Emir. J. Agric. Sci. 2004. 16 (2): 48-57
http://www.cfs.uaeu.ac.ae/research/ejas.html

Short Communication

Optimization of seed hardening techniques for rice seed invigoration

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Abstract: Seed hardening techniques were optimized for vigor enhancement in fine and coarse rice types by evaluating the germination and seedling vigor, and analyzing the electrical conductivity of seed leachates. Seeds were hardened for 12 h (1cycle), 12 h (2cycles), 18 h (1cycle), 18 h (2cycles), 24 h (1 cycle) or 24 h (2 cycles). All the seed treatments resulted in vigor enhancement in both fine and coarse rice types except seeds hardened for 24 h (2 cycles) that behaved similar to that of control. Maximum vigor enhancement as indicated by high germination and seedling vigor, and lower electrical conductivity of seed leachates was noted in seeds hardened for 24 h (1 cycle), which was similar to that of seeds hardened for 12 h (2 cycles) in both rice types.

Key words: fine rice, coarse rice, vigor enhancement, hardening

Abbreviations: T_{50}= Time taken for 50% germination, MGT = Mean germination time, MET= Mean emergence time, GI= MET Germination index, GE= Energy of germination, FGP= Final germination percentage, EC= Electrical conductivity.

كفاءة تقنيات تقسية بذرة الأرز

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ملخص: تم تحسين كفاءة تقنيات تقسية بذور الأرز من حيث تعظيم قوة النبات في أنواع الأرز الناعمة والخشنة بواسطة تقدير الإنبات وقوة النبتة وتحليل التوصيل الكهربي لافرازات البذور. تم تقسية البذور لمدة ١٢ ساعة (دورة واحدة)، ١٢ ساعة (دورتين)، ١٨ ساعة (دورة واحدة)، ١٨ ساعة (دورتين)، ٢٤ ساعة (دورة واحدة)، أو ٢٤ ساعة (دورتين). أنتجت كل المعاملات تحسين في قوة النبات في كل من نوعي الأرز الناعم والخشن فيما عدا البذور التي تم تقسيتها لمدة ٢٤ ساعة (دورتين) والتي كانت نتائجها تشبه العينة القياسية. إن أقصى قوة نبت تم قياسها بنسبة الإنبات العالية وقوة النبت، وأقل توصيل كهربي لإفرازات البذور تم ملاحظتها عن التقسية لمدة ٢٤ ساعة (دورة واحدة)، وهو يشابه البذور التي تم تقسيتها لمدة ١٢ ساعة (دورتين) في كلاً من نوعي الأرز.

الكلمات المفتاحية: تقسية، زيادة الجودة، أرز خشن، أرز ناعم

Introduction

Hardening, also called wetting, drying or hydration dehydration, refers to repeated soaking in water and drying at 15-25^{o}C (Pen Aloza and Eira, 1993). This hydration-dehydration cycle may be repeated twice, thrice and so on (Lee et al., 1998; Lee and Kim, 2000). The beneficial effects of seed hardening are primarily due to pre-enlargement of the embryo (Austin et al., 1969), biochemical changes like enzyme activation (Lee et al., 1998; Lee and Kim, 2000; Basra et al., 2005), and improvement of germination rate particularly in old seeds (Lee et al., 1998).

Hardening treatment of 24 h proved itself the best in vigor enhancement of rice (Basra et al., 2003; 2005) compared with osmopriming (-1.1 MPa KNO_3) for 24 and 48h and traditional soaking. Increased α-amylase activity and sugar contents were also reported in the hardened seeds (Basra et al., 2005). Basra et al., (2003) found hardening for 24 h the most effective technique for vigor enhancement in fine rice seeds. Hardening of normal and naturally aged coarse rice seeds improved the germination and seedling vigor. Total sugar contents and α-amylase activity of normal seeds were higher than in the aged seeds (Lee and Kim, 2000). The α -amylase activity was positively correlated with the total sugars and germination rate (Lee and Kim, 2000).

