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The Effect of Ageing (Using Controlled Deterioration) on the Germination at 21°C as an Indicator of Physiological Quality of Seed Lots of Fourteen Bangladeshi Rice (*Oryza sativa* L.) Cultivars

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Abstract: The effect of seed ageing of 14 Bangladeshi rice cultivars was investigated to aid the identification of rice genotypes tolerant of low temperature during germination. This would facilitate breeding cultivars suitable for direct wet-seeding in the cooler *Boro* season in Bangladesh. The present study was carried out at the University of Aberdeen, UK during 1999. The results of the experiment on temperature gradient plate at a range of constant temperatures (13.7-37.3°C) revealed a number of cultivars (BR1, KS and KG) were to be of lower physiological quality than the rest. It was therefore, necessary to confirm whether the reason for their relatively poor performance was physiological deterioration. Seed survival curves of all cultivars at 24% moisture content (mc) and 45°C for up to 96 h tested at 21°C showed a clear separation in germination after 48 h ageing. Cultivars BR1, KS and KG were identified as the lowest quality seed lots with 0, 35 and 17% germination, respectively. Cultivar samples had different K_i (initial seed quality) after probit transformation with a range 79.30% (e.g. cv. KG) to 99.36% (e.g. cv. BR29), but surprisingly, had different slopes. The steepest slope was found for cv. BR11 (-0.046) and shallowest of that was for cv. BR24 (-0.017). The rates of germination of the faster germinating cultivars (8 cultivars, around 0.30 seed d^{-1}) declined more rapidly and at 72 h ageing the rates of germination of all cultivars were closer. Cultivars KS and KG had the least rates of germination (around 0.15 seed d^{-1}). Only when the lower quality cultivars (BR1, KS and KG) were included, were significant relationships found between measures of physiological age (48 h ageing germination, K_i and viability period) and final germination at lower temperature. The results of the study suggested that seed quality as well as genotype might be responsible for reducing final germination of cultivars. The present study also revealed that germination of seed lots of 14 rice cultivars in low temperature was influenced more by genotype than seed quality.

Key words: Ageing, germination, physiological quality and rice

Introduction

There are four criteria which normally determine seed quality: physical purity, seed health, germination capacity and seed vigour. The germination capacity and seed vigour are the most important physiological properties of the seed which determine the response to stress conditions (e.g. low and high temperatures). When seed lots with high germination percentage are sown, large differences in emergence have been observed (Ellis and Roberts, 1980a). These differences in field emergence of seed lots with high germination capacities have been attributed to differences in seed vigour (Matthews, 1980). The major cause of differences in seed germination and vigour is seed ageing or physiological deterioration (Powell, 1988; Naylor and Gurmu, 1990; Powell and Matthews, 1992; Matthews, 1994). Seeds may deteriorate both on the mother plant and in storage and the rate of deterioration is highly dependent upon the environmental conditions, particularly temperature and relative humidity (RH). Thus, the prevailing hot and humid climates in the rice growing regions of the world including Bangladesh also favour rapid deterioration of conventional seeds, result in lower percentage germination.

When this declination in germination percentage of seeds is plotted against storage period, it follows a sigmoidal survival curve which represents a negative cumulative normal distribution (Ellis and Roberts, 1980b). The viability of a seed lot is affected by a given period of ageing and this viability is also dependent upon the initial position of the seed lot on this survival curve. When the survival curve is converted into probits and then plotted this against time the results is a straight line with a negative slope. The intercept on the y-axis is termed K_i, a constant for the seed lot and is probit percentage

germination at zero time which is an indicator of initial seed quality and the slope is $1\sigma^{-1}$, where σ is the standard deviation of the distribution of seed death in time. The intercept K_i, is specific to the seed lot and its value is dependent on the pre-storage environment, genotype and the interaction between these two factors. Storage conditions affect only the value of σ and not K_i. Therefore, the value of K_i can be determined using any set of storage condition; and so can be carried out quite quickly by storing seeds under poor conditions where viability is lost fairly rapidly and sampling several times (Ellis and Roberts, 1980b).

