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Effect of seed priming on the grain quality characters of rice cultivars in direct- seeded rice

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Abstract
The study aimed to evaluate the effect of seed priming on the grain quality characters of rice cultivers in direct-seeded rice. The experiment design was completely randomized with 3 replications and 4 treatments. Seeds of 2 rice genotypes IR64 and IET21214 were primed with water (hydro priming), Potassium Chloride (0.45M), Calcium Chloride (0.60M) and control (untreated paddy) for 48 hours and were sown in the field using direct-seeded rice technique. The crop was harvested at maturity and air-dried to 14% moisture content. It was stored in cloth bags for three months at room temperature before the evaluation of physicochemical quality characteristics. The physical quality characters include kernel size, shape, hulling, milling and head rice recovery. The chemical quality is mainly determined by amylose content, gelatinization temperature, gel consistency and cooking behaviour. The cooking quality of the rice genotypes did not vary with seed priming treatments. Amylose content did not change while a small but significant decrease was observed in the protein content and maximum decrease in the protein content was observed with hydropriming in both the cultivars under study. Milling, head rice recovery and 1000-grain weight increased with priming. The maximum increase was observed with hydropriming in both the cultivars followed by priming with $CaCl_2$ and KCl.

Keywords: Direct-seeded rice, seed-priming, milling quality, physicochemical quality.

Introduction
For more than half of humanity, rice (*Oryza sativa* L.) is the staple diet and one of the most important cereals in the world. It is the principal source of food for more than half of the world population, especially in South & South-East Asia and Latin America (Prasad 2011) [26].
It is a semi-aquatic tropical C_3 crop and requires high level of soil moisture for growth (Mckersie and Lesham 1994) [24]. Rice production is characterized by low water-use efficiency and growing of rice requires large amounts of water. Recent research indicates higher crop water and labour productivity from dry-direct seeded rice (DSR) compared to puddle transplanted rice particularly in Malaysia and Thailand (Pandey and Velasco 2005) [25]. Direct seeding of rice is involves establishing of rice crop from seeds sown directly in the field rather than by transplanting seedlings from the nursery. The adoption of a direct-seeded method for lowland rice culture would significantly decrease costs of rice production (Flinn and Mandac 1986) [15]. However, weeds, crop establishment and low yield are the major impediments in the large scale adoption of direct- seeded rice technology.
Seed priming is a pre-sowing imbibition treatment in which the seeds are partially hydrated such that germination processes begin, but radical emergence does not occur (Farooq *et al.* 2009b) [12]. One of the techniques widely used to enhance seed performance with respect to rate and uniformity of germination is seed priming. Priming allows the metabolic processes necessary for germination to occur without actual germination. Primed seeds usually exhibit an increased germination rate, greater germination uniformity and at times, greater total germination percentage (Basra *et al.* 2005) [3]. Seed priming treatment may be one of the useful techniques used to promote the production of DSR. Increased germination rate and uniformity have been attributed to metabolic repair during imbibition (Bray *et al.* 1989) [7], buildup of germination - enhancing metabolites (Basra *et al.* 2005) [3], osmotic adjustment and for seeds that are not redried after treatment a simple reduction in imbibition lag time (Bradford

1986) [6]. Rice is the only cereal crop cooked and consumed mainly as whole grains and quality consideration are much more important than for any other food crop (Hossain *et al.* 2009) [17]. Although production, harvesting and postharvest operations affect overall quality of milled rice, variety remains the most important determinant of market and end-use qualities. Quality desired in rice vary from one geographical region to another and consumers demand certain varieties and favour specific quality traits of milled rice for home cooking (Juliano *et al.* 1964, Azeez and Shafi 1966) [19, 2]. Seed priming techniques are a promising solution to poor stand establishment in DSR (Farooq *et al.* 2006a) [11]. Seed priming techniques, such as hydropriming (Farooq *et al.* 2006c) [13]; on-farm priming (Harris *et al.* 2002) [16]; osmohardening (Farooq *et al.* 2006a) [11]; hardening (Farooq *et al.* 2004) [6]; and priming with growth promoters like growth regulators and vitamins have been successfully employed in rice to hasten and synchronise emergence, achieve uniform stands and improve yield and quality (Du and Toung 2002, Basra *et al.* 2006) [4, 9]. These treatments protect seeds from biotic and abiotic stresses during the critical phase of seedling establishment (Shakirova *et al.* 2003) [27]. One of the major concerns in rice production has to do with seed and grain quality (Traore 2005). The physico-chemical characteristics of rice grains are important indicators of grain quality. Grain quality is mainly determined by the combination of many physical as well as chemical characters. The physical quality characters include kernel size, shape, hulling, milling and head rice recovery. The chemical quality is mainly determined by amylose content, gelatinization temperature, gel consistency and cooking behaviour (Khatun *et al.* 2003) [21]. These characters can be used as selection indices for the improvement in grain quality characteristics of rices. Surveys showed that consumer acceptance of a variety depend primarily on its cooking and eating quality (Juliano *et al.* 1964) [19].