Although the benefits associated with seed hardening techniques have been reported by the previous work still no comprehensive study has been made to find the most optimum technique for vigor enhancement both in fine and coarse rice. Keeping in view all these facts the present study was therefore planned to optimize the seed hardening treatments for vigor enhancement in both fine and coarse rice.

Material and Methods

Seed materials

Seeds of coarse rice cultivar KS-282 and of fine rice cultivar Super-Basmati were used as the medium of experiment. The seeds were obtained from the Rice Research Institute, Kala Shah Kakoo, Pakistan. The initial seed moisture contents were 8.04% and 8.43% in the coarse and fine rice respectively.

Seed treatments

Hardening

A weighed quantity of seeds (250 g) were soaked in tap water at 27 ^{0}C for 12, 18 or 24 h followed by redrying to initial moisture under shade with forced air. This hydration-dehydration cycle was was repeated once (Basra et al., 2003, 2005) or twice (Lee et al., 1998).

Post treatment operations

After treating, the seeds were dried to original weight with forced air under shade (Basra et al., 2002). These seeds were then sealed in polythene bags and stored in a refrigerator for further use.

Germination test

15 seeds were sown in a Petri dish between the layers of moist whatman 45 at 27^{o}C in an incubator. The Petri plates were arranged in completely randomized design (CRD) with four replications Germination was observed daily according to the AOSA method (AOSA, 1990). The time to get 50% germination (T_{50}) was calculated according to the following formula of Coolbear et al., (1984) modified by Farooq et al. (2005):

$$T_{50} = t_i + \frac{\left(\frac{N}{2} - n_i\right)(t_j - t_i)}{n_j - n_i}$$

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

Mean germination time (MGT) was calculated according to the equation of Ellis and Roberts (1981):

$$MGT = \frac{\sum Dn}{\sum n}$$

where n is the number of seeds which were germinated on day D, and D is the number of days counted from the beginning of germination.

The germination index (GI) was calculated as described in the Association of Official Seed Analysts

(AOSA, 1983) as the following formulae:

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of first count}} + - - - + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

The energy of germination was recorded on the 4^{th} day after planting. It is the percentage of germinating seeds 4 days after planting, relative to the total number of seeds tested (Ruan et al., 2002).

Seedling Emergence

Control and treated seeds were sown in plastic trays (25 in each) having moist sand, replicated four times, and were placed in a growth chamber (Windon, England). Day and night lengths were kept at 15 and 9 h with 30^0C and 24^0C temperatures respectively. The relative humidity was maintained at 70%. Emergence was recorded daily according to the seedling evaluation Handbook of Association of Official Seed Analysts (AOSA, 1990). Mean emergence time was calculated according to the method described earlier.

Electrical conductivity of seed leachates

After washing in distilled water, 5 g seeds were soaked in 50 mL distilled water at 25ºC. Electrical conductivity of steep water was measured 0.5, 1.0, 1.5, 2.0, 6.0, 12.0 and 24.0 h after soaking using a conductivity meter (Model Twin Cod B-173) and expressed as µS/cm/g (Basra et al., 2003).

Results

Seed hardening treatments significantly ($P < 0.05$) affected the germination vigor of both fine and coarse rice seeds (Table 1).

