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Responses to seed dormancy-breaking treatments in rice species (*Oryza* L.)

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Summary

Seed germination tests of 18 germplasm accessions representing 16 rice species (*Oryza* L.) were conducted under a series of dormancy-breaking treatments. Hulled and intact seeds were exposed to (1) constant temperatures of 26 °C, 31 °C, and 36 °C or left at room temperature (28±1 °C); and (2) alternating temperatures of 40/25 °C, 40/30 °C, and 45/30 °C (12 h/12 h), or left at room temperature. In a third treatment, intact seeds were exposed to dry heat at 50 °C for 7 or 14 d before germination of hulled and intact seeds under the optimum temperature regimes for each species determined from Treatments 1 or 2. Species that showed less than 85% germination in Treatment 3 were exposed to various chemical treatments, namely germination of intact seeds in 0.001 M potassium nitrate (KNO_3), or presoaking the seeds for 24 or 48 h in 0.1 or 0.2 M nitric acid (HNO_3), 1 M hydrogen peroxide (H_2O_2), 1000 ppm gibberellic acid (GA_3), and a combination of 0.1 M HNO_3 and 1 M H_2O_2 or 0.2 M HNO_3 and 1 M H_2O_2. Four conclusions emerge from this work: (1) removal of the seed hull is extremely effective for breaking seed dormancy; (2) species respond differently to various constant or alternating temperature regimes, and no single temperature regime is consistently effective to break seed dormancy across all *Oryza* species; (3) heat treatment generally promotes germination of the species that respond to certain constant or alternating temperature treatments, but no consistent differences were observed in seed germination of most species between heat treatments at 50 °C for 7 or 14 d; and (4) some species respond to certain chemical treatments effectively under the optimum temperature regimes. An appropriate combination of seed hull removal, dry heat or chemical treatments, and germination under the optimum temperature regimes for individual species provides the best results for breaking seed dormancy of rice species.

Introduction

Continued improvement of rice varieties relies on the evaluation and utilization of germplasm in the rice genepool for agronomically important traits. This genepool includes two domesticated species (*Oryza sativa* L. and *O. glaberrima* Steud. from Asia and West Africa, respectively), and the 22 wild species in the genus *Oryza* L. Over 3,000 accessions of these wild rices are conserved in the International Rice Genebank (IRG) at the International Rice Research Institute (IRRI) in the Philippines. This important germplasm is held in trust under the auspices of the Food and Agriculture Organization in an International Network of *Ex Situ* Collections, and is freely available to researchers worldwide.

Rapid and full germination of seeds is an essential first step towards effective utiliza-

tion of rice germplasm. However, seed dormancy – a condition that temporarily suspends visible growth of meristems – is one of the factors that hamper germination. In nature, dormancy can be an advantage for some species because it renders resistance to preharvest sprouting and prevents germination until favourable conditions for plant development prevail (Das, 1989). However, it is problematic when utilizing rice species in research and breeding.

Variation in seed dormancy has been reported in different varieties of *O. sativa* (Chang and Yen, 1969; Panchaksharaiah *et al.*, 1976; Agrawal, 1981; Siddique *et al.*, 1988; Seshu and Dadlani, 1991), and several studies have been undertaken to break seed dormancy of cultivated rices (Misra and Misro, 1970; Singh *et al.*, 1973; Ellis *et al.*, 1983; Zhang, 1990). However, information on dormancy and dormancy breaking in wild species of rice is rather limited, although previous research (Takahashi, 1961; Cohn and Hughes, 1981; Cohn and Butera, 1982; Das, 1989) and our own experiences have indicated that, in general, wild *Oryza* species possess stronger seed dormancy than cultivated rice varieties.

The objectives of this study were (1) to evaluate the dormancy of wild *Oryza* species and *O. sativa* and their response to different dormancy-breaking treatments, such as removal of the seed hull, germination under different constant and alternating temperature regimes, dry heat treatment of seeds, and chemical treatments, and (2) to determine the most appropriate dormancy-breaking procedure for each species.

Materials and methods

Seed materials

Seeds of 18 germplasm accessions representing 16 *Oryza* species (Table 1) were harvested between October 1994 and February 1995 in the IRG screenhouse at IRRI, Los Baños (121° 15' E and 14° 13' N), Philippines. The seeds were dried at 15 °C and 15% RH for 14 d, and subsequently stored at about 2 °C and 40% RH in the Active Collection storage vault of the genebank until needed. Thereafter, seed samples were allowed to equilibrate at room temperature (28 ± 1 °C and 68-80% RH) for 7 d before being subjected to dormancy-breaking treatments.

Seed viability, germination, and dormancy determination

Tetrazolium tests (Chalam *et al.*, 1967) were conducted to estimate seed viability of all accessions before proceeding with dormancy-breaking treatments. Ten hulled seeds (i.e., with the hull removed) from each species were preconditioned by soaking in distilled water at 28 °C for 3 h, and then dissected longitudinally and medially through the embryo. They were soaked in 1% tetrazolium solution for 1 h at 40 °C in the dark, and then washed several times with distilled water to remove excess solution. Seeds were considered viable when the embryo was completely stained, or when the only extremities of the scutellum and/or the tip of the radicle remained unstained.

