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STUDIES ON APPROPRIATE CHITOSAN TYPE AND OPTIMUMP CONCENTRATION ON RICE SEED STORABILITY

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ABSTRACT

Chitosan can be used as seed coating for stimulating germination and extending storability in many crop species. The objective of this work was to determine the optimum concentration and appropriate type of chitosan to use as seed coating before storage for maintaining seed quality. The experiment was conducted using completely randomized design with seven methods of seed soaking: seed soaked in water, seed soaked in oligomeric chitosan solution at the concentration of 50, 100, 150 ppm and seed soaked in polymeric chitosan solution at the concentration of 50, 100, 150 ppm, before storage at various time periods. It was performed during November 2010 to September 2011 in laboratory of Plant Science department, Faculty of Agricultural Technology and Agro-industry, Rajamangala University of Technology Suvarnabhumi. The results indicated that seed moisture content, shoot length, germination percentage, electrical conductivity and speed of germination were significantly influenced by seed soaking with different types and concentrations of chitosan solution in some months of storage. Soaking the rice seeds with polymeric chitosan at the concentration between 50-150 ppm showed positive effects on rice seed storability over than soaking in oligomeric chitosan and water. In this study, it can be recommended that the most effective concentration to use as seed coating before storage was 50 ppm of polymeric chitosan.

Keywords: seed soking, chitosan concentration, rice seed, storability.

INTRODUCTION

Rice is very important economic crops in Thailand. Most areas of the country grow rice plants as main crop. Therefore, Thailand has been one of the biggest rice exporter for many years. In 2011, Thai rice is exported about 1000 million-ton with the values of 6000 million US dollars. Normally, directed - seeding rice growing system has been performed in Thailand; however one problem of its system is poor seed quality due to seed deterioration during storage in open-air (room temperature) which may be caused by various factors, particularly fungal and store pest infection. In the past chemical seed treatments were often utilized for protecting and solving these problems, consequently, negative impact on both environment and human was seriously occurred. Hence, the ways to solve seed deterioration from storage and be also friendly to environment and human should be considered. Chitosan, a natural biopolymer, derived from chitin which is the structural component in the exoskeleton of crustaceans (crabs and shrimp) and cell walls of fungi. It has been used in agriculture as a natural seed treatment and plant growth promotor. Several previous works reported about chitosan as extending vase life of gladiolus and keeping quality of sweet pepper (Ramos-Garcia *et al*., 2009; Xihong Li *et al*., 2011), enhancing seed germination rate and seedling growth (Guan *et al*., 2009; Kananont *et al*., 2010; Ziani *et al*., 2010; Thobunluepop *et al*., 2009; Zeng and Luo, 2012) and inducing immune system to resist to several plant diseases, and tolerate to environmental stress (Sharathchandra *et al*., 2004; Rodriguez *et al*., 2007; Yang *et al*., 2009). Furthermore, chitosan acts as antifungal agent for protecting seed during storage (Thobunluepop, 2008) and also promotes plant growth and increases yields in many crop species (Boonlertnirun *et al*., 2012; Lizarraga-Paulin *et al*., 2011; Ghoname *et al*., 2010; Abdel-Mawgoud *et al*., 2010). In this investigation, we were to assess the efficiency of chitosan by focusing on optimum concentration and appropriate type of chitosan to be used as seed coating for maintaining seed quality (viability and vigor) during storage times.

MATERIALS AND METHODS

Experiment design

Completely randomized design with 7 treatments and 4 replications was performed. The treatment details are as follows: soaking rice seeds in water (control), oligomic chitosan at the concentration of 50, 100, 150 ppm and polymeric chitosan at the concentration of 50, 100, 150 ppm.

Chitosan and seed preparation

Both oligomeric and polymeric chitosan solutions used in this experiment extracted from shrimp shell with 90% level of deacetylation. Rice seeds cv. Pathum Thanee 1 were soaked in chitosan solution in different types and various concentrations according to the mentioned treatment details for 3 hours, and then dried at 35^0C with 10± 2% seed moisture content, after that placed that seeds in sealed plastic and kept in room temperature for 10 months. Seed quality and vigor assessment were performed every 2 months.

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Seed quality and vigor assessment

Seed moisture content was tested by hot air oven method and calculated to be seed moisture content by the following equation:

$$\text{Seed moisture content (\%)} = \frac{\text{wet seed weight - dry seed weight x 100}}{\text{wet seed weight}}$$

Seed viability was determined as normal seedling percentage in top of paper (TP) at 25^{o}C for 14 days by standard germination test and calculated as the following equation:

$$\text{Standard germination} = \frac{\text{Normal seedling x100}}{\text{Total used seed number}}$$

Dehydrogenase activity of rice seeds was detected by Tetrazolium Test (TZ Test) (ISTA, 2006).

Seed vigor was assessed by measuring shoot length, electrical conductivity and speed of germination.

