

Improving the performance of transplanted rice by seed priming

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Abstract Transplanting is the major method of rice cultivation in the world, in which seedlings are raised in nursery and then transplanted into well puddle and prepared fields. The traditional nursery sowing method is tedious and produces weak seedlings that reduce the final yield due to high mortality. The potential of seed priming to improve the nursery seedlings and thus the transplanted rice was evaluated in the present study. The experiment was conducted in the rice growing area (31.45° N, 73.26° E, and 193 m) of Pakistan, during 2004–2005. Seed priming tools employed during the investigation included traditional soaking, hydropriming for 48 h, osmo-hardening with KCl or CaCl₂ (Ψ_s -1.25 MPa) for 24 h (one cycle), 10 ppm ascorbate for 48 h or seed hardening for 24 h. Priming improved the initial seedling vigor and resulted in improved growth, yield and quality of transplanted fine rice while traditional soaking behaved similar to that of untreated control. Osmohardening with CaCl₂ resulted in the best performance, followed by hardening, ascorbate priming and osmohardening with KCl. Osmohardening with CaCl₂ produced 3.75 t ha⁻¹ (control: 2.87 t ha⁻¹) kernel yield,

11.40 t ha⁻¹ (control: 10.03 t ha⁻¹) straw yield and 24.57% (control: 22.27%) harvest index. The improved yield was attributed due to increase in the number of fertile tillers. Significant positive correlation was found between mean emergence time of nursery seedlings and kernel yield, nursery seedling dry weight and kernel yield, fertile tillers and kernel yield, and leaf area duration and kernel yield.

Keywords Fine rice · Hardening · Nursery raising · Osmohardening · Quality · Transplanting · Yield

Abbreviations

MET	Mean emergence time
HI	Harvest index
LAI	Leaf area index
LAD	Leaf area duration
CGR	Crop growth rate
NAR	Net assimilation rate
Ψ_s	Osmotic potential
RL	Seedling root length
SL	Seedling shoot length
FW	Seedling fresh weight
DW	Seedling dry weight

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Introduction

Half of the world's population subsists wholly or partially on rice. It is the grain that shaped the

cultures, diets, and economies of billions of people in the world. For them, life without rice is simply unthinkable. It is arguably one of the most important cereals in the world feeding well in excess of 4 billion people and occupies a conspicuous position in the agro-based economy of Pakistan. Besides meeting the dietary requirements of the people, it has emerged as a major export commodity contributing about 13% to the total foreign exchange earnings of the country (Anonymous 2005). Rice accounts for 6.6% value added in agriculture and 1.6% in GDP in the country. In Pakistan, it is grown on an area of 25,000 ha with the average yield of 2.94 t ha⁻¹ (Anonymous 2005).

Transplanting is the major method of rice cultivation in the world, in which nursery seedlings are raised and then transplanted into flooded fields. For decades, farmers in Pakistan have been using traditionally pre-germinated seeds for rice nursery sowing resulting in poor and delayed germination. Not only is it very difficult to handle the pre-germinated seeds but it also makes nursery sowing a tedious job. Because of delayed emergence and lower growth rate, nursery seedlings thus raised can be transplanted when they are 40–45 days old. While, 30-day-old seedlings are considered ideal for transplanting as older seedling results in lower tillering capacity thus reducing the final yield. Sub-optimum plant population and uneven crop stand resulting from poor nursery seedlings are among the most important yield limiting factors in the traditional rice production system (Reddy 2004).

Seed priming techniques viz., hydro priming, osmoconditioning, osmohardening, hardening, and hormonal priming have been used to reduce the seedling emergence time, for synchronized emergence, improved emergence rate, and better seedling stand production in many horticultural (Khan 1992; Jett et al. 1996) and field crops like wheat (Chowdhary and Baset 1994), maize (Dell Aquilla and Tritto 1990) and rice (Basra et al. 2005; Farooq et al. 2004, 2006a–e).

