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Effects of Rice Seeds Coated with Calcium Peroxide on Rice Seedlings Establishment

[1]*V. Jeyanny*, [2]*S.R. Syed Omar*, [3]*A.S. Juraimi and* [4]*M. Azmi*

[1]Forest Research Institute of Malaysia, 52109 Kepong, Selangor, Malaysia
[2]Department of Land Management, [3]Department of Crop Science,
Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[4]Rice Research Division, Malaysia Agricultural Research and Development Institute (MARDI),
Seberang Perai, Mail Box 203, 13200 Kepala Batas, Pulau Pinang, Malaysia

Abstract: Rice seeds coated with calcium peroxide have been reported to increase germination and seedling growth in flooded paddy fields. However, studies on the binding agent which plays an important role in maintaining the adhesion of the coating materials to rice seeds and the various coating techniques are relatively limited. The objective of this study was to investigate whether a suitable calcium peroxide coating technique could be developed and to evaluate the coating strength, germination and seedling establishment of coated seeds using three different binders in rice water seeding practice. MR219 rice seeds were weighed and soaked in water for 24 h before coating with 40% calcium peroxide, 30% gypsum and 30% starch flour (filler), of seed weight. Seeds were sprayed with binders according to treatments. These treatments were water (control), sodium lignosulfonate and sodium silicate. The coating strength of the dried coated seeds was tested using the TA-XT2i model Texture Analyser, after which their germination and seedling establishment were evaluated. Sodium silicate binder significantly increased the coating strength (33 folds) compared to control. Germination, emergence score and seedling height for control was higher compared to water, sodium silicate and sodium lignosulfonate. No significant differences were recorded for root length for all treatments tested. Sodium silicate improved the coating strength, which determines the durability of the coated seeds. However, the use of coated seeds needs further investigation in highly reduced soils with the possibilities of brief oxidizing effect and other anaerobic toxic products affecting the overall establishment.

Key words: Rice seed · calcium peroxide · rice seedling establishment · binders

INTRODUCTION

Asian farmers in the tropics are shifting gradually from transplanting techniques to direct seeding, as it is less labor intensive and cheaper. In direct seeding, particularly in flooded soils, the rice seed germination and subsequent growth is hindered because of hypoxia [1]. Thus, studies on promoting germination of rice seeds have been carried out by supplying seeds with oxygen that may have positive effects on seed germination and establishment. Studies have shown that the utilization of zinc peroxide [2], magnesium peroxide [3] and calcium peroxide [4, 5] were able to improve oxygen supply to rice seeds. However, the materials used for coating seeds and the coating process differ from one another. The maximum emergence of rice seedlings requires a 40% calcium peroxide coating on seed weight [6]. A chemical with 60% calcium peroxide was also able to supply 1000 μg of oxygen with 35% coating rate of seed weight [3]. In Japan, 100% coating of dry seed weight was recommended using a calcium peroxide and gypsum formula combined with water spraying [7]. In some studies, seeds were soaked 24 h [8] or 48 h [9] before the coating process took place. The coating process is also facilitated using a mechanical rotating basin [4, 6, 7], particularly for a uniform coating of the rice seeds.

Binders play an important role in coating seeds. Binders such as water [7] or polyvinyl alcohol [6] have been used to increase adhesion strength. Some workers prefer using sodium silicate [10] and lignosulfonate [11]

Corresponding Author: Dr. Syed Omar Syed Rastan, Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor Malaysia

as binders in rice seed coating. Sodium silicate or 'water glass' as a 50% viscous solution in water was able to coat rice seeds. Sodium lignosulfonate which is usually used as a food binder [12] has also been used due to its gel forming properties [13]. The method of coating rice seeds with calcium peroxide plays an essential role in further improving rice seedling establishment in flooded soils. Thus, the objectives of this study were: 1) To investigate whether a suitable calcium peroxide coating technique could be developed and 2) To evaluate the coating strength, germination and seedling establishment of coated seeds using three different binders in rice water seeding practice.

