
The Effect of Pre-Sowing Treatments on Germination and Vigor of Upland Rice (*Oryza sativa* L.)

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High quality-seeds are required for successful germination, and seedling establishment however, deterioration decreases quality of all crop species seed. This experiment was conducted to determine the effectiveness of seed priming on germination, vigor and stand establishment of an upland rice (*Oryza sativa* L.). The deteriorated seeds were subjected to different priming as follow; traditional soaking for 24 h, hardening for 24 and 48 h, hydropriming for 24 and 48 h, osmohardening for 24 and 48 h, and a non-primed was control treatment. The seed qualities were assessed in both laboratory and field conditions. All 7 primed treatments markedly increased germination, emergence, and all the tested vigors. Germination and emergence of all primed were ranked from 90.50 to 97.00% and 90.00 to 95.75%, while the non-primed were 78.00 and 80.00%, respectively. In addition, those primed treatments showed significantly higher seedling performance comparing with the control. The seedling establishment of all primed were ranked from 84.50 to 94.25% while the non-primed was 78.25%. However, among those primed treatments, hardening for 48 h and hydropriming for 24 and 48 h had a greater tendency to enhance seed quality as well as seedling performance.

Keywords: pre-sowing treatment, seed quality, seedling establishment, upland rice.

Introduction

Direct seeding of a crop seed often germinates slowly and poor seedling performance in the field. This would be due to low seed vigor identified as seed deterioration or aging (Dornbos, 1995; Finch-Savage, 1995). Furthermore, the environmental constraints especially common in the tropics such as high temperature, inadequate or excess water or weed infestation are not conducive to rapid seed emergence and seedling growth. However, there have been many attempts to develop pre-sowing treatments to enhance seedling performance in the field. Pre-sowing treatment such as priming have been reported to be one of an effective method in improving germination percentage, uniformity of germination, and seedling establishment under field conditions (Haigh *et al.*,

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1986; Karssen *et al.*, 1989; McDonald, 1999; Chong *et al.*, 2002; Corbineau and Come, 2006). Seed priming consists of a controlled hydration in water or osmotic solutions that sufficient to permit pregerminative metabolism to occur but not sufficient to allow radicle emergence through the seed coat (Bewley, 1997; McDonald, 2000) and followed by redrying to attain their original moisture content prior planting. Primed seed normally shows higher in germination percentage, germination rate, and uniform in seedling establishment under a wider range of environmental conditions than a non-priming seed (McDonald, 2000; Corbineau and Come, 2006; Matsushima and Sakagami, 2013).

Among seed priming techniques, the hardening; repeated cycle of soaking with water and redrying of seeds), osomhardening; repeated cycle of soaking in osmotic solution and redrying, hydropriming; soaking in aerated water, and traditional soaking; soaking in a tap water were proved to be successful in improving seed quality of various species particularly, rice (Andoh and kobata, 2000; Lee and kim, 2000; Basra *et al.*, 2005; Farooq *et al.*, 2006; Matsushima and Sakagami, 2013). Among these studied, the osmohardening and hardening were the most commonly used, Therefore, osmohardening and hardening may be used to improve seed quality of upland rice and leading to better seedling performance under field conditions. Although, many reported were available to direct-seeded rice for seed enhancement by using such method (Basra *et al.*, 2005; Farooq *et al.*, 2006; Matsushima and Sakagami, 2013), published data on naturally aged seed of upland rice is scarce especially in response to the wide range of seed priming treatments for enhancement of seed quality and seedling establishment.

Objectives of this study was to determine the appropriate pre-sowing treatment in aged-seed of an upland rice for increase germination, vigor, emergence and seedling establishment in the field.

Materials and methods

Seed material

Seed of an upland rice (*Oryza sativa* L.) cv. Nuch Sara with 9.0% moisture content were kept at 6 °C in a refrigerator for 18 months prior to be use. Before pre-sowing seed treatment, the germination capacity of this stored seed was 70.25%.