Different priming tools have also been successfully integrated (Farooq et al. 2006a). While seed hardening is a alternate wetting and drying of seeds in tap or distilled water and this wetting and drying may be repeated once, twice or thrice

(Farooq et al. 2004), seed priming is a controlled hydration technique in which, seeds are soaked in solutions of low-osmotic potential (Ψ_s) before the actual germination take place and then re-dried near to their original weight to permit routine handling. Seeds are primed in a single cycle of wetting and drying (Basra et al. 2005). Most recently, we (Farooq et al. 2006b) introduced a new technique for rice seed invigoration in which both seed hardening and osmoconditioning have been successfully integrated and is named as osmohardening. Osmohardening with CaCl₂ (Ψ_s – 1.25 MPa) solution was found to be the most effective for both coarse and fine rice.

Although, very useful informations have been reported for enhancing the performance of direct seeded rice (Du and Toung 2002), still no study has been accomplished to improve the performance of transplanted rice. The present study was therefore, aimed to standardize appropriate invigoration technique/s for fine rice in transplanted rice cultures, to evaluate their effect on the quality of harvested paddy and to investigate the biochemical and physiological changes associated with the seed priming.

Materials and methods

Seed source and general experimental details

Seed of a widely grown fine rice (*Oryza sativa* L.) cultivar Super-Basmati was obtained from the Rice Research Institute, Kala Shah Kakoo, Sheikhpura, Pakistan. The initial seed moisture content and germination percentage were 8.65 and 84%, respectively. The field experiments were conducted at a progressive farmer's field at Kalar tract, Sialkot district (31.45° N, 73.26° E, and 193 m MSL) during 2004–2005. The experiment was laid out in the randomized complete block design (RCBD) with three replications.

Soil was sandy clay loam having pH 8.1, total exchangeable salts 0.30 mS cm⁻¹ and organic matter 0.75%. Wetland preparation method was used for land preparation for transplanting. Four cultivations with a tractor drawn cultivator followed by two plankings were given to achieve the desirable soil structure. The field was flooded with

water and puddled with the help of a tractor drawn cage-wheel puddler in standing water. The puddled field was left for a day for the soil particles to settle.

Seed treatments

A series of experiments were conducted to optimize different priming treatments for a widely grown fine rice cv. Super-Basmati (Basra et al. 2005; Farooq et al. 2004, 2006a–c, e). For all pre-sowing seed treatments, 300 g of healthy seeds were used. The ratio of seed weight to solution volume was 1:5 (Farooq et al. 2006b). The detail of the seed treatments is as follows:

- a. C = Control (untreated seeds).
- b. T1 = Traditional soaking treatment was achieved by placing the seeds between two layers of saturated jute mats till the appearance of radicles, which took about 48 h (Basra et al. 2005).
- c. T2 = Hydropriming was carried out by soaking seeds in distilled water under aerated conditions for 48 h (Farooq et al. 2006e).
- d. T3, T4 = For osmohardening, the seeds were hardened in solutions of KCl or CaCl₂ with osmotic potential of -1.25 MPa for 24 h, dried back and cycle was repeated once (Farooq et al. 2006b).
- e. T5 = For priming with ascorbate, seeds were soaked in 10 mg l^{-1} ascorbate solution for 48 h.
- f. T6 = For hardening treatment seeds were soaked in tap water at room temperature for 24 h, dried back and cycle was repeated once.

After each soaking treatment, seeds were given three surface washings with distilled water. Except for the traditional soaking treatment, the soaked seeds were dried close to their original moisture level under shade with forced air at $27^{\circ}\text{C} \pm 3$ (Farooq et al. 2006d). The dried seeds were sealed in polythene bags and stored in a refrigerator until use.

Crop husbandry

Transplanting was done in the puddled field manually in the first week of July using 30-day-old

nursery seedlings in the standing water. Two seedlings per hill were planted by keeping a row-to-row and plant-to-plant distance of 22 cm. The missing hills were gap filled within a week after transplanting. Fertilizers were applied as to provide 150 kg N, 90 kg P₂O₅, 75 kg K₂O, and 10 kg Zn ha⁻¹ in the form of urea (46%), single super phosphate (18% P₂O₅), sulphate of potash (50% K₂O), and ZnSO₄ (35% Zn). The whole quantity of P, K, and Zn, and half of the N were applied and incorporated into the soil as basal dose. The remaining N was applied in two equal splits each at tillering and panicle initiation.

