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Different treatment of rice seed dormancy breaking, germination of both wild species and cultivated varieties (*Oryza sativa L.)*

Abdul Waheed, Habib Ahmad and Fida M. Abbasi[1]
Genetics Department, Hazara University, Mansehra (KPK) Pakistan[1]

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* Corresponding author : email: abdulw900@gmail.com

Abstract:
The present study was conducted at Genetics Department, Hazara University Garden Campus and during 2008-09 as a part of PhD study. Seed dormancy breaking, germination tests of 2 wild speciesi.e., *O. longistaminata and O. rufipogon* and 3 local cultivated rice varieties/ germplasm i.e., *Swat-1, F. Malakand and JP-5* were conducted under different treatments. Hulled seeds were exposed to: constant temperatures of 28 degree celsius to 38 degree celsius & room temperature (25#1 degree celsius); and alternating temperatures of 40/30 degree celsius to 45/35 degree celsius (12 hours/12 hours) and at a room temperature(25# degree celsius). Third treatment ,intact seeds were exposed to dry heat at 50 degree celsius for 7 and 14 days before germination of hulled and intact seeds under the maximum temperature for each wild species determined from treatments 1 - 2 species that showed minimum % of germination , various chemical treatment, for germination of intact seeds in 0.001M potassium nitrate, presoaking the seeds for 24 or 48 hours in (0.1 or 0.2 M Nitric acid , I M Hydrogen peroxide , 1000 ppm Gibberellic acid (GA_3) and in-combination of 0.1 M HNO, and 1 M H , 0.2 MHNO_2O_2 , 0.2 M HNO_3 and 1 $M_3H_2O_2$ were conducted . It was observed that: removal of the seed hull is extremely effective for breaking seed dormancy while species respond differently to various constant / alternating temperatures and heat generally promotes germination of the species that respond to certain constant and alternating temperature but no consistent differences observed in seed germination of most species between heat treatments at 50 degree celsius for 7 or 14 days. *O rufipogon* and *Swat-1* both 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 individual species provides the best results for braking seed dormancy of rice species.

Key words : Rice , Seed dormancy , Treatments , KPK, Pakistan.

Introduction

Rice is one of the agronomically and nutritionally important cereal crops and is the second principle staple food after wheat in Pakistan. Pakistan growing area with an average production of 5.156 million tones with a mean yield of 2.056 T ha^{-1} [3]. There is a remarkably rich diversity in cultivated rice; however, a series of biotic and abiotic stresses continue to limit its productivity. Thus there is an urgent need to identify diverse sources of genes for tolerance to various stresses and to broaden the rice gene pool. Wild species of *Oryza* are an important reservoir of useful genes and can be exploited both to broaden the existing narrow genetic base and to enrich the existing varieties with desired agronomically important traits. Wild rice related to cultivated rice, *Oryza sativa*, has been recognized as an important constituent in any rice genetic resource conservation program.

About 22 wild rice species have been reported from various parts of the world and they belong to different genomic groups [1]. Accordingly *O. sativa, O. nivara, O. rufipogon* and *O. glaberrima* belong to genomic group AA and *O. eichingeri* and *O. rhizomatis* belong to genomic group CC. Species of the genus *Oryza* are distributed throughout tropical and subtropical regions of the world. Some of these species are diploid with 2n = 24, while others, with 2n = 48, are tetraploid. The genus *Oryza* comprises two cultivated rice species, *Oryza sativa* and *O. glaberrima*, and about 20 wild species. The common cultivated rice *O. sativa* is grown all over the world while *O. glaberrima* is limited to West Africa.

A seed represents the end of flowering process and the beginning of a new plant in miniature means, for dispersal, survival, renewal and germination. The seed germinate even if required condition for germination viz H_2O or O_2 available renders than dormant [15] Rapid and full germination of seeds is an essential firs step towards effective utilization of rice germplasm. However, seed dormancy - a condition that temporarily suspends visible growth of meristem is one of the factors that hamper germination. In nature, dormancy can be an advantage for some species because it renders resistance to pre-harvest sprouting and prevents germination until favorable conditions for plant development prevail [8] However, it is problematic when utilizing rice species in research and breeding. Continued improvement of rice varieties relies on the evaluation and utilization of germplasm in the rice gene pool for agronomically important traits.