Thus, for any seed lot stored under constant conditions:

$$V = K_i - p\sigma^{-1}$$

Where, V is the probit percentage viability, p is the storage period (days). If the period of seed deterioration required for viability to become zero, the storage period or the survival period (p), can be determined as:

$$\begin{aligned} 0 &= K_i - p^{-}\sigma^{1} \\ p\sigma^{-1} &= K_i \\ p &= K_i \times \sigma \end{aligned}$$

As relative differences in longevity between seed lots are maintained in all storage environments, differences in survival period can be used as a method for ranking the vigour of different seed lots. However, a little was known about the seed quality of Bangladesh Rice Research Institute (BRRI)- released rice cultivars regarding low temperature germination. The present study was, therefore, carried out to assess the physiological quality of the seed lots of 14 rice cultivars using ageing test (Controlled Deterioration) so as to separate the influence of seed quality and genotype on low temperature germination which would facilitate cultivar selection for winter sown *Boro* rice in Bangladesh.

Materials and Methods

Cultivars: Seeds of fourteen indica rice cultivars were produced in Bangladesh between March and December 1997 (Table 1). The well dried and pure seed samples were brought to the United Kingdom (UK) from BRRI, Bangladesh and were stored in the Department of Agriculture and Forestry, University of Aberdeen, UK in sealed aluminum foil packets at 4°C and were used to carry out the present experiment, during 1999.

Seed moisture content (mc) measurement: The moisture content of the seed lot of each cultivar was determined by the Oven Dry method (ISTA, 1999) and expressed on a wet weight basis. Five replicate samples of 25 seeds in each replication were ground to a fine texture using an electrical grinding mill and weighed to four decimal places before and after oven drying at 130°C for 1 h. Seed moisture content was then determined as a percentage of seed wet weight.

Table 1: List of rice cultivars used in the study with initial seed moisture content (mc) and initial final germination at 21°C

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 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

$$\text{Seed moisture content (\%)} = \frac{\text{(Wet weight of seed - Oven dry weight of seed)}}{\text{Seed wet weight}} \times 100$$

Ageing tests: Seed samples were screened under a low magnification binocular microscope and broken seeds were removed. Initial seed moisture content was determined as previously described.

The weight of the seed at the required moisture content was calculated by using the following formula (ISTA, 1995).

$$W_2 = \frac{100 - A}{100 - B} \times W_1$$

Where

A = initial seed moisture content (%) wet weight basis,

B = required seed moisture content which is 24%,

W_1 = initial seed weight (g),

W_2 = seed weight (g) at the required moisture content.

Seed moisture content was raised to 24% by placing a weighed sample of seeds of each cultivar on a filter paper moistened with distilled water in a 90 mm plastic petridish (with lid) to allow imbibition. Seeds were regularly removed from the filter paper and quickly surface-dried

with a paper tissue and weight was taken until required weight was reached.

When the seed samples reached the required weights they were placed inside individual aluminum foil packets and heat-sealed. The packets were kept in the refrigerator overnight at a temperature of 5°C for moisture to equilibrate through out the seeds. The foil packets were then placed in a water bath at 45±0.5°C for periods of 0, 24, 48, 72 or 96 h. The packets were taken out from water bath after the relevant deterioration time and washed with cool running tap water for 2 min. Seeds were removed from packets, placed in a petridish and put in a germination room maintained at 21±1°C. Weights of samples were taken regularly until the samples had dried back to their initial weight indicating that the samples had returned to their initial moisture content.

Germination test: A standard germination test using petri dishes was carried out using 4 replicates of 25 seeds for each period of deterioration for each cultivar with a control of 4 replicates of 25 undeteriorated seeds. The test was conducted in a germination room at 21±1°C in dark. Germination counting was done at 24 h interval and seeds were considered as germinated when radicles were extended more than 2 mm. Number of normal and abnormal seedlings were recorded at final counting (14 days).

Data analyses: Analyses of variance were carried out on all data collected by using MSTAT statistical programme. The correlations and regressions were studied by using graphical routines in Microsoft Excel (Version 5). The data were examined by probit analysis (Ellis and Roberts, 1980b) and K_i, σ and p were calculated for each cultivar. The mean germination time (MGT) was calculated as

$$MGT = (\Sigma nd)N^{-1}$$

where

n = number of germinated seeds on each day,
d = number of days from beginning of the test,
N = total number of germinated seeds and the rate of germination was determined as $1(MGT)^{-1}$.

