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Effect of a Range of Constant Temperatures on Germination of Fifteen Bangladeshi Rice (*Oryza sativa* L.) Cultivars

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Abstract: Germination of 15 rice cultivars were investigated on a temperature gradient plate with a range of temperatures 13.7-37.3°C to identify rice genotypes tolerant of low temperature which would facilitate in cultivar selection for winter sown rice (*Boro* rice) in Bangladesh. The results demonstrated a plateau of more than 90% final germination between 18 and 33°C for eleven cultivars. Three cultivars had a plateau of less than 90% germination. At the lowest temperature seven cultivars achieved 80% germination whereas lower quality cultivars showed less than 8% germination. The rest of cultivars were intermediate with about 30-50% germination. The rate of germination (the reciprocal of median germination time) for all cultivars increased linearly from 13.7 up to 30.9°C and then declined rapidly between 30.9 and 37.3°C. At optimum temperature cultivars with lower quality seed sample (BR1, KS, KG and BR30) had the lowest rates of germination along with cv. BR5. Rates of germination at higher temperatures (20.1 and 30.9°C) were significantly related to the rates of germination at lower temperature (13.7 and 15.8°C). The base temperature differed little between cultivars (range 12.6–13.9°C) and optimum temperature ranged from 29.9 to 33.5°C (mean 32.1°C). Thermal time to achieve 50% germination at sub-optimal temperatures ranged from 526 to 1667°C h and differed significantly between cultivars. Cultivars with lower quality seeds and BR5 required than above 900°C h. The application of these findings to the development of routine methods to identify rice genotypes able to germinate at lower temperature is emphasized. Measurements of rates of germination at higher temperatures (20 and 30°C) could provide a relatively rapid screening method indicative of low temperature performance for large numbers of genotypes.

Key words: Germination, rice, temperature, thermal time

Introduction

Rising labour costs and the need to intensify rice production through double cropping provide economic incentives for a switch to direct-wet seeding (De Datta, 1986) instead of traditional transplanting method in Asia. Simultaneously, the availability of high yielding, short duration cultivars of rice and chemical weed control methods has made such a switch technically feasible.

In Bangladesh there is a vast area under irrigated rice cultivation where *Boro* rice intensively transplanted (about 40% of total cultivated area) in the winter season. There is a great scope to increase the productivity and economic return of this *Boro* cultivated lands by adopting the direct wet-seeding method instead of transplanting by saving a considerable amount of labour and costly irrigation water. During *Boro* transplanting period (November-February) the temperature remains low about 11–29°C (minimum–maximum) (Anonymous, 1999). Therefore, an optimum stand establishment by directs wet seeding in *Boro* season (i.e., in winter) is not always attained due to the low temperature can reduce the percentage and timing of radicle protrusion. (Kaneda and Beachell, 1994; Scott *et al.*, 1984). The poor stand that results due to cool weather in winter may decrease the rice yield in the final harvest (De Datta, 1981).

In Bangladesh, the modern cultivars bred for transplanting used in direct-wet-seeding are not well suited for these low temperature conditions. Thus the development of the direct wet-seeding method of rice culture is limited by the lack of suitable low temperature tolerant modern cultivars. Therefore, there is a need to identify the cultivars traits especially seed germination that confer tolerance to low temperature. For BRRI-developed rice cultivars, limited information is available regarding cultivar differences during seed germination in low temperature. Therefore, the objective of this study is to compare the responses of 15 rice cultivars to a range of constant temperatures with respect to final germination, rate of germination and cardinal temperatures to enable temperature characteristics of the cultivars to be related to germination at low temperature.

Materials and Methods

Thirteen modern rice cultivars (*Oryza sativa* L.) developed by the Bangladesh Rice Research Institute (BRRI) along with two traditional cultivars (Table 1) were tested for their germinations on a temperature gradient plate at a range of constant temperatures 13.7-37.3°C. First the temperature gradient plate was cleaned with ethanol and covered it with a single piece of plastic-backed filter paper, called "Benchkote". Then 250 ml of distilled water was added to the Benchkote which was placed with absorbed side upper-most. The removable grid was then placed in position. A filter paper was folded and placed in each cell. An additional 3.5 ml of distilled water was added to each cell at the start of the experiment to ensure that filter papers were kept moist at all time. The temperature gradient plate was run to give a desired range of constant temperatures. The temperatures were checked by an electronic thermometer in the front, middle and back rows. After temperatures had equilibrated, the seeds were placed on the filter paper in each cell. Seeds of each cultivar were placed in a randomized complete block design (RCBD) with 3 replicate lines of the 14×14 grid on the temperature gradient plate leaving the outer lines of the grid empty. Each replicate line consisted of 12 separate cells, each containing 30 seeds. The top of the temperature gradient plate was then covered with a piece of Benchkote (plastic-side down) to prevent excessive evaporation and covered with perspex sheets. Temperature monitoring, seed inspection and germination recording were carried out approximately every 12 h. This was continued until no further germination was recorded for 5 consecutive days in each seed batch (in total 4 batches). Seeds were taken as germinated when radicle extension was 2 mm or more. Germinated seed were counted and removed and the ungerminated seeds remaining were counted at the end of the experiment.