The chosen pre-sowing treatments were employed as follows:

1. Traditional soaking, seed were soaked in 2,000 ml tap water for 24 h at room temperature.

2. Hardening, seed were soaked in 2,000 ml of continuously aerated distilled water at 18 °C for 24 h and 48 h followed by forced air drying under shade to the initial moisture. The cycle was repeated twice (Farooq *et al.*, 2006).
3. Hydropriming, seed were soaked (for 24 h and 48 h) and dried in the same manner as previously described for hardening, except for the repetition.
4. Osmohardening, seeds were soaked in 1.0% KNO₃ solution for 24 h and 48 h, and followed by the same practiced to hardening (Basra *et al.*, 2005; Ruttanaruangboworn, 2016), and a non-priming was controlled treatment

Seed quality testing

All of the studied datas were tested for 4 replications of 50 seeds.

1. Laboratory tested

1.1 Germination tested

The tested was conducted according to ISTA (1993) on filter paper moisture with distilled water, and then kept at 25 °C in darkness. Germination was counted daily according to the AOSA method (AOSA, 1990).

1.2 Seed vigor

The vigor tested as follows:

1. Germination index (GI) was calculated as described by AOSA (1990) using the following formula

$$GI = \sum \left(\frac{Nt}{Tt} \right)$$

Where Nt is the number of seeds which were germinated on day t, and Tt is the number of days counted from the beginning of germination.

2. Mean germination time (MGT) was calculated according to the formula of Ruan *et al.* (2002).

$$MGT = \frac{\sum (Nd)}{\sum N}$$

where n is the number of seeds that germinated on day d, and d is the number of days counted from the beginning of germination, and N is the final number of germinated seeds.

3. Time to get 50% germination (T_{50G}) was calculated according to Coolbear *et al.* (1984).

$$T_{50G} = t_i + \frac{(N + 1)/2 + n_i}{n_j - n_i} (t_j - t_i)$$

where N is the final number of germinants, and n_i and n_j are the cumulative number of seeds germinated by adjacent counts at times t_i and t_j , respectively, when $n_i < N/2 < n_j$.

4. Germination energy (GE) was recorded on the 4th day after planting as the percentage of germinated seeds within 4-days after planting relative to the total number of seeds tested (Ruan et al., 2002).

2. Field tested

Control and treated seeds were planted 2 cm depth 1 seed for each hole in sandy-loam soil in a plastic pots, 50 seeds each pot, and the pots were placed in the field condition. Tap water was supplied daily to achieve and keep appropriate moist soil consistantly. Air temperature was recorded using maximum-minimum thermometer from the first planting day until the 21st day after planting (Figure 1).

2.1 Seedling emergence (E) tested

The emergence was recorded when the coleoptile appeared above soil surface. The number of emerged seedlings was recorded daily.

2.2 Seedling vigor

The seedling vigor was tested as follows: Emergence index (EI), mean emergence time (MET), time to get 50% emergence (T_{50E}) and emergence energy (EE). All of the tested datas were calculated the same as previously described for the laboratory tested.

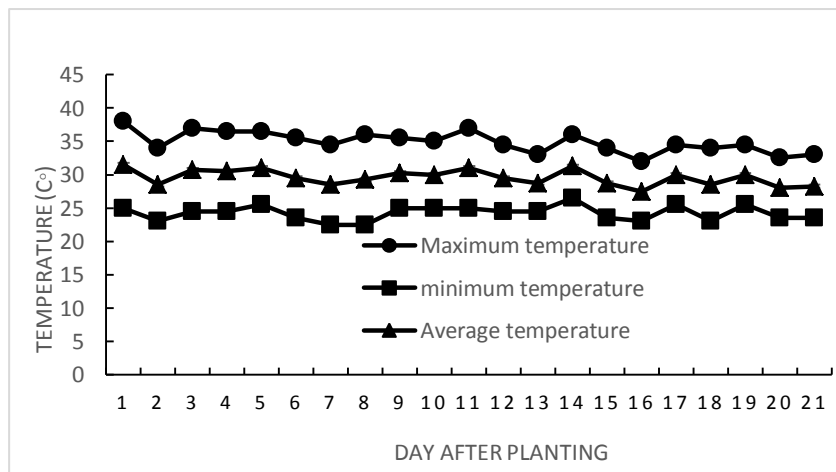


Figure 1. Temperature in the field during the field tested of an upland rice treated seeds.

