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Variation in seed longevity of rice cultivars belonging to different isozyme groups

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Summary

The storage potential of seeds harvested at different stages of maturity was studied in ten cultivars of *Oryza sativa*, representing six known isozyme groups, and in two cultivars of *O. glaberrima*. Mass maturity (the end of the seed-filling period) was attained between 14.2 and 20.2 days after anthesis (DAA). A comparison of the estimates of p_{50} (time in storage for viability to decline to 50%) of seeds harvested at 21, 28 and 35 DAA and stored at 35°C with 15% moisture content showed that maximum longevity was attained between 28 and 35 DAA in most cultivars. Cultivars belonging to isozyme group II survived longer than other cultivars with estimates of p_{50} nearly doubled. On the other hand, the floating rices of Group IV had shorter longevity. Within group VI, the upland cultivar survived longer than the lowland cultivar. Both *O. glaberrima* cultivars survived reasonably well, showing that African rice cultivars also differ in longevity.

Introduction

Rice is the staple food for half the world's population. *Oryza sativa* L. is grown all over Asia, and *O. glaberrima* Steud. is indigenous to West Africa. The Asian rice cultivars have been classified into three major ecogeographic groups, namely indica, japonica and javanica, based on morphological and ecogeographic characteristics (Chang, 1985). Biochemical tools have been used to add to this classification and, based on isozyme polymorphism at five diagnostic loci, rice cultivars can be classified into six varietal groups. Groups I and VI represent the majority of rice cultivars, II and V are minor groups, and III and IV are considered as satellite groups (Glaszmann, 1987).

Group I encompasses the tropical lowland indica rices, while group VI comprises the typical japonica types from temperate regions. This group also includes the upland rices from tropical regions and the bulu rices from Indonesia, which were previously classified as indica and javanica types, respectively. The minor groups II–V consist of varieties with special characteristics and adaptation to specific ecological niches, but are nonetheless classified as indica types based on morphology. Thus, group II encompasses the aus and boro rices of Bangladesh, group III includes rices adapted to deep water habitats, group IV consists of Rayada rices with floating ability from Bangladesh, and group V comprises the high quality and aromatic rices of South and Southeast Asia.

The broad morphological and ecogeographic variation in rice germplasm conserved in the International Rice Genebank (IRG) at International Rice Research Institute (IRRI), Los Baños, Philippines, presents a unique opportunity to study genetic diversity in seed longevity. Previous investigations revealed marked variation in longevity of rice cultivars originating from different ecogeographic regions (Kameswara Rao & Jackson, 1996a, b). For example, japonica cultivars from lowland ecosystems showed poorer storage characteristics than those from upland ecosystems. Similarly, large-seeded accessions and *O. glaberrima* showed shorter longevity compared to other cultivars. Those studies covered a broad spectrum of variation, but cultivars from only isozyme groups I and VI were included. We present further studies to understand the develop-

Table 1. Classification and special characteristics of the 12 rice cultivars under study.

Variety name	IRGC accession number	Origin	Subspecies	Isozyme group	Days to flower	Remarks
I-geo-tze	120	Taiwan	Indica	I	78	
Dular	636	India	Indica	II	70	Aus
Dholi boro	27513	Bangladesh	Indica	II	68	Boro rice
Bhadoia 233	6541	Bangladesh	Indica	III	105	Deep water
Rayada 16-04	27590	Bangladesh	Indica	IV	103	Floating rice
Basmati 370	3750	India	Indica	V	84	Aromatic
ARC 10497	12485	India	Indica	V	91	Aromatic
Ta mao tao	8194	China	Japonica	VI	98	
Rikuto norin 21	7697	Japan	Japonica	VI	93	Upland
Plah sew	32042	Thailand	Javanica	VI	75	
O. glaberrima	103334	Senegal			65	
O. glaberrima	103975	Nigeria			65	

ment of longevity in cultivars with special characteristics belonging to the four minor isozyme groups. We also included upland japonica and *O. glaberrima* cultivars to test previous findings.