Data analysis: The data for each cell were analyzed to provide estimates of final germination percentage, median germination time (t_{50}) and rate of germination ($1/t_{50}$) in GENSTAT. Analysis of variance to compare cultivar and regressions to determine relationships with temperature was also done in GENSTAT.

The base temperature (T_b) for each cultivar was calculated by extrapolation of a linear regression of the rate of germination in the sub-optimal temperature range to its intercept of the x-axis (temperature). An estimate of maximum temperature (T_{max}) was made using a regression through the germination rate at the two highest temperatures. Using the linear regression data for germination rate for sub-optimal and supra optimal temperatures, an optimum temperature (T_{opt}) of final germination was estimated from the intersection of two regressions. The thermal time (θ_1) or the accumulated temperature required for germination was calculated as the reciprocal of the slope of the rate of germination verses temperature graph between T_b and T_{opt}.

Table 1: List of rice cultivars used in the study with initial seed moisture content (mc) and initial final germination

Rice cultivars	Designated as	Initial seed mc (%)	Initial germination (%)
BR1	BR1	9.36	89.00
BR5	BR5	8.75	96.00
BR11	BR11	9.14	97.00
BR14	BR14	9.17	94.00
BR23	BR23	10.17	96.00
BR24	BR24	8.41	86.00
BR26	BR26	8.31	86.00
BRRI dhan28	BR28	9.18	100.00
BRRI dhan29	BR29	10.26	99.00
BRRI dhan30	BR30	10.09	30.00
BRRI dhan31	BR31	9.46	95.00
BRRI dhan32	BR32	10.48	95.00
BRRI dhan33	BR33	10.15	98.00
Kartiksail*	KS	12.29	83.00
Kalaghora*	KG	12.41	74.00

* Traditional cultivars

Results

Final germination percentage: For most cultivars final germination progressively increased from lower temperatures, maintained a plateau at optimum temperatures and then declined as temperature increased further (Fig. 1a-c).

There was a plateau of final germination of above 90% between 18 and 33°C for eleven cultivars (BR5, BR11, BR14, BR23, BR24, BR26, BR28, BR29, BR31, BR32 and BR33) (Fig. 1a-c). A lower plateau of just less than 90% was seen for cv. BR1. In contrast, the traditional cultivars KS and KG did not show a plateau but a peak of over 90% germination. The low germinating cv. BR30 gave a plateau at around 25%.

At lower temperatures the final germination percentages contrasted more. At 13.7°C seven cultivars (BR14, BR24, BR26, BR28, BR29, BR31 and BR32) achieved close to 80% germination, while less than 8% was recorded for cvs. BR1, BR30, KS and KG. The cultivars BR5, BR11, BR23 and BR33 showed an intermediate germination of between 30 and 50% at this temperature (Fig. 1a-c). At the highest temperature (37.3°C) all cultivars (except cv. BR23 with a germination of over 80%) showed less than 41% germination and low germinating cv. BR1 did not germinate at all. Two-way Analysis of variance revealed highly significant ($P<0.001$) effects of temperature for all cultivars and a significant interaction between cultivars and temperatures (Table 2).