Statistical analysis

All data were subjected to analysis of variance according to experimental design used in this study (CRD) by MSTAT programme and treatment means were compared by the Least Significant Difference (LSD).

RESULTS AND DISCUSSIONS

Seed moisture content

Seed moisture content of rice seeds was not significantly different after soaking the seeds in different types and various concentration of chitosan solution and then stored in room temperature for 2, 4, 6 and 10 months. However, at 8 months of storage, seed moisture content was significantly different among treatments. Soaking the seeds in water showed higher seed moisture content than soaking those in chitosan solution at different concentrations and types. Moisture content of rice seeds soaked in polymeric chitosan solution tended to be lower than those in oligomeric chitosan, particularly, at the concentration of 100 ppm had the lowest seed moisture content (Table-1).

Table-1. Effect of chitosan on seed moisture content after storage at various time periods.

Seed soaking (in)	**Seed moisture content** (%)				
	Months after storage (month)				
	2	**4**	**6**	**8**	**10**
Water (control)	5.36	6.11	5.71	7.20 a	6.78
Oligomeric chitosan at 50 ppm	5.26	6.19	5.56	5.67 ab	5.70
Oligomeric chitosan at 100 ppm	5.28	6.08	5.59	5.70 ab	5.39
Oligomeric chitosan at 150 ppm	5.56	5.94	5.10	6.89 a	5.63
Polymeric chitosan at 50 ppm	5.55	5.78	5.23	5.68 ab	5.53
Polymeric chitosan at 100 ppm	5.77	5.88	5.65	4.66 b	6.21
Polymeric chitosan at 150 ppm	5.38	5.06	6.34	5.03 b	5.45
LSD 0.05	ns	Ns	ns	1.65	ns
CV (%)	5.93	13.40	14.50	19.24	16.08

Shoot length

Soaking the seeds in water and different types and concentrations of chitosan had significant effects on shoot length after storage at 2, 6 and 8 months. The highest shoot length was achieved from seed soaking in polymeric chitosan, especially at the concentration of 150 ppm. Seeds soaked in water before storage showed negative effect on shoot length after 2 and 8 months of storage. No significant difference on shoot length was detected after storage at 4 and 10 months, however seeds soaked in in polymeric chitosan at the concentration of 150 ppm tended to promote shoot length greater than the other treatments (Table-2). Ziani *et al*. (2010) reported that artichoke seed coated with chitosan resulted in highly developed seedlings compared with the control. Seed priming with chitosan 0.5 % enhanced shoot length of maize (Guan *et al.*, 2009).

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Table-2. Effect of chitosan on shoot length of rice seedling after storage at various time periods.

Seed soaking (in)	**Shoot length** (cm)				
	Months after storage (month)				
	2	**4**	**6**	**8**	**10**
Water	9.96 b	10.62	10.69 bc	10.53 c	10.92
Oligomeric chitosan at 50 ppm	10.01 b	10.45	10.43 c	10.88 bc	11.09
Oligomeric chitosan at 100 ppm	10.67 ab	10.64	10.85 abc	10.75 c	11.01
Oligomeric chitosan at 150 ppm	10.86 a	10.54	10.95 ab	11.16 ab	10.87
Polymeric chitosan at 50 ppm	11.18 a	10.52	11.33 a	11.34 a	11.18
Polymeric chitosan at 100 ppm	11.12 a	10.83	11.12 ab	11.45 a	11.16
Polymeric chitosan at 150 ppm	11.34 a	11.08	11.34 a	11.55 a	12.26
LSD 0.05	0.77	Ns	0.46	0.38	ns
CV (%)	4.91	4.26	2.87	2.37	6.79

Germination percentage

Soaking the seeds in different types and concentrations of chitosan solution significantly affected germination percentage after storage at 2, 6 and 8 months but did not significantly differ when stored at 4 and 10 months. It was found that soaking the seeds in polymeric chitosan at the concentration of 50 ppm promoted germination percentage over than the other treatments whereas the lowest germination percentage almost resulted from soaking the seeds in polymeric chitosan at the concentration of 150 ppm (Table-3). Kananont *et al*. (2010) found that oligomeric chitosan with 70% level of deacetylation or polymeric chitosan with 80% level of deacetylation can enhance seed germination rate of *Dendrobium bigibbum* var. *compactum* when compared to untreated chitosan. Chili seeds treated with chitosan at 0.8% increased seedling survival to 77% whereas without chitosan was about 33% and also improved seedling performance. (Photchanacha *et al.*, 2006).

Table-3. Effect of chitosan on germination percentage of rice seed after storage at various time periods.