Results

In the experiment on temperature gradient plate conducted at a range of constant temperatures, seed lots of a number of rice cultivars (BR1, KS and KG) were thought to be of lower physiological quality than the rest because germination at the optimum temperature was close to 80%. These cultivars (BR1, KS and KG) were also consistently lower in their final germination and rate of germination at the lower temperatures of 13.7 and 15.8°C (Ali, 2001). It was therefore, necessary to confirm whether the reason for their relatively poor performance was physiological deterioration. Seeds of 14 cultivars (Table 1) were aged for 0, 24, 48, 72 and 96 h at 24% mc and 45°C and subsequently germinated at 21°C. Distinct differences in germination between cultivars began to be seen at and after 48 hours ageing (Table 2). Three assessments of physiological quality related to position on the seed survival curve (Fig. 1) were determined: germination after 48 h ageing, K_i and viability period. Germination after 48 h ageing identified cvs. BR1, KS and KG as the lowest quality seed lots (Table 2). These were also the seeds with a high number of abnormal seedlings (Table 2). The initial viability (K_i) also identified the four poorest cultivars as KS (80.92%), KG (79.30%), BR24 (91.65%) and BR26 (93.43%). K_i could not be calculated for cv. BR1. Three cultivars (BR11, BR28 and BR29) had K_i's above 99% (Table 3).

Table 2: Percentage germination at 21°C of fourteen rice cultivars as affected by five levels of seed ageing at 24% mc and 45°C

	Levels of ageing (h)				
Cultivars	0	24	48	72	96
BR1	80 (43)	3 (100)	0	0	0
BR5	95 (20)	97 (14)	84 (26)	10 (42)	0
BR11	97 (4)	97 (5)	56 (10)	11 (37)	0
BR14	94 (18)	97 (3)	87 (13)	46 (15)	22
BR23	96 (1)	96 (3)	81 (5)	34 (20)	0
BR24	86 (22)	89 (9)	84 (13)	43 (18)	0
BR26	88 (8)	90 (8)	69 (14)	27 (26)	17
BR28	99 (3)	87 (6)	80 (10)	38 (18)	4
BR29	99 (4)	97 (3)	91 (11)	55 (13)	0
BR31	95 (14)	96 (5)	81 (5)	15 (40)	0
BR32	97 (15)	94 (1)	89 (2)	38 (24)	0
BR33	98 (1)	96 (3)	90 (9)	78 (21)	0
KS	83 (37)	52 (44)	35 (49)	13 (62)	0
KG	78 (33)	56 (39)	17 (42)	8 (50)	0
Mean	93 (13)	88 (11)	73 (16)	32 (30)	3

$LSD_{(0.05)}$ for ageing = 1.05 cultivar = 1.70
ageing × cultivar = 2.30 CV (%) = 4.76
Number (s) in parentheses are the percentages of germinating seeds that gave abnormal seedlings

Surprisingly the slopes of all the lines were different despite the seeds being aged under the same conditions (24% mc and 45°C). The steepest slope was found for cv. BR11 (-0.046) followed by cvs. BR5 and BR28 (both -0.040), the shallowest slopes were seen for cvs. BR24 (-0.017) and BR33 (-0.018) (Table 3). These differences in slope combined with differences in K_i led to a wide range in calculated viability period (p) from as low as 24.2 h for cv. KG to as high as 117.3 for cv. BR33 (Table 3).

Rate of germination: The rate of germination decreased significantly and linearly (R^2 = 0.9961, P <0.001) with increasing ageing level (up to 72 h ageing) in all cultivars (pooled data) (Fig. 2). Initial rates of germination were found to be higher in cvs. BR11, BR14, BR23, BR28, BR29, BR31, BR32 and BR33 (around 0.30 seed d^{-1})

Table 3: Seed vigour parameters from probit analysis of controlled deterioration for 13 rice cultivars. The average viability period, p is based on the survival of seeds incubated at 24% moisture content and 45° C