Both in fine and coarse rice, earlier germination was recorded as indicated by lower values of T_{50} and MGT in seeds hardened for 24 h (1, cycle). That was similar to those of seeds hardened for 12 h (2 cycles). Maximum T_{50} was noted in untreated seeds. That was similar to T_{50} of seeds hardened for 24 h (2 cycles) (Table 1). Statistically maximum MGT was observed in seeds hardened for 24 h (2 cycles), followed by the control seeds. All other seeds treatments resulted in lower T_{50} and MGT (Table 1). Statistically maximum and similar FGP, GI and GE were noted in seeds hardened for 24 h (1 cycle) and 12 h (2 cycles). Minimum FGP, GI and GE were observed in untreated seeds, similar to those of seeds hardened for 24 h (2 cycles). All other seed treatments resulted in higher FGP, GI and GE (Table 1). Statistically maximum and similar radicle length were recorded in seeds hardened for 24 h (1 cycle) and 12 h (2 cycles), while minimum radicle length was noted in control. All seed treatments resulted in higher radicle length than that of control (Table 1). Maximum plumule length was noted in seeds hardened for 24 h (1 cycle). That was followed by seeds hardened for 12 h (2 cycles). Minimum plumule length was recorded in seeds hardened for 24 h (2 cycles), which was similar to that of control. All other seed treatments resulted in longer plumule than control (Table 1).

Significant ($P < 0.05$) effect of seed hardening treatments was seen on the seedling vigor of both fine and coarse rice seeds (Table 2).

In both fine and coarse rice, statistically highest MET was noted in control. That was statistically similar to that of hardening for 12 h (1 cycle), 18 h (2 cycles) and 24 h (2 cycles), while the rest of the treatments resulted in lower MET than that of control (Table 2). However, minimum MET was noted in seeds hardened for 24 h (1 cycle) (Table 2). Maximum FEP was seen in seeds hardened for 24 h (1 cycle), which was statistically similar to that of seeds for hardened 12 h (2 cycles).

Statistically maximum and similar root and shoot length were noted in seeds hardened for 24 h (1 cycle) and 12 h (2 cycles). Minimum root and shoot length were observed in untreated seeds. That was similar to those of seeds hardened for 24 h (2 cycles). All other seed treatments resulted in higher FEP, GI and GE (Table 2). Maximum root/shoot ratio was recorded in seeds hardened for 24 h (2 cycles), which is similar to that of seeds hardened for 18 h (2 cycles) and control; other treatments resulted in a lower ratio than that of control (Table 2). Minimum seedling fresh and dry weight was recorded in seeds hardened for 24 h (2 cycles) which was similar to that of control. The rest of the treatments resulted in higher seedling fresh and dry weights than that of control (Table 2). However, maximum fresh and dry weight was recorded in seeds hardened for 24 h (1 cycle), which is similar to that of seeds hardened for 12 h (2 cycles) in the case of seedling fresh weight (Table 2).

In both rice types, the highest rate of seed leachates was noted in untreated seeds, which was followed by seeds hardened for 24 h (2 cycles). The significantly lowest rate of seed leachates was noted in seeds hardened for 24 h (1 cycle), which was statistically at par with that of seeds hardened for 12 h (2 cycles) (Fig. 1, 2).

Discussion

Seed hardening treatments had a significant effect on the germination and seedling vigor in both fine and coarse rice seeds (Tables 1, 2,). The response of both rice types to different hardening treatments was similar (Tables 1, 2; Fig. 1, 2).

This study revealed that employing hardening treatments could invigorate both fine and coarse rice seeds. Earlier and more uniform germination and emergence was observed in seeds hardened for 24 h (1 cycle) and 12 h (2 cycles) as indicated by lesser time to start to germination, T_{50}, higher GI, GE and MGT and higher radicle and plumule length (Tables 1, 2). Lesser T_{50} and MGT indicate the earlier and rapid germination, while higher GI and GE express the power of germination i.e. germination spread over time (Table 1). These findings support the earlier work on asparagus (Evan and Pill, 1989), pepper (Smith and Cobb, 1991), canola (Zheng et al., 1994), wheat (Nayyar

Emir. J. Agric. Sci. 2004. 16 (2): 48-57
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Table 1. Effect of hardening treatments on the germination vigor of fine and coarse rice