Materials and methods

Ten cultivars of *O. sativa*, representing the six isozyme groups and two cultivars of *O. glaberrima* were studied (Table 1). They were grown on the IRRI Upland Farm Block UC 3 during the dry season 1993–94. The seedlings were raised in wet seed beds and transplanted into 3 × 5 m plots arranged in a randomized block design with two replications. The row to row and plant to plant distances were 0.3 and 0.25 m, respectively.

Panicles were tagged with the date of anther dehiscence and, commencing on the seventh day at weekly intervals until 35 days, about 50–60 panicles were harvested from alternate rows for each sampling time. The seeds were threshed gently by hand and samples were drawn for dry mass, moisture content and germination determinations. The remaining seeds were dried for 24 h in a mechanical convection incubator at 30°C and 20–30% RH and then stored at 1–2°C in sealed aluminum foil packets until longevity determinations began.

Dry weight observations were based on two 100 seed samples from each plot, dried in a ventilated oven at 80°C for 3 d. Moisture content determinations were made on two 3–5 g samples from each plot using the high constant temperature oven method (International Seed Testing Association, 1985a, b). Germination tests were conducted on 200 seeds (from each plot) as four replicates of 50 seeds each on top of two moist filter papers in 9.0 cm Petri dishes at an alternating temperature regime of 30/20°C (16/8 h). The first counts of germination were made on the seventh day. Ungerminated but firm seeds were dehulled to remove dormancy and tested for another 7 days before final counts were taken. Seeds which produced seedlings with normal root and shoot were considered as germinated (normal germination), while seeds which remained ungerminated but became soft at the end of testing period were considered dead.

Within each cultivar, the potential longevity of seeds harvested at 21, 28 and 35 days after anthesis (DAA) from each plot was determined by artificial aging at 35°C with 15 ± 0.2% moisture content following Kameswara Rao and Jackson (1996a). The data on seed survival were analyzed by probit analysis, where a regression of transformed percentage normal germination was calculated against time in storage.

Results

The dry mass of the seeds increased rapidly until it reached maximum by 14 DAA in cvs. Dular, Dholi boro, Rayada 16-04, Basmati 370 and *O. glaberrima* – 103975; and by 21 DAA in cvs. I-geo-tze, Bhadoia 233, ARC 10497, Ta mao tao, Rikuto norin 21, Plah sew and *O. glaberrima* – 103334. Subsequent changes in dry mass were not significant ($P > 0.05$). Mass