Seed dormancy was determined by germination of intact seeds in 9 cm Petri dishes lined with filter paper and moistened with distilled water, in germination chambers at

31 °C constant temperature, 99% RH and 12 h light. Germination was monitored daily over a period of 14 days. Based on previous studies, *O. sativa* seeds gave the best germination at this temperature (Hall, 1966 a, b; Ellis *et al.*, 1983). Germination was scored as the emergence of the radicle. Species with seed germination ≤ 25% were considered to be strongly dormant, whereas those with seed germination ≥ 50% were considered to be weakly dormant (Das, 1989).

Dormancy-breaking treatments

Three replications, each containing 25 seeds, were used in the different treatments throughout all experiments. For treatments 1 and 2 described below, seeds of each species were germinated at room temperature (28 ± 1 °C) and 12 h light, without control of relative humidity, to monitor the possible loss of dormancy over time. Germination was monitored daily over a period of 14 days, and root and shoot lengths were measured at the 2nd, 4th, and 6th d after germination.

1. *Germination under constant temperatures.* Intact and hulled seeds were germinated under either of the constant temperature regimes 26 °C, 31 °C, or 36 °C, 99% RH and 12 h light.
2. *Germination under alternating temperatures.* Intact and hulled seeds were germinated under one of the alternating temperature regimes, 40/25 °C, 40/30 °C, or 45/30 °C (12 h/12 h) with light at the upper temperature of each cycle, and 99% RH.
3. *Dry heat treatment.* Two sets of intact seeds were incubated at 50 °C for either 7 or 14 d. One set from each treatment was hulled after equilibration to room temperature for 2 d. Both hulled and intact seeds were germinated in the best constant or alternating temperature regime for each species determined from previous germination tests. When more than one favourable temperature regime was observed in seed germination of the same species, the availability of a germination chamber was taken into consideration regarding the choice of the temperature regime to be used.
4. *Chemical treatments.* Chemical treatments were only applied to species that did not reach 85% germination in previous treatments. The four chemical treatments were (a) 0.001 M potassium nitrate (KNO_3), (b) 0.1 M and 0.2 M nitric acid (HNO_3), (c) 1 M hydrogen peroxide (H_2O_2), and (d) 1000 ppm gibberellic acid (GA_3). Intact seeds were germinated directly in sterilized Petri dishes lined with filter paper moistened with 6 ml KNO_3. All subsequent treatments involved presoaking intact seeds in 0.1 M or 0.2 M HNO_3, 1 M H_2O_2, or 1000 ppm GA_3 for 24 or 48 h, prior to germination. A combination of presoaking for 24 or 48 h each successively in 0.1 M HNO_3 and 1 M H_2O_2 or in 0.2 M HNO_3 and 1 M H_2O_2 was also included in the chemical treatments. After drying at room temperature for 30 min to 1 h, all treated seeds were germinated under the best constant or alternating temperature regime for each species determined from the previous germination tests.

Statistical analysis

Analyses of variance were carried out using IRRISTAT Version 3.1 (Gomez *et al.*, 1994). The arcsin transformation was used for percentage germination data. For con-

stant and alternating temperatures, the analysis was based on a split-split plot design, with temperature as the main plot, seed condition (intact vs. hulled) as sub-plot, and species as sub-subplot. With the 50 °C heat treatment, the analysis was based on a split plot design, with duration of heat treatment as the main plot, and seed condition as the subplot. For chemical treatments, the analysis was based on a randomized complete block design.

Duncan's Multiple Range Test (DMRT) was used to compare mean values using transformed means, and transferred to the original mean values (Gomez and Gomez, 1984). However, the least significant difference (LSD) was used for pair-wise comparison of mean values between intact and hulled seeds for the 50 °C heat treatment.

Results

Seed viability and dormancy of Oryza *species*

Seed viability in all *Oryza* species studied was high based on the tetrazolium test, ranging from 70 to 100% (Table 1). However, all species except *O. grandiglumis*, had strong dormancy and germination varied from 0 to 9.3%.

Germination under different temperature regimes

Germination of intact seeds of most *Oryza* species was low at constant temperatures, except for *O. grandiglumis*, with germination between 64.0 and 76.0% in different temperature regimes (Table 2). Most of the other species showed germination lower than 15.0%. In contrast, hulled seeds showed substantially higher germination of all species than the intact seeds under all constant temperatures, although there was significant variation within species. Eight species had a mean germination of hulled seeds higher than 85% under constant temperatures.

Germination of intact seeds under alternating temperatures was higher in most species. An average of 12.9% germination was observed over all species at different alternating temperatures, but with large variation from 0 to 85.3% between species. Exposure of the hulled seeds of all species under different alternating temperature regimes promoted an average of 59.8% germination, but with great variation from 1.3% (*O. ridleyi* at 45/30 °C) to 100% (*O. grandiglumis* at 40/25 °C and 40/30 °C) between different species. As with the constant temperature, variation in germination was also found within the same species at different alternating temperatures. In general, hulled seeds exposed to alternating temperatures had similar or slightly higher germination, compared with that under the constant temperatures for most species. The germination data also indicated that species which showed good response to the constant temperatures also gave a good result under alternating temperatures.

Combination of dry heat treatment and germination under optimum temperatures

Dry heat treatments at 50 °C both for 7 and 14 d in general gave a substantial increase in germination of intact seeds for most species at the optimum temperature regimes, compared with the germination responses of intact seeds under their optimum temperature

Table 1. Origin, seed dormancy, and viability of the *Oryza* species used in dormancy breaking tests.