Irrigation water at the time of transplanting was maintained at 3–4 cm depth. One week after transplanting a constant water depth of 5–6 cm was maintained to keep the field continuously flooded. Overall, 16 irrigations were applied during the crop growth period. Irrigation was withheld about 1 week before harvesting when the signs of physiological maturity appeared. For weed control, Butachlor @ 800 ml ha⁻¹ was applied at 7 days after transplanting in standing water (Reddy 2004). Carbofuran was broadcasted @ 25 kg ha⁻¹ at 55 days after transplanting to protect the crop from stem borers and leaf folders. Harvesting was done manually at harvest maturity and the approximate seed moisture content was 23% and threshing was done separately for each plot.

Nursery seedling characteristics

Mean germination time (MGT) was calculated according to Ellis and Roberts (1981). On days 30 after sowing, the seedlings were tested for vigor after carefully removing from the soil. Shoot length (SL) and root length (RL) were recorded of ten seedlings per replicate and averaged. Seedling fresh weight (FW) was determined immediately after harvest while dry weight (DW) was taken after drying at 70°C for 2 days (Basra et al. 2005).

Agronomic traits, yield components, and growth analysis

At harvesting, observations regarding agronomic traits and yield components were recorded following the standard procedures. Leaf area

was measured with a leaf area meter (Licor, Lincoln, NE, USA, Model 3100). Leaf area index (LAI) was calculated as the ratio of leaf area to land area (Watson 1947). Leaf area duration (LAD), crop growth rate (CGR) and net assimilation rate (NAR) were estimated following the formulae of Hunt (1978).

Kernel quality

A common electric lamp with a flexible stand was used as a source of light. A panicle was positioned in front of the lamp so that light may pass through it. Sterile spikelets, abortive and opaque kernels were separated. The chalky kernels were visually separated from normal kernels on the basis of chalky area present in different parts of the kernel with the help of high-power magnifying glass.

Protein content of rice kernels was determined by first carrying out Micro-Kjeldahl digestion and ammonia distillation, and then using titration or colorimetric ammonia assay of the digest to determine nitrogen concentration which was converted to protein by multiplying with the factor 5.95 (Shaw and Beadle 1949). Milled rice grains were ground on a Restsch mill equipped with 100 mesh sieve for the determination of amylose content according to the method reported by Juliano (1971). Kernel dimension, i.e. length and breadth were taken on 100-normal kernels from each replication with the help of a digital calipers and thereafter length/breadth ratio was calculated.

The water absorption ratio was determined by the formula of Juliano et al. (1965).

Statistical analysis

The data were statistically analyzed using the computer software MSTAT-C. Analysis of variance (ANOVA) technique was employed to test the overall significance of the data, while the least significant difference (LSD) test (at $p = 0.05$) was used to compare the differences among treatment means. Regression analysis was carried out to establish the relationship between various characteristics and quantify the same (Farooq et al. 2006c).

Results

Nursery seedling characteristics

All the priming treatments showed significant affect on the mean emergence time (MET), while final emergence percentage (FEP), and seedling FW and DW (Table 1). Comparison of priming treatments revealed that osmohardening with CaCl_2 (T4) was the most effective in reducing MET and increasing FEP, SW, and DW followed by osmohardening with KCl (T3) (FW) and hardening (T6) (DW). Nonetheless, all the priming treatments were better than the control (C). Maximum SL was measured in T6, which was not statistically different from the control and other treatments. Minimum SL was seen in T3. None of the treatments resulted in improved RL as compared with control. Minimum RL was measured in traditionally soaked seeds (T1), which was similar to T3 and T4.

Table 1 Effect of seed priming on the nursery seedling in fine rice

Treatments	MET (days)	FEP (%)	Seedling fresh weight (g)	Seedling dry weight (g)	Root length (cm)	Shoot length (cm)
C = Control	5.81 ^a	56.00 ^g	8.08 ^c	1.38 ^f	9.52 ^a	25.04 ^{ab}
T1 = Traditional soaking	5.39 ^b	66.33 ^f	9.03 ^d	2.18 ^e	4.25 ^e	23.75 ^b
T2 = Hydropriming	5.04 ^c	71.00 ^d	11.86 ^b	2.83 ^c	6.23 ^c	23.98 ^b
T3 = Osmohardening (KCl)	4.23 ^d	74.67 ^c	12.78 ^a	3.41 ^b	4.45 ^e	20.32 ^c
T4 = Osmohardening (CaCl_2)	3.83 ^e	80.00 ^a	12.96 ^a	3.82 ^a	4.53 ^e	22.89 ^{bc}
T5 = Ascorbate priming	4.23 ^d	69.33 ^e	9.33 ^d	2.50 ^d	5.20 ^d	24.41 ^b
T6 = Hardening	3.92 ^e	77.33 ^b	11.29 ^c	3.64 ^{ab}	6.77 ^b	27.86 ^a