Variation in seed dormancy has been reported in different varieties of *O. sativa* [5; 23; 1; 25; 24] , and several studies have been undertaken to break seed dormancy of cultivated rice [21 ; 26 ; 10 ;31]. However, information on dormancy and dormancy breaking in wild species of rice is rather limited, a previous research [28; 7; 6; 8] and it was noted that in general, wild *Oryza* species possess stronger seed dormancy than cultivated rice varieties. While reported by [30] that seed were soaked in tap water for 24 hours proved the best treatment for early emergence and seed breaking dormancy in tea seed after sun drying for 24 h. they further reported that the use of H_2So_4 damaged the embryo of the seed completely.

The objectives of this study were to evaluate the dormancy of wild *Oryza* species and local cultivated varieties of rice , their response to different dormancy-breaking treatments, by removal of seed hull, germination under different constant and alternating temperature , heat treatment of seeds, and using chemical, with most appropriate dormancy-breaking procedure for each species of rice.

Materials and methods

Seeds of both the wild species *O. longistaminata and O. rufipogon* and local cultivated rice *Swat-1, F. Malakand and JP-5* (Table I) were collected from gene bank of PGRI, NARC (PARC) Islamabad .The dried seeds were subsequently stored at 2°C and 40% RH in the active collection storage vault of the gene bank until needed. Thereafter, seed samples were allowed to equilibrate at room temperature (25 ± I degree celsius and 68-80 % RH) for 7 days before being subjected to dormancy-breaking treatments.

Viability of rice seed, dormancy determination and germination

Tetrazolium tests [3] were conducted to estimate seed viability for all the rice species and cultivated rice 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 degree celsius for 3 hours , and then dissected longitudinally and medially through the embryo were soaked in 1% tetrazolium solution for I hour at 4°C in the dark, and then washed several times with distilled water to remove excess solution. Seeds we 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 normal size of Petri dishes 3 lined with filter paper and moistened with distilled water, in germination chambers at 31°C constant temperature, 99% RH and 12 hours light. Germination was monitored daily over a period of 14 days. It was reported by different authors that seed of *O. Sativa* gave the best germination at this temperature [12; 13; 9]. Germination was scored as the emergence of the radicle. Species with seed germination $\leq$ 25% were considered to be strongly dormant, whereas those with seed germination $\geq$ 50% were considered to be weakly dormant [7].

Dormancy-breaking treatments

Three replications, each containing 25 seeds, were used in the different treatments throughout all experiments. For treatments I and II described below, seeds of each species were geminated at room temperature (25± 1 degree celsius) in 12 hours 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 days after germination.

a. Germination under constant temperatures. Intact and hulled seeds were germinated under the constant temperature i.e., 28°C, 32 °C, and 38°C, with 99% RH in 12 hours light.

b. Germination under alternating temperatures. Intact and hulled seeds were germinated under one of the alternating temperature i.e., 40/30°C 40/35°C and 45/35°C for 12 hours with light at the upper temperature of each cycle, and 99% RH.

c. Dry heat treatment. Two sets of intact seeds were incubated at 50 °C for 7 and 14 days. Each of one set from each treatment was hulled after equilibration to room temperature for 2 days. 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 favorable 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 he used.

d. *Chemical treatments*. Chemical treatments were only applied to *O. rufipogon* and *Swat -1* that did not reach above 80% germination in former treatments. 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) I M hydrogen peroxide (H_2O_2) and (d) 1000 ppm gibberellic acid (GA_3) applied accordingly. Intact seeds were germinated directly in sterilized Petri dishes lined with 3 layer filter paper moistened with 6 ml KNO_3. All subsequent treatments involved presoaking intact seeds in 1 M or 0.2 M HNO, M H_2O_2, and 1000 ppm GA_3 for 24 and 48 hours, prior to germination. A combination of presoaking for 24 and 48 hours each successively in 0.1 M HNO_3 and 0.1 M H_2O_2 and in 0.2 M HNO_3 and 0.I M H_2O_2 was also included in the chemical treatments. After drying at room temperature for 30 minutes to 01 hours, all treated seeds were germinated under the best constant or alternating temperature regime for each species determined from the former germination tests.