Species	IRGC[1] accession number	Seed viability (%)[2]	Germination at 31 °C (%)[3]
***Sativa* complex**			
O. glumaepatula	104386	100	4.0
O. longistaminata	94-10530[4]	90	1.3
O. nivara	82015	90	9.3
O. rufipogon	81981	90	0
O. sativa	101174	80	2.7
O. sativa f. *spontanea* [5]	82027	100	0
***Officinalis* complex**			
O. alta	101395	70	0
O. australiensis	103303	70	2.7
O. eichingeri (tetraploid)	105182	90	8.0
O. grandiglumis	105669	100	76.0
O. latifolia	100962	80	1.3
O. minuta	101097	100	1.3
O. officinalis (diploid)	100176	100	0
O. officinalis (tetraploid)	105223	90	0
O. punctata (tetraploid)	104975	90	0
O. rhizomatis	105448	80	0
***Ridleyi* complex**			
O. ridleyi	106471[4]	100	3.0
Species not assigned to any complex			
O. brachyantha	81950	80	0

[1] International Rice Genebank Collection.
[2] Based on the tetrazolium test conducted before the dormancy breaking experiment.
[3] % germination of intact seeds at constant 31 °C was used to determine dormancy level in this study. Germination ≤25% indicates strong dormancy, and germination ≥50% indicates moderate or weak dormancy.
[4] This is a temporary identification number before an IRGC accession number has been assigned.
[5] Referred to subsequently as *O. spontanea*.

regimes without heat treatments (Table 3). Except for *O. alta* and *O. punctata*, there were no significant differences between germination of intact seeds treated at 50 °C for 7 or 14 d.

There was a marked increase in germination of hulled seeds after heat treatment of most species under their optimum temperature regimes, compared with the germination responses of intact seeds. All species that showed dormancy loss in previous treatments (Table 2) under constant or alternating temperatures responded similarly to the heat treatments. In other words, species that showed high germination in the previous treatments also had high germination responses in this treatment. Hulled seeds of 11 species had germination higher than 85% under their appropriate temperature regimes after the heat treatments.

Table 2. Germination of intact and hulled seeds of *Oryza* species under constant and alternating temperatures.

Species	Constant temp. (°C)	Germination (%)		Alternating temp. (°C)	Germination (%)	
		Intact	Hulled		Intact	Hulled
***Sativa* complex**						
O. glumaepatula	26	8.0 a[1]	46.7 b	40/25[2]	28.0 a	88.0 a
	31	4.0 a	52.0 b	40/30	24.0 a	85.3 a
	36	5.3 a	88.0 a	45/30	32.0 a	96.0 a
	RT[3]	5.3 a	62.7 ab	RT	18.7 a	54.7 b
O. longistaminata	26	0 a	26.7 a	40/25	6.7 b	6.7 c
	31	1.3 a	41.3 a	40/30	4.0 bc	25.3 b
	36	4.0 a	40.0 a	45/30	24.0 a	60.0 a
	RT	0 a	16.0 a	RT	0 c	10.7 bc
O. nivara	26	0 a	37.3 a	40/25	1.3 b	52.0 a
	31	9.3 a	48.0 a	40/30	4.0 b	68.0 a
	36	6.7 a	38.7 a	45/30	25.3 a	72.0 a
	RT	6.7 a	53.3 a	RT	5.3 b	48.0 a
O. rufipogon	26	0 a	21.3 ab	40/25	0 a	4.0 c
	31	0 a	10.7 ab	40/30	0 a	10.7 bc
	36	0 a	6.7 b	45/30	4.0 a	40.0 a
	RT	0 a	37.3 a	RT	0 a	28.0 ab
O. sativa	26	4.0 b	74.7 b	40/25	49.3 a	85.3 a
	31	2.7 b	93.3 a	40/30	52.0 a	93.3 a
	36	29.3 a	90.7 a	45/30	12.0 b	92.0 a
	RT	2.7 b	88.0 ab	RT	8.0 b	93.3 a
O. spontanea	26	0 a	80.0 b	40/25	2.7 b	92.0 ab
	31	0 a	97.3 a	40/30	6.7 b	94.7 a
	36	13.3 a	97.3 a	45/30	85.3 a	97.3 a
	RT	0 a	74.7 b	RT	4.0 bc	62.7 b
***Officinalis* complex**						
O. alta	26	1.3 a	68.0 ab	40/25	18.7 a	98.7 a
	31	0 a	57.3 b	40/30	1.3 c	96.0 a
	36	0 a	86.7 a	45/30	10.7 ab	70.7 b
	RT	0 a	61.3 ab	RT	4.0 bc	62.7 b
O. australiensis	26	1.3 a	28.0 b	40/25	2.7 a	6.7 c
	31	2.7 a	66.7 a	40/30	2.7 a	36.0 b
	36	0 a	57.3 ab	45/30	12.0 a	69.3 a
	RT	1.3 a	45.3 ab	RT	8.0 a	30.7 b
O. eichingeri	26	14.7 ab	74.7 b	40/25	14.7 ab	96.0 a
	31	8.0 b	90.7 a	40/30	13.3 b	94.7 a
	36	1.3 b	86.7 ab	45/30	32.0 ab	33.3 b
	RT	30.7 a	92.0 ab	RT	34.7 b	88.0 a
O. grandiglumis	26	64.0 a	82.7 ab	40/25	81.3 ab	100.0 a
	31	76.0 a	98.7 a	40/30	73.3 b	100.0 a
	36	70.7 a	97.3 ab	45/30	2.7 c	64.0 b
	RT	69.3 a	81.3 b	RT	93.3 a	98.7 a

Table 2. continued.

