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Role of Embryo and Endosperm in Rice Seed Deterioration

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Embryos of rice seeds deteriorated to different degrees by storing under ambient conditions for 0–3 years were transplanted on to endosperms of freshly-harvested (high-vigour) seeds. Age-induced deterioration adversely affected the germinability of embryo and its subsequent growth; storage for three years under ambient conditions resulted in complete non-viability of the embryo. In the reciprocal transplantation experiment, high-vigour embryos were transplanted on to differentially aged endosperms. The condition of the endosperm considerably influenced the growth of the embryo. However, the endosperms of non-viable seeds could support the growth of high-vigour embryo to a certain extent. While the effect of ageing on the sprouting of the embryo is direct, that of the endosperm on seedling growth is indirect. Our results suggest that the damage to the aleurone cells of the endosperm by ageing reduced the capacity of the endosperm to support the development of the sprouted embryo. Growth of high-vigour embryos transplanted on to endosperms of high-vigour seeds was proportional to the amount of aleurone tissue retained on the latter and the embryos completely failed to grow on aleuroneless endosperms. Root and shoot growth of transplants was inversely related to the induction of α-amylase in the endosperms. Ageing or artificial removal of aleurone cells reduced the growth of transplants because of failure of GA-induced synthesis of amylase and possibly other enzymes in the endosperm.

Key Words: **Seed Deterioration, Transplantation, Embryo, Endosperm, Aleurone**

Introduction

In cereal seeds, both embryo and endosperm age with time but their respective rates of ageing may not be synchronous. It is the embryo which is the basic determinant of seed viability; the conditions of the endosperm would, however, considerably influence the capacity of a seed to produce a normal healthy seedling.

The relative role of embryo and endosperm in the ageing of wheat seed was investigated by Floris (1970) who concluded that metabolic events in both the embryo and

endosperm determined the extent of seed ageing. The inhibitory compounds produced in the endosperm would therefore induce deterioration in the embryo. Subsequently, Floris and Anguillesi (1974) showed that age-induced nuclear damage in the embryo was due to the ageing of the embryo itself and was not a consequence of endosperm ageing.

In the present investigation, we have studied the effects of embryo and endosperm deterioration brought about by ageing in reciprocal embryo-endosperm transplantation experiments to verify the relative roles of embryo and endosperm in rice seed senescence and more specifically to elucidate the role of endosperm deterioration on the performance of transplanted embryos.

Materials and Methods

Reciprocal transplantation studies on differentially deteriorated embryo and endosperm of rice seeds

Harvest-fresh and 1-, 2-and 3-year-old seeds of rice (*Oryza sativa* L. CV. Jaya) were obtained from the Calcutta University Experimental Farm. The seeds were graded, selected for uniformity and tested for germinability in the laboratory. The germination percentage and shoot and root growth of a sample of each seed lot were recorded. About 10 g dehusked of each lot were soaked in 0.05% mercuric chloride solution for 4 min followed by thorough washing with sterilized distilled water. After final washing, the seeds were soaked in distilled water for 2 hr and then the embryos were carefully isolated from the endosperm with a sharp needle. The embryos and endosperms of the variously deteriorated seeds were then used in reciprocal embryo-endosperm transplantations as shown in figure 1. Firstly, the embryos of the differentially aged seeds were transplanted on high-vigour endosperms of fresh seeds and then high-vigour embryos were transplanted on to the differentally aged endosperms. The transplants were aseptically planted on an agar medium in slanted tubes which were placed in a temperature controlled room at 24 ± 1°C. Percentage of germination and growth of shoot and root of transplants and differentially deteriorated full seeds were recorded after 7 days. Three replications were provided for each set of experiment.

Studies on the role of aleurone in the growth of rice seedling

The aleurone cells of high-vigour endosperms were removed partially (3/4, 1/2, 1/4-th) or fully (0) by very carefully scraping off the monolayer with the flattened tip of a needle. Seeds were sterilized with 0.05% mercuric chloride solution and after washing it was planted in agar medium. Each experiment was repeated 5 times and the germination percentage and growth of shoot and root were recorded after 7 days (figure 2).