MATERIALS AND METHODS

Procedure of coating rice seeds with calcium peroxide: A 60% calcium peroxide in CALPER 60C was selected as oxygen supplier. The chemical reactions which usually take place are as follows [7]:

$$2CaO_2 + 2H_2O \rightarrow 2Ca\,(OH)_2 + O_2$$

$$Ca\,(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$$

When coated seeds are sown in water, the oxygen derived from calcium peroxide is believed to supply sufficient oxygen for germination and seedling emergence. Incorporation of more than 25% of gypsum [7] and 40% calcium peroxide [6, 9] in the coating material of rice seeds was found to enhance rice seed germination and growth. Based on these studies and our preliminary trials, a coating material containing 40% calcium peroxide, 30% gypsum and 30% starch flour (filler material) was developed to facilitate the coating process. From the preliminary trials, 12% sodium lignosulfonate solution and 50% sodium silicate solution [10] provided a cohesive coating. Thus, these two binders were tested alongside water (control).

Rice seed coating procedure: A procedure for coating rice seeds was developed for small scale seed coating. Instead of a rotating basin [6], a 500 ml beaker (9.5 cm diameter x 12 cm height) was used during the coating process. The beaker was manually rotated to facilitate the coating process. During our preliminary trials, we observed that the coating was uneven and unsatisfactory. Thus, rice seeds were soaked for 24 h [5] before coating with calcium peroxide. The dry weight of rice seeds and soaked rice seeds were taken to determine the amount of water absorbed by rice seeds.

Table 1: Amount of coating material used during coating

Coating material	40% CaO_2	30% Gypsum	30% Starch flour (filler)
Amount (g)	6.0	4.5	4.5

Table 2: Amounts of binders used during coating

Binders	Water	Sodium lignosulfonate	Sodium silicate
Amount (ml)	5.49	5.51	8.05

Dry weight of rice seeds (*approximately* 630 seeds): 15 g

- Weight of soaked rice seeds: 19.52 g
- Water absorbed in 24 h soaking: 4.52 g

Water, sodium lignosulfonate and sodium silicate were used as binding agents in this study. Weighed portions of calcium peroxide, gypsum and starch flour were manually mixed in a beaker to form a uniform powdered material. The amounts of coating material and binders used are shown in Tables 1 & 2, respectively.

MR219 soaked rice seeds were transferred into a 500 ml beaker in small portions. During the coating process, water mist was sprayed unto the seeds using a hand held mist sprayer and the mixed coating material in powder form was sprinkled alternately, while rotating the beaker to ensure uniform coating. The coating was done until all seeds were coated with the appropriate amount of coating material and without the seeds sticking to each other. The coated seeds were dried at room temperature (25-30°C; [5]) for 48 h to allow the hardening of the coating material to the rice seeds. The coating procedure was repeated for sodium lignosulfonate and sodium silicate binders. The coating process took approximately 5 min per replication. All treatments were replicated four times.

Determination of coating strength: Coated seeds were left in the open to equilibrate to constant weight at room temperature (25-30°C) before testing [1]. Coating strength of 5 seeds from each replication of each treatment was tested using the TA-XT2i (Microstable Systems, UK) Texture Analyser. Specimen was placed in the centre of the supporting plate such that the rupture point was measured to be the centre of the coated rice seeds. A 2 mm diameter cylindrical probe at a speed of 2.0 mm s^{-1} in a compression mode with a rupture distance of 0.7 mm was used. The rupture distance was standardized for all treatments as it was the average thickness of the coating layer. The increase in rupture distance resulted in the rupture of the natural husk of the rice grain. The peak force was measured in Newton (N). Treatment effects were analyzed using analysis of variance and Duncan's New Multiple Range Test (DNMRT) was used for means comparison using Statistical Analysis System (SAS) software [15].

Evaluation of germination and emergence of coated seeds: One hundred seeds from each replication were sown in an 8.5 cm diameter petri dish lined with moistened filter paper. The filter paper was moistened with 4 ml of distilled water and kept saturated throughout the germination period. A batch of uncoated seeds was included as control. All treatments were kept for 7 days at room temperature (25-30°C) to germinate [16] and germination percentage was calculated [17].