Species	Constant temp. (°C)	Germination (%)		Alternating temp. (°C)	Germination (%)	
		Intact	Hulled		Intact	Hulled
O. latifolia	26	0 a	50.7 a	40/25	14.7 a	78.7 a
	31	1.3 a	56.0 a	40/30	21.3 a	88.0 a
	36	12.0 a	72.0 a	45/30	33.3 a	68.0 a
	RT	2.7 a	73.3 a	RT	1.3 b	78.7 a
O. minuta	26	4.0 a	76.0 a	40/25	0 a	86.7 b
	31	1.3 a	86.7 a	40/30	0 a	92.0 ab
	36	2.7 a	84.0 a	45/30	2.7 a	6.7 c
	RT	5.3 a	85.3 a	RT	2.7 a	97.3 a
O. officinalis (diploid)	26	0 a	42.7 a	40/25	5.3 a	66.7 ab
	31	0 a	57.3 a	40/30	1.3 a	80.0 a
	36	0 a	60.0 a	45/30	2.7 a	45.3 b
	RT	0 a	60.0 a	RT	0 a	53.3 b
O. officinalis (tetraploid)	26	0 a	36.0 b	40/25	0 a	57.3 a
	31	0 a	44.0 b	40/30	1.3 a	54.7 a
	36	0 a	78.7 a	45/30	5.3 a	26.7 b
	RT	0 a	65.3 ab	RT	0 c	46.7 ab
O. punctata	26	1.3 a	73.3 b	40/25	2.7 a	36.0 b
	31	0 a	92.0 a	40/30	4.0 a	53.3 b
	36	1.3 a	69.3 b	45/30	9.3 a	32.0 b
	RT	4.0 a	69.3 b	RT	13.3 a	97.3 a
O. rhizomatis	26	0 a	21.3 a	40/25	0 a	30.7 a
	31	0 a	42.7 a	40/30	0 a	29.3 a
	36	0 a	29.3 a	45/30	0 a	46.7 a
	RT	0 a	40.0 a	RT	0 a	48.0 a
***Ridleyi* complex**						
O. ridleyi	26	0 a	34.0 a	40/25	0 a	13.3 b
	31	3.0 a	15.7 ab	40/30	0 a	14.7 b
	36	0 a	8.0 b	45/30	0 a	1.3 c
	RT	4.7 a	25.0 a	RT	0 a	46.7 a
Species not yet assigned to any complex						
O. brachyantha	26	0 a	22.7 c	40/25	0 b	57.3 b
	31	0 a	50.7 ab	40/30	0 b	53.3 b
	36	0 a	73.3 a	45/30	12.0 a	81.3 a
	RT	0 a	30.7 bc	RT	0 b	33.3 b

Within each species and within constant or alternating temperature, means followed by a common letter are not significantly different at 5% level by Duncan's Multiple Range Test (DMRT).
12/12 h, with light at the upper temperature of each cycle.
RT = Room temperature (28 ± 1 °C)

Table 3. Germination at optimum temperature regimes of wild *Oryza* species subjected to heat treatments.

Species	Optimum temp. (°C)	Germination (%)					
		50 °C for 7 days			50 °C for 14 days		
		Intact	Hulled	Difference[1]	Intact	Hulled	Difference
***Sativa* complex**							
O. glumaepatula	45/30[2]	74.7	98.7	24.0**	76.0	97.3	21.3**
O. longistaminata	45/30	49.3	88.0	38.7**	46.7	86.7	40.0**
O. nivara	45/30	53.3	78.7	25.3**	61.3	69.3	8.0**
O. rufipogon	45/30	16.0	56.0	40.0*	10.7	28.0	17.3*
O. sativa	40/30	90.7	98.7	8.0ns	98.7	100.0	1.3ns
O. spontanea	36	58.7	100.0	41.3ns	86.7	100.0	13.3ns
***Officinalis* complex**							
O. alta	40/30	10.7	89.3	78.7**	30.7	98.7	68.0**
O. australiensis	45/30	32.0	74.7	42.7*	50.7	76.0	25.3*
O. eichingeri	40/30	53.3	93.3	40.0**	72.7	97.3	24.7**
O. grandiglumis	40/30	62.7	98.7	36.0**	73.3	100.0	26.7**
O. latifolia	40/30	37.3	84.0	46.7**	58.7	85.3	26.7**
O. minuta	RT[3]	48.0	92.0	44.0**	58.7	94.7	36.0**
O. officinalis (diploid)	40/30	5.3	84.0	78.7**	2.7	80.0	77.3**
O. officinalis (tetraploid)	36	2.7	68.0	65.3**	0	76.0	76.0**
O. punctata	RT	58.7	98.7	40.0**	88.0	98.7	10.7**
O. rhizomatis	RT	0	36.0	36.0**	0	41.3	41.3**
***Ridleyi* complex**							
O. ridleyi	RT	30.7	72.0	41.3**	21.3	80.0	58.7**
Species not yet assigned to any complex							
O. brachyantha	45/30	32.0	78.7	46.7**	13.3	85.3	72.0**

[1] ** = significant at 1% level; * = significant at 5% level; ns = not significant, by LSD.
[2] 12/12 h, with light at the upper temperature of each cycle.
[3] RT = room temperature (28±1 °C)

Combination of chemical treatments and germination under optimum temperatures regimes

Seven species which had seed germination lower than 85% in the previous treatments were subjected to chemical treatments, and only intact seeds were used for all species. Table 4 presents germination data of various species under their optimum temperature regimes after pre-soaking in different chemicals, except for KNO_3 on which the seeds were directly germinated.