Cultivar	Intercept K_i (probits)	Initial seed quality K_i (%)	Slope ($1\sigma^{-1}$)	Distribution of seed deaths (σ)	Viability period, p (h) ($K_i \times \sigma$)	Vigour ranking (based on p)	Ranking of germination at 13.7°C
BR5	2.260	98.81	-0.040	24.8139	56.1	10	9
BR11	2.329	99.01	-0.046	21.7391	50.6	11	11
BR14	2.064	98.05	-0.028	36.2319	74.8	4	3
BR23	1.693	95.48	-0.024	42.5532	72.0	6	10
BR24	1.382	91.65	-0.017	59.8802	82.7	3	5
BR26	1.508	93.42	-0.026	39.0625	58.9	8	2
BR28	2.365	99.10	-0.040	25.0627	59.3	7	7
BR29	2.490	99.36	-0.030	33.5570	83.5	2	4
BR31	2.147	98.41	-0.037	26.8817	57.7	9	1
BR32	2.122	98.31	-0.029	34.8432	73.9	5	6
BR33	2.112	98.27	-0.018	55.5556	117.3	1	8
KS	0.875	80.92	-0.028	35.9712	31.5	12	13
KG	0.817	79.30	-0.034	29.6736	24.2	13	12

Notes: (1) The initial seed quality is the percentage detransformed from the probits K_i
(2) The viability period is calculated using K_i (probits)

Table 4: Intercept, slope and R^2 values of the fitted linear curves of germination rate fitted against ageing for different rice cultivars

Cultivars	Intercept (seed d^{-1})	Slope (seed d^{-1} ageing h^{-1})	R^2
BR5	0.1696	-0.0008	0.7386
BR11	0.3046	-0.0028	0.9615
BR14	0.2703	-0.0021	0.9893
BR23	0.2856	-0.0024	0.9962
BR24	0.1833	-0.0009	0.9475
BR26	0.2415	-0.0020	0.9610
BR28	0.2609	-0.0022	0.9963
BR29	0.2769	-0.0021	0.9714
BR31	0.2697	-0.0022	0.9633
BR32	0.3209	-0.0024	0.9397
BR33	0.2792	-0.0019	0.9445
KS	0.1525	-0.0010	0.7624
KG	0.1745	-0.0013	0.9030

(Fig. 3, Table 4) and lower initial rates were found in cvs. BR5, BR24 and BR26 (around 0.20 seed d^{-1}) and the least were seen for cvs. KS (0.15 seed d^{-1}) and KG (0.17 seed d^{-1}) (Fig. 3, Table 4). Up to 24 h ageing, the differences in rates between germination of these three groups of cultivars were maintained but as ageing progressed, the rates of germination of the faster germinating cultivars declined more rapidly and at 72 h ageing the rates of germination of all cultivars were closer.

Assessments of physiological quality in relation to low temperature germination: The three assessments of physiological quality (germination at 48 h ageing, K_i and viability period) were examined for their relationship to final germination at the lower temperatures of 13.7°C and 15.8°C. The relationships were examined both including the clearly identified cultivars of low physiological quality (cvs. BR1, KS and KG) and excluding them.

When the low quality cultivars were included, final germination at 13.7 and 15.8°C was significantly and positively related to germination at 48 h ageing (Fig. 4a, c).

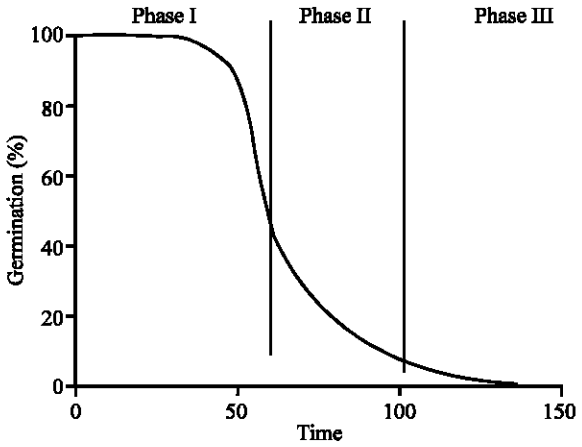

Fig. 1: The seed survival curve showing the pattern of decline in viability (% germination) of individual seeds in a population of orthodox seeds under constant temperature and moisture content (Priestley, 1986)

The cultivars BR1, KS and KG were lower in their low temperature germination and in the assessment of their positions on the survival curve as indicated by germination at 48 h ageing. When the lower cultivars were excluded the significant relationships were lost (Fig. 4b, d) although there was a range of germination after ageing from 56% (cv. BR11) to 91% (cv. BR29) (Table 2). When K_i (initial germination) was used as an assessment of physiological quality, a significant relationship between K_i and germination was found only at 15.8°C when cvs. KS and KG were included (Fig. 5c). This was also the case when seed viability period (p) was related to germination.

