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Dormancy and Cardinal Temperatures during Seed Germination of Five Weedy Rice (*Oryza* spp.) Strains

Adam B. Puteh*, Roziah Rosli and Rosli B. Mohamad

Department of Crop Science,
Faculty of Agriculture, Universiti Putra Malaysia,
43400 UPM, Serdang, Selangor, Malaysia
**E-mail: aputeh@yahoo.com*

ABSTRACT

Temperature during seed imbibition has been found to influence germination rate and final percent germination. Seeds of one cultivated variety and five weedy rice strains, collected from different localities in Peninsular Malaysia, were used to determine their degree of dormancy and cardinal temperatures. Meanwhile, standard germination and tetrazolium chloride (TTC) tests were used to evaluate the percentage of seed viability and degree of dormancy. Seed germination test at six different constant temperatures (between 10 and 35°C) was applied to determine the cardinal temperatures estimated by linear regression models, base temperature, T_b, optimum temperature, T_o, and maximum temperature, T_c. The TTC test was found to be a simple and quick test to determine the degree of seed dormancy among different weedy rice strains, when used together with a standard germination test. Germination rate was found to be related to the degree of dormancy but it had no influence on the range of cardinal temperatures. The T_b among the five weedy rice strains was in the range of 2-7.3°C. The T_o varied between 28.1 and 37.5°C, with an average of 32.5°C. This temperature (T_o) was higher than that of the cultivated MR73 variety (24.3°C), whereas the range of T_c was 42.2-43.3°C. The study indicated that the non-dormant cultivated rice seed had lower T_b and T_o values than the dormant seed of weedy rice.

Keywords: Weedy rice, germination, seed dormancy, cardinal temperatures

INTRODUCTION

Weedy rice (*Oryza* spp.) infestation occurs in most major rice-growing areas of the world. The invasive nature of weedy rice has become a major concern, especially in direct seeded rice cultivation. In Malaysia, the presence of weedy rice in a direct-seeded field can reduce yield up to 74% (Bakar *et al.*, 2000). Early shattering has enabled them to escape harvest, while seed dormancy ensures their survival in the soil seed bank. The degree of infestation through soil seed bank germination varies between production years. Nonetheless, the reasons behind these observations are not known. Thus, determining the temperature range at which weedy rice seed germinates will help to predict seedling emergence.

Temperature is an important single factor affecting the capacity for germination by regulating dormancy, and it also critically determines the rate of progress toward completion of germination once a seed is stimulated (Alvarado and Bradford, 2002; Bradford, 2002). The degree of seed dormancy influences the temperature range, at which seed will germinate, with the range increasing as seeds loose dormancy (Benech-Arnold *et al.*, 2000; Vegis, 1964). These critical temperatures,

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*Corresponding Author

which are commonly referred to as cardinal temperatures, consist of the base and maximum temperatures, below or above which germination will not occur, while the optimum temperature is where germination is the most rapid (Bradford, 2002). This concept was initially proposed on the whole plant basis, but it is also applicable to seed during germination. Bewley and Black (1994) described cardinal temperatures as the range of temperatures over which seeds of a particular genotype could germinate. In cultivated rice varieties, a high percentage of germination is attained in two days at 27-37°C, while no germination is found to occur at 8°C and 45°C (Yoshida, 1981). However, the cardinal temperatures for seed germination have never been reported in weedy rice strains, which are helpful for predicting the degree of seed dormancy and infestation in the field.

Models which describe seed germination behaviour, in response to a range of temperatures during imbibition, have been proposed and developed (Covell *et al.*, 1986; Ellis and Butcher, 1988; Alvarado and Bradford, 2002; Hardegree, 2006). The model commonly known as the thermal time model has been extensively used and successfully applied to describe germination timing and seedling emergence in crops (Finch-Savage and Phelps, 1993) and weed species (Roman *et al.*, 2000). This particular thermal time model predicts germination rate at sub-optimal temperatures (from the minimum temperature to the optimum temperature) and supra-optimal temperature (i.e. from the optimum temperature to the maximum temperature) in a linear function (Hardegree, 2006). The thermal time model has also been successfully used to predict weeds seedling emergence in the field under temperate growing conditions (Forcella *et al.*, 2000; Vleeshouwers and Kropff, 2000).