Seed germination in 0.001 M KNO_3 solution was suppressed completely in all species. Presoaking seeds in HNO_3, 1M H_2O_2, or 1000 ppm GA_3 promoted seed germination of most species. The different species responded very differently to various

Table 4. Germination of intact seeds of *Oryza* species subjected to chemical treatments at optimum temperature regimes.

Treatments	Species						
	O. australiensis [1]	*O. nivara*	*O. rufipogon*	*O. officinalis* (2x)	*O. officinalis* (4x)	*O. rhizomatis*	*O. ridleyi*
KNO_3	0 c	0 c	0 d	0 e	0 d	0 b	0 c
0.1M HNO_3							
24 h	34.7 ab	66.7 a	42.7 ab	84.0 abc	13.3 bcd	1.3 b	46.7 b
48 h	37.3 ab	29.3 b	37.3 abc	93.3 a	26.7 bc	0 b	41.3 b
0.2M HNO_3							
24 h	54.7 a	25.3 b	48.0 a	90.7 ab	73.3 a	6.7 ab	38.7 b
48 h	13.3 bc	1.3 c	14.7 cd	78.7 abc	45.3 ab	14.7 ab	54.7 b
1M H_2O_2							
24 h	40.0 ab	36.0 b	17.3 bc	37.3 d	5.3 cd	0 b	0
48 h	42.7 a	22.7 b	34.7 abc	48.0 cd	13.3 bcd	0 b	0
1000 ppm GA							
24 h	32.0 ab	48.0 ab	26.7 abc	56.0 bcd	42.7 ab	9.3 ab	65.3 ab
48 h	26.7 ab	41.3 ab	40.0 abc	82.7 abc	42.7 ab	4.0 b	84.0 a
0.1M HNO_3+1M H_2O_2							
24 h	9.3 c	0 c	1.3 d	0 e	22.7 bc	14.7 ab	0 c
48 h	0 c	0 c	0 d	0 e	0 d	16.0 ab	0 c
0.2M HNO_3+ 1M H_2O_2							
24 h	0 c	0 c	0 d	0 e	4.0 cd	33.3 a	4 c
48 h	0 c	0 c	0 d	0 e	0 d	1.3 b	0 c

[1] In a column, within each species, means followed by a common letter are not significantly different at the 5% level by DMRT.

chemical treatments, but in general, no consistent differences in germination were observed between the treatments with different concentrations of or soaking duration in the chemicals. The data from germination of various rice species after chemical treatments did not exceed the amount of germination of hulled seeds under their optimum temperature regimes without chemical treatment, except for the diploid *O. officinalis*, which achieved germination of 93.3% in 0.1 M HNO_3. This is the only species to reach germination higher than 85% under the optimum temperature regime after chemical treatments. *O. ridleyi* responded best to treatment in 1000 ppm GA for 48 h.

The combination of presoaking successively in HNO_3 and H_2O_2 did not induce better germination than using HNO_3 or H_2O_2 alone. However, the combination treatment yielded a much better result in *O. rhizomatis* than the other chemical treatments, where germination of 33.3% was achieved.

Root and shoot growth in different temperature regimes

Under constant temperature regimes, root and shoot growth did not differ significantly

at 2 d after germination (Figs. 1 and 2). Growth was affected starting at about 4 d after germination. At this point, most of the species showed maximum growth at 31 °C. The effects of 26 °C, 36 °C, and room temperature treatments on growth varied from species to species. For instance, exposure to 36 °C resulted in poor seedling growth of the following tetraploid species: *O. alta, O. grandiglumis, O. latifolia, O. officinalis*, and *O. ridleyi*. At this temperature, the growth of *O. brachyantha, O. australiensis, O. glumaepatula, O. longistaminata*, and *O. spontanea* was similar, although slightly slower than at 31 °C. However, under alternating temperatures, significant differences in growth were observed soon after germination (Figs. 1 and 2). Maximum growth was generally observed at 40/30 °C, where the roots and shoots in most of the species were generally longer than 20 mm. Growth under the 40/25 °C temperature regime was slightly slower than at 40/30 °C. Exposure to 45/30 °C alternating temperatures always resulted in slow growth of roots and shoots for all species, although higher germination was achieved in some species. Under the 45/30 °C temperature regime, roots and shoots were generally shorter than 5 mm 6 d after germination.

