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Full Length Research Paper

Improving germination, seedling establishment and biochemical characters of aged hybrid rice seed by priming with KNO_3 + PVA

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Using naturally aged F_1 seed and artificially aged F_1 seed of hybrid rice cultivar "Shan-you 63" as materials, 20 traits (10 traits measured in laboratory and 10 traits measured in paddy field) were compared between primed seed and corresponding control (non-primed seed). Results showed that germination percentage increased by 11.7% for naturally aged F_1 seed after primed with 0.5% PVA (polyvinyl alcohol) + 1.0% KNO_3 and by 10.3% for artificially aged F_1 seed after treated with 1.0% PVA+ 1.0%KNO_3. Germination energy and vigor index of primed seeds were significantly enhanced. Activities of SOD, POD and CAT of primed seeds were significantly increased. Content of MDA and soluble sugar in seed leakage of primed seeds were significantly decreased. Compared with corresponding non-primed seeds, seedling establishment rate, seedling height and seedling weight significantly increased, and mean emergence time shortened obviously on-farm in 2006 and in 2007 in primed seeds. Under transplanting condition, there was no significant difference between primed seed and non-primed seed in plant height, panicle number per plant, panicle length, panicle weight and grain yield.

Key words: Hybrid rice, aged seed, priming technology, germination, seedling establishment, biochemical property, yield related traits.

INTRODUCTION

Fifty percent of the world's population lives on rice. Rice is grown on an area of 150 million hectares in the world. In China, 33 million hectares is planted with rice, which occupies 22% of the world's rice area. Since 1976, hybrid rice has been planted in commercial in China. Up to 2005, more than 350 million hectares of hybrid rice was cultivated totally and more than 500 million tons of rough rice increased in China (Peng et al., 2007). Currently, the area of hybrid rice planted annually is 15.3333 million hectares, occupying 50% of total rice area in China (Lu and Hong 1999; Gai 2006). Since 2002, hybrid rice technology has been applied in more than 2 million hectares outside China (Peng et al., 2007). Hybrid rice F_1 seed must be produced every year as which only be used one generation in farmers' field. Owing to the influence of climatic factors and combination substitutions, areas and yields of F_1 seed production varied with year (Hu et al., 2008), which resulted in periodic fluctuation of hybrid rice seed price. When demands surpass supply, the price will be up, area for hybrid rice seed production will be expanded next year. When bumper harvest occurs for years running and F_1 seed supplies surpass demands, the seeds have to be stored for two or more years. Although the seeds are stored in low temperature storeroom, they will be gradually aging naturally and germination percentage down below 80%. According to "Quality Standards for Agricultural Seed (GB 4404.1-2008)" published by Administration of Technique Supervision of the People's Republic of China in 2008, hybrid rice seed with germination percentage below 80% is unqualified. Unqualified seeds can not be sold as seeds and must be sold as foodstuff. Because the cost for hybrid rice seed production is 4~5 times of those for foodstuff, and thus the

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seed price is 7~8 times of that for foodstuff. Once hybrid rice seed is sold as foodstuff, the losses will be heavy.

Seed priming is a controlled hydration technique, in which, seeds are soaked in solution of low-osmotic potential before the actual germination take place and then re-dried near to their original weight to permit routine handling. In rice, substances used for seed priming included PEG (Xiong 1998), KCl, $CaCl_2$ (Farooq et al., 2006, 2007) Cerium Nitrate (Hong et al., 1999b, 2000a), Lanthanum Nitrate (Hong et al., 1999a, 2001a), wet sand (Hu et al., 2005), and so on. Types of cultivar used for seed priming in rice included conventional cultivars (Li et al., 2000; He et al., 2002; Hu et al., 2005) and hybrid cultivars (Xiong 1998; Hong et al., 1999a-b, 2000a-b, 2001). Seeds used for priming in rice included natural ageing seed (Hong et al., 1999a-b, 2000a-b, 2001; Li et al. 2000), artificial ageing seed (Ma et al., 1991; Tan et al., 1997) and normal seed (Xiong 1998; Hu et al., 2005; Farooq et al., 2006, 2007). Purposes of seed priming included enhancing germination percentage and seedling esta- blishment for direct-seeded rice (Hu et al., 2005; Farooq et al., 2006), improving performance of transplanted rice (Farooq et al., 2007), enhancing tolerance to stress of rice (Li et al., 2000; He et al., 2002; Ruan et al., 2003), improving seed vigor and germination percentage of aged rice seed (Ma et al., 1991; Tan et al., 1997; Xiong 1998; Hong et al., 1999a-b, 2000a-b, 2001; Li et al., 2000).