In another transplantation experiment, the role of aleurone on the growth of high-vigour embryos have been studied using isolated high-vigour embryos and aleurone-less endosperms. The high-vigour embryos were transplanted on to high-vigour aleurone-less endosperms as well as on endosperms with full aleurone (table 2). Transplants were made in the same way as described earlier and after 7 days, data on germination percentage and growth of shoot and root were recorded. Three replications were employed for each treatment.

α-Amylase induction studies

GA-induced α-amylase induction was studied in endosperms of dehusked rice seeds stored under ambient conditions for different durations and in high-vigour endosperms having full, three-fourth, one-half, one-fourth and no (complete removal) aleurone tissue. For

the assay of α-amylase activity of rice seeds, the method described by Ogawa (1967) was followed with minor modifications. Seeds were sterilized with 1% sodium hypochlorite solution. To avoid microbial contamination, 0.2 mg/ml streptomycin sulphate was used in the incubation solution. Incubation of full seeds and embryo-less half-seeds was done in the presence of gibberellic acid (GA_3, 5×10^{-6}M) or in its absence. After 48 hr of incubation at 25 ± 1°C, α-amylase was extracted and its acivity was measured by the extent of hydrolysis of a standard starch solution (Punjabi & Basu 1978). The residual starch was measured colorimetrically by developing the blue starch-iodine colour with a standard iodine solution.

Results and Discussion

The germinability of the seed was adversely affected by ageing, and storage for three years under ambient conditions resulted in complete loss of viability. Two-year-old seed, however, showed 90% germination but the shoot and root length were reduced to about half of that noted in harvest-fresh seeds (table 1).

Table 1 *Germination percentage and shoot and root growth of differentially deteriorated rice seeds**

	Age of seed (Year)			
	0	1	2	3
Germination (%)	100	100	90	0
Mean shoot length (mm)	29.1 ±0.60	20.6 ±0.17	15.9 ±0.13	0
Mean root length (mm)	49.5 ±2.06	40.3 ±3.15	22.8 ±2.27	0

*Data computed from three replications of 30 seeds each

The sprouting of embryos of 1-, 2-and 3-year-old seeds transplanted on endosperms of high-vigour (harvest-fresh) seeds, was the same as the germination percentage of whole seeds. On the other hand, high-vigour embryos transplanted on deteriorated endosperms showed very good sprouting in all the cases. These results would, in general, confirm the highly predominant role of embryo in seed germination (Hallam 1972, 1973, Hallam et al. 1972a). The growth of shoot and root of embryos from variously aged seeds transplanted on high-vigour endosperms was less than that in whole seeds but the decrease with ageing showed a similar trend to that noted in whole seeds. Thus, longer storage under warm-humid conditions greatly reduced shoot and root growth of embryos (figure 1A).

The controlling effect of the endosperm on the growth of shoot and root cannot, however, be overlooked. While the capacity to germinate rests with the embryo, the endosperm is responsible for supporting the subsequent growth. Thus when the embryo was dead, the endosperm was functionless. But within limits, the endosperm of the non-viable seed (3-year-old) would still be capable of supporting the growth of high-vigour embryos transplanted on them (figure1B). The endosperm appears to be more resistant to the deteriorative process than the embryonic tissues; but if its damage is too much, the growth of the embryo, even if it is highly vigorous, would be affected. The aged (3-year-old) endosperm, in fact, greatly reduced shoot and root length of transplants (figure 1B).

Our results suggest that the damage to the aleurone cell of the endosperm is one of the basic reasons of its failure to support embryo growth. When the aleurone cells of the endosperm of high-vigour seeds are removed partially by carefully scraping off the monolayer, it would reduce seedling growth (figure 2). Complete removal of