Discussion

The results of the experiment on temperature gradient

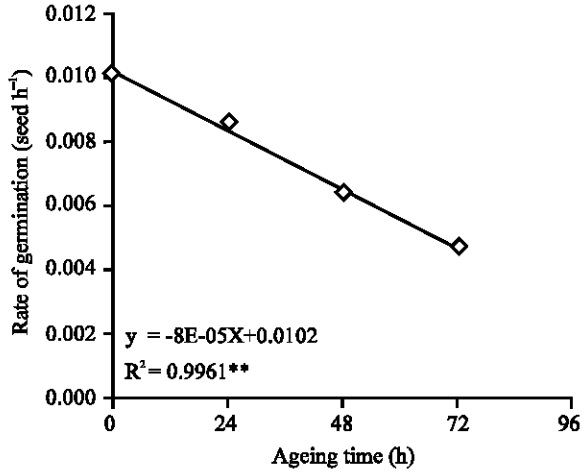

Fig. 2: Effect of rapid ageing at 24% mc and 45°C on germination rate (pooled data) of 13 rice cultivars (fitted linear regression) at 21°C

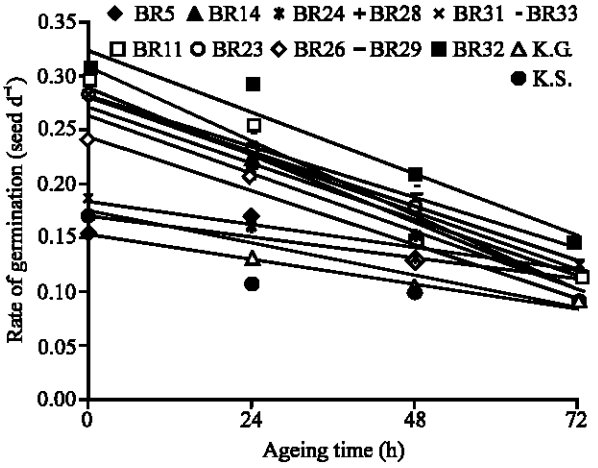

Fig. 3: The effect of ageing at 24% mc and 45°C on germination rate at 21°C of 13 rice cultivars

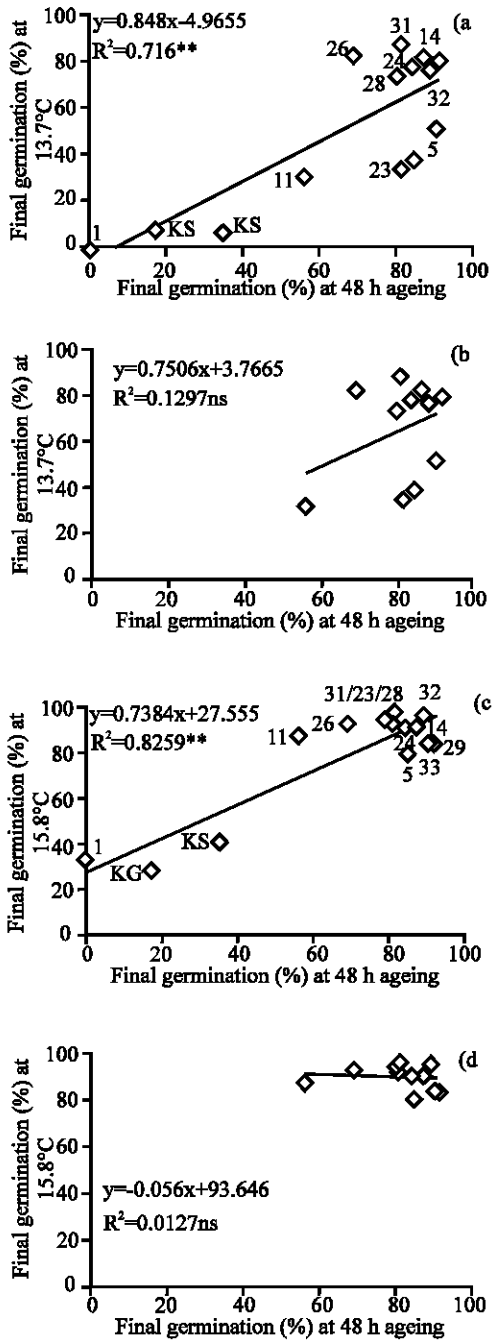

Fig. 4: Relationship between final germination at 21°C after 48 h ageing (24% mc and 45°C) and final germination at 13.7°C and 15.8°C, (a) and © including all cultivars, (b) and (d) excluding cvs. BR1, KS and KG