	Treatments	T_{50} (days)	MGT (days)	FGP (%)	GI	GE (%)	Radicle length (mm)	Plumule length (mm)
Fine Rice	Control	1.50 a	2.90 b	63.33 c	17.50 d	30.25 d	45.90 c	41.56 d
	Hardening 12 h (1 cycle)	1.47 a	2.867 b	86.67 b	22.50 c	55.57 c	51.50 b	51.73 b
	Hardening 12 h (2 cycles)	0.97 c	2.233 d	100.00 a	36.50 a	95.45 a	58.41 a	56.54 b
	Hardening 18 h (1 cycle)	1.27 b	2.600 c	83.33 b	26.00 b	75.28 b	52.34 b	51.66 b
	Hardening 18 h (2 cycles)	1.33 a	2.632 c	86.67 b	27.32 b	70.00 b	52.16 b	49.57 bc
	Hardening 24h (1 cycle)	0.90 c	2.133 d	100.00 a	36.00 a	90.00 a	58.73 a	65.46 a
	Hardening 24h (2 cycles)	1.53 a	3.13 a	65.56 c	18.34 d	34.45 d	50.10 b	44.26 d
	LSD at 0.05	0.204	0.178	12.36	2.33	8.23	4.24	6.06
Coarse Rice	Control	1.40 a	2.70 b	66.33 c	19.50 d	33.25 d	40.90 c	36.56 d
	Hardening 12 h (1 cycle)	1.37 a	2.66 b	89.67 b	24.50 c	58.57 c	46.50 b	46.73 b
	Hardening 12 h (2 cycles)	0.87 c	2.03 d	100.00 a	38.50 a	97.45 a	53.41 a	51.54 b
	Hardening 18 h (1 cycle)	1.17 b	2.40 c	86.33 b	28.00 b	78.28 b	45.34 b	46.66 b
	Hardening 18 h (2 cycles)	1.23 a	2.42 c	89.67 b	29.32 b	73.00 b	47.16 b	44.57 bc
	Hardening 24h (1 cycle)	0.80 c	1.91 d	100.00 a	38.00 a	93.00 a	53.73 a	60.46 a
	Hardening 24h (2 cycles)	1.43 a	2.91 a	68.56 c	20.34 d	37.45 d	45.10 b	39.26 d
	LSD at 0.05	0.204	0.178	12.36	2.33	8.23	4.24	5.06