PEG and rare earth elements (cerium, lanthanum) had good effects on enhancing germination percentage of aged rice seed, but they were difficult to be applied in large area because of the price. In order to enhance seed sowing quality of aged F_1 seed and alleviate the economic losses derived from transferring F_1 seed to foodstuff, we reported results of seed priming with 0.5% PVA + 1.0% KNO_3 or 1.0% PVA + 1.0% KNO_3 (Zhu and Hong, 2007) on germination percentage, activities of enzymes, agronomic traits and grain yield in paddy rice field in this paper.

MATERIALS AND METHODS

Experiment 1: Seed priming

Seed source and germination percentage prior to priming

Naturally aged F_1 seed, artificially aged F_1 seed and normal F_1 seed of hybrid rice "Shan you 63" were used in this study. The naturally aged F_1 seed, produced in 2003 and stored in short-term storehouse for 3 years, was got from Nanjing Shenzhou Seed Company, Limited, which was established in 1997 and located within the campus of Nanjing Agricultural University. Germination percentage of the naturally aged F_1 seed was 63.0%. The artificially aged F_1 seed was made by us (Zhu and Hong, 2008) from the normal F_1 seed which was bought from the same seed company as mentioned above. The normal F_1 seed was produced in 2005 and its germination percentage was 83.7% prior to artificial aging operation in 2006. Germination percentage of the artificially aged seed was 74.7%.

Priming process

For each treatment per replication, samples of 100 seeds were put into a beaker (100 ml). And then 10 ml of solution of 0.5% PVA + 1.0% KNO_3 or 1.0% PVA+ 1.0% KNO_3 was poured into the beaker. After stirred by a glass bar, the beaker was put in an artificial climate incubator at 28°C with 12 h light /12 h dark. In order to make oxygen into the solution during the soaking process, the solution was stirred with a glass bar every 6 h. After one day soaking finished, the primed seeds were washed with tap water and blotted up with absorbent paper. Then the primed seeds were re-dried at room temperature (25±1°C) for 24 h or more, and re-dried near to their original weight. Moisture content of seed was about 13.5%. The re-dried seeds were used immediately for study of germination and biochemical properties.

Germination and measurements of seed vigour

The primed and non-primed seeds were sterilized in solution of bactericide (Methylenedithiocyanate, $C_3H_2N_2S_2$) for 24 h, soaked in water for another 24 h, and then germinated in artificial climate incubator (RXZ-300A) at 28°C with 12 h light /12 h dark.

Number of germinated seeds was recorded every day. Germination percentage of the first 3 days was designated as germination energy (GE). In the seventh day, germination percentage (GP) was calculated, and seedling height (SH) and seedling fresh weight (SFW) were measured (10 plants each treatment). Vigor index was calculated as $VI=S\times\sum(Gt/Dt)$ (Zhu and Hong, 2008), where S is seedling height of the seventh day, Gt is number of germinated seeds in the "t th" day, Dt is number of days from the first day to the "t th" day.

Experiment 2: Comparison of biochemical properties between primed and non-primed seeds

Content of soluble sugar

Twenty grains of primed seed and 20 grains of non-primed seed were soaked in 20 ml of distilled water respectively for 24 h. One milliliter of the leakage solution from each sample was taken out for measuring content of soluble sugar. Measurement was carried out following the protocol described by Yuan and Yang (1983).

Activities of SOD, POD, CAT and content of MDA

Twenty grains of primed seed and 20 grains of non-primed seed were weighted respectively. After soaked in water for 24 h at 28°C, the two samples were ground in a mortar (pre-cold in -20°C icebox) and homogenized in 6 ml of 0.05 mol/l phosphate buffer (pH 7.0) followed by centrifuging at 15000×g for 20 min at 4°C. The supernatant was used for measurement of activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and content of malondialdehyda (MDA). For measurement of SOD activity, method described by Huang et al. (1990) was used. For measure- ments of POD and CAT activities and content of MDA, methods described by Li et al. (2000) were used.