Natural selection on germination responses to seasonal environmental cues in some species has been proposed as a significant determinant for the genotype to establish in a given seasonal environment (Donohue, 2005). However, little information is available on the germination responses of weedy rice strains to temperature during imbibition, and there has been no record of its cardinal temperatures to date. Thus, understanding the variation in temperature during seed imbibition may establish germination responses of weedy rice strains seedling emergence in the field. The aims of this study were to (1) determine the cardinal temperatures, base (T_b), optimum (T_o) and maximum (T_c) of the different weedy rice strains, and (2) determine the germination rate within these cardinal temperatures.

MATERIALS AND METHODS

Plant Material

For the purpose of this study, five weedy rice strains and one cultivated variety were used. Seeds of the cultivated variety MR 73 were obtained from the Malaysian Agriculture Research and Development Institute (MARDI). The seeds of five weedy rice strains were randomly collected from several locations in Malaysia, namely Seberang Perak, Kuala Pilah, Besut, Perlis, and Kemubu Agricultural Development Authority in Peninsular Malaysia. Hereafter, these five strains are termed as SP, KP, Besut, Perlis and KADA strains, respectively. The seeds were collected in January and February 2008 and stored at 0°C in double sealed plastic bags for two months before conducting the study. Seed moisture was kept in the range of 9-11% prior to storage and at the start of the experiments.

Seed Viability Tests

For standard germination test, the imbibing seeds were left at ambient temperature in the laboratory at 25±3°C. The seeds were germinated on double layered moistened (±10mL distilled water) filter papers (Whatman, no. 1, in 80 mm diameter by 10 mm deep disposable plastic Petri dishes. The seeds were soaked in 10% Clorox for surface sterilization for 3 min. prior to the testing.

Seedling evaluation on the number of germinated seeds was done daily, starting on day 2 after imbibition for 14 days. Seeds which did not germinate after 14 days were considered as dead seeds. In contrast, seeds were considered as germinated when the radical emergence was >5 mm. The test was replicated twice with 50 seeds per replication.

For the tetrazolium chloride test, two replicates of 50 seeds per replicate were imbibed in distilled water at room temperature for 24 h. The middle portion of the seeds was pierced with a needle before soaking them in 0.1% of 2, 3, 5-triphenyl tetrazolium chloride (TTC) salt solution. The seeds imbibing in the TTC solution were exposed to 35°C in the oven for 2 h. Viable seed was evaluated based on the topographical staining pattern on the embryo, as described in the ISTA procedure (ISTA, 1993).

Seed Germination Test at Different Temperature

A germination test at different temperatures for all the seeds was conducted in the germination chamber in darkness. Nonetheless, the preliminary works did not show any seed sensitivity to light during germination (Rosli, 2008). Meanwhile, the preparation of seeds and imbibition media are similar to the procedures used for the standard germination test, as described above. The imbibing seeds were exposed to the constant temperatures of 10, 15, 20, 25, 30, or 35°C. This experiment was performed in two replications consisting of 50 seeds per replicate. Seeds were considered as germinated when the radicle was >5 mm. The evaluation was done daily for 20 days, beginning on Day 2 after sowing.

Statistical and Data Analysis

All the collected data were subjected to the analysis of variance using the Statistical Analysis System (SAS) Software, version 8.2. When ANOVA indicated a significant effect, the least significant difference (LSD) was performed to determine significant differences among the means of the treatments.

Meanwhile, the germination rates were calculated as the inverses of times to radicle emergence (Alvarado and Bradford, 2002). The reciprocals of the time to germination were plotted to estimate the optimum temperature, at which the rate of germination was maximum (T_o). The rates of germination were also subjected to the linear regression analysis to describe cumulative germination response of temperature (SAS Institute, 2005). The cumulative percentage germination (CGP), obtained from the germination tests at different temperatures, were used to calculate the cardinal temperatures. Intersected-line models were used as proposed by Garcia-Huidobro *et al.* (1982). The equation used to describe the rates of germination between base and up to optimum temperatures is as follows:

$$1/t = (T - T_b)/\theta_1 \qquad (1)$$

In order to describe the germination responses above T_o, but below the maximum temperature (T_c), equation (2) was used:

$$1/t = (T_c - T)/\theta_2 \qquad (2)$$

where t is the time taken in days for the CGP to reach a given percentage, T is the temperature, while T_b, T_o and T_c are the base, optimum and maximum temperatures, respectively. These models predict the germination rate for a given seed fraction (sub-optimal and supra-optimal range) in a linear function of temperature. The intercepts of the fitted linear regression lines on the temperature axes were used to estimate T_b and T_o. T_o, was calculated as the intercept of sub-optimal and supra-optimal temperature function (Hardegree, 2006).