For the emergence test, ten seeds (based on the surface area of experimental pot) from each replication were sown in experimental containers on soil surface containing 2.5 cm depth soil (450 g). The soil used in this study was a riverine alluvial soil (Typic Paleaquults) [18]. Uncoated pre-sprouted seeds were also included as control. Water level was maintained at 5 cm above soil surface to simulate a flooded condition. Seedling emergence [19] and emergence score whereby 0 = no emergence, 1 = coleoptile emerged, 2 = 1st leaf emerged, 3 = 2nd leaf emerged [5] were determined 14 days after sowing (DAS). The experimental design used was a Randomized Complete Block Design (RCBD). ANOVA was used to test treatment effect and DNMRT used to compare mean difference using SAS.

Evaluation of redox potential and pH measurement: The redox potential (Eh) and pH of the soil were measured every 24 h for 14 DAS using a pH/ORP transmitter (Pro-series model P3, GLI International, Colorado, America). The probe of the transmitter was inserted into the soil in the seed vicinity (2 cm depth) until stable readings were obtained. The probe was calibrated in a known standard solution (220 mV) before readings were taken. The pH sensor was calibrated using pH 4.0 and pH 7.0 buffer solutions. All readings were expressed as the means of three observations in every pot [20].

Evaluation of seedling height and root length: The seedling heights for all treatments were taken using a measuring tape by measuring the height of the plant from the base of the soil to the tip of the longest leaf [1]. The root length was also measured by washing away excess soil from the roots and measuring them from the base of the plant to the longest root tip.

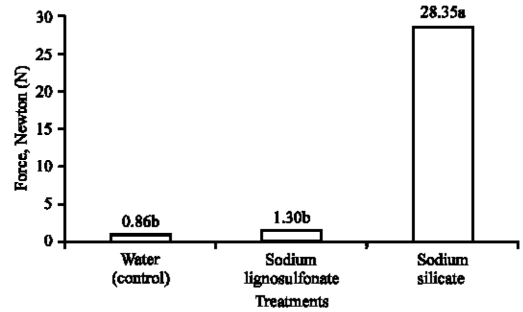

Fig. 1: Force tested on coated rice seeds using three different binders

Note: Individual bars with the same letters are not significantly different at $p<0.05$ according to DNMRT

RESULTS AND DISCUSSION

Coating rice seeds with calcium peroxide using sodium silicate as a binder significantly increased the coating strength compared to water (control) and sodium lignosulfonate (Fig. 1). In this study, the performances of water and sodium lignosulfonate binders were not statistically significant (Fig. 1). The force tested on seeds coated with sodium silicate binder was 33 times greater compared to the control (Fig. 1). The effect of sodium silicate was 22 times greater compared to sodium lignosulfonate.

Silicates were observed to bond by chemical binding or matrix reactions, developing high adhesion strengths [21]. Incorporation of a filler might have enhanced the effectiveness of the binder. Laboratory studies proved that 20% starch in diluted sodium silicate improved the green bond strength [21]. The presence of calcium source from gypsum [19] and calcium peroxide has also been observed to enhance the adhesion strength of the coating material to the rice seeds. Silicate portions are able to react chemically with positive cations such as Ca^{2+}, Mg^{2+} and Zn^{2+} [21]. High amounts of Ca^{2+} cations may have bonded chemically with silicates and acted as a chemical binder to improve the coating strength of the materials used. This was further explained when higher concentration of gypsum resulted in the reduction of setting time and increased the compressive strength of sodium silicate-based alkali activated slag binder [22].

The germination for control was similar to water, but significantly different compared to sodium lignosulfonate and sodium silicate (Table 3). The values for germination were in the order of: control (99.3%) > water (92.3%) > sodium silicate (82.5%) > sodium lignosulfonate (79.0%).