Seed soaking (in)	**Germination percentage** (%)				
	Months after storage (month)				
	2	**4**	**6**	**8**	**10**
Water	74.50 ab	71.25	71.25 bc	64.50 abc	59.75
Oligomeric chitosan at 50 ppm	76.25 ab	78.25	71.00 bc	61.50 bc	58.25
Oligomeric chitosan at 100 ppm	79.00 a	78.00	77.00 ab	68.75 ab	65.50
Oligomeric chitosan at 150 ppm	72.50 ab	69.75	77.25 ab	60.25 c	59.75
Polymeric chitosan at 50 ppm	80.75 a	77.50	82.00 a	70.75 a	58.25
Polymeric chitosan at 100 ppm	69.25 b	71.00	77.75 ab	59.50 c	59.25
Polymeric chitosan at 150 ppm	68.75 b	70.00	67.50 c	60.00 c	62.00
LSD 0.05	7.90	ns	8.47	7.79	ns
CV (%)	7.22	7.80	7.70	8.33	8.77

Seed electrical conductivity

Storage time at 2 and 6 months did not significantly affect electrical conductivity of rice seeds soaked in different types and various concentrations of chitosan solution. Considering at 4, 8 and 10 months after storage, indicated that the highest electrical conductivity was detected from rice seeds soaked in water before storage. At 10 months of storage, particularly, electrical conductivity value was not significantly different between soaking the seeds in water and all concentrations of oligomeric chitosan. The lowest electrical conductivity value was observed in the seeds soaked in polymeric chitosan at all concentrations, therefore it can be used to soak the seeds before storage to maintain seed quality (Table-4). Yang *et al*. (2009) revealed that pretreatment of apple seedling leaves with chitosan solution prior to drought stress significantly decreased electrolyte leakage and malondialdehyde in the leaves.

Table-4. Effect of chitosan on seed electrical conductivity of after storage at various time periods.

Seed soaking (in)	**Seed electrical conductivity** (μS/cm/g. seed)				
	Months after storage (month)				
	2	**4**	**6**	**8**	**10**
Water	5.85	7.05 a	5.32	6.04 a	5.94 a
Oligomeric chitosan at 50 ppm	5.50	6.61 ab	5.54	5.51 ab	5.60 a
Oligomeric chitosan at 100 ppm	5.63	6.24 bc	5.86	5.24 bcd	5.77 a
Oligomeric chitosan at 150 ppm	5.62	6.38 bc	5.73	5.33 abc	5.50 a
Polymeric chitosan at 50 ppm	5.78	6.37 bc	5.60	4.67 cd	4.57 b
Polymeric chitosan at 100 ppm	5.39	5.76 c	5.82	4.55 d	4.42 b
Polymeric chitosan at 150 ppm	5.89	5.81 c	5.64	4.70 cd	4.77 b
LSD 0.05	ns	0.59	ns	0.69	0.70
CV (%)	7.28	6.44	6.71	9.12	9.22

Speed of germination

Soaking the seeds at different chitosan concentrations and types had significantly positive effects on speed of germination after storage at 4, 8 and 10 months. Soaking the seeds in oligermeric chitosan at 50 ppm showed the highest speed of germination after storage at 4 months and did not differ from those soaking in water whereas soaking in polymeric chitosan at 50 ppm had the highest speed of germination after 8 months storage. At 10 months of storage, the highest speed of germination was achieved from soaking the seeds in polymeric chitosan at 100 ppm; however it was not significantly from soaking in polymeric chitosan at 50 ppm. Ramos-Garcia *et al*. (2009) reported that the gladiolus corms var. 'Blanca Borrego' dipped in commercial chitosan, Biorend®, at 10.5% accelerated corn emergence in approximately 4 days. At 2 and 6 months of storage, soaking the seeds at different chitosan concentrations and types was not significantly different in terms of speed of germination ((Table-5). Guan *et al*. (2009) found that application of chitosan solution at 0.5% for maize seed priming can improve speed of germination under cold stress.

Table-5. Effect of chitosan on speed of germination after storage at various time periods.

Seed soaking (in)	**Speed of germination** (plant/day)				
	Months after storage (month)				
	2	**4**	**6**	**8**	**10**
Water	10.26	9.67 ab	8.51	5.00 c	5.50 cd
Oligomeric chitosan at 50 ppm	8.40	10.28 a	8.54	4.83 c	4.97 d
Oligomeric chitosan at 100 ppm	8.21	7.80 c	8.82	6.69 b	6.64 bc
Oligomeric chitosan at 150 ppm	8.52	8.15 c	9.08	6.49 b	5.97 bcd
Polymeric chitosan at 50 ppm	8.03	8.49 bc	8.98	7.88 a	7.02 ab
Polymeric chitosan at 100 ppm	7.90	7.53 c	9.64	7.43 ab	8.01 a
Polymeric chitosan at 150 ppm	9.07	8.20 c	9.18	6.94 ab	6.09 bcd
LSD 0.05	ns	1.32	ns	0.94	1.28
CV (%)	12.34	10.45	5.87	9.90	13.84

CONCLUSIONS

From this study, it can be recommended that soaking rice seed with polymeric chitosan at the concentration of 50-150 ppm for 3 hours and dried it at 35^0C with 10±2% seed moisture content before storage in room temperature can preserve viability and vigor of rice seed storaged during 2 to 8 months greater than untreated seeds.

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