Seedling performance

1. Seedling establishment (SES)

Seedling establishment was the percentage of seedling established within 21 days or seedling with the 4th leaf denoting plant establishment (Dunand and Soichuk, 2009) after sowing relative to the numbers of seeds sown (Yamaushi and Winn, 1996)

2. Seedling growth

The growth of establishment seedlings was characterized with measurements of shoot height, root length, dry weight of shoot and root. Shoot height was the distance between the soil surface and the tip of the longest leaf. Shoot and root dry weight were obtained by drying at 80 °C for 24 h.

Statistical analysis

All data were subjected to the analysis of variance (ANOVA) and followed by Duncan's new multiple range test. A completely randomized design with four replicates was used. To assess the relationships of various seed quality and seedling performance, correlation coefficients were computed.

Results and discussion

It is recognized that seeds stored for any last long time gradually lose their quality due to deterioration or vigor, leading to delayed germination, decreased tolerance to suboptimal field conditions during emergence and low seedling performance (Finch-Savage, 1995; McDonald, 1999). Seed deterioration is therefore fundamental cause for a major constraint for successful in rice production

In this study, even of keeping seed in the refrigerator, we found that the percentage germination of upland rice cv. Nuch Sara seeds tested in the laboratory was only 78.0% (Table 1), whereas that of normal fresh rice seeds was more than 85.0% (Basra *et al.*, 2003; Basra *et al.*, 2005) this would generally indicated that the seed had begun to deterioration in some degree. This study, however, clearly revealed that all pre-sowing seed treatments could significantly improved seed qualities of aged seeds over that of the control not only in the laboratory but also in the field tested (Table 2). This implies that seed priming is able to restore the adverse effects of aging on seed quality (McDonald, 2000; Corbineau and Come, 2006). Among seed priming treatments studied, although no significant difference was observed for germination (Table 1) and seedling emergence (Table 2). However, These

treatments significantly improved all vigor parameters both in the laboratory (Table 1) and in the field (Table 2) over that of the control. Among treatments used in this studied, hardening 48 h and hydropriming 48 h were most pronounced seed vigor in all parameter tested (Table 1 and Table 2). Hardening 48 h showed better seedling vigor, although they had no significant difference in vigor with each other. This was consistent with the work of Andoh and Kobata (2000) in seeds of some lowland rice varieties and in seeds of fine rice (Basra *et al.*, 2003; Basra *et al.*, 2005) thus, seed hardening treatment can be used as an alternate method instead of traditional soaking which being used for decades due mainly to greater emergence and seedling vigor (Table 2).

All seed priming treatments including traditional soaking significantly improved rice seedling establishment (SES) with high temperature variation in the field as compared with the control (Table 3). Result also showed that hardening 48 h and hydropriming 48 hr gave the highest SES. This seems that seedling vigor would be responsible for improvement of SES (Tekrony and Egli, 1991; Yamauchi and Winn, 1996; Ruan *et al.*, 2002). The high correlation of all parameters of seedling vigor with SES (Table 4) would confirm such evidence. In addition, the seed priming treatments also increased seedling growth in all cases as compared with the control which was consistent with Basra *et al.* (2003) who founded that among seed priming treatments, hardening for 24 h resulted in higher shoot and root length and shoot dry weight. Similarly, Farooq *et al.* (2006) report that all priming treatments improved seedling dry weight and length of shoot and root.

Increasing of all the parameters of seedling growth in the study would depend on seedling vigor. The correlation of seedling vigor and seedling growth (Table 4) would support such evidence. Among the priming treatments, hardening 48 h increasing of SES and all the seedling growth (Table 3). Therefore, seed priming treatments increased not only seedling vigor but seedling performance in the field.