Figures not sharing the same letters differ significantly at $P \leq 0.05$

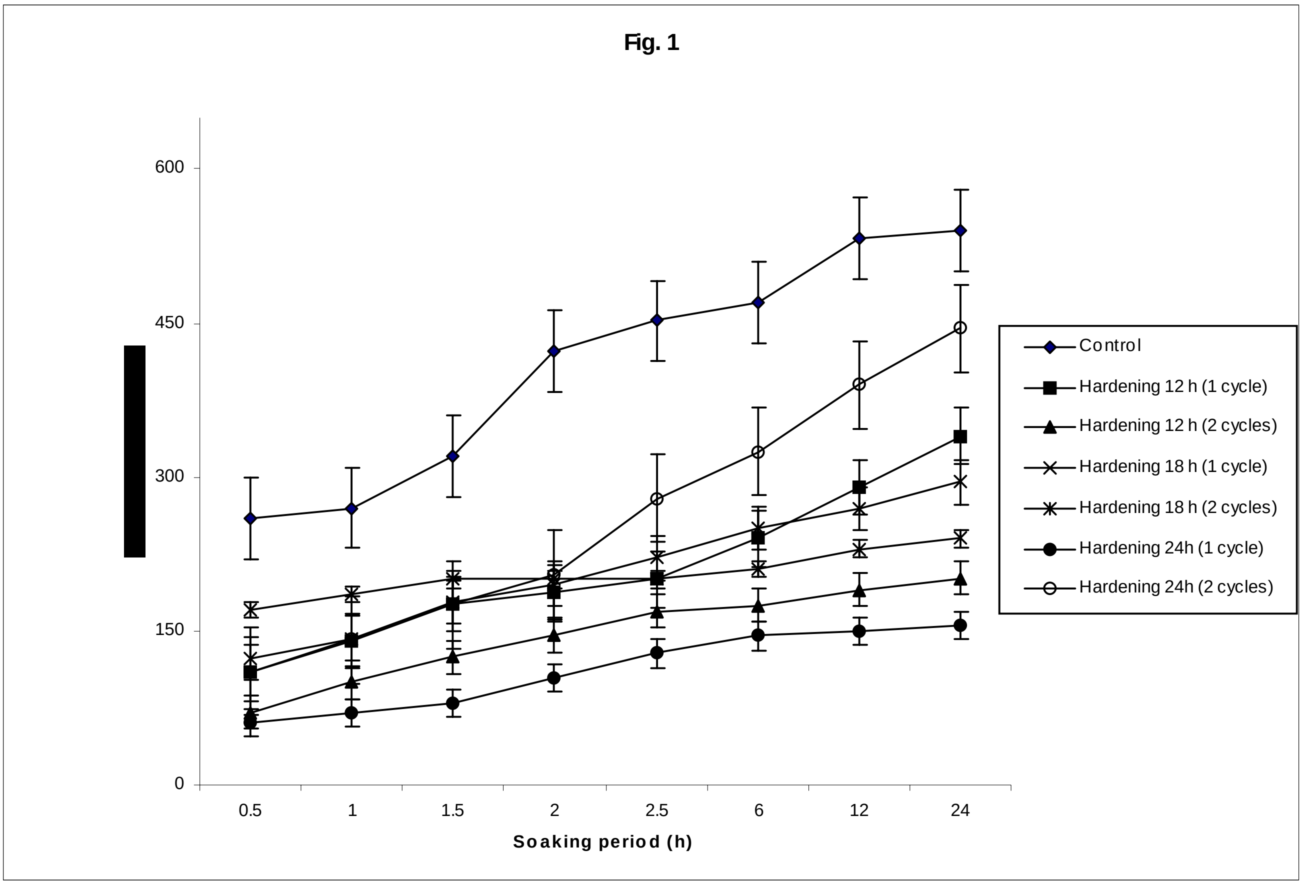
Table 2. Effect of hardening treatments on the seedling vigor of fine and coarse rice

	Treatments	(days)	FEP (%)	Root length (cm)	Shoot length (cm)	Root/Shoot ratio	Seedling fresh weight (mg)	Seedling dry weight (mg)
Fine Rice	Control	7.51 a	41.41	45.05 d	30.88 d	1.45 a	20.29 d	5.33 e
	Hardening 12 h (1 cycle)	7.33 a	70.03 b	54.45c	49.77 c	1.09 b	27.77 b	7.65 c
	Hardening 12 h (2 cycles)	5.15 d	84.19 a	74.52 a	69.29 a	1.07 b	30.03 a	8.61 b
	Hardening 18 h (1 cycle)	5.87 c	64.97 c	57.87 c	57.75 b	1.02 b	27.05 b	8.10 b
	Hardening 18 h (2 cycles)	6.93 ab	74.56 b	65.05 b	44.51 c	1.46 a	24.90 c	7.00 d
	Hardening 24h (1 cycle)	4.64 e	88.19 a	77.87 a	65.55 a	1.18 b	32.87 a	9.86 a
	Hardening 24h (2 cycles)	7.21 a	64.75 c	44.85 d	28.34 d	1.58 a	20.29 d	5.43 e
	LSD at 0.05	0.595	4.342	6.112	5.126	0.213	0.712	0.675
Coarse Rice	Control	7.51 a	41.41	45.05 d	30.88 d	1.45 a	20.29 d	5.33 e
	Hardening 12 h (1 cycle)	7.33 a	70.03 b	54.45c	49.77 c	1.09 b	27.77 b	7.65 c
	Hardening 12 h (2 cycles)	5.15 d	84.19 a	74.52 a	69.29 a	1.07 b	30.03 a	8.61 b
	Hardening 18 h (1 cycle)	5.87 c	64.97 c	57.87 c	57.75 b	1.02 b	27.05 b	8.10 b
	Hardening 18 h (2 cycles)	6.93 ab	74.56 b	65.05 b	44.51 c	1.46 a	24.90 c	7.00 d
	Hardening 24h (1 cycle)	4.64 e	88.19 a	77.87 a	65.55 a	1.18 b	32.87 a	9.86 a
	Hardening 24h (2 cycles)	7.21 a	64.75 c	44.85 d	28.34 d	1.58 a	20.29 d	5.43 e
	LSD at 0.05	0.595	4.342	6.112	5.126	0.213	0.712	0.675