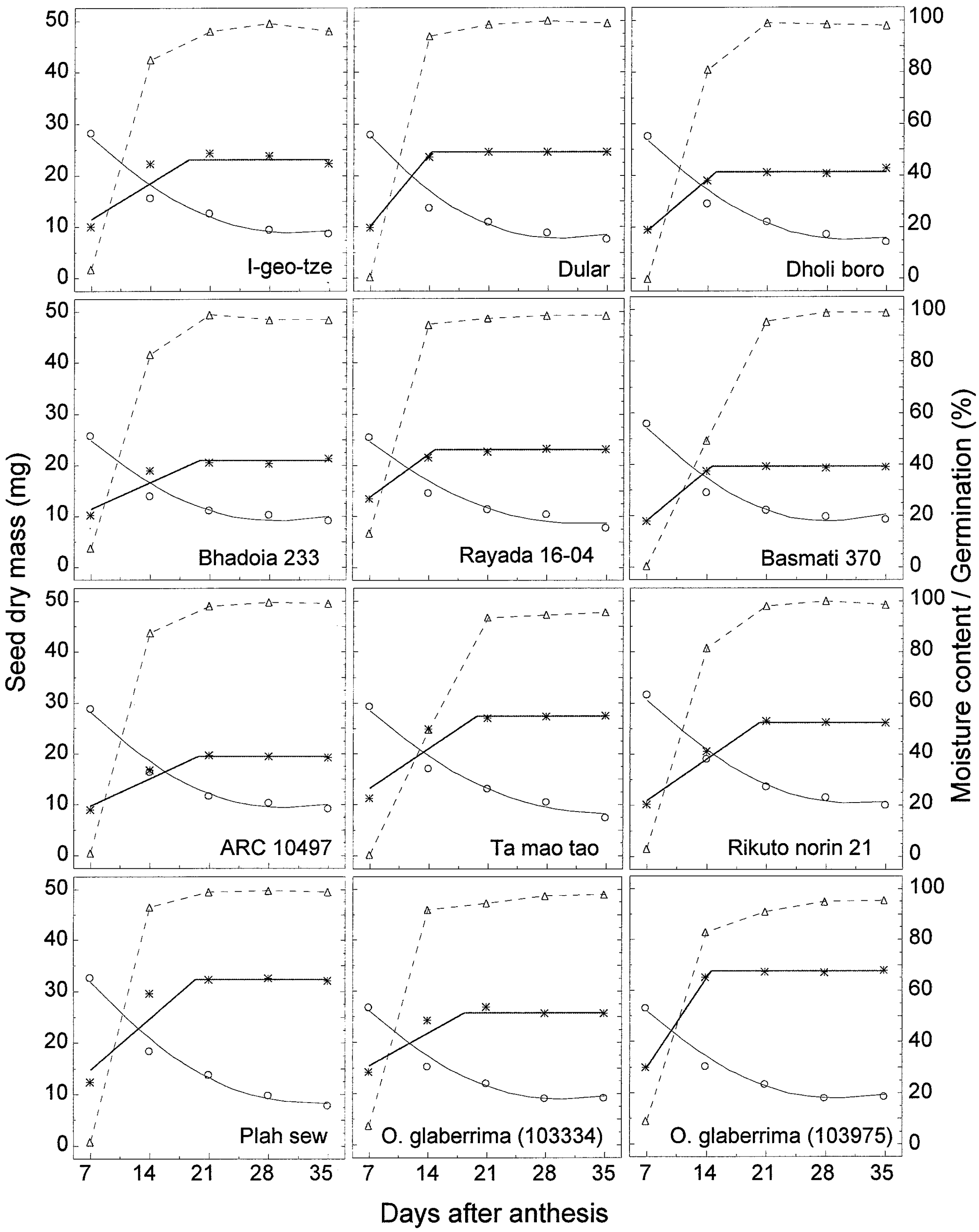

Figure 1. Mean dry weight (∗) moisture content (○) and germination (△) of seeds during development in twelve cultivars of rice grown in the dry season 1993–94 at Los Baños, Philippines.

maturity or the end of the grain filling period was estimated for each cultivar by fitting a positive relation to the dry mass observations between 7 and 14 or 21 days (where differences were significant) and a horizontal line thereafter and then assessing the day on which the two lines intercepted (Fig. 1). The estimates of mass maturity varied between 14.2 and 20.2 days, with a mean of 17.4 $\pm$ 0.7 days. Due to lack of sufficient data points, it was not possible to derive mass maturity more objectively, therefore, these estimates should be treated with caution. The moisture content of seeds started decreasing from 7 DAA. Derived from a quadratic relationship fitted to the data, the moisture content ranged between 22.9% (Bhadoia 233) and 33.3% (*O. glaberrima* – 103975) with a mean of 28.9 $\pm$ 0.94% at mass maturity (Fig. 1).

In all cultivars the ability to germinate varied in the initial stages of seed development and increased to a maximum approaching mass maturity. The increase was more rapid in Dular, Rayada 16-04, Plah sew and *O. glaberrima* (IRGC 103334), achieving maximum germination at 14 DAA. Mean maximum germination of 98% was attained at 28 DAA (Fig. 1).