Pre-sowing seed treatments, especially hardening and hydropriming considerably improved various seedling vigor in terms of increased EI and EE, shortened MET and T50E (Table 2). This would lead to apparent early in seedling growth with increased emergence and seedling establishment (Tekrony and Egli, 1991; Finch-Savage, 1995). The earlier and faster emergence in treated seeds leading to the better seedling growth and seedling establishment might apparently involve the completion of early metabolic steps of germination process during imbibition period (Bewley, 1997). The occurred metabolism during priming such as increases in macromolecule synthesis, enzymes and available mitochondria is the basis events participating in the mobilization of seed reserves for rapid cell differentiation and growth (Farooq

et al., 2006; Matsushima and Sakagami, 2013) as evident by increase in shoot and root length and shoot and root dry weight (Table 3).

In conclusion, based on our results, seed performance of upland rice can be enhanced by different pre-sowing treatments; however, both seed hardening and hydropriming for 48 h were more effective in all eviation of seed deterioration or naturally aged seeds as evident by increasing in seed germination, emergence and seedling vigor. Furthermore, both of these two treatments can increasing in seedling growth and seedling establishment which relate to seedling vigor.

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Table 1. Effect of pre-sowing seed treatments on the germination and seed vigor of the Nuch Sara upland rice seeds in the laboratory.

Treatment	<i>Seed qualities</i>				
	Germination (%)	GI	MGT (days)	T50G% (days)	GE (%)
Control (non-priming)	78.00 ±4.08 c	23.34 ±1.27 f	3.45 ±0.06 a	2.78 ±0.06 a	50.75 ±4.11 d
Traditional soaking	90.50 ±1.91 b	38.67 ±1.52 de	2.44 ±0.05 cd	1.90 ±0.08 d	90.50 ±1.91 bc
Hardening 24 h	93.00 ±3.42 ab	39.17 ±1.58 cde	2.47 ±0.12 c	1.98 ±0.18 cd	89.50 ±7.72 b
Hardening 48 h	95.50 ±1.15 a	42.17 ±2.52 ab	2.27 ±0.04 e	1.75 ±0.06 e	93.00 ±1.15 abc
Hydropriming 24 h	97.00 ±2.58 a	39.75 ±1.10 cd	2.54 ±0.08 bc	2.07 ±0.13 c	97.00 ±2.58 a
Hydropriming 48 h	96.00 ±2.83 a	43.75 ±1.91 a	2.13 ±0.11 f	1.69 ±0.04 e	96.00 ±2.83 ab
Osmohardening 24 h	94.50 ±1.91 ab	37.08 ±1.13 e	2.65 ±0.04 b	2.23 ±0.06 b	94.50 ±1.91 abc
Osmohardening 48 h	93.00 ±1.15 ab	41.33 ±0.54 bc	2.33 ±0.06 de	1.76 ±0.07 e	93.00 ±1.15 abc
F-test	**	**	**	**	**
C.V. (%)	2.79	4.06	3.05	4.68	4.04

Data were represented as mean ± standard error; means in each column followed by the same letter were not significantly different ($p < 0.01$) by Duncan's multiple range test.

Table 2. Effect of pre-sowing seed treatment on the emergence and seedling vigor of the Nuch Sara upland rice seeds in the field.