Experiment 3: Comparisons of seedling establishment, agronomic traits and grain yield between primed and non-primed seeds

Seedling establishment

Experiments for seedling establishment investigation were conducted at Experiment Farm of Nanjing Agricultural University in 2006 (from August 21 to September 4) and in 2007 (from May 9 to 23), respectively. Five types of seeds were used in the experiment. They were P_1 (primed seed with 0.5% PVA+1.0% KNO_3 for aged F_1 naturally), P_2 (primed seed with 1.0% PVA+1.0% KNO_3 for aged F_1 artificially), C_1 (non-primed seed of aged F_1 naturally), C_2 (non-primed seed of aged F_1 artificially) and C_3 (non-primed seed of normal F_1). Prior to sowing, the seeds were sterilized for 24 h with the same method mentioned above, and then soaked in water for another 12 h. After that, the seeds were sown in polystyrene boxes (54 × 27 ×7 cm) contained 4 cm of fine soil in depth, with 3 replicates, 100 grain per replicate, and covered with a thin layer of fine soil containing pesticide and compound fertilizer. When the soil dried, water was added in the morning or in the evening. Within 5 days after sowing, plastic shed was made to prevent seeds from washing away by rainwater. Number of germinated seed was recorded every day. Seedling height (SH) was measured every 7 days with a ruler. Total number of seedlings in each replicate, seedling fresh weight (SFW) and seedling dry weight (SDW) were investigated 14 days after sowing (DAS). SFW was the average weight of 10 plants sampled randomly. SDW was the average weight of the same 10 plants dried at 70°C for 24 h in an oven. Seedling establishment percentage was calculated as (total number of seedlings/ total number of seeds sowed) × 100%. Mean emergence time (MET) was calculated as $MET=\sum(Gt \times Dt)/\sum Gt$ (Guan et al., 2009), where Gt is number of germinated seeds in the "t th" day, Dt is number of days from the first day to the "t th" day.

Agronomic traits and grain yield under transplanting condition

Both primed seeds and non-primed seeds were sowed on seedling raise bed on May 9, 2007. The seedlings were transplanted on June 13. For each replicate of each treatment, 88 hills were transplanted at space 16.7 × 20.0 cm with 1 seedling per hill. Common cultivated management was adopted. Plant height (PH), panicle length (PL), main panicle weight (MPW) and panicles per plant (PP) were investigated from 10 plants on September 24. After that, all plants of the plots were harvested manually and threshed by a small thresher, and the grains were sun dried and weighted.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Micro- soft Excel 2003 software. Percentage data were arcsine-transformed before analysis according to $\hat{y}$ = arcsin $\sqrt{x/100}$. Significant level P=0.05 was used.

RESULTS

Priming effects on naturally aged F_1 seed and artificially aged F_1 seed after treating 1 day with 0.5% PVA + 1.0% KNO_3 and 1.0% PVA + 1.0% KNO_3, respectively

For naturally aged F_1 seed of "Shan you 63" cultivar, germination percentage increased by 11.7% after treating with P_1 (0.5% PVA+1.0% KNO_3) for 1 day compared with the non-primed seed. For artificially aged F_1 seed, germination percentage increased by 10.3% after treating with P_2 (1.0% PVA+1.0% KNO_3) for 1 day. All the priming treatments showed significant effect on germination energy (GE), vigor index (VI), seedling height (SH) and seedling fresh weight (SFW) (Table 1).

Priming effects on five biochemical properties of the aged seed

Activities of SOD, POD and CAT in primed seed increased

For naturally aged F_1 of "Shan you 63", activities of SOD and CAT in seeds primed with P_1 (0.5% PVA+1.0% KNO_3) increased significantly, compared with non-primed seed (Table 2). Activity of POD in seed primed with P_1 also increased but not significant. For artificially aged F_1 of "Shan you 63", all the activities of SOD, POD and CAT in seeds primed with P_2 (1.0% PVA + 1.0% KNO_3) increased significantly, compared with non-primed seed (Table 2).

Content of soluble sugar in seed leakage and content of malondialdehyda (MDA) in primed seed decreased

For both naturally aged F_1 and artificially aged F_1, content of MDA in seeds primed with P_1 (0.5% PVA + 1.0% KNO_3) or with P_2 (1.0% PVA + 1.0% KNO_3) decreased significantly at α = 0.05 probability level, compared with that of non-primed seed (Table 3). Similar results were obtained in content of soluble sugar in seed leakage (Table 3).