Means sharing the same letters in a column do not differ significantly at $p = 0.05$ according to LSD test

Agronomic traits, yield components, and growth analysis

All the treatments lowered the duration to heading and maturity except in T1, which behaved similar to that of control (Table 2). Minimum days to heading and heading to maturity were recorded in T4 and T6, respectively. Similar observations were recorded with ascorbate priming (T5) and T6 for transplanting to heading days, and with T3, T5, and T4 for heading to maturity days.

Maximum plant height was recorded in T6, which was similar to that of T4 and T5 (Table 2). Minimum number of tillers, panicle bearing tillers, straw and kernel yield, and harvest index were recorded from plants raised from CaCl₂ osmohardened seeds. The effect of priming techniques on number of branches per panicle and number of kernels per panicle and 1,000-kernel weight was ineffective.

Growth analysis

All the seed priming treatments resulted in improved LAI except T1 which behaved similar to that of control at first, third, and final harvest (Fig. 1). Maximum LAI was measured in T4, which was comparable to that of T6, T3, and hydro priming (T2). Maximum LAD (296 days) was recorded in T4 as compared to 292 days (T6), 290 days (T3), 287 days (T5), and 286 days (T2) (Table 2).

All treatments resulted in improved CGR except T1 and T2 at all three harvests (Fig. 2). Maximum crop growth rate was recorded in seeds subjected to T4 followed by T3, T6, and T5 in first and final harvest. Maximum NAR was recorded in seeds subjected to T4 as compared T3 in all the harvests and hardening in the final harvest (Fig. 3).

Kernel quality

The effect of seed priming treatments on the kernel quality was also significant (Table 3). Seed priming resulted in less sterile spikelets, opaque and chalky kernels, and more normal kernels except with traditional soaking, which behaved similar to that of control in case of chalky.

Table 2 Effect of seed priming on the agronomic traits and yield components of transplanted fine rice

Treatments	Days to heading	Heading to maturity (days)	Plant height (cm)	Tillers (m ⁻²)	Panicle bearing tillers (m ⁻²)	Branches/panicle	No. of kernels/panicle	1,000 kernel weight (g)	Straw yield (t ha ⁻¹)	Kernel yield (t ha ⁻¹)	HI (%)	LAD (days)
C = Control	39.00 ^{ab}	38.00 ^a	115.7 ^{cd}	548.0 ^e	448.7 ^d	12.12	81.00	15.33	10.03 ^e	2.85 ^e	22.27 ^d	280 ^e
T1 = Traditional soaking	40.67 ^a	38.33 ^a	113.0 ^d	606.0 ^d	459.7 ^d	12.85	83.33	15.00	10.33 ^{de}	3.06 ^e	22.85 ^c	279 ^e
T2 = Hydropriming	37.33 ^{bc}	32.67 ^b	118.0 ^{bc}	666.0 ^{bc}	522.0 ^c	12.37	85.00	15.00	10.84 ^c	3.17 ^d	22.62 ^c	286 ^b
T3 = Osmohardening (KCl)	35.33 ^c	29.33 ^{bc}	119.3 ^b	669.3 ^b	538.7 ^b	14.77	85.33	15.67	10.84 ^c	3.57 ^b	24.77 ^a	290 ^b
T4 = Osmohardening (CaCl ₂)	29.67 ^d	29.30 ^{bc}	123.3 ^a	707.0 ^a	587.7 ^a	14.57	86.33	17.00	11.40 ^a	3.75 ^a	24.57 ^a	296 ^a
T5 = Ascorbate priming	32.00 ^d	31.33 ^{bc}	123.7 ^a	672.3 ^b	569.7 ^a	13.81	83.33	16.00	10.91 ^{bc}	3.41 ^c	23.81 ^b	287 ^b
T6 = Hardening	30.00 ^d	28.67 ^c	124.7 ^a	670.7 ^b	543.7 ^b	13.99	84.33	16.00	11.34 ^{ab}	3.58 ^b	22.48 ^{cd}	292 ^{ab}