Table 2. Changes in longevity (expressed as the half-viability period, p_{50} in weeks) of seeds harvested at different stages of maturity in twelve cultivars of rice grown during the dry season 1993–94 at Los Baños, Philippines.

Cultivar	Maturity (days after anthesis) 21	28	35	Mean
I-geo-tze	4.6 f	6.0 fg	6.5 d	5.7
Dular	11.0 a	11.6 b	12.5 a	11.7
Dholi boro	11.6 a	12.8 a	12.4 a	12.3
Bhadoia 233	6.1 cd	7.1 de	7.3 c	6.8
Rayada 16-04	5.2 ef	5.2 g	5.3 e	5.3
Basmati 370	7.6 b	8.1 c	8.8 b	8.2
ARC 10497	5.5 de	6.6 ef	6.9 cd	6.9
Ta mao tao	5.5 de	6.1 fg	6.5 d	6.1
Rikuto norin 21	5.2 ef	7.0 de	7.1 cd	6.4
Plah sew	6.6 c	7.4 d	6.8 cd	6.9
O. glaberrima (103334)	4.8 ef	6.0 fg	7.4 cd	6.2
O. glaberrima (103975)	3.8 g	6.0 fg	7.2 cd	5.7
Mean	6.5	7.6	7.9	7.3

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.
LSD (5%) to compare two maturity means within each cultivar = 0.71

During storage all seed lots gradually lost viability, but differences were apparent among seeds harvested at different stages of maturity. The seed survival curves were sigmoid (Fig. 2), which therefore were described as negative cumulative normal distributions of seed deaths with time following Ellis and Roberts (1980). Within each cultivar, the slopes of the survival curves of seed lots harvested at different times were similar ($P > 0.05$) except in Dholi boro, where complete survival curves were not obtained because sufficient seeds were not available.

Seed longevity was estimated as the half-viability period (p_{50}), which is the number of weeks in storage for viability to decline to 50% (Roberts, 1972). Analysis of variance of the estimates of p_{50} showed significant differences among cultivars and harvest times ($P < 0.01$). The interaction between the two was also significant ($P < 0.01$). Maximum seed longevity was attained at 28 DAA in most cultivars and changes thereafter were not significant (Table 2). In I-geo-tze, Dular, Basmati 370 and the two accessions of *O. glaberrima*, however, longevity was maximum at 35 DAA, while in Rayada 16-04, it was highest at 21 DAA. Mean longevity at 35 DAA was marginally higher (7.9) than at other times of harvest (Table 2).

Considerable differences were observed in absolute seed longevity of cultivars. Thus, Dular and Dholi boro survived longer than all other cultivars. The values of p_{50} were nearly twice as high as those for the other cultivars. On the other hand, Rayada 16-04 lost viability faster than the other cultivars, and therefore had steeper survival curves. With the exception of Basmati 370, which had a reasonably longer longevity, differences among other cultivars were only marginal (Fig. 1 and Table 2).

Discussion

The results show that rice seeds in general attain maximum longevity one or two weeks after mass maturity. Thus they confirm previous observations in rice (Ellis et al., 1993; Ellis & Hong, 1994; Kameswara Rao & Jackson, 1996a, b) and other cereals (Kameswara Rao et al., 1991; Pieta Filho & Ellis, 1991; Ellis & Pieta Filho, 1992) that maximum potential longevity continues to improve after mass maturity. This contradicts the hypothesis that seed quality is maximum at physiological maturity and that vigour and viability decline thereafter (Harrington, 1972).