RESULTS

Viability and Degree of Dormancy

Based on the standard germination test, the initial quality of the five weedy strains was in the range of 19-86% (Table 1). The germination percentage of the cultivated variety (MR73) was found to be the highest (98%). Meanwhile, the lowest germination percentage among all the weedy rice strains was observed in the KADA strain and the highest was in the SP strain, with 19% and 86%, respectively.

The TTC test used was to determine the percentage viability, as well as the degree of dormancy among the weedy strains and for MR 73. The highest percentage of viability was also recorded in MR73 with 99% (Table 1). This suggests that no seed dormancy is present in this particular cultivated variety. Based on the TTC test, a higher percentage of seed viability was observed in all the weedy rice strains, except for the SP strain, as compared to the percentage of viability based on the standard germination test. Based on the TTC test, the viability percentage was found to be 70-83%. Based on this test, the seed of SP strain was not dormant. As for Besut and Perlis strains, the seeds appeared to have a slight dormancy. The data indicate that there is a variation in the degree of seed dormancy among the weedy strains used in this study.

TABLE 1
Percentage viability of the cultivated rice variety and weedy rice strains based on the standard germination and tetrazolium chloride (TTC) tests

Strain/Variety	Cumulative germination (%)	TTC (%)
MR 73[†]	98	99
Seberang Perak	86	83
Kuala Pilah	51	76
KADA	19	74
Besut	65	71
Perlis	74	80
LSD	10	2

[†] cultivated rice variety

TABLE 2
The germination percentage of the cultivated rice variety and weedy rice strains at constant temperatures

Variety/strain	Percentage germination					
	10˚C	15˚C	20˚C	25˚C	30˚C	35˚C
MR 73[†]	58	78	94	98	94	29
Seberang Perak	8	10	54	62	82	54
Kuala Pilah	20	32	50	54	60	34
KADA	0	0	0	8	22	12
Besut	0	0	34	28	48	22
Perlis	6	8	20	24	50	36
LSD	26.2	16.6	17.0	14.1	20.2	19.6

[†] cultivated rice variety

Germination and Germination Rate at Constant Temperatures

The increase in the temperature (i.e. from 10°C to 30°C) during imbibition enhanced the germination percentage of both the cultivated rice variety and weedy strains (Table 2). All the weedy rice strains were found to have low germination percentages (≤82%) as compared to MR73 (>90%) within the range of constant temperatures. Increasing the imbibition temperature to >30°C was found to cause a rapid decline in the germination percentage of both the cultivated variety and weedy strains. Meanwhile, the maximum germination percentage of the cultivated variety was observed at 25°C, whereas this was observed at 30°C for all the weedy rice strains. No germination was observed in KADA weedy strain at temperature 20°C and below, indicating that this strain is highly dormant.