Means sharing the same letters in a column do not differ significantly at $p = 0.05$ according to LSD test

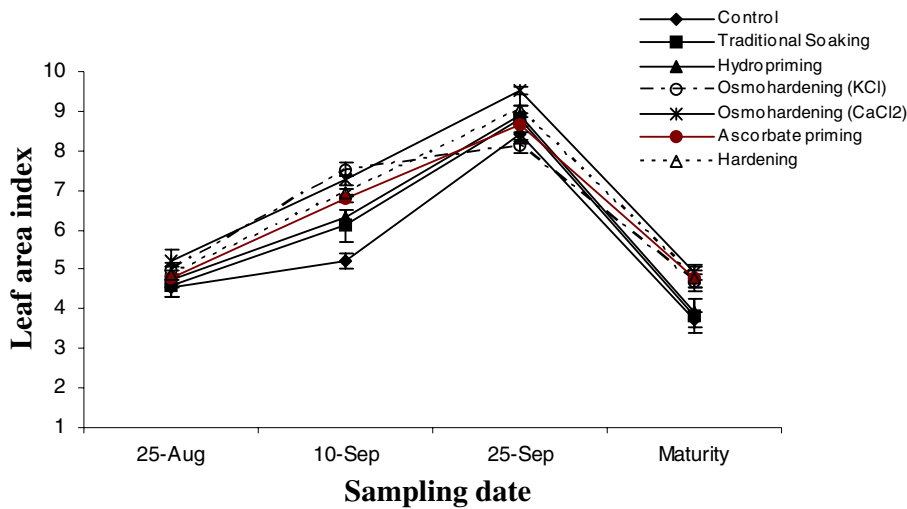


Fig. 1 Influence of seed priming on the leaf area index (LAI) in transplanted fine rice \pm standard error. Date of nursery sowing: June 1, 2004; Date of transplanting: July 1, 2004

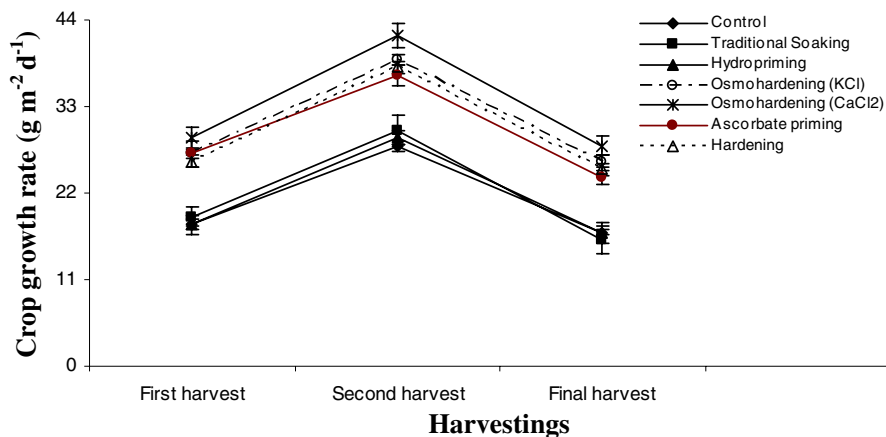


Fig. 2 Influence of seed priming on the crop growth rate (CGR) in transplanted fine and rice \pm standard error. Date of nursery sowing: June 1, 2004; Date of transplanting: July 1, 2004

Minimum sterile spikelets, and opaque kernels and maximum normal kernels were recorded in T4, which was similar to that of hardening in case of sterile spikelets, and T3, T5, and T6 in case of normal kernels. Minimum chalky kernels were recorded in T6, which was similar to T3, T4, and T5. The effect of seed priming on abortive kernels and kernel width was non-significant. Lower kernel amylose contents was observed in T6, which was similar to T3, T4, and T5 (Table 3). Maximum kernel proteins were recorded from untreated seeds, which was similar to T1 and T2, whereas other treatments resulted in lower kernel

proteins being minimum in T4. Maximum kernel length and kernel water absorption ratio was recorded in T6, which was similar to T3, T4, and T5. The effect of seed priming techniques on the kernel width was non-significant.