Analyses of variance were carried out using IRRISTAT Version 3.1 [11]. The arcs in transformation were used for percentage germination data. The experiments were conducted in randomized complete block design, while arrangements were made as split plot. Duncan's Multiple Range Test (DMRT) was used to compare mean values using transformed means, and transferred to the original mean values [12]. 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 and discussion

Seed viability and dormancy of Oryza species

Seed viability in both the *Oryza* species studied was high based on the tetrazolium test, ranging with both were same at 92 % (Table 1). While the other cultivated rice was 72 to 98 % had strong dormancy and germination at 32°C %. Both *Oryza* species included in this experiment showed same seed viability of 92% based on the tetrazolium test. The low germination of intact seeds under swat-1 was observed i.e., 72 % with 0 % germination at 32 °C indicated strong dormancy while *F. Malakand* showed 98 % with 75 % germination at 32 °C among others.

Table I:- Seed dormancy, and viability of *Oryza* Species and cultivated rice varieties used in dormancy breaking tests

Wild Species	Seed Viability (%) [1]	Germination at 32 °C (%) [2]
O. longistaminata	92	1.2
O. rufipogon	92	0
O. sativa cultivated varieties		
Swat -1	72	0
F. Malakand	98	75.0
JP-5	81	1.4

[1] Tetrazolium test conducted before the dormancy breaking experiment.

[2] Percent germination and intact seeds at constant 32 °C was used to determine dominancy level. Germination ≤ 25% strong and ≥50% indicates weak dominancy.

Germination under different temperatures

Table-2 showed that germination of intact seeds in both the *Oryza* species was low at constant temperatures with germination of 0 - 4.1 % in different temperatures followed by 1.2 at 32 °C *JP-5* showed 1.2 % at 32 °C where as maximum was observed in 78 % in *F. Malakand* at 32 °C In contrast, hulled seeds showed substantially higher germination of all species than the intact seeds under constant temperatures, although there was significant variation within both the wild species. Maximum 96.2 % at 38 °C while lowest 6.9 % at 38 °C was recorded in *O. rufipogon*. Germination of hulled seeds higher than intact seeds under constant temperatures.

Germination of intact seeds under alternating temperatures was higher in cultivated rice as compared to wild species .Minimum 0 in w. species in case of *O. longistaminata* while in case of *O. rufipogon at 40/30, 40/35 & room temp.* and 1.2 in cultivated rice at 40/35 temperature was noted in both *JP-5* and *Swat -1* respectively .

Table 2:- Germination of intact and hulled seeds of *Oryza* species cultivated rice varieties under constant and alternating temperatures**.**

Wild Species	Constant temp. (°C)	Germination (%) Intact	Hulled	Alternating temp (°C) 12/12 hrs with light	Germination (%) Intact	Hulled
O. longistaminata	28	0 a	27.7 a	40/30	6.8 b	6.9 c
	32	1.3 a	42.2 a	40/35	4.1 bc	25.4 b
	38	4.0 a	42.0 a	45/35	23.0 a	62.0 a
	RT	0 a	17.1 a	RT	0 a	11.2 bc
O. rufipogon	28	0 a	20.3 ab	40/30	0 a	4.2 c
	32	0 a	10.9 ab	40/35	0 a	10.9 bc
	38	0 a	6.9 b	45/35	3.9 a	40.3 a
	RT	0 a	38.3 a	RT	0 a	29.0 ab
O. sativa cultivated varieties						
Swat-1	28	1.4 a	69.0 ab	40/30	18.9 a	97.8 a
	32	0 a	58.2 b	40/35	1.2 c	98.0 a
	38	0 a	88.3 a	45/35	10.9 ab	71.1 b
	RT	0 a	62.2 ab	RT	4.2 bc	63.7 b
F. Malakand	28	62.0 a	83.4 ab	40/30	81.3 ab	98.0a
	32	78.0 a	95.3 a	40/35	73.3 b	97.0a
	38	72.7 a	96.2 ab	45/35	2.7 c	63.4b
	RT	70.3 a	81.5 b	RT	93.3 a	93.7a
JP-5	28	0 a	51.7 a	40/30	14.7 a	79.7 a
	32	1.2 a	58.0 a	40/35	21.3 a	87.0 a
	38	13.0a	75.0 a	45/35	33.3 a	70.0 a
	RT	2.9 a	73.1 a	RT	1.3 b	80.0 a

Means followed by a same letter are not significantly different at 5% level of probability (Duncan's Multiple Range Test (DMRT).

