88 ^{de}	35.20
1:25	1.12 ^{bc}	8.75 ^{de}	58.69 ^{cd}	36.13
1:5	1.28 ^a	10.59 ^c	66.82	33.07
1:2	1.16 ^{ab}	12.26 ^b	61.56 ^{bcd}	30.99
1:1	0.85 ^e	17.88 ^a	50.97 ^{ef}	30.62
F-test				
A	**	ns	**	ns
T	**	**	**	ns
A × T	**	**	**	ns

v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at $P = 0.05$.

ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

rice seedlings may have come from an exogenous plant nutrient source. It is postulated that the rice root produces root exudate comprised of organic acids and these may have the ability to extract a portion of the P and micronutrients from the growing medium (Bhattacharyya *et al.*, 2013). The different root length of the rice seedlings due to the accelerated ageing treatments may have introduced differences in the ability to extract plant nutrient from the sand-growing medium and caused the differences in plant nutrient content of the rice seedlings. However, the alterations were not significant regarding differences in seedling vigor

including root and shoot length, and the seedling dry weight of rice, except where accelerated ageing significantly decreased the seedling fresh weight of rice (Table 2). Seshu *et al.* (1988) reported that seed ageing was always associated with a decline in the seedling vigor, respiration rate and sugar content accompanied by a complete decline of alpha amylase activity as well as a reduction in the concentration and the number of amino acids and the tRNA and DNA contents with higher RNAase activity. The results of the current study confirmed that there were changes in the rice seed metabolism resulting in significant changes in the chlorophyll

Table 6 Effect of seed priming with concentrated swine manure extract (CSME) on boron, nickel, molybdenum and sodium concentrations of accelerated ageing of rice seedlings and non-accelerated ageing of seedlings at 14 d after sowing.

Factor	B (mg.kg ⁻¹)	Ni (mg.kg ⁻¹)	Mo (mg.kg ⁻¹)	Na (g.kg ⁻¹)
Ageing (A)				
Non-ageing	13.96	48.56 ^b	8.57 ^a	3.36 ^b
Ageing	13.21	54.25 ^a	7.19 ^b	3.79 ^a
Treatment (T)				
Non-priming	12.68 ^{cd}	51.31 ^{abc}	7.46 ^{bcd}	3.15 ^c
Hydropriming	12.82 ^{bcd}	47.66 ^c	8.04 ^{abc}	2.38 ^d
CSME solution (v:v)				
1:200	13.09 ^{bcd}	56.65 ^{ab}	7.95 ^{abc}	4.02 ^a
1:100	14.99 ^a	58.09 ^a	10.24 ^a	3.92 ^a
1:50	13.02 ^{bcd}	53.39 ^{abc}	9.05 ^{ab}	3.51 ^b
1:25	14.50 ^{abc}	49.8 ^{6bc}	8.65 ^{ab}	3.86 ^a
1:5	14.68 ^{ab}	47.96 ^c	7.84 ^{bc}	3.96 ^a
1:2	13.55 ^{abcd}	48.05 ^c	5.77 ^{cd}	3.89 ^a
1:1	12.10 ^d	48.69 ^c	5.40 ^d	3.50 ^b
F-test				
A	ns	**	**	**
T	**	*	**	**
A × T	ns	*	ns	**

v:v = Volume to volume.

Means (n = 4) within a column followed by the same lowercase superscript letter are not different according to Duncan's new multiple range test at $P = 0.05$.

ns = Not significant; * = Significant at $P < 0.05$; ** = Significant at $P < 0.01$.

content and the nutrient accumulation in the rice seedlings. However, the accelerated ageing treatment effected only a significant decrease of seedling fresh weight but not the root and shoot length and the seedling dry weight of rice. The results of the study are in contrast to Kapoor *et al.* (2011) who reported that accelerated ageing of five cultivars of rice seeds at 45°C, 100% RH for 96 hr significantly ($P < 0.05$) reduced the germination rate, shoot length and root length of the seedlings. Furthermore, there was variation in the responses to accelerated ageing among the cultivars of rice studied. Yamauchi and Winn (1996) reported that there is a rice cultivar (ASD1) in Myanmar that resists the seed ageing treatment. The results of the current study indicated that Pathum Thani 1 rice seed also has the property of ageing resistance.

In general, the results of the study indicated that seed priming either by hydropriming or CSME dilution could not only improve the seed germination, seedling vigor under unfavorable conditions associated with high humidity and temperature but could also enhance nutrient homeostasis in the rice seedlings. Farooq *et al.* (2006a) reported that an increase in the hydropriming period of rice seeds significantly ($P < 0.05$) increased the seed germination rate and the seedling vigor of both fine rice (*Oryza sativa indica* L.) and coarse rice (*Oryza sativa japonica* L.), with an optimal hydropriming period of 48 hr. Hydropriming of the rice seeds in their study significantly increased ($P < 0.05$) the shoot length and dry weight of the seedlings but not the root length and fresh weight compared to the non-priming group. Hydropriming of the rice seeds also significantly increased ($P < 0.05$) the P, K, Ca, Mg, Fe and Na contents, but not any of the chlorophyll contents nor the contents of N, Cu, Mn, Zn, B, Ni and Mo of the seedlings compared to the non-priming group. Hydropriming of rice seeds was reported to result in the breakdown of seed dormancy and to increase metabolic activities in rice seeds (Soon *et al.*, 2000). Although the

effects of hydropriming on the plant nutrient content in rice seedlings have been previously reported, the results of the current study indicated that hydropriming significantly increased the contents of P, K, Ca, Mg, Fe and Na and may be involved in increasing the metabolic activities in the rice seedling.