Treatment	<i>Seed quality</i>				
	E (%)	EI	MET (day)	T50 (day)	EE (%)
Control (non-priming)	80.00 ± 3.27 c	19.73 ± 0.79 d	4.17 ± 0.06 a	3.68 ± 0.07 a	53.00 ± 3.83 d
Traditional soaking	90.00 ± 2.31 b	23.65 ± 0.28 c	3.90 ± 0.10 b	3.44 ± 0.09 b	78.00 ± 4.00 c
Hardening 24 h	92.25 ± 1.71 ab	24.40 ± 0.78 bc	3.88 ± 0.08 b	3.41 ± 0.11 b	80.75 ± 3.77 bc
Hardening 48 h	95.75 ± 1.50 a	26.57 ± 0.34 a	3.68 ± 0.03 d	3.26 ± 0.04 c	93.00 ± 1.63 a
Hydropriming 24 h	94.50 ± 1.29 a	25.18 ± 0.16 b	3.84 ± 0.04 b	3.36 ± 0.05 bc	85.25 ± 1.50 b
Hydropriming 48 h	95.50 ± 2.08 a	26.50 ± 0.50 a	3.69 ± 0.08 cd	3.24 ± 0.09 c	89.75 ± 2.36 a
Osmohardening 24 h	92.75 ± 1.50 ab	24.83 ± 0.21 b	3.82 ± 0.03 b	3.42 ± 0.16 b	84.00 ± 3.27 b
Osmohardening 48 h	93.25 ± 3.40 ab	25.24 ± 0.72 b	3.80 ± 0.11 bc	3.31 ± 0.03 bc	81.00 ± 2.00 bc
F-test	**	**	**	**	**
C.V. (%)	2.47	2.17	1.92	2.61	3.67

Data were represented as mean ± standard error; means in each column followed by the same letter were not significantly different ($p < 0.01$) by Duncan's multiple range test.

Table 3. Effect of pre-sowing seed treatment on seedling performance of the Nuch Sara upland rice seeds in field tested.

Treatment	<i>Seedling performance</i>				
	SES (%)	Shoot length (cm)	Root length (cm)	SDW (mg/shoot)	RDW (mg/root)
Control (non-priming)	78.25 ± 2.75 c	18.83 ± 1.48 c	7.96 ± 0.31 c	24.02 ± 3.81 a	6.61 ± 1.62 b
Traditional spaking	84.50 ± 3.70 b	18.82 ± 1.10 c	8.51 ± 0.33 c	27.58 ± 4.43 a	7.89 ± 1.30 ab
Hardening 24 h	89.75 ± 1.71 a	20.71 ± 2.38 abc	8.59 ± 0.21 c	33.31 ± 3.96 a	6.75 ± 2.50 b
Hardening 48 h	94.25 ± 2.87 a	24.15 ± 2.85 ab	10.49 ± 0.74 a	32.50 ± 15.11 a	11.93 ± 6.79 a
Hydropriming 24 h	92.25 ± 2.06 a	21.52 ± 1.12 abc	8.92 ± 0.85 bc	32.63 ± 6.49 a	6.51 ± 1.83 b
Hydropriming 48 h	94.25 ± 2.06 a	24.32 ± 1.04 a	10.12 ± 0.85 ab	36.28 ± 1.71 a	9.60 ± 2.02 ab
Osmohardening 24 h	90.50 ± 1.29 a	22.47 ± 2.05 abc	8.83 ± 0.81 bc	29.40 ± 10.51 a	8.45 ± 2.30 ab
Osmohardening 48 h	90.75 ± 4.57 a	20.34 ± 3.15 bc	8.95 ± 1.14 bc	27.60 ± 6.55 a	6.62 ± 3.23 b
F-test	**	*	**	ns	ns
C.V. (%)	3.16	11.08	9.27	25.38	39.26

Data were represented as mean ± standard error; means in each column followed by the same letter were not significantly different ($p < 0.05$ / $p < 0.01$) by Duncan's multiple range test.

Table 4 Coefficient of simple correlation between seedling vigor and seedling performance in the field.

	SES	shoot length	root length	SDW	RDW
EI	0.880**	0.589**	0.622**	0.378*	0.343ns
MET	-0.680**	-0.554**	-0.576**	-0.361*	-0.395*
TE50	-0.668**	-0.446*	-0.529**	-0.282ns	-0.357*
EE	0.755**	0.359*	0.401*	0.293ns	0.133ns

*, ** Significant at $p < 0.05$ and $p < 0.01$, respectively; ns= not significant