Rate of germination: For all cultivars the rate of germination increased linearly from the lowest temperature

Table 2: Two-way analysis of variance of final germination of 15 rice cultivars at a range of constant temperatures

Source of variation	df	SS	MS	F	P
Replication	2	202.4	101.2	3.73	0.025
Cultivar	14	197646.9	14117.6	520.30	<0.001
Temperature	11	217620.4	19783.7	729.12	<0.001
Cultivar×Temperature	154	70183.8	455.7	16.80	<0.001
Error	358	9713.8	27.1		
Total	539	495367.2			

Table 3: Two-way analysis of variance of rate of germination of 15 rice cultivars at a range of constant temperatures

Source of variation	df	SS	MS	F	P
Replication	2	0.0000012	0.0000006	0.18	0.834
Cultivar	14	0.0096658	0.0006904	211.46	<0.001
Temperature	11	0.0324190	0.0029472	902.66	<0.001
Cultivar×Temperature	154	0.0038400	0.0000249	7.64	<0.001
Error	358	0.0011689	0.0000033		
Total	539	0.0470948			

(13.7°C) with increasing temperature to a well defined peak at optimum temperature (around 30.9°C) then it declined rapidly between 30.9 and 37.3°C (Fig. 2a-c). For all cultivars, at the lowest temperature, the slowest rate of germination was observed. But at the overall optimum temperature, cv. BR23 gave the highest rate of germination. In contrast, cvs. BR1, BR5, BR30, KS and KG showed lower rates of germination. Two-way Analysis of variance for data of all cultivars presented in Fig. 2a-c for the sub-optimal temperature range (13.7-30.9°C) showed highly significant (P<0.001) differences between cultivars (Table 3).

The differences in response of rate of germination to temperature between cultivars is clear when the rate of germination were regressed against sub-optimal temperature range and slopes and R^2 values are presented in Table 4. The lower germinating cultivars (BR1, BR30, KS and KG) had shallower slopes (ranged from 0.0006 to 0.0011) as did cv. BR5 which was 0.0008. This indicated that for these cultivars the increase in germination rates

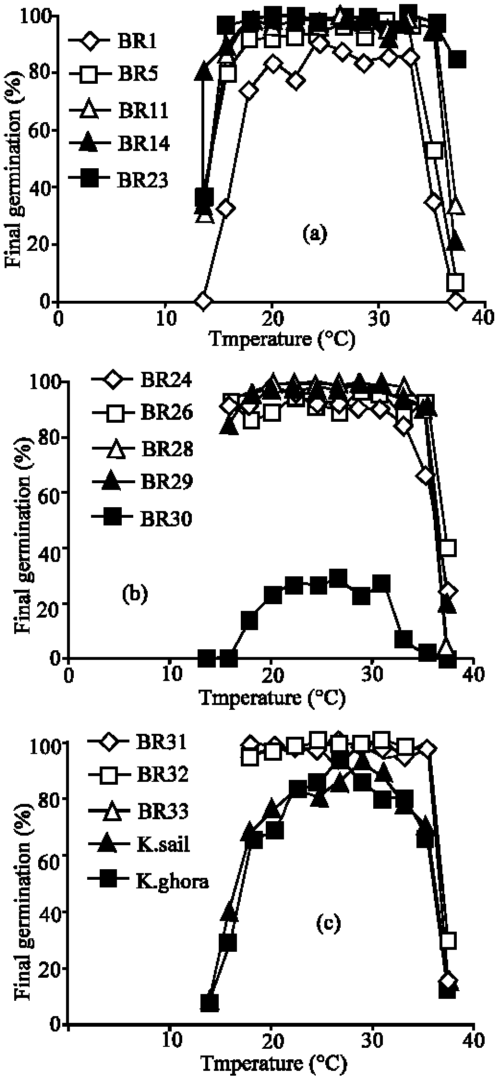

Fig. 1a-c: Effect of temperature on final germination of 15 rice cultivars

Table 4: Slopes and R^2 values of linearly fitted regression lines of germination rate in the suboptimal temperature range and germination rate ($1/t_{50}$) at lower and high temperatures, and final germination at 13.7°C for 15 rice cultivars (arranged in order of decreasing response)

			Germination rate (seed h^{-1})		
Rice cultivars	Slope	R^2	At lower (15.8°C) temperature	High temperature response	Final germination (%) at 13.7°C
BR23	0.0019	0.9896	0.0053	0.0369	34.47
BR31	0.0018	0.9766	0.0056	0.0309	86.67
BR32	0.0017	0.9669	0.0046	0.0303	76.67
BR11	0.0016	0.9782	0.0046	0.0291	30.87
BR14	0.0016	0.9810	0.0037	0.0276	81.10
BR26	0.0016	0.9826	0.0049	0.0282	82.20
BR24	0.0014	0.9913	0.0040	0.0264	77.97
BR29	0.0014	0.9743	0.0040	0.0270	78.90
BR33	0.0014	0.9738	0.0040	0.0270	51.10
BR28	0.0013	0.9715	0.0040	0.0270	73.30
KG	0.0011	0.9830	0.0026	0.0211	7.73
KS	0.0009	0.9811	0.0028	0.0189	6.70
BR1	0.0008	0.9764	0.0018	0.0119	0.00
BR5	0.0008	0.9697	0.0024	0.0157	37.77
BR30	0.0006	0.9753	0.0010	0.0100	0.00