Table 3: Treatment effects of three different binders on germination, seedling emergence and emergence score of coated rice seeds

Treatments	Germination (%)	Emerged seedlings	Emergence score
Uncoated seeds (control)	99.3a	8.50a	4.50a
Water	92.3ab	9.25a	3.40c
Sodium Lignosulfonate	79.0c	8.50a	3.70bc
Sodium Silicate	82.5bc	8.25a	3.85b

Note: Individual values in column with the same letters are not significantly different at p<0.05 according to DNMRT

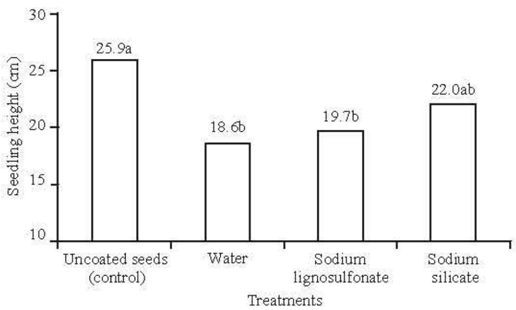

Fig. 2: Treatment effects on seedling height

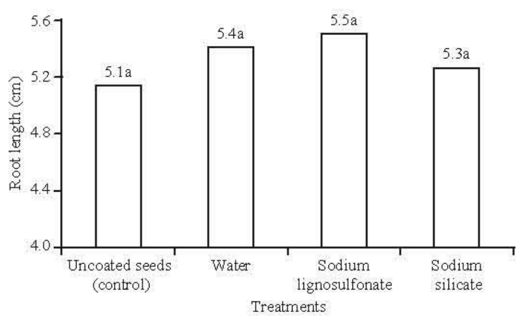

Fig. 3: Treatment effects on root length

Note: Individual bars with the same letters are not significantly different at p<0.05 according to DNMRT

Germination more than 80% is acceptable for rice cultivation [23]. From the results, sodium lignosulfonate coated seeds were in the borderline as the germination level was only 79%. But the performances of the other treatments were consistent with the recommended value [23]. The reduced germination in coated seeds compared to control may be due to an alkaline medium developed from the solution of calcium peroxide in water [24]. From our observations, we believe that the coated layer of the coated seeds might have delayed the emergence of the tip of the coleoptile to some extent compared to control.

The number of emerged seedlings in a pot out of 10 seeds sown was the highest for water (9.25) (Table 3). Besides, it was also the second highest in terms of germination. Since water spray was used during the coating process, it may have had a priming effect on coated seeds [4] and as a result increased germination and number of emerged seedlings. Nevertheless, in our findings, the coating strength of seeds treated with water was not satisfactory. The coating material peeled off easily and was very fragile to handle. The seeds were also not coated evenly as the differences in the sizes of the coated seeds varied.

The emergence score for control was significantly higher compared to the other treatments (Table 3). The emergence score for control was 17, 22 and 32% higher than sodium silicate, sodium lignosulfonate and water, respectively. Sodium silicate and sodium lignosulfonate showed no significant differences (Table 3). The lowest value for this variable was recorded for water.

In terms of seedling height, control was significantly different compared to water and sodium lignosulfonate, but not sodium silicate (Fig. 2). Regardless of different treatments, root length was not significantly affected (Fig. 3). This demonstrates that calcium peroxide coating effects were more significant for seed germination, hence its effect on seedling height.

The soil pH increased for all treatments from one DAS and two DAS (Table 4). Two days after sowing, the pH for T2 and T4 ranged between 6.2 to 6.8 while that of T1 was between 6.1 to 6.6. Nevertheless, the soil pH of T1 was significantly lower than the other treatments from three DAS onwards except for ten DAS where T1 and T3 were similar.

The redox potential (Eh) of the submerged soil decreased steadily for all treatments from one to four DAS and generally leveled off 5 DAS till the end of study (Fig. 4). Highly negative redox potential was observed for all treatments. The increasing negative potentials (4 DAS) was in the following order: T2 > T4 > T3 > and T1. At 3 DAS, Eh of the coated seeds (T2, T3 and T4) were relatively lower (-203 mV to -234 mV) compared to control (-166 mV).