Discussion

All *Oryza* species included in this experiment showed high seed viability of 70–100% based on the tetrazolium test. The low germination of intact seeds under 31 °C, as well as other constant and alternating temperature regimes, indicated strong dormancy in all rice species studied, except for *O. grandiglumis* which had high germination under most of the temperature regimes. This result agrees largely with the previous reports

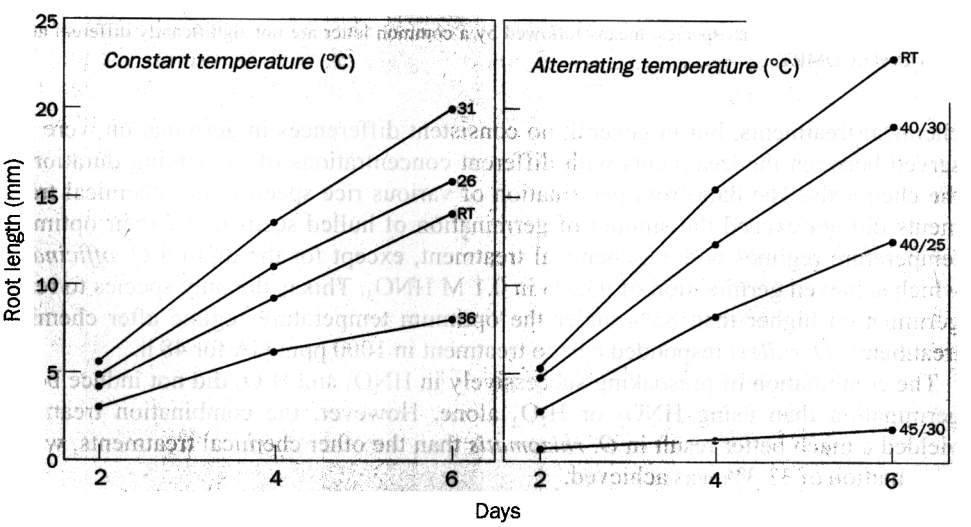

Figure 1. Average root growth rate of different rice species measured at 2, 4, and 6 d after germination (radicle emergence) under different temperature regimes. *RT = Room temperature (28 ± 1 °C).

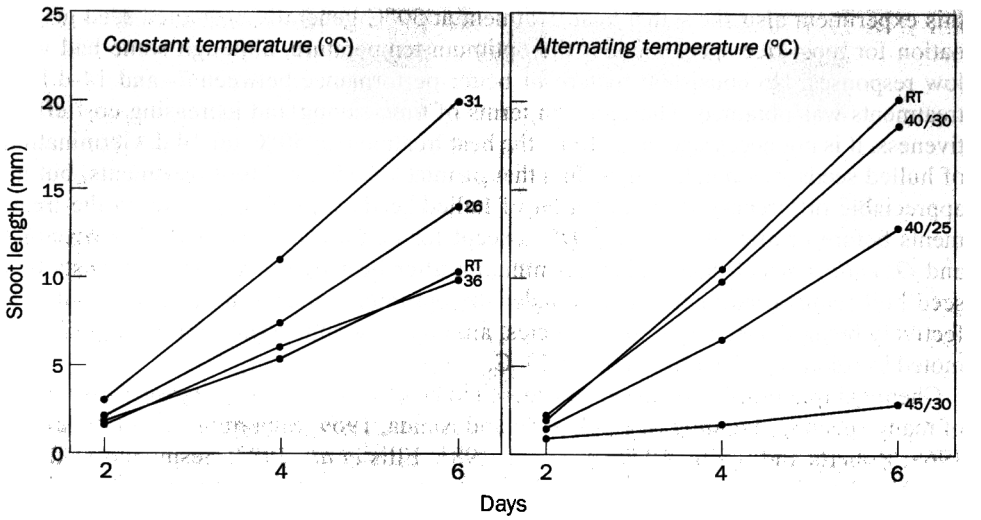

Figure 2. Average shoot growth rate of different rice species measured at 2, 4, and 6 d after germination (radicle emergence), under different temperature regimes. *RT = Room temperature (28±1 °C).

that wild species or weedy rices in general have strong dormancy (Cohn and Hughes, 1981; Hyakutake and Zungsontiporn, 1985; Das, 1989), and also indicates a remarkable diversity of dormancy intensity between the rice species.

The germination data obtained from intact seeds under different constant temperature regimes indicate a rather weak effect on dormancy breaking for rice species. Alternating temperatures seem to promote somewhat higher germination of some species than the constant temperatures, but the impact is very limited, confirming the previous results reported in *O. sativa* (Nakamura, 1963; Hayashi and Morifuji, 1972; Ellis *et al.*, 1983). In general, germination under the different temperature regimes alone does not provide an effective method for breaking seed dormancy of wild rice species which usually possess strong dormancy.

Hull removal broke seed dormancy of all rice species to a considerable extent, with a remarkable increase in seed germination under both constant and alternating temperature regimes, supporting previous conclusions (Karivaratharaju and Sakharam Rao, 1973; Seshu and Dadlani, 1991). However, diversity of responses to different germination conditions was found between species. For example, taxa in the *Sativa* complex had excellent germination (>90%) at an alternating temperature of 45/30°C, but most species of the *Officinalis* complex and *O. ridleyi* showed reduced germination at these temperatures. Therefore, it is evident that no single germination temperature regime is ideal to break dormancy of all *Oryza* species.