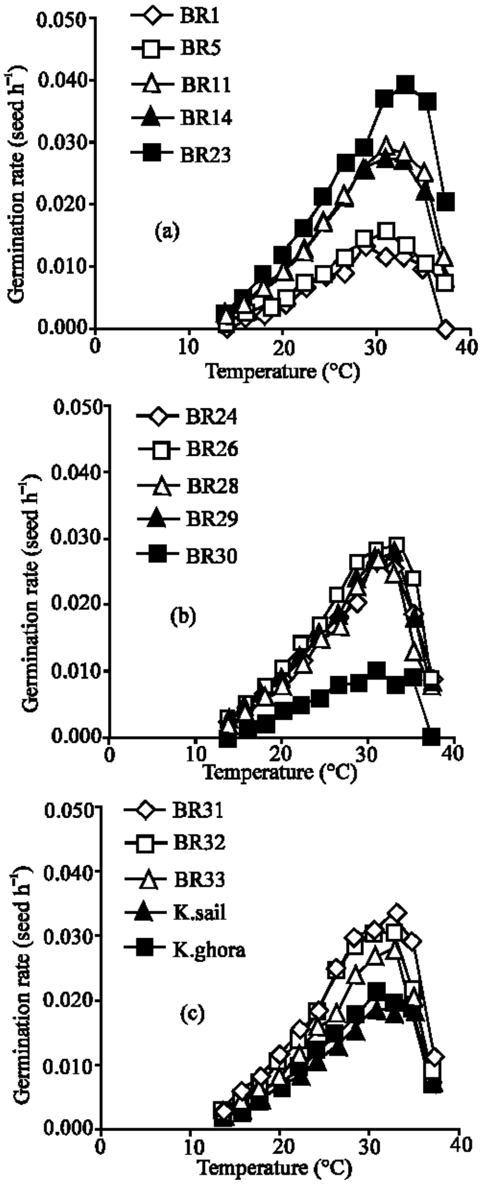

Fig. 2a-c: Effect of temperature on germination rate of 15 rice cultivars

with temperature were lower than for other cultivars which had slopes ranging from 0.0013 to 0.0019 (Table 4).

Highly significant positive regressions between rates of germination at 30.9 and 20.1°C with the rate of germination at 13.7°C were observed (Fig. 3). Similar significant regressions were seen between rates of germination at 30.9 and 20.1°C with that at 15.8°C (Fig. 4).

Base, optimum and maximum temperatures (cardinal temperatures): The base temperatures (T_b) ranged from 12.6 to 13.9°C: a small range of only about 1°C between cultivars. Cultivars BR14, BR26, BR29 and BR31 had low

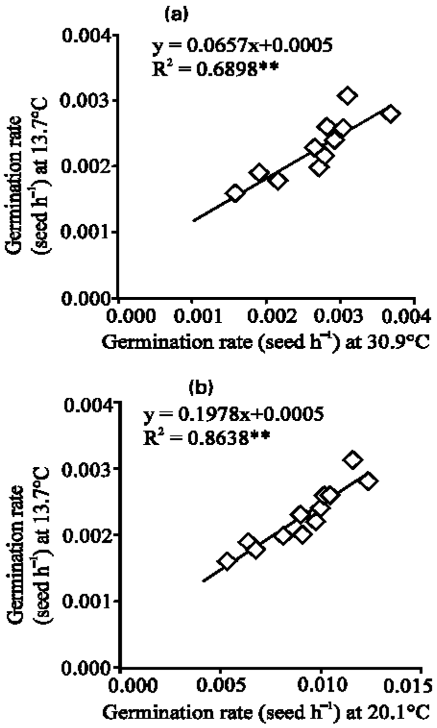

Fig. 3: Relationship between rate of germination ($1/t_{50}$) at 13.7°C and rate of germination ($1/t_{50}$) at 30.9°C (a) and 20°C (b)