Considerable differences in longevity were observed. Dular and Dholi boro belonging to the isozyme group II, with loci coding for phosphoglu-

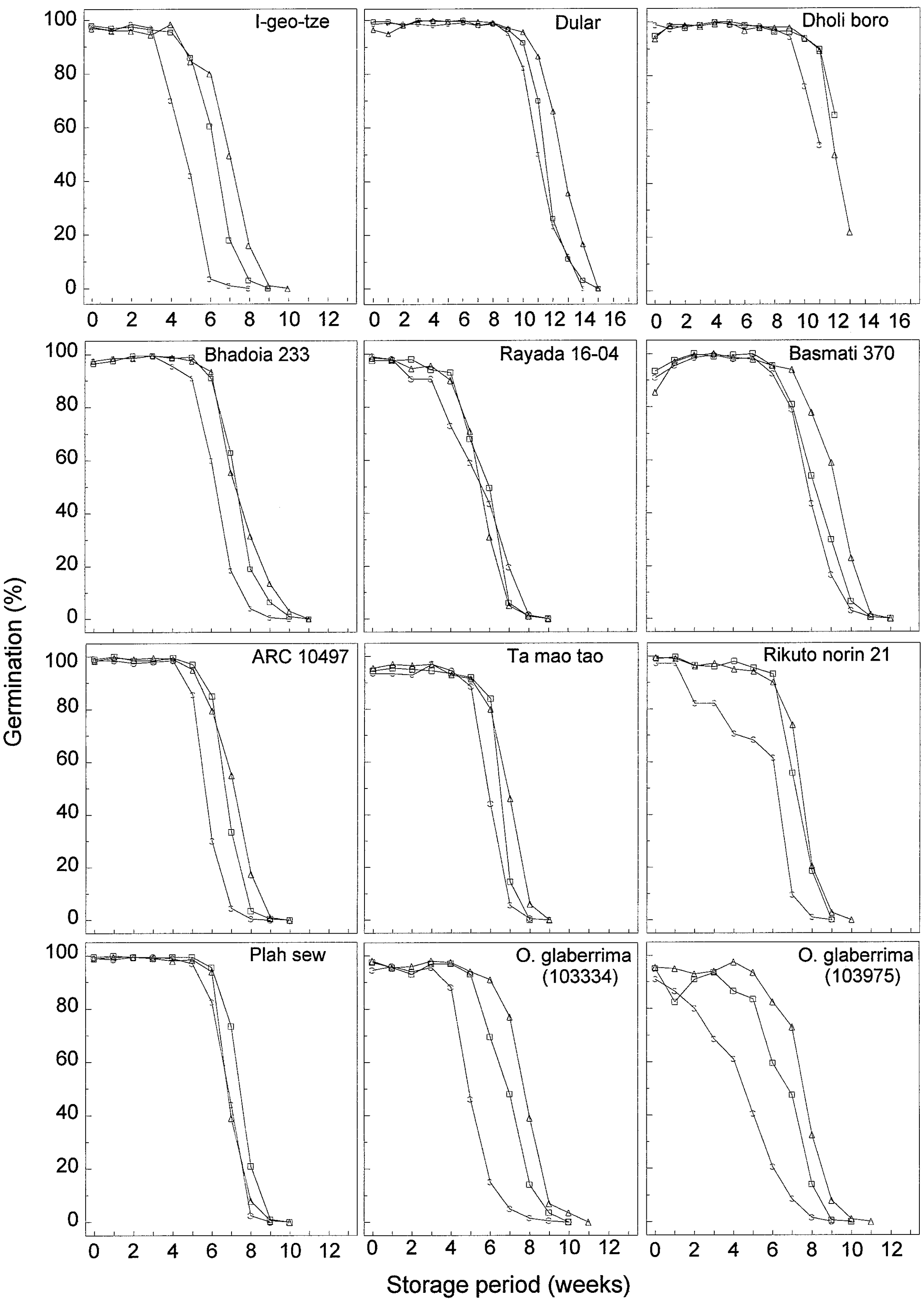

Figure 2. Seed survival curves (% normal germination plotted against time in storage) in twelve cultivars of rice. The seeds were harvested at 21 (○), 28 (□), and 35 (△) days after anthesis and stored hermetically in laminated aluminium foil packets at 35°C with 15% moisture content.