Priming effect on seedling establishment, agronomic traits and grain yield of aged seed

Seedling establishment, seedling height, seedling fresh weight and seedling dry weight of primed seed were improved compared with non-primed seed in field

In the two experiments conducted in 2006 and 2007, seedling establishment rate (SER), seedling height (SH), seedling fresh weight (SFW) and seedling dry weight (SDW) from primed seeds were significantly greater than those from corresponding non-primed seeds for both naturally aged F_1 and artificially aged F_1 (Table 4). Mean emergence time (MET) of primed seed was significantly shorter than that of non-primed seed in 2006, while was only a little shorter in 2007. These revealed that seed priming treatment not only increased seedling establish-ment, but also improved uniform of emergence of aged seed in field as indicated by lower MET. Furthermore, SER, SH, SFW and SDW from primed seed were even significantly greater than those from non-primed normal F_1 seed (Table 4).

Agronomic traits studied and grain yield of plants grown from primed seed showed no significant difference from those non-primed seed under transplanting condition

Table 5 showed that four agronomic traits, that is, plant

Table 1. Comparisons of 5 seedling traits between primed seed and non-primed seed.

Treatments	Germination percentage (%)	Germination energy (%)	Vigor index	Seedling height (cm)	Seedling fresh weight (mg)
C_1	63.0b	61.7b	252.9b	6.69b	25.37b
P_1	74.7a	73.0a	399.5a	7.99a	33.70a
C_2	74.7b	69.0b	330.3b	6.58b	23.97b
P_2	85.0a	81.3a	498.2a	7.95a	29.10a

Note: C_1= Control (non-primed seed of naturally aged F_1); C_2=Control (non-primed seed of artificially aged F_1); P_1= seed primed with 0.5% PVA + 1.0% KNO_3 for naturally aged F_1; P_2= seed primed with 1.0% PVA + 1.0% KNO_3 for artificially aged F_1. Within columns, values with different letters between C_1 and P_1 or between C_2 and P_2 are significantly different at $P < 0.05$.

Table 2. Priming effect on activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of aged seed subjected to various treatments.

Treatments	superoxide dismutase (SOD) U/g	Peroxidase (POD) $A_{470}\cdot g^{-1}\cdot min^{-1}$	Catalase (CAT) $mg\cdot g^{-1}\cdot min^{-1}$
C_1	102.95b	5.51a	54.87b
P_1	181.43a	5.76a	97.49a
C_2	175.35b	5.62b	83.48b
P_2	399.37a	6.30a	105.47a

Note: C_1, C_2, P_1 and P_2 had the same meaning as Table 1. Within columns, values with the same letters between C_1 and P_1 or between C_2 and P_2 are not significantly different at $P < 0.05$.

Table 3. Comparison of content of soluble sugar in seed leakage and content of MDA in seed between primed seed and non-primed seed.

Treatments	Malondialdehyda (MDA) ($nmol\cdot g^{-1}$)	soluble sugar ($mg\cdot g^{-1}$)
C_1	16.32a	0.039a
P_1	11.09b	0.035b
C_2	14.95a	0.044a
P_2	7.140b	0.026b

Note: C_1, C_2, P_1 and P_2 had the same meaning as Table 1. Within columns, values with different letters between C_1 and P_1 or between C_2 and P_2 are significantly different at $P < 0.05$.

height, panicle length, panicle weight and panicles per plant, and grain yield per unit area had no significant differences between primed seed and corresponding non-primed seed. This result obtained under transplanting condition could be explained by the reason that there was no difference intrinsically after transplanting because the genetic basis was the same in primed seed and non-primed seed.

DISCUSSION AND CONCLUSION

According to "Quality Standards for Agricultural Seed (GB 4404.1-2008)" published by Administration of Technique Supervision of the People's Republic of China in 2008, it is illegal to sold hybrid rice seed with germination percentage lower than 80%. Our results showed that germination percentage of primed seed increased by 11.7% (from 63.0 to 74.7%) after treated with 0.5% PVA + 1.0% KNO_3 for naturally aged F_1 seed of "Shan-you 63" (Table 1). Similar result was obtained for artificially aged F_1 seed treated with 1.0% PVA + 1.0% KNO_3 (Table 1). This means that germination percentage of aged seed of "Shan you 63" can be increased approximately by 10% through our priming technology. This result suggests that if germination percentage of aged F_1 seed of "Shan you 63" was higher than 70%, it may be enhanced to more than 80% by priming with 1.0% PVA + 1.0% KNO_3 for 1 day.