Correlation studies

There was a positive correlation between nursery seedling MET and kernel yield, nursery seedling DW and kernel yield, leaf area duration and kernel yield (Fig. 3), and panicle bearing tillers and kernel yield (Table 4).

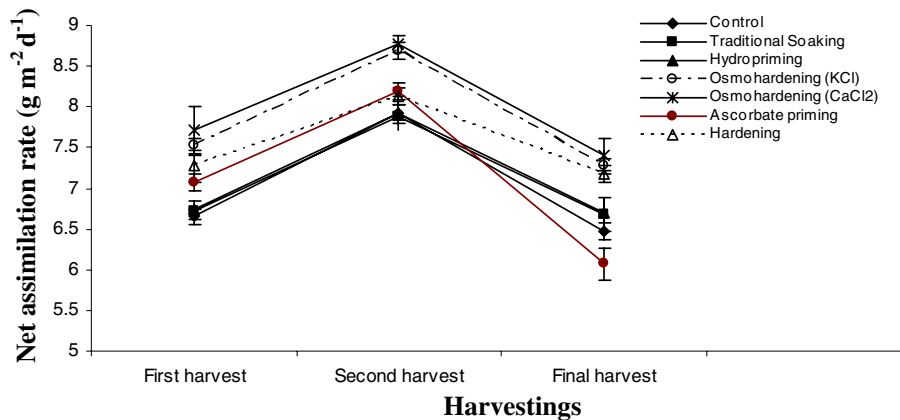


Fig. 3 Influence of seed priming on the net assimilation rate (NAR) in transplanted fine rice ± standard error. Date of nursery sowing: June 1, 2004; Date of transplanting: July 1, 2004

Table 3 Effect of seed priming on the kernel quality of transplanted fine rice

Treatments	Sterile spikelets (%)	Opaque kernels (%)	Abortive kernels (%)	Chalky kernels (%)	Normal kernels (%)	Kernel protein (%)	Kernel amylose (%)	Kernel length (mm)	Kernel width (mm)	Kernel water absorption ratio
C = Control	7.32 ^a	15.00 ^a	1.65	24.33 ^a	58.72 ^c	7.87 ^a	27.16 ^a	6.34 ^c	1.51	4.25 ^b
T1 = Traditional soaking	7.03 ^b	15.33 ^a	1.66	24.33 ^a	58.48 ^c	7.87 ^a	26.91 ^b	6.58 ^b	1.52	4.26 ^b
T2 = Hydropriming	6.85 ^{cd}	14.00 ^{ab}	1.65	19.67 ^b	64.58 ^b	7.86 ^a	26.38 ^c	6.57 ^b	1.46	4.26 ^b
T3 = Osmohardening (KCl)	6.98 ^{bc}	12.00 ^c	1.69	18.00 ^{bc}	68.31 ^a	7.69 ^b	26.15 ^d	6.92 ^a	1.40	4.30 ^a
T4 = Osmohardening (CaCl ₂)	6.65 ^c	10.67 ^c	1.62	16.33 ^{bc}	71.38 ^a	7.28 ^c	25.97 ^d	6.93 ^a	1.34	4.31 ^a
T5 = Ascorbate priming	6.91 ^{bc}	12.33 ^{bc}	1.64	16.33 ^{bc}	69.29 ^a	7.56 ^b	26.14 ^d	6.96 ^a	1.40	4.30 ^a
T6 = Hardening	6.71 ^{de}	12.33 ^{bc}	1.63	15.67 ^c	70.36 ^a	7.61 ^b	26.09 ^d	6.96 ^a	1.37	4.31 ^a

Means sharing the same letters in a column do not differ significantly at $p = 0.05$ according to LSD test

Table 4 Correlation between various attributes and kernel yield as affected by seed priming transplanted fine rice

X-variable	Y-variable	Regression equation	Coefficient of correlation
MET	Kernel yield	$y = -0.4107x + 5.2451$	0.981
DW	Kernel yield	$y = 0.3481x + 2.3589$	0.941
PBT	Kernel yield	$y = 0.0056x + 0.4073$	0.907
LAD	Kernel yield	$y = 0.0491x - 10.781$	0.949