Dry heat treatment has been reported to be effective to remove dormancy of rice seeds (Agrawal and Nanda, 1969; Hayashi and Morifuji, 1972; Jalote and Vaish, 1976; Dolago *et al.*, 1977; Siddique *et al.*, 1988). The germination data of intact seeds from

this experiment also show that heat treatment at 50 °C generally promoted seed germination for most rice species under the optimum temperature, although some had very low responses. No consistent pattern of better performance between 7- and 14-d heat treatments was obtained. Therefore, in terms of time-saving and increasing cost effectiveness, it is not necessary to continue the heat treatment at 50 °C for 14 d. Germination of hulled seeds was much higher than that of intact seeds after heat treatments, but no appreciable differences in germination of hulled seeds were found between the treatments before or after heating at 50 °C, except for *O. longistaminata*, *O. brachyantha*, and *O. ridleyi* which had higher germination after heat treatment. This suggests that seed hull removal and germination under the optimum temperature regimes could effectively break dormancy in most species, although maximum germination can be promoted by additional heat treatment at 50 °C.

Chemical treatments have been considered to be effective in removing seed dormancy of many species, including rice (Agrawal and Nanda, 1969; Subramoney and Abraham, 1969; Roberts and Smith, 1977; Agrawal, 1981; Ellis *et al.*, 1983; Seshu and Dadlani, 1991), although contradictory results have also been reported (Hayashi and Morifuji, 1972; Hayashi, 1980; Agrawal, 1981). In this experiment, KNO_3 totally inhibited germination of all *Oryza* species. We, therefore, do not recommend direct germination in KNO_3 for breaking dormancy of *Oryza* species. Our data demonstrated that HNO_3, H_2O_2, and GA_3 treatments were generally effective to break seed dormancy of *Oryza* species under the optimum temperature regimes, but with large variation between different species. The diploid *O. officinalis* reached maximum germination by HNO_3 treatment. H_2O_2 only promoted germination of some species to a limited extent and was not effective to break dormancy of *O. rhizomatis* and *O. ridleyi*. GA_3 largely promoted germination of most species, and *O. ridleyi* reached maximum germination under GA_3 treatment. It is important that optimum chemical concentration and duration of treatment should be determined when applying to different species. A combination of presoak in HNO_3 and H_2O_2 suppressed germination of most species except *O. rhizomatis*, and is different from a previous study on cultivated rice (Ellis *et al.*, 1983). Therefore, the combination of presoak in HNO_3 and H_2O_2 successively is not recommended to break seed dormancy of *Oryza* species.

To achieve quick and full germination of different *Oryza* species for the purpose of research or use of germplasm, ideal or standard procedures are necessary for breaking seed dormancy. This was at least one of our goals when we initiated this experiment. However, data from a series of dormancy-breaking treatments, i.e. removal of seed hull, germination in different temperature regimes, as well as heat and chemical treatments, have shown that none of the treatments alone can provide an ideal method to break seed dormancy of all *Oryza* species. A combination of different treatments and exposure of seeds to the optimum temperature regimes for individual species will provide the best results for breaking seed dormancy of *Oryza* species. Table 5 lists simple and effective seed dormancy-breaking procedures for each rice species based on our experiment.

It should be understood that each treatment has advantages and disadvantages. For example, removal of the seed hull is very effective in breaking seed dormancy, but it is

Table 5. Recommended dormancy breaking procedures for different *Oryza* species.

Species	Pre-germination treatments	Germination temperature (°C)
***Sativa* complex**		
O. glumaepatula	hull removal	45/30[1]
O. longistaminata	heat treatment at 50 °C for 7 days and hull removal	45/30
O. nivara	heat treatment at 50 °C for 7 days and hull removal	45/30
O. rufipogon	heat treatment at 50 °C for 7 days and hull removal	45/30
O. sativa	heat treatment	45/30
O. spontanea	heat treatment at 50 °C for 7 days or hull removal	36
***Officinalis* complex**		
O. alta	hull removal	40/30
O. australiensis	heat treatment at 50 °C for 14 days and hull removal	45/30
O. eichingeri	hull removal	40/30
O. grandiglumis	hull removal	40/30
O. latifolia	hull removal	40/30
O. minuta	hull removal	RT[2]
O. officinalis (diploid)	0.1MHNO_3 for 48 h	40/30
O. officinalis (tetraploid)	hull removal	36
O. punctata	heat treatment at 50 °C for 14 d or hull removal	RT
O. rhizomatis	hull removal	RT
***Ridleyi* complex**		
O. ridleyi	1000 ppm GA_3 for 48 h	RT
Species not yet assigned to any complex		
O. brachyantha	heat treatment at 50 °C for 14 d and hull removal	45/30

[1] 12 h/12 h, with light at the upper temperature of each cycle. Transfer to suitable growth medium immediately after radicle emergence is recommended when this temperature is used as a dormancy breaking treatment.
[2] RT = Room temperature, (28 ± 1 °C)

labour-intensive and may also have the risk of damaging embryos. Alternating temperatures of 45/30°C are generally effective in breaking dormancy of species in the Sativa complex, but seedling growth is adversely affected. We recommend that seeds are transferred to culture solution (Yoshida *et al.*, 1976) or soil, immediately after radicle emergence to permit maximum seedling growth. Dry heat treatment, although promoting moderate germination, provides an easy method when handling a large number of samples. Likewise, chemical treatment markedly promotes germination for some *Oryza* species, and at the same time, it is also an easily applied method. Therefore, the choice of an appropriate dormancy-breaking treatment depends to a considerable extent on the species, the amount of seeds, and the available conditions.

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References

Agrawal, P. K. (1981). Genotypic variation on seed dormancy of paddy and simple methods to break it. *Seed Research*, **9** (1), 20–27.