However the role of seed priming on grain quality characters in direct-seeded rice is poorly known. Therefore the present study was performed for the evaluation of physico-chemical characters of rice grains under the influence of seed priming in direct-seeded rice.

Materials and Methods

Paddy of rice genotypes IR 64, a pure line variety released by IRRI and IET 21214, a pure line variety suitable for aerobic rice cultivation promoted by Directorate of Rice Research, Hyderabad, were primed with water (hydropriming), potassium chloride (KCl 0.45 M), calcium chloride ($CaCl_2$ 0.60 M) and control (untreated paddy) for 48 hours and were sown in the first week of June 2011 in the experimental fields of the Department of Plant Breeding and Genetics using aerobic/direct-seeded method of crop establishment. The crop was harvested at maturity and the paddy obtained was dried to 14% moisture content and stored in cloth bags for three months at room temperature before the evaluation of the milling, physicochemical and cooking quality characteristics.

1) Physical Properties

1.1 Dehusking and milling:

To determine milling characters of crop grains, weighed samples (125 g at 14 percent moisture content) of cleaned paddy were dehusked in a laboratory sheller equipped with rubber roller (Satake Rice Sheller, Satake Engg. Co., Japan). The shelled rice (brown rice) was weighed and expressed as the percentage of rough rice. Brown rice samples were polished in the Mc Gill Miller No. 2 (USA) in such a way to obtained 6 per cent degree of polish in all the samples and expressed as the per cent of rough rice which gives the value of milled rice recovery. The laboratory model Satake Test Rice Grader (Satake Engg. Co. Ltd., Japan) was used to separate the broken rice from the polished rice. The kernels with more than three- fourth length were considered as head rice. The head rice recovery and broken rice yields were calculated as the percentage of rough rice.

1.2 Thousand Kernel weight

One thousand head rice kernels of paddy, brown and milled rice were counted randomly in triplicate and weighed separately. Mean of three replications was reported.

1.3 Length–Breadth ratio (L/B)

Length and breadth of milled rice was measured with a dial gauge and their additive measurements (in mm) were taken. L/B ratio was determined by dividing length to breadth. A mean of 10 replications was calculated.

2) Chemical Properties

2.1 Extraction and estimation of amylose (Juliano 1971) [71]

Extraction

100 mg of ground grain sample was weighed accurately in duplicate and 1 ml 95% ethanol and 9 ml 1 N NaOH was added. The sample was heated for 10 minutes in a boiling water bath to gelatinize the starch. Cool and transferred to 100 ml volumetric flask. The total volume was made upto 100 ml with distilled water and mixed well.

Estimation

5 ml of the above solution was pipetted into 100 ml volumetric flask. 1 ml of 1 N acetic acid and 2 ml of iodine solution was added and the final volume was made 100 ml with distilled water. The solution was shaken well and left undisturbed for 20 minutes. The colour developed was read at 620 nm against a reagent blank. The standard curve was prepared by using potato amylose in the range of 40-200 µg.

2.2 Water uptake ratio: (Bhattacharya and Sowbhagya 1971) [5]

Water uptake ratio is inversely related to gelatinization temperature in rice of diverse quality. 2.0 g of whole grain rice is cooked in 20 ml of distilled water in a test tube for 20 minutes in a thermostatically controlled (80.5°C) water bath and a boiling water bath (96°C). The increase in weight of the rice divided by the original weight gives the apparent water uptake (g water absorbed/ g rice). The ratio of the values determined at the two temperatures is then calculated and expressed as a percent.

2.3 Alkali Spreading Value (ASV) (Little *et al.* 1958) [23]

Select duplicate sets of six milled kernels without cracks and put in small petri-dishes. Add 10 ml of 1.7% KOH (store for at least 24 hours before use) to each petri dish. Arrange kernels to provide enough space between them to allow for spreading. Cover the petri dishes and let them stand for 23 hours at ambient temperature. A rating for spreading of 1-3 is classified as high, 4-5 as intermediate and 6-7 as low gelatinization temperature. Clearing values were usually 1 unit lower than spreading values.