cose isomerase 2 (PGI 2) and aminopeptidase 3 (Amp 3) (Glaszmann, 1987) survived longer than other cultivars. These are representative cultivars of the aus and boro rices from Bangladesh. Apart from these, group II also includes varieties originating from the foothills of the Himalayas and from Iran to Assam, India. These are short duration cultivars grown under irrigated to dry land conditions. The results also confirm that the upland japonica rices differ in longevity from the lowland japonica types. Upland japonica Rikuto norin 21 survived longer than lowland japonica Ta mao tao, although both belong to group VI. Longevity of the two *O. glaberrima* cultivars was similar to most other cultivars. However, one of the *O. glaberrima* cultivars studied earlier (Kameswara Rao & Jackson, 1996a) had shorter longevity, which indicates that there are also differences in longevity of African rice cultivars.

Seed dormancy does not appear to have a relationship with longevity. On the one hand, Rayada 16-04 was strongly dormant, but lost viability faster than the nondormant cultivars as Plah sew and I-geo-tze. On the other hand, Ta mao tao was strongly dormant and showed similar longevity as other indica cultivars, despite being a lowland short-lived japonica cultivar (Kameswara Rao & Jackson, 1996a).

The significantly greater longevity of Dular and Dholi boro offers scope for biochemical investigations. The inheritance and genetic control and of this trait should be determined for incorporation into the genetic background of high yielding cultivars in order to control seed deterioration, which is important in tropical climates.

As reported (Kameswara Rao & Jackson, 1996a, b) it is important to take account of the seed production environment for rice during multiplication or regeneration of germplasm. Since many genebanks have access to only one or a few sites for germplasm regeneration, it is important to understand the relationship between production environment and seed quality in terms of storage potential, as well as the diversity of the germplasm *per se*. This study has focused on rice germplasm accessions from a wide geographical area regenerated in one location in the Philippines, it may also hold for the conservation of other crops.

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References

Chang, T.T., 1985. Crop history and genetic conservation: rice-a case study. Iowa State J. Res. 59: 425–455.

Ellis, R.H. & T.D. Hong, 1994. Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). Ann. Bot. 73: 501–506.

Ellis, R.H., T.D. Hong & M.T. Jackson, 1993. Seed production environment, time of harvest and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). Ann. Bot. 72: 583–590.

Ellis, R.H. & C. Pieta Filho, 1992. Seed development and cereal seed longevity. Seed Sci. Res. 2: 9–15.

Ellis, R.H & E.H. Roberts, 1980. Improved equation for the prediction of seed longevity. Ann. Bot. 45: 13–30.

Glaszmann, J.C., 1987. Isozymes and classification of Asian rice varieties. Theor. Appl. Genet. 74: 21–30.

Harrington, J.F., 1972. Seed storage and longevity. In: T.T. Kozlowski (Ed.), Seed Biology, Vol. III., pp. 142–245. Academic Press, New York.

Kameswara Rao, N. & M.T. Jackson, 1996a. Seed longevity of rice cultivars and strategies for their conservation in genebanks. Ann. Bot. 77: 251–260.

Kameswara Rao, N. & M.T. Jackson, 1996b. Seed production environment and storage longevity of japonica rices (*Oryza sativa* L.). Seed Sci. Res. 6: 17–21.

Kameswara Rao, N., S. Appa Rao, M.H. Mengesha & R.H. Ellis, 1991. Longevity of pearl millet (*Pennisetum glaucum* R. Br.) seeds harvested at different stages of maturity. Ann. Appl. Biol. 119: 97–103.

Pieta Filho, C. & R.H. Ellis, 1991. The development of seed quality in spring barley in four environments. I. Germination and longevity. Seed Sci. Res. 1: 163–177.

Roberts, E.H., 1972. Seed storage environment and the control of viability. In: E.H. Roberts (Ed.), Viability of Seeds, pp. 14–58. Chapman & Hall, London.