plate demonstrated that cultivars differed significantly in their response to constant temperature. The cultivars that showed a particularly low rate of germination and final germination (BR1, KG and KS) at low temperature had a relatively low level of germination at higher temperatures suggesting low physiological quality (Ali, 2001). The present experiment was, therefore, conducted to confirm this and to ascertain how much physiological quality and not genotype was the explanation for differences in temperature response.

Controlled Deterioration (CD), a standard vigour testing method (ISTA, 1995) was carried out for the assessment of seed physiological age. According to the vigour concept described by Delouche (1969) there could be differences between seed lots in the degree of deterioration. Germination after additional standard

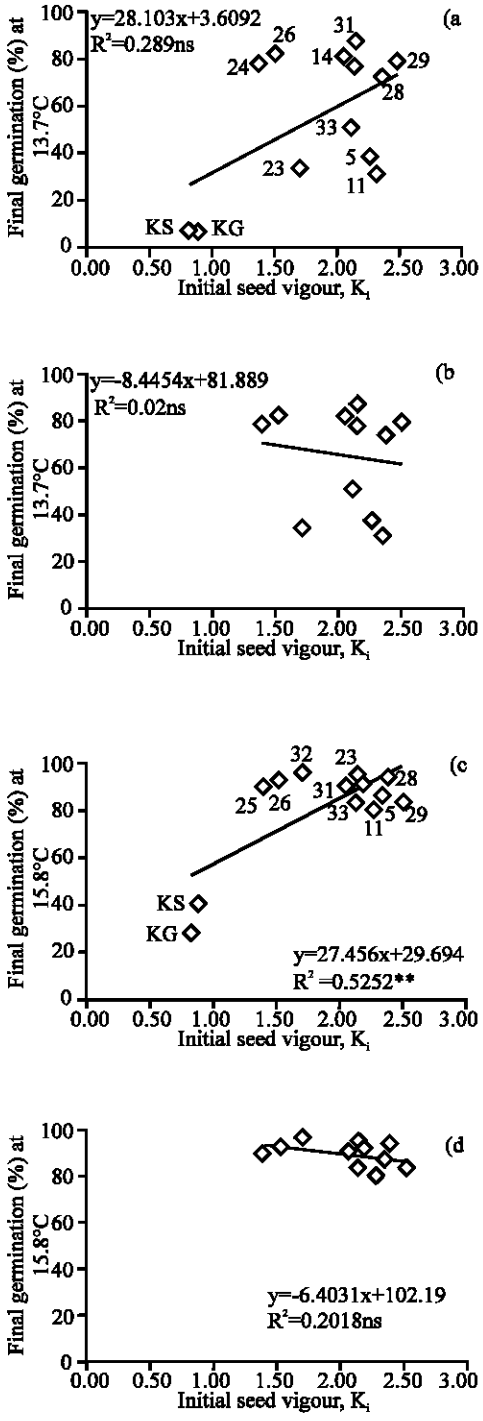

Fig. 5: Relationship between initial seed vigour (K_i) and final germination at 13.7°C and 15.8°C, (a) and © including all cultivars, (b) and (d) excluding cvs. BR1, KS and KG. K_i was calculated for each lot from the survival curve determined during ageing at 24% mc and 45°C followed by germination test at 21°C

amounts of ageing would therefore reflect the relative positions of the seed lots in the deterioration process before the ageing was imposed. Therefore, CD was designed to force seeds out from phase I of seed survival curve where they are similar in final germination (Fig. 1) into phase II where they can be discriminated. In this test, seed lots with low final germination percentage after deterioration or ageing are said to be a low vigour with poor field emergence and storage potential.