Table 1: Characterstics regarding spreading and clearing of rice kernels and corrosponding score

Score	Spreading	Clearing
1	Kernel not affected	Kernel chalky
2	Kernel swollen	Kernel chalky, collar powdery
3	Kernel swollen, collar complete or narrow	Kernel chalky, collar cottony or cloudy
4	Kernel swollen, collar complete and wide	Centre cottony, collar cloudy
5	Kernel split or segregated, collar complete and wide	Centre cottony, collar clearing
6	Kernel dispersed merging with collar	Centre cloudy, collar clear
7	Kernel completely dispersed and intermingled	Centre and collar clear

2.4 Gel consistency test (Cagampang *et al.* 1973) [8]
Weigh 100 mg of powder, in duplicate in culture tubes (12 x 100 mm). Add 0.2 ml of 95% ethanol containing 0.025% thymol blue. Add 2.0 ml of 0.2 N KOH and mix well with a cyclomixer/vortex mixer. Cover the tubes with glass marbles and heat in vigorously boiling water bath for 8 minutes, making sure that the tube contents reach 2/3rd the height of the tube. Remove the tubes from the water bath and let stand for 5 minutes. Cool in an ice water bath for 20 minutes. Lay the tubes horizontally over a graph on a table. Do not disturb for 1 hour. Measure the total length of the gel (mm) from the bottom of the tube to the gel front.

2.5 Volume expansion ratio (Juliano *et al.* 1965) [65]
Took 50 ml graduated centrifuge tubes and added 15 ml distilled water. Added 5 gm rice sample and soaked it for 10 minutes. Noted the volume again ('y' ml). The increase in volume before cooking was 'y-15' ml. Cook the grains on a boiling water bath for 20 minutes. The cooked rice were put on a blotting paper to remove excess of water. Then dipped the rice in 50 ml water taken in 100 ml measuring cylinder. The volume risen was recorded as 'x'. The increase in volume after cooking is 'x-50' ml. The ratio between the increase in volume after cooking (x-50) and that before cooking (y-15) was calculated and expressed as volume expansion ration.

2.6 Extraction And Estimation Of Crude Protein (Micro Kjeldahl method, A.O.A.C 2000) [1]
Weighed 100 mg of powdered sample was digested in Kjeldahl digestion tubes with 15 ml concentrated sulphuric acid and 5 g of digestion mixture until clear solution was obtained. The contents were cooled to room temperature and volume was made to 100 ml with distilled water. The 100 ml aliquot was distilled with 50ml of 40% sodium hydroxide solution using Kjeldahl distillation unit (FOSS Kjeltec 2100). About 100-150 ml of the distillate was collected in 25 ml of 4% boric acid solution and titrated against 0.01N hydrochloric acid till a grey colour was obtained as end point. Nitrogen content of the sample was calculated from the following formula:

% N = [{(X-Blank) x 0.00014 x Dilution factor}/Y] X 100

Where X- titre value
Y – Weight of sample (g)

Dilution factor – Volume made/volume taken for distillation
The crude protein content was calculated by multiplying %N with a factor 5.95.

Statistical Analysis: The data was statistically analyzed by using Analysis of Variance (ANOVA). For significant mean effects, means were separated using least significant test (LSD).

Results and Discussion
The cooking quality of the rice genotypes did not vary with the priming treatments expect that slight variations were found in the water uptake ratio (Table 1). Priming treatments did not cause significant change in the amylose content while a small but significant decrease was observed in the protein content. Maximum decrease in the protein content was observed with hydropriming in both the cultivars under study. Gelatinization temperature also did not exhibit a significant change as reflected from the alkali spreading values (Table 1).

Table 2: Chemical and cooking quality in the grains of direct-seeded rice

Treatments	Variety	Water uptake ratio	Volume expansion ratio	Gel consistency (mm)	Amylose content (%)	Crude protein (%)	Alkali spreading value
Control	IR 64	23.42±0.04	4.50±0.50	78.00±2.00	22.22±0.63	6.14±0.08	4.0
	IET 21214	16.08±0.05	4.21±0.46	80.50±0.50	16.82±0.32	6.81±0.08	3.0
H_2O	IR 64	23.48±0.33	4.54±0.79	88.00±2.00	23.17±0.32	5.52±0.12	4.0
	IET 21214	16.68±0.06	4.33±0.33	80.75±0.25	17.46±0.32	5.60±0.12	3.0
KCl	IR 64	22.91±0.20	4.33±0.33	79.50±1.50	22.54±0.95	5.85±0.04	4.0
	IET 21214	16.06±0.10	4.33±0.33	84.50±0.50	17.46±0.32	6.18±0.04	3.0
$CaCl_2$	IR 64	23.36±0.21	4.33±0.50	75.50±4.50	22.54±0.32	5.85±0.12	3.5
	IET 21214	16.00±0.09	3.63±0.13	73.00±3.00	17.14±0.63	6.23±0.08	3.5
LSD5%							
A=Rice	A	0.14	NS	NS	0.39	0.08	0.33
varieties	B	0.20	NS	2.50	NS	0.12	NS
B= Treatments	A*B	0.28	NS	3.50	NS	0.18	NS