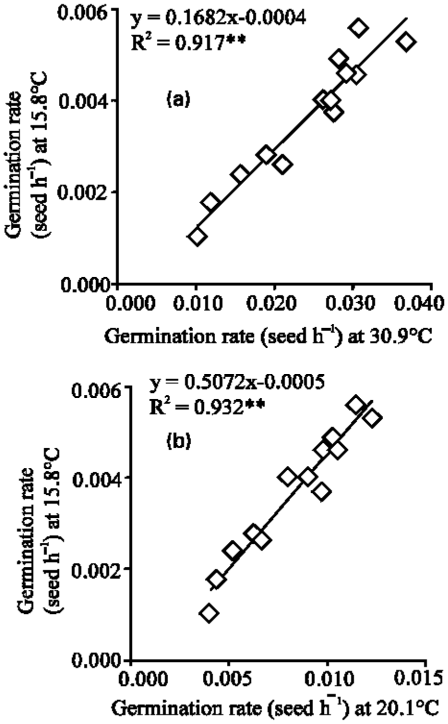

Fig. 4: Relationship between rate of germination ($1/t_{50}$) at 15.8°C and rate of germination ($1/t_{50}$) at 30.9°C (a) and 20°C (b)

Table 5: Base temperature (T_b), optimum temperature (T_{opt}), maximum temperature (T_{max}) and thermal time at suboptimal temperature range (θ_1) for 15 rice cultivars

Rice cultivars	T_b (°C)	T_{opt} (°C)	T_{max} (°C)	θ_1 (°C h)
BR1	13.9	29.9	38.1	1111
BR5	13.5	32.2	43.6	1250
BR11	13.1	31.2	40.8	625
BR14	12.7	31.6	43.0	625
BR23	13.3	32.6	48.3	526
BR24	12.6	32.9	41.4	833
BR26	12.7	31.7	42.2	625
BR28	13.2	32.5	40.2	769
BR29	12.8	31.8	39.8	714
BR30	13.5	31.8	40.2	1667
BR31	12.7	31.2	42.3	556
BR32	13.3	32.0	41.5	588
BR33	13.1	32.4	40.4	714
KS	13.2	33.5	45.6	1111
KG	13.1	32.3	42.9	909
Mean	13.1	32.0	42.0	842
Range	12.6-13.9	29.9-33.5	38.1-48.3	526-1667
S. E.	0.0945	0.2127	0.6484	
CV (%)	2.79	2.58	5.9	

T_b (Table 5). The optimum temperature (T_{opt}) for rate of germination showed differences among the cultivars, but these differences were less than 4°C. Cultivars BR1, BR11, BR14, BR26, BR29, BR29 and BR31 showed lower T_{opt}. The range of variation in maximum temperature (T_{max}) was greater (about 10°C) and the mean T_{max} was 42°C (Table 5).

Thermal time to germination at sub-optimal temperatures: Thermal time at sub-optimal temperatures (θ_1) ranged from 526 to 1667°C h (Table 5). The thermal time requirement differed significantly between cultivars. The highest (1667°C h) thermal time was recorded with cv. BR30 and the lowest (526°C h) was that of cv. BR23. The low germinating cultivars (BR1, BR30, KS and KG) as well as cv. BR5 required higher thermal time to reach t_{50}.

Discussion

The fifteen test cultivars could be categorized into three distinct groups according to their germination response to temperature. The first group comprised eleven cultivars with a broad plateau of 90% final germination between 18 and 33°C. In the second group cv. BR1 had a lower plateau of just less than 90% germination and two traditional cultivars KS and KG did not have a plateau but had a distinct peak of over 90% germination at a specific optimum temperature. The third group consisted of solely cv. BR30 which had only about 25% germination.

When tested at 13.7°C seven cultivars had close to 80% germination while four cultivars (BR1, BR30, KS and KG) showed less then 8% germination. These were the cultivars previously identified as in group 2 or 3 with poor peak germination. The rest of the cultivars (BR5, BR11, BR23 and BR33) showed an intermediate germination of between 30% and 50% at this temperature. Thus the results demonstrated that low temperature reduced germination (Fig. 1a-c). These findings agree with those of Kamaha and Maguire (1992), Naylor (1993), Ali *et al.* (1994) on wheat; Kasalu *et al.* (1993) and Dunbabin *et al.* (1994) on Songhum; Hall (1966) and Chaudhary and Ghildyal (1970) on rice. Tanida (1996) has similarly found intra-specific variation for low temperature tolerance in germination in rice. Thus, the results with different rice cultivars showed the overall response of germination to temperature and suggested that some cultivars performed better at lower temperatures than others.