MET nursery seedling mean emergence time, DW seedling dry weight, PBT panicle bearing tillers, LAD leaf area duration

Discussion

Priming rice seeds resulted in enhancement of initial seedling vigor, which resulted in early and uniform emergence of seedlings in nursery (Hampton and Tekrony 1995) as indicated by lower MET (Table 1). Similarly, primed seeds

showed enhanced vigor levels (Basra et al. 2005), and also a positive correlation was found between seed vigor and field performance in rice (Yamauchi and Winn 1996). Improved seedling establishment in primed seeds was understood to be due to rapid and regulated production of emergence metabolites (Basra et al. 2005).

Improved plant height, reduced duration to heading and heading to maturity seem to be the result of healthy and more vigorous nursery seedlings (Table 2), which gave a vigorous start. Healthy nursery seedlings resulted in higher number of tillers per m² and number of fertile tillers per m² (Reddy 2004). Number of branches per panicle remained statistically unaffected by seed priming treatments, which resulted in non-significant effect of priming on number of kernels per panicle and 1,000-kernel weight. Seed priming techniques resulted in improved straw yield that might be due to healthier and vigorous seedlings, which resulted in improved plant height, crop growth rate and net assimilation rate (Table 4), which ended in increased straw yield. Enhanced yield from primed seeds might be due to healthier and vigorous seedlings as confirmed by positive correlation between seedling MET and kernel yield (Table 4), and seedling DW and kernel yield (Table 4) and also due to improved number of panicle bearing tillers and leaf area duration as is confirmed by the positive correlation between number of panicle bearing tillers and kernel yield (Table 4), and leaf area duration and kernel yield (Table 4). Improved harvest index by seed priming in direct seeded rice might be the result of enhanced dry matter partitioning toward the panicles that resulted in improved kernel yield. The performance of traditional soaking was similar to that of control, which might be due to poor nursery seedlings (Table 1), where there was higher mortality of seedlings. Kathiresan et al. (1984) observed enhanced growth and yield in ascorbic acid and CaCl₂ treated sunflower seeds, which confirms with the present results. Findings of Paul and Choudhary (1991) also supported present observations as higher wheat grain yield was obtained with seeds primed with potassium salts. Kurdikeri et al. (1995) reported that soaking maize seeds in water and 0.5% CaCl₂ increased field emergence and grain yield compared with dry seed.

Improved CGR is possibly the result of strong and energetic start from vigorous nursery seedlings, which resulted in improved LAI that ended in NAR. This interpretation suggests that vigorous nursery seedlings by seed priming could result in earlier and enhanced resource capture than poor nursery seedlings from non-primed seeds.

Improved LAI, LAD, CGR, and NAR had been observed in wheat seeds primed with potassium salts than the untreated seed (Paul and Choudhary 1991), which supports the present study.

The improved nutrient and moisture supply in seedlings raised from primed seeds might have resulted in enhanced fertilization, which might have ended in lower number of sterile spikelets. Uniform photosynthetic assimilate throughout the panicles might have resulted in increased normal kernels and lower opaque, abortive and chalky kernels. It is very important to note here that increased kernel yield was accompanied with lower protein contents that might be attributed to growth dilution factor. Essentially the concentration of nitrogen and other nutrients was diluted when there was higher kernel yield. This diluted kernel nitrogen resulted in lower kernel proteins. Improved kernel length from primed seeds might be the result of improved net assimilation rate (Fig. 3) that resulted in improved photo assimilation and its translocation and partitioning toward the kernels. These results support the findings of Thakuria and Choudhary (1995) who reported improved kernel quality of rice seeds primed with potassium salts. Improved kernel quality had been observed in rice seeds osmoprimed with KCl and CaCl₂ (Zheng et al. 2002), which are in confirmatory with the present findings. Osmohardening with CaCl₂ and KCl were the most effective techniques for fine and coarse rice, respectively, as were earlier concluded by Farooq et al. (2006b).

In conclusion, employing seed priming improved the rice nursery seedlings, which after transplanting improved the growth, yield and quality of the harvested paddy. Osmohardening with CaCl₂ performed better than all other treatments, followed by hardening, ascorbate priming, and osmohardening with KCl.

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