Seedling establishment rate in field increased by 10.3~16.7% in primed seed compared with non-primed aged seed (Table 4). This had practical significance for direct-sowed rice, a practice which is becoming more and more popular in Asia. Increase of germination energy, vigor index, seedling height and seedling fresh weight (Table 1), and shorting of mean emergence time (Table 4), might be the cause of improved seedling stand rate.

Table 4. Priming effects on seedling establishment rate, seedling height, seedling fresh weight, seedling dry weight and mean emergence time in field.

Year	Treatments	Seedling establishment rate (%)	Seedling height (cm)	Seedling fresh weight (mg)	Seedling dry weight (mg)	Mean emergence time (day)
2006	C_1	63.7b	26.01b	198.62b	41.82b	2.87a
	P_1	74.7a	27.32a	251.61a	55.64a	2.23b
	C_2	71.7c	25.33ab	170.93b	34.71b	2.87a
	C_3	78.0b	24.50b	174.20b	37.82b	2.53b
	P_2	88.3a	26.44a	237.21a	55.34a	2.03c
2007	C_1	62.7b	10.40b	80.30b	22.51b	5.82a
	P_1	73.0a	12.10a	93.44a	32.52a	5.30a
	C_2	69.0c	10.84b	81.95c	24.14c	5.09a
	C_3	78.7b	10.80b	99.20b	33.75b	4.62a
	P_2	85.7a	12.50a	117.07a	39.55a	4.60a

Note: C_1, C_2, P_1 and P_2 had the same meaning as Table 1. C_3= Control (non-primed normal F_1 seed of "Shan-you 63"). Within columns, values with the same letters between C_1 and P_1 or among C_2, C_3 and P_2 are not significantly different at $P < 0.05$.

Table 5. Priming effects on agronomic traits and grain yield.

Treatments	Plant height (cm)	Panicle length (cm)	Main panicle weight (g)	Panicles per plant	Grain yield (t ha^{-1})
C_1	117a	28.5a	5.9a	14.1a	8.130a
P_1	116a	29.4a	5.9a	13.4a	8.460a
C_2	117a	27.9a	4.8a	11.8a	7.680a
P_2	118a	26.7a	4.6a	10.6a	7.935a

Note: C_1, C_2, P_1 and P_2 had the same meaning as Table 1. Within columns, values with the same letters between C_1 and P_1 or between C_2 and P_2 are not significantly different at $P < 0.05$.

Hu et al. (2005) and Farooq et al. (2006, 2007) reported similar results in rice. Significantly increase of activities of SOD, POD, CAT, and decrease of content of MDA, soluble sugar in leakage, in primed seed, might account for the improvement of germination percentage, germination energy and vigor index studied.

For agronomic traits and grain yield investigated in this study, there was no significant difference between primed seed and non-primed seed. This was because the data was obtained from transplanting rice field. Priming did not change the seed genotype. After transplanting at the same density, difference of seedling establishment percentage between primed seed and non-primed seed disappeared.

Prices of PVA and KNO_3 are 1.25 and 2.65 US dollars per kilogram, respectively. Priming 1 kg of aged F_1 seed with 1.0% PVA + 1.0% KNO_3 costs 0.303 US dollars for the chemicals. Price of normal F_1 seed of "Shan-you 63" in 2005 was 2.0 US dollars per kilogram. When the F_1 seed aged naturally (duo to store for more than 1 year) and its germination percentage lowered less than 80%, it had to be transferred to foodstuff at the price of 0.25 US dollars per kilogram, which was 12.5% of the original price. If the aged F_1 seed of "Shan you 63" with germination percentage more than 70% was treated with 1.0% PVA + 1.0% KNO_3 and enhance the germination percentage to more than 80%, the seed could be sold normally and 86.8% of the original income would be returned.

Although seed companies can sell aged seed at a lower price than that of normal seed and farmers can use the aged seed by increasing the amount of sowing in countries where no limitation was set for germination percentage of market seed, the resource losses were inevitable due to the lower germination percentage. Therefore, increasing germination percentage of the aged seed by priming technology was also necessary in these areas.

We concluded that, after treating with 0.5% PVA + 1.0% KNO_3 or 1.0% PVA + 1.0% KNO_3 for 1 day, germination percentage, germination energy, vigor index and seedling emergence percentage were significantly enhanced, activities of SOD, POD and CAT were significantly increased, content of MDA and soluble sugar in seed leakage were significantly decreased, compared with non-primed seed. Under transplanting condition, there was no significant difference between primed seed and non-primed seed on agronomic traits studied and grain yield.

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