Figures not sharing the same letters differ significantly at $P \leq 0.05$

et al., 1995) and rice (Lee and Kim 2000; Basra et al., 2003; 2005), who reported improved germination rate and percentage in seeds subjected to seed hardening for 24 h. The higher germination percentage in hardened seeds [except hardening for 24 h (2 cycles)] might be the result of dormancy breakdown (Basra et al., 2005) as fresh seeds were used during the investigations. The earlier and better synchronized germination may be the result of increased metabolic activities in the hardened seeds as reported by Lee and Kim (2000) and Basra et al. (2004). Hardening treatments not only improved the germination rate and time but also enhanced the seedling vigor as indicated by lower values of MET and higher FEP, root and shoot length and seedling fresh and dry weight (Table 2). Faster emergence rate after hardening (Table 2) might be the result of induced replications in the root tips as was

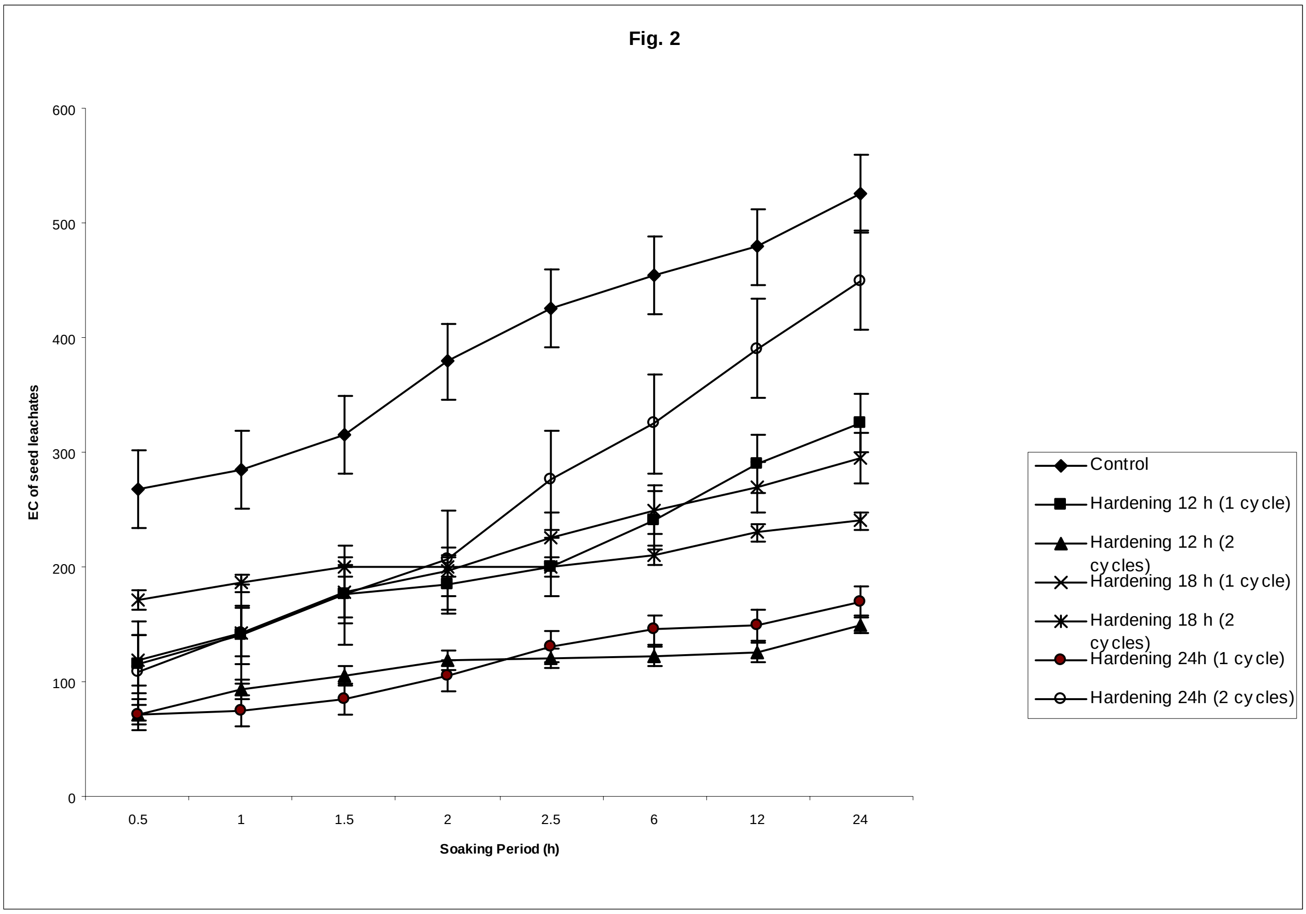
Figure 1. Effect of seed treatments on the EC of seed leachates in fine rice ± s.e.