Not only final germination, but also the rate of germination was influenced by temperature. The present study revealed that for all cultivars, up to about 33°C, there was a decreasing t_{50} (median germination time) with temperature but above this temperature more time to germinate was required. The rate of germination (as the reciprocal of t_{50}) of all cultivars increased linearly with temperature from a base temperature of about 13°C to an optimum temperature and then declined up to maximum temperature (Fig. 2a-c). Similar results were reported by Ong and Monteith (1985), Covell *et al.* (1986) and Dunbabin *et al.* (1994) working on sorghum. In this study, contrasting responses of the rate of germination to temperature were seen between cultivars. For example, cv. BR23 had highest rate of germination at optimum temperature whereas cvs. BR1, BR5, BR30, KS, and KG showed lower rates of germination.

The regression of rate of germination against sub-optimal temperature range also revealed differences between cultivas. The low germinating cultivars (BR1, BR30, KS and KG) had shallower slopes, indicating that for these cultivars the increase in germination rates with temperature were lower than the other cultivars. Although the rate of germination of the cultivars that germinated at the lower temperatures of 13.7 and 15.8°C showed a narrow range at these temperature, the relative differences between the cultivars in rates of germination remained the same (Table 4).

Thus the rates of germination of the cultivars at the higher temperature (20.1 and 30.9°C) were highly indicative of their rates of germination at lower temperature 13.7 and 15.8°C (Fig. 3 and 4). For example, cv. BR23, which was the most rapid germinator at 30.9°C was also of the most rapid at 13.7 and 15.8°C. Thus rankings obtained at standard laboratory test temperatures (20-30°C) were the same as at lower temperatures. The significant relationship between rate of germination at higher temperature (20.1 and 30.9°C) with that at lower temperatures (13.7 and 15.8°C) held it appeared to be genetically based.

Lafond and Baker (1986) working with nine spring wheat cultivars stated that since the relative rates of germination were not affected by temperature (as was seen in the present study), it should be possible to select for rate of germination at any temperature in the range of 5 to 30°C. They further suggested that selection at 5°C would take longer than at 25°C. Use of higher temperature would take less time to perform the experiment, but would require a minimum of three readings per day in order to characterize the germination response curves. Therefore, measurements of rate of germination of rice genotypes at higher temperatures which would predict rates at lower temperatures would also be much more quickly completed for large scale routine screening.

The cardinal temperatures presented in Table 5 allowed the quantification of the temperature responses for each cultivar. The variation in base temperature (T_b) between each cultivar was small. Six cultivars had the lower T_b. Low T_b might have an important role in adaptation to lower temperature (Ali *et al.*, 1994). The research results reported by Ellis *et al.* (1987) suggested that base temperature may be a species characteristics but they did find different base temperatures between genotype groups of faba bean (*Vicia faba*).

In this study, mean optimum germination temperature (T_{opt}) was recorded at around 32°C. However, some authors (Adair, 1968; Chaudhary and Ghildyal, 1970; Yoshida, 1981) reported that the optimum temperature range for seed germination and early seedling growth of rice is 20-35°C, whereas most rapid germination of rice seeds occurred at 30°C (Krishnasamy and Seshu, 1989).

Plants of a genotype grown throughout their lives at different temperatures have a similar base temperature and thermal time between growth stages; there seems little adaptation to temperature. Much crop phenology modeling is based on quantifying the response of plants to thermal time. Even if temperature occasionally falls below T_b, the response is unchanged when temperature returns above T_b (Squire, 1990). In this study differences in thermal times to germination were noted between the cultivars. The low germinating cultivars (BR1, BR30, KS and KG) and cv. BR5 had relatively high thermal time requirements for germination (Table 5) compared to other cultivars. The range of thermal time to reach t_{50} was 526 to 1667°C h. In comparison, in different cultivars of sorghum, the values were about 552 to 670°C h (Dunbabin *et al.*, 1994). Thus thermal time varied with crop species and with cultivars. This reflects the different responses of rate of germination to temperature.

The overall picture from this work on rice and previous work on a range of species seems to be that the cardinal temperatures are not helpful in selecting genotypes with the ability to germinate at extreme temperatures. However, rate of germination seems to be a distinct feature of genotypes and differences in rates of germination are maintained at a range of temperatures. In the case of rice in this work the range was 13.7 to 30.9°C and in wheat 5 to 39°C (Lafond and Baker, 1986).

Thus, in this study differences in the low temperature response of different rice cultivars in final germination and rates of germination were noted. The differences could be of use to plant breeders for selection of low temperature germination tolerant cultivars. However, the indications from this work is that the physiological age of seed reflecting the conditions of production and storage can also influence rates of germination and final germination at low temperature.

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