Table 2: Physical and milling characteristics in the grains of direct-seeded rice

Treatments	Variety	1000 Grain Weight (g)			Grain length (mm)	Grain breadth (mm)	L/B ratio	Hulling (%)	Milling (%)	Head rice (%)
		Paddy	Brown rice	Milled rice						
Control	IR 64	23.07 ±0.09	19.46±0.07	17.70±0.07	6.97±0.17	1.89±0.03	3.68±0.14	80.74±0.27	69.75±0.17	55.51±0.37
	IET 21214	22.79±0.22	19.23±0.18	17.50±0.16	5.94±0.03	1.81±0.03	3.28±0.03	80.00±0.55	73.78±0.36	59.29±0.05
H_2O	IR 64	26.10±0.01	21.88±0.008	19.91±0.007	7.13±0.10	1.89±0.01	3.76±0.07	81.49±0.18	72.63±0.48	59.89±0.73
	IET 21214	24.69±0.26	20.75±0.26	18.88±0.23	6.00±0.05	1.86±0.005	3.23±0.04	80.79±0.50	73.16±0.16	64.78±0.04
KCl	IR 64	24.75±0.14	20.80±0.11	18.92±0.10	7.16±0.02	1.89±0.002	3.79±0.009	79.77±0.11	71.76±0.30	57.33±0.43
	IET 21214	23.16±0.65	19.53±0.52	17.77±0.47	6.12±0.002	1.81±0.03	3.38±0.07	80.66±0.23	72.72±0.40	61.69±0.43
$CaCl_2$	IR 64	25.80±0.22	21.64±0.17	19.69±0.16	7.15±0.16	1.88±0.005	3.79±0.06	80.46±0.05	72.13±0.10	56.79±0.12
	IET 21214	23.85±0.05	20.08±0.04	18.27±0.04	5.96±0.01	1.81±0.004	3.30±0.01	80.59±0.24	72.95±0.07	61.28±0.38
LSD5%										
A= Rice	A	0.21	0.16	0.15	0.09	0.02	0.62	NS	0.24	0.27
Varieties	B	0.30	0.24	0.22	0.12	NS	NS	0.40	0.35	0.38
B=Treatments	A*B	0.42	0.34	0.31	NS	NS	NS	0.57	0.49	0.54

A significant increase was observed in the 1000-grain weight of paddy, brown rice and milled rice in both IR 64 and IET 21214 (Table 2). The maximum increase was observed with hydropriming in both the cultivars followed by priming with $CaCl_2$ and KCl. The grain length improved significantly with the priming techniques while the grain shape was unaffected. The milling quality was also found to improve with priming with a maximum increase in milling and head rice recovery observed with hydropriming (1.57% and 5.19% respectively). Head rice recovery (HR) is the proportion of the intact grain which is more than or equal to 3/4th its length. HR indicates that percent of whole grains obtained after milling processing. For quality evaluation, HR recovery is one of the most important characters and more than 65% of HR recovery is desirable. In the present study, priming increased the percentage of head rice recovery with maximum inccrease was observed with hydropriming while there was no significant difference was found in case of priming with KCl and $CaCl_2$. Priming with KCl and $CaCl_2$ increased milling quality as compared to control but there was no significant difference between these two priming techniques. Improved kernel quality of direct-seeded rice primed with salts of potassium and calcium chloride have been reported by Zheng *et al.* (2002) [29]. Improved kernel length from primed seeds have been attributed to improved net assimilation rate that resulted in improved photoassimi lation and its translocation and partitioning towards the kernels (Lee *et al.* 1999, Farooq *et al.* 2007) [12, 29]. Seed priming treatments in direct-seeded fine rice have been reported to resulted in increased kernel proteins and lower amylose content (Farooq *et al.* 2006a) [11]. However, effect of seed priming treatments on the kernel width was non-significant. All the priming treatments resulted in higher kernel water absorption ratio compared with control.

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