concluded by earlier research for wheat (Bose and Mishra 1992, Basra et al., 2002) and rice (Basra et al., 2003; 2005). Higher root and shoot length and seedling fresh and dry weight from hardened seeds [except hardening for 24 h (2 cycles)] are an indication of early emergence and more rapid rate of emergence of vigorous seeds. Earlier work of Bose and Mishra (1992) and Basra et al. (2002) on wheat and Basra et al. (2003; 2005) on rice supports the present findings. Delayed and poor germination, and emergence in seeds subjected to hardening for 24 h (2 cycles), are probably due to overpriming, as was reported by Lee and Kim (1999) for rice.

The low vigor seeds have been shown to possess poor membrane integrity as a result of mechanical injury and storage deterioration when seeds are subject to imbition. Cells having poor membrane structure release cytoplasm solutes into the imbibing medium. These solutes with electrolytic properties increase the electrical conductivity of the seed leachates. It means seed membrane damage may be repaired during hardening treatments. That could stimulate the germination percentage and develop the vigor. The lower EC of steep water for the pre-sowing seed treatments is an indication of better membrane repair during controlled hydration. Greater membrane integrity in treated seeds of eggplant and radish (Rudrapal and Nakamura, 1988), wheat (Basra et al., 2002) and rice (Basra et al., 2003) has been reported. The Lower EC value

Figure 2. Effect of seed treatments on the EC of seed leachates in coarse rice ± s.e.



indicates that seed hardening did not damage the seed structure. Rather it allowed the better membrane repair. Low EC induced by seed hardening [except hardening for 24 h (2 cycles)] was accompanied with lower T_{50} and MGT, and higher GI and GE. This suggests successful membrane and genetic repair and trigger of metabolic activities promised by seed hardening.

From the present investigations, it may be concluded that seed hardening is an effective vigor enhancement tool both in fine and coarse rice seeds. Hardening for 24 h (1 cycle) was found the most effective technique. However, hardening for 24 h (2 cycles) resulted in excess priming.

We acknowledge the Higher Education Commission, Govt. of Pakistan for financial support of the present project.

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