CSME dilution priming produced various responses on the seedling vigor, chlorophyll content and plant nutrient content of the rice seedlings. Different CSME dilution priming levels showed maximum and significantly higher seedling vigor and plant nutrient contents than those of the non-priming group with: the 1:1 solution regarding shoot length, seedling fresh weight and Cu content; the 1:2 solution regarding Mg content; the 1:25 solution regarding root length; and the 1:100 solution regarding seedling dry weight, and the contents of P, K, Ca, Fe, Mn, Zn, B, Ni, Mo and Na. However, a CSME dilution of 1:100 produced no significantly different effects in the shoot length, root length, seedling wet weight and Mg and Cu contents than those of CSME dilutions 1:1, 1:25, 1:25 and 1:2, respectively. The results indicated that a CSME dilution of 1:100 is appropriate for rice seed priming as this level not only provided significantly higher seedling vigor but also provided significantly higher plant nutrient accumulation in the rice seedlings. Farooq *et al.* (2012) reviewed the beneficial effects of micronutrient applications through rice seed priming on improving crop growth and grain nutrient enrichment. CSME contains a full profile of plant nutrients consisting of both macro- and micro-nutrients (Kanto *et al.*, 2012), therefore providing for plant nutrient accumulation in the rice seeds and seedlings. A CSME dilution of 1:100 priming produced no significant differences in the shoot and root length, seedling wet and dry weight, and the N, P, K, Ca and Mg contents, but a significantly higher ($P < 0.05$) micronutrient content including the Fe, Cu, Mn, Zn, B, Ni and Mo contents in the rice seedlings when compared

to those of hydropriming. There was a trend for a higher concentration of CSME dilution to cause lower seedling vigor and lower accumulation of plant nutrients in the seedlings which was due to cell leakage. A high concentration of CSME may cause toxicity in the cell membrane resulting in poor plasma membrane permeability, reducing the imbibition performance of the nutrient solution and resulting in low germination and low seedling vigor (Robert, 1948; Memon *et al.*, 2013). The mineral nutrient status of the plant plays a crucial role in enhancing plant resistance to environmental stress factors (Marschner, 1995) particularly the potassium status, as it plays a special role in contributing to the survival of crops under environmental stress conditions (Cakmak, 2005). Moreover, Ahmad *et al.* (2009) demonstrated that sunflower seed exposed to nickel at low concentrations of 10 and 20 mg.L⁻¹ significantly ($P < 0.01$) improved early seedling growth such as plumule, radical length, fresh and dry weight of seedling, while seed exposed to higher levels of nickel (40–60 mg.L⁻¹) showed significantly reduced seed germination and seedling growth. However, it is important to mention that the nutrient concentration accumulation in rice seedlings was dependent on the plasma membrane permeability which was measured via relative injury during the seed priming treatment. The results from the present study showed that the plasma membrane integrity has a positive relationship to the concentrations of P ($r = 0.4643$, $P = 0.0074$), Mg ($r = 0.3763$, $P = 0.0338$), Fe ($r = 0.4055$, $P = 0.0213$), B ($r = 0.3834$, $P = 0.0303$), and Mo ($r = 0.4955$, $P = 0.0039$) in the rice seedlings. Seed priming with CSME at a low concentration could potentially restore the membrane stability to transport nutrient to the seed during imbibition.

Seed priming by both hydropriming and CSME dilution priming provided significantly higher shoot length, root length and seedling dry weight of the rice seedlings than did non-priming. This was due to seed priming with water and

CSME improving the nutrient status in the rice seedling (Table 4-6). The improvement in the rice root and shoot lengths, as well as the seedling fresh and dry weights may be explained by an increased rate of cell division in the root tips. Ca²⁺ is a constituent part of the newly developed cells for cell elongation (Farooq *et al.*, 2006b). The present study provides the first evidence that CSME seed priming could significantly elevate the plant nutrient uptake, especially of molybdenum by rice seedlings (Table 5). However, seed vigor is always affected by the genetic constitution of the seed, the external conditions under which the mother plant is grown, the nutrition applied during that period, the maturity of the seed at harvest, the seed weight and its size (Milošević *et al.*, 2010). CSME is a cheap source of micronutrients for rice seed priming and rice seedling production. CSME seed priming may be an alternative method to soil application for the supplementation of limited nutrients. However, appropriate CSME dilution is required for the optimal rice seed priming result.

CONCLUSION

Rice seed ageing significantly increased the contents of chlorophyll b, a+b, carotenoids and the Car:Chl ratio, N, Mn, Ni and Na in rice seedlings, but significantly decreased their contents of chlorophyll a, P, Mg, Fe and Mo when compared to the non-ageing group. However, the alteration caused no significant differences in rice seedling vigor, except the seedlings subjected to accelerated ageing had a significantly lower seedling fresh weight than those of the non-ageing group. Seed priming using either hydropriming or CSME dilution improved the seedling vigor, photosynthetic pigment content and nutrient status of the rice seedlings. Seed priming with a CSME dilution of 1:100 provided the optimal results for improved seedling vigor and plant nutrient accumulation in the rice seedlings which were superior to those of hydropriming and non-priming.

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