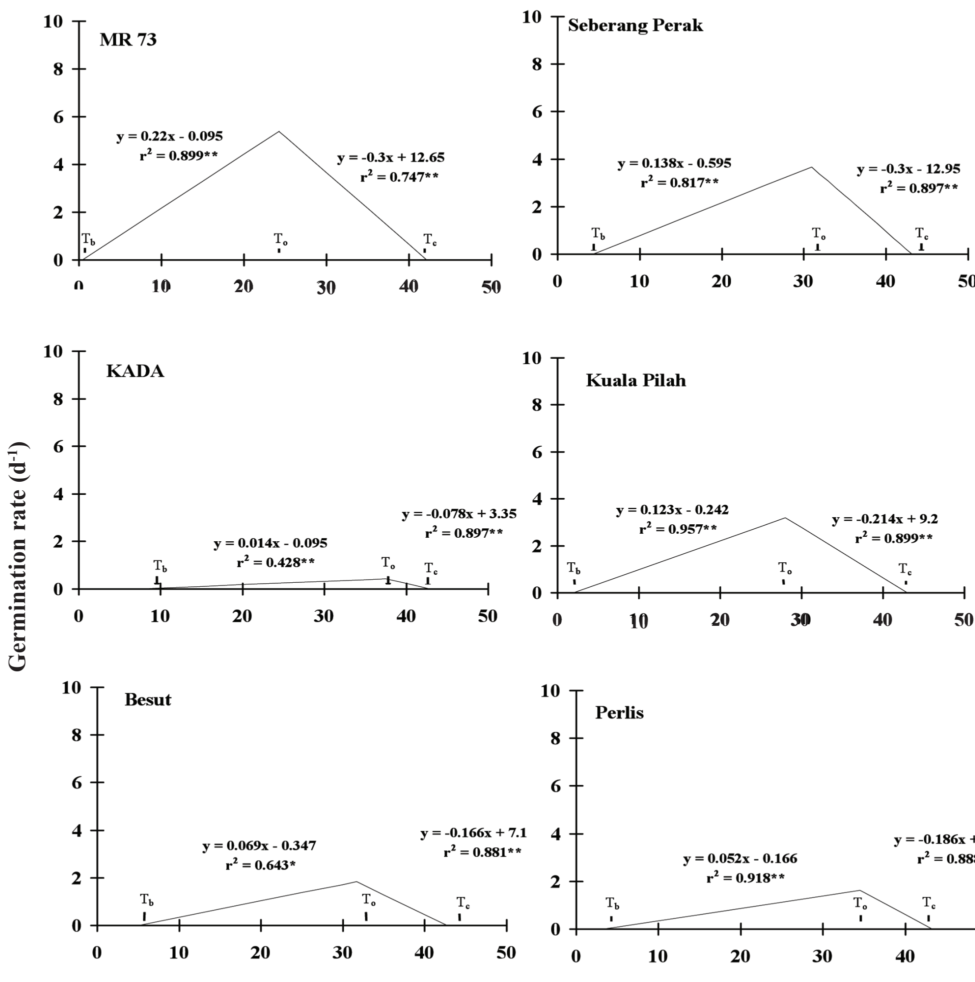

*Fig. 1: Germination rates at the suboptimal and supraoptimal temperature range in response to the different temperatures for MR73 cultivated rice variety, Seberang Perak, Kuala Pilah, KADA, Besut and Perlis weedy rice strains. T_b, T_o, and T_c indicate base, optimum and maximum temperature, respectively. * and ** , significant at P<0.05 and <0.01, respectively*

The higher germination percentage in the cultivated variety at different constant temperatures (10-30°C) could be attributed to the relatively higher germination rate (Table 3). Similarly, a lower germination percentage in weedy rice strains was due to the lower germination rate, particularly at 25°C and lower. The highest germination rate in the cultivated variety and the weedy strains was observed at 25 °C and 30 °C, respectively.

Germination Rate and Cardinal Temperatures Based on the Linear Model

The estimated germination rates, within the sub-optimal and supra-optimal range of temperatures, vary between the weedy rice strains. All the germination rates, which were calculated from the estimated germination time course, showed a significant correlation with temperature at both the sub-optimal and supra-optimal ranges of temperatures (*Fig. 1*). The highest estimated germination rate was recorded for MR73, which was 0.226 day^{-1} in the sub-optimal range. On the contrary, the lowest estimated germination rate was observed in the KADA strain (0.014 day^{-1}), while the highest was in SP strain with 0.128 day^{-1}, based on the linear regression model in the sub-optimal range.

The decline in the germination rate within the supra-optimal range for the weedy rice strains was between -0.078 day^{-1} to -0.33 day^{-1}. Meanwhile, the cultivated rice variety and SP weedy strains had similar germination rate (within the supra-optimal range of temperature), suggesting that the weedy rice strain has a similar germination characteristic with the cultivated rice variety (MR73) at higher temperature. Within this supra-optimal range of temperature, the KADA weedy strain was found to have the lowest estimated germination rate of -0.078 day^{-1}.