Agrawal, P. K. and Nanda, J. S. (1969). A note on dormancy in rice. *Riso*, **18**, 325–326.

Chalam, G. V., Singh, A. and Douglas, J. E. (1967). Seed Testing Manual, pp. 118.

Chang, T. T. and Yen, S. T. (1969). Inheritance of grain dormancy in four rice crosses. *Botanical Bulletin Academia Sinica*, **10** (1), 1–8.

Cohn, M. A. and Butera, D. L. (1982). Seed dormancy in red rice (*Oryza sativa*). II. Response to cytokinins. *Weed Science*, **30**, 200–205.

Cohn, M. A. and Hughes, J. A. (1981). Seed dormancy in red rice (*Oryza sativa*). I. Effect of temperature on dry-after ripening. *Weed Science*, **29**, 402–404.

Das, T. (1989). Inheritance of seed dormancy in rice. PhD dissertation, Department of Botany, *The University of Dhaka*, Bangladesh.

Dolago, A. A., Furlani, P. R. and Azzini, L. E. (1977). Effect of the temperature of 50 °C on the breaking of dormancy of rice seeds. *Marco*, **36**, 6–8.

Ellis, R. H., Hong, T. D. and Roberts, E. H. (1983). Procedures of the safe removal of dormancy from rice seed. *Seed Science & Technology*, **11**, 77–112.

Gomez, K. A., Bartolome, V. I., Calinga, R. T. and Cosico, A. B. (1994). IRRISTAT Release 3.1. International Rice Research Institute, Manila, Philippines.

Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for agricultural research, second edition. John Wiley & Sons, New York.

Hall, V. L. (1966a). Temperature and the germinating rice seed. I. Minimum to maximum temperature for growth in four days. *Rice Journal*, **69**, 40–42.

Hall, V. L. (1966b). Temperature and the germinating rice seed. II. Effect of temperature on germination and eight days growth of aerated seed. *Rice Journal*, **69**, 22–23.

Hayashi, M. (1980). Studies on the dormancy and germination of rice seed. IX. The effect of oxygen and moisture upon the release of the rice seed dormancy and upon the inactivation of inhibitors in the dormant seed. Bulletin of the Faculty of Agriculture, *Kagoshima University*, **30**, 1–9.

Hayashi, M. and Morifuji, N. (1972). Studies on the dormancy and germination of rice seed. I. The influences of temperatures and gaseous conditions on dormancy and germination in rice seeds. *Japanese Journal of Tropical Agriculture*, **16**, 115–120.

Hyakutake, H. and Zungsontiporn, S. (1985). Effect of temperature and chemicals on breaking seed dormancy in wild rice. In: Proceeding of 10th Asian-Pacific Weed Science Conference, Thailand 1985, Vol. 2, pp. 419–424. Bangkok.

Jalote, S. R. and Vaish, C. P. (1976). Dormancy behavior of paddy varieties in U.P. Seed Research, **4**, 187–190.

Karivaratharaju, T. V. and Sakharam Rao, J. (1973). Possible role of germination inhibitors on dormancy in rice. *Madras Agricultural Journal*, **60** (1), 6–11.

Misra, P. K. and Misro, B. (1970). Seed dormancy in the African cultivated rice (*Oryza glaberrima* Steud.) *Indian Journal of Agricultural Science*, **40**, 23–16.

Nakamura, S. (1963). Short communication on dormancy of rice seed. *Proceedings of International Seed Test Association*, **28**, 57–59.

Panchaksharaiah, S., Veeraraj, Y. S., Maharudruppa, U. K. and Mahadevappa, M. (1976). Dormancy study in rice. *Mysore Journal of Agricultural Science*, **10** (2), 180–184.

Roberts, E. H. and Smith, R. D. (1977). Dormancy and the pentose phosphate pathway. In Khan, A. A. (ed.) The physiology and biochemistry of seed dormancy and germination. pp. 385–411. Elsevier/North-Holland Biomedical Press, Amsterdam.

Seshu, D. V. and Dadlani, M. (1991). Mechanism of seed dormancy in rice. *Seed Science Research*, **1**, 187–194.

Siddique, S. B., Seshu, D. V. and Pardee, W. D. (1988). Classification of dormancy in rice and release from dormancy by moist heat treatment. *Sabrao Journal*, **20** (1), 11–18.

Singh, P. V., Singh, M. B. and Khanna, A. N. (1973). Note on the temperature requirements of germinating rice seeds under controlled conditions. *Indian Journal of Agricultural Science*, **43**, 426–427.

Subramoney, N. and Abraham, A. (1969). A new method of seed treatment against bacterial leaf blight. *International Rice Communication Newsletter*, **18**, 33–34.

Takahashi, N. (1961). Studies on the dormancy of wild species. *Part 2. Roles of seed coat, embryo and endosperm in dormant seeds*. The 21st report of the Institute for Agricultural Research, Tohoku University, pp., 75–85.

Yoshida, S., Forno, D. A., Cock, J. H. and Gomez K. A. (1976). Routine procedures for growing rice plants in culture solution. In: Laboratory Manual for Physiological Studies of Rice. pp. 61–66. International Rice Research Institute, Manila, Philippines.

Zhang, X. G. (1990). Physicochemical treatments to break dormancy in rice. *International Rice Research Notes*, **15** (1), 22.