The profound decrease in emergence score (Table 3) and seedling height (Fig. 2) of the coated seeds compared to uncoated seeds (control) may be attributed to soil biochemical processes such as soil pH and redox potential.

Neutral pH increased soil reduction [25] and the average soil pH recorded for control and coated seeds (T2, T3 and T4) were 6.3 and 6.6 onwards, respectively

Table 4: Changes in soil pH in the soil solution

	Days after sowing (DAS)														
Treatments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Average
T1	5.9b	6.2a	6.3b	6.3b	6.3c	6.1b	6.4c	6.5b	6.4c	6.6b	6.2b	6.1b	6.1b	6.2b	6.3
T2	5.9b	6.5a	6.6a	6.6a	6.6b	6.7a	6.7b	6.8a	6.8a	6.8a	6.7a	6.7a	6.7a	6.7a	6.6
T3	6.0ab	6.7a	6.6ab	6.4b	6.7a	6.7a	6.7b	6.8b	6.6b	6.6b	6.6a	6.6a	6.6a	6.6a	6.6
T4	6.0a	6.2a	6.5ab	6.7a	6.6b	6.8a	6.8a	6.7a	6.8a	6.8a	6.8a	6.7a	6.7a	6.7a	6.6

Note: Same letters in column are not significantly different at $p = 0.05$ according to DNMRT

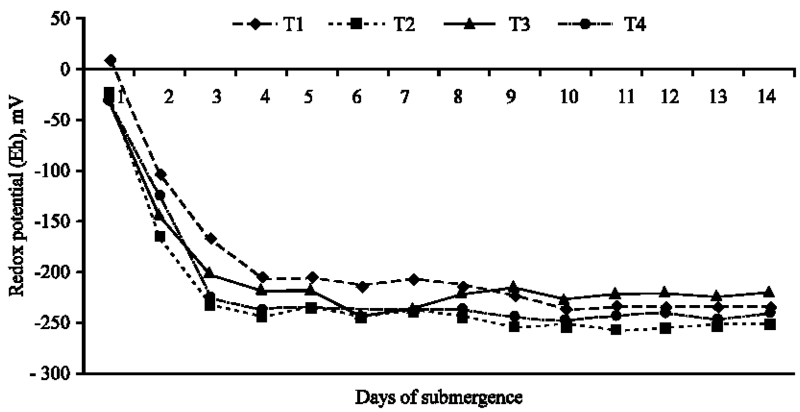

Fig. 4: Changes in redox potential in the soil solution

(Table 4). The addition of Ca^{2+} may have caused an alkaline effect for the coated seeds [24], causing pH increase towards neutral (pH 7.0) levels.

It was reported that the oxidation of the soil around the seed is more important than the supply of oxygen to a seed [4]. Malaysian paddy soils are usually in reduced state (flooded conditions). The area around the coated seeds is oxidized first and then reduced rapidly [25]. The oxygen supplied from the calcium peroxide might have been used up in the reduction process, causing an anaerobic effect for rice seeds. Since soil-oxidizing effect of calcium peroxide only lasted until germination (3-5 days after sowing) and was more reduced thereafter [26], the performance of coated seeds for emergence score and seedling height compared to control were associated with rapid oxygen uptake for reduction processes due to calcium peroxide coating.

Moreover, the surrounding conditions such as pH range between 6.5 to 7.5, temperature above 30°C [25], Eh value of -200 mV and prolonged incubation time more than 8 days [27] favoured rapid reduction processes.

Regardless of different treatments, root length was not significantly affected (Fig. 3). This demonstrates that calcium peroxide coating effects were more significant for seed germination and the subsequent seedling establishment in terms of seedling height.

CONCLUSIONS

Although all the binders may be considered in future rice seed coatings, the use of sodium silicate as a binder significantly improved the coating strength, which is associated with the durability of the coated seeds. Sodium silicate also gave satisfactory effects on seed germination and emergence. However, the performance of coated seeds in flooded soils, especially in highly reduced soils needs further investigations on the possibility of a brief oxidizing effect and other anaerobic toxic products affecting the overall establishment of rice seedlings.

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