The germination rate for the weedy rice strains and the cultivated variety increased linearly with the increase in the germination temperature (*Fig. 1*). Meanwhile, the lowest estimated T_b for MR73 was 0.4°C (Table 4 and *Fig. 1*). The range of the estimated T_b for the weedy rice strains was between 2.0 - 7.3°C, while the KP strain had the lowest T_b. The T_o for the

TABLE 3
The germination rate at different imbibition temperatures of a cultivated rice variety and weedy rice strains

Variety/strain	Germination rate (d^{-1})					
	10°C	15°C	20°C	25°C	30°C	35°C
MR 73†	2.9	3.9	4.7	4.9	4.7	1.5
Seberang Perak	0.4	0.5	2.7	3.1	4.1	2.7
Kuala Pilah	1.0	1.6	2.5	2.7	3.0	3.0
KADA	0	0	0	0.4	1.1	0.6
Besut	0	0	1.7	1.4	2.4	1.7
Perlis	0.3	0.4	1.0	1.2	2.5	2.8
LSD	1.3	0.8	0.9	0.7	1.1	1.0

† cultivated rice variety

seed germination ranged from 28.1 to 37.5°C for the weedy rice strains (Table 4). The estimated T_o for MR73 was found to be the lowest (24.3°C) as compared to the weedy rice strains, whereas the highest T_o of 37.5°C was observed in the KADA strain. Nonetheless, T_c did not differ much between the weedy rice strains and MR73. The narrow range of T_c among the two varieties was between 42.2 – 43.3°C, suggesting that the non-dormant rice seed will not germinate above 43°C.

DISCUSSION

Under ideal germination environments, such as in the laboratory condition, seed dormancy is strongly imposed in some weedy rice strains. Weedy rice strains have often been associated with seed dormancy (Gu *et al.*, 2005). The results indicated that the seed of the KADA strain is highly dormant relative to the seeds of other strains. However, the data presented in this study are still insufficient to determine the type or class of dormancy involved in the tested weedy rice strains. Reducing the percentage of viability between the TTC test and the standard germination test will indicate the degree of dormancy of a seed lot. The SP strain does not have seed dormancy, suggesting that not all weedy rice strains have seed dormancy. It appears that the strain of SP is closely related to the cultivated variety. Therefore, those weedy rice strains producing non-dormant seeds will result in a more widespread infestation in the field throughout the year.

The degree of seed dormancy in weedy rice varies between the strains. Since the strains used in this study were collected from different locations, environmental conditions are therefore suggested to play important roles in determining the degree of dormancy. The variation in the degree of seed dormancy is not only influenced by the environment at the location where the plants are grown, but it is also influenced by genetic factors (Li and Foley, 1997; Gu *et al.*, 2005). The rate of germination appears to be related to the degree of dormancy, but it does not seem to be related to the range of cardinal temperatures. The seed of the KADA strain was found to be very dormant and thus had the lowest germination rate. However, the range of the cardinal temperatures, T_b to T_c, was quite similar to the non-dormant seed of the SP strain. The results indicated that for the non-dormant seed (e.g. in cultivated MR73 variety), T_b would be shifted to a much lower temperature relative to the weedy strains. Meanwhile, T_o for the weedy strains, except for the KP strain, was above 30°C. In this study, the increase in the degree of seed dormancy does not shift T_c in rice, but it changes T_o to a much higher temperature.

T_c was almost similar among the weedy rice strains and the cultivated variety. This suggests that the maximum temperature limit for seed germination is species specific, and it is not influenced by seed dormancy. It is interesting to note that the degree of seed dormancy will change, i.e. either shorten or widen, the sub-optimal and supra-optimal ranges of temperature. This study has clearly indicated that the sub-optimal range of the temperature of the dormant seed is widened by 26-31°C temperature points compared to the non-dormant cultivated variety and the SP strain. The supra-optimal range of temperature is concurrently shortened with the increase in seed dormancy. The results also provide evidence that there is an ecotypic variation in the base and optimum temperatures for seed germination in weedy rice.

Fluctuation in soil temperatures is commonly associated with weed seed emergence in several species (Foncella *et al.*, 2000; Vleeshouwers and Kropff, 2000). However, in tropical growing environments, fluctuation in soil temperature may be negligible, yet higher weedy rice infestation is commonly observed when temperature during preceding growing season is higher. Higher T_o requirement for the weedy rice strains observed in this study could possibly trigger the germination process and the reason for high weedy rice infestation when the temperature during preceding growing season was above normal.

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