Maximum germination was observed in *F. Malakand* 95.2 % followed by 81 .4 % at 28 °C and room temp as well. Maximum germination of 23.0 % at 45/35 in *O. longistaminata* while minimum 0 at room temperature in both the wild species was recorded. But variation of germination was observed over all in all the both species at different alternating temperatures. Hulled exposure responded well of all species under different a temperature regimes promoted with minimum 10.9 to maximum 98.0 %. Whereas wild species performed 68.0 % maximum 11.2 minimum in *O. longistaminata*, 40.3 and 4.2 was recorded in *O. rufipogon.* 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 temperature as well. Wild species or weedy rice in general have strong dormancy [15], and also indicates a remarkable diversity of dormancy intensity between the rice species.

Heat treatment

Heat treatments(dry) at 50°C both for 7 and 14 days in general gave a substantial increase in germination of intact seeds for both species and cultivated rice varieties at the optimum temperature regimes, compared with the germination responses of intact seeds under their optimum temperature regimes without heat treatments (Table-3). There were no significant differences in *O. longistaminata and O. rufipogon* for both treatments at 50°C in 7 & 14 days of observance in between germination of intact seeds treated at 50°C for 7 or 14 days at 1 % and 5 % level of probability.

There was an increase in germination of hulled seeds after heat treatment of cultivated rice species under their optimum temperature regimes as compared with the germination responses of intact seeds. The results are more or similar as per (Table-2). Hulled seeds of both the wild species and cultivated rice varieties had germination higher than 18-28 % and 18 - 98.9 % under their appropriate temperature regimes after the heat treatments.(Table-3)

Table 3:- Heat treatment of both wild species and cultivated rice

Wild Species	Optimum temp.(°C)	Germination (%)					
		50°C for7 days			50°C for 14 days		
		Intact	Hulled	Difference[1]	Intact	Hulled	Difference
O. longistaminata	45/30[2]	50.3	84.0	39.8**	48.7	87.7	41.0**
O. rufipogon	45/30	18.0	54.0	42.0*	12.8	28.0	18.2*
Cultivated rice varieties							
Swat-1	40/30	10.2	90.3	80.7**	30.6	98.7	70.0**
F.Malakand	40/30	63.7	97.7	35.2**	74.4	98.9	27.5**
JP-5	40/30	38.2	86.7	47.1**	60.0	88.1	26.9**

[1]** = significant at 1% level;* = significant at 5% level ns = not significant

[2] 12/12h, with light at the upper temperature of each cycle.

Chemical treatments and germination under optimum temperatures regimes

Only *O. rufipogon* and *Swat-1* which had seed germination lower in the previous treatments were subjected to chemical treatments, and only intact seeds were used for both. Table-4 presents germination data of both wild and cultivated rice variety under their optimum temperature regimes after presoaking 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 both species. Presoaking seeds in HNO_3, IM H_20_2, at 1000 ppm GA_3 promoted seed germination (Table-4). Both were responded very differently to various concentrations of treatment.

Table 4:- Germination of intact seed of *Oryza* species treated chemically

Treatments	**Species cultivated rice variety**	
	O rufipogon	*Swat-1*
KNO_3	0 d	0 e
0.1M HNO_3		
24 h	48.2 ab	86.0 abc
48 h	38.2 abc	92.1 a
0.2M HNO_3		
24 h	49.0 a	91.1 ab
48 h	16.4 cd	77.3 abc
1M H_2O_2		
24 h	18.2 bc	35.9 d
48 h	36.1abc	47.8 cd
1000 ppm GA		
24 h	25.7 abc	55.9 bcd
48 h	40.3 abc	83.3 abc
0.1M HNO_3+1M H_2O_2		
24 h	1.2 d	0 e
48 h	0 d	0 e
0.2M HNO_3 + 1M H_2O_2		
24 h	0 d	0 d
48 h	0 d	0 e

Means followed by same letter are no significantly different at the 5% level of probability. But in general, no consistent differences in germination were observed among 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 *Swat -1* , which achieved germination of 91.1% in 0.2 M HNO_3. This is the only CRV to reach germination higher than 85% under the optimum temperature regime after chemical treatments. *O. rufipogon* responded best treatments in 0.2 M HNO for 14 hours followed by 40 in 1000 ppm GA in 48 hours respectively. The combination of presoaking successive in HNO_3 and H_2O_2 did not induce better germination than using HNO_3, and H_2O_2 alone.

Root and shoot growth

Under constant temperature regimes root and shoot growth did not differ significantly at 2 days after germination (Fig. 1). Growth was affected starting at about 4 days after germination at this point it showed maximum growth at 31°C the effects of 26°C, 36°C, and room temperature treatments on growth varied from each others . However, under alternating temperatures, significant differences in growth were observed soon after germination (Fig.1). 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 alternating temperatures always resulted in slow growth of

roots and shoots, Under all 4 temperature regime, roots and shoots were generally < 5 mm 6 days after germination.

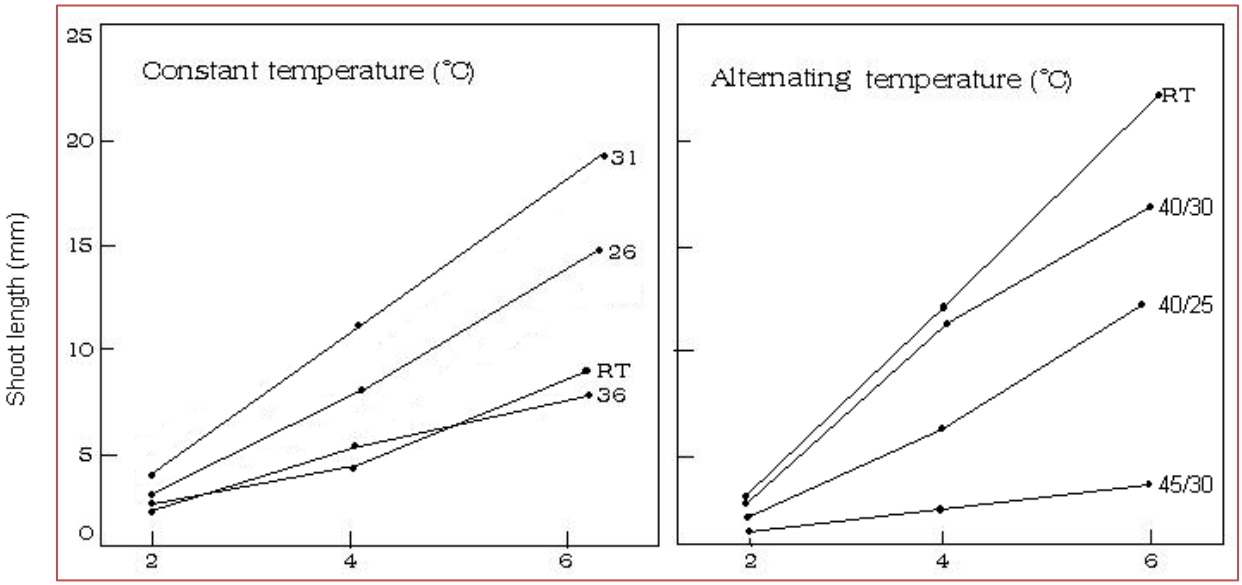

Fig 1. Average root growth rate all 5 (2) Wild species and (3)cultivated rice measured at 2, 4, and 6 days after germination regimes.*RT = Room temperature (28± 1 ^{0}C).

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 showed the simple and effective seed dormancy-breaking procedures for each rice species and variety based on experimental results used. There was also have an advantages and disadvantages, e.g., removal of the seed hull is very effective in breaking seed dormancy, but it is labor-intensive and may also have the risk of damaging embryos.

Table 5:- Suitable dormancy breaking procedures for *Oryza sp.*

Species	**Pre-germination treatments**	**Germination Temperature (°C)**
O. longistaminata	heat treatment at 50°C for 7 days and hull removal	45/35[1](12/12h)
O. rufipogon	heat treatment at 50 °C for 7 days and hull removal	45/35
O. sativa cultivated varieties		
Swat-1	hull removal	40/30
F. Mlakand	hull removal	40/35
JP-5	hull removal	40/35

Recommendation

It is recommended that seeds are transferred to culture solution 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. Like chemical treatment promotes germination for

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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