Three assessments of physiological quality of seed of all cultivars were determined from the seed survival curves at 25% mc and 45°C: germination after 48 h ageing, initial seed quality (K_i) and viability period (p). All assessments reflected the position of the seed lots of the cultivars on the seed survival curve before the ageing treatments. The relationships between germination at low temperatures (13.7 and 15.8°C) and the three assessments of physiological quality were only positive and significant when all cultivars were included. When the lower germinating cultivars BR1, KG and KS were excluded, no significant relationships were seen. This confirmed the low physiological quality of the seed of these cultivars and suggested that seed quality differences were not the explanation for differences in low temperature germination in the other cultivars.

There were some differences in seed quality seen in cultivars other than BR1, KS and KG, but these differences did not seem to influence the level of germination at the lower temperatures. For example, cv. BR11 was identified as the lowest quality and also had a relatively low germination at 13.7°C. In contrast, cv. BR5 appeared to be better quality seed but exhibited a relatively low and slow germination at 13.7 and 15.8°C (Ali, 2001). Thus the implication might be that once cvs. BR1, KS and KG were excluded; differences in low temperature germination performance were primarily the result of genotypic differences. There were no significant relationships between seed quality as measured for example by germination after 48 h ageing and low temperature germination even though relatively small differences in physiological quality were seen (Fig. 4). This would have resulted from the effect of genotype having greater influence on low temperature germination than quality. For example, the germinations of cvs. BR23 and BR5 are lower than might be expected from the 48 h ageing germination (Fig. 4) suggesting they were genotypically less able to germinate at 13.7°C. In contrast, cvs. BR26 and BR31 germinated at 13.7 and 15.8°C (Fig. 4) to a higher level than have been expected from 48 h ageing germination suggesting a genotypically determined ability to germinate at lower temperatures.

The increases in percentage germination and decrease in

abnormal seedlings (Table 2) which occurred mostly in intermediate vigour seeds after mild ageing (24 h) in this study have been interpreted as a demonstration of repair mechanism resulting in invigoration of seeds (Naylor, 1989). Some authors similarly explained invigoration of seeds as metabolic repair which enhanced germination and this has been observed in wheat (Lush *et al*., 1981), onion (Ward and Powell, 1983) and Brussels sprouts (Burgass and Powell, 1984). Naylor and Syversen (1988) showed enhanced germination in Italian ryegrass which was subjected to ageing at high moisture content and high temperature. They also noted that lots with intermediate proportions germinating or intermediate germination times showed most improvement. This could be because in a high vigour lot there is little scope for improvement while in a low vigour lot deterioration has proceeded too far. The explanations for the improvement achieved have focused on the metabolic repair of previously sustained deterioration (in aged seed) and germination advancement (in high vigour seed) (Powell *et al*., 2000). Thornton and Powell (1992) provided physiological evidence of repair by increased germination after controlled deterioration in cauliflower and in Brussels sprouts seed following aerated hydration.

Ageing consistently reduced the rate of germination of all cultivars (Fig. 3). Comparisons of the rate of germination of unaged seeds showed that seeds clearly identified as being of lower physiological quality (BR1, KS and KG) all had slower rates of germination at all temperatures relative to most other cultivars (Ali, 2001). One clear exception was cv. BR5 which germinated slowly but was one of the better seed lots in terms of physiological quality (Table 3). This suggests that cv. BR5 is a slow germinating genotype.

Since seed longevity is strongly affected by seed quality (Tang *et al*., 1999), the deterioration rate may also be influenced by initial seed quality and possibly by genotype. Thus, the differences in seed longevity among seed lots of rice cultivars may result from a difference in initial seed quality, seed deterioration rate, or both. The differences in the rate of deterioration in this study were not very large and would, therefore, not result in dramatically different estimates of seed longevity. Thus, the vigour ranking (Table 3) which was done on the basis of initial seed quality and rate of seed deterioration was supported by the findings in other parameters, such as final germination percentage (Table 2), germination rate (Fig. 3, Table 4) and percentage abnormal seedlings (Table 2).

The results of the present study have shown that the differences in germination after ageing is the indication of the differences in initial seed quality and seed vigour of the cultivars. The results also revealed that germination of seed lots of fourteen rice cultivars in low temperature was influenced more by genotype than seed quality. There is no doubt that reduced seed vigour decreases field emergence. The magnitude of the decrease, however, depends on the severity of the stresses encountered in the field. It is desirable that a vigour test ranks different seed lots in order of superiority. In this way, it gives some rational measure of the absolute standard, so that relative difference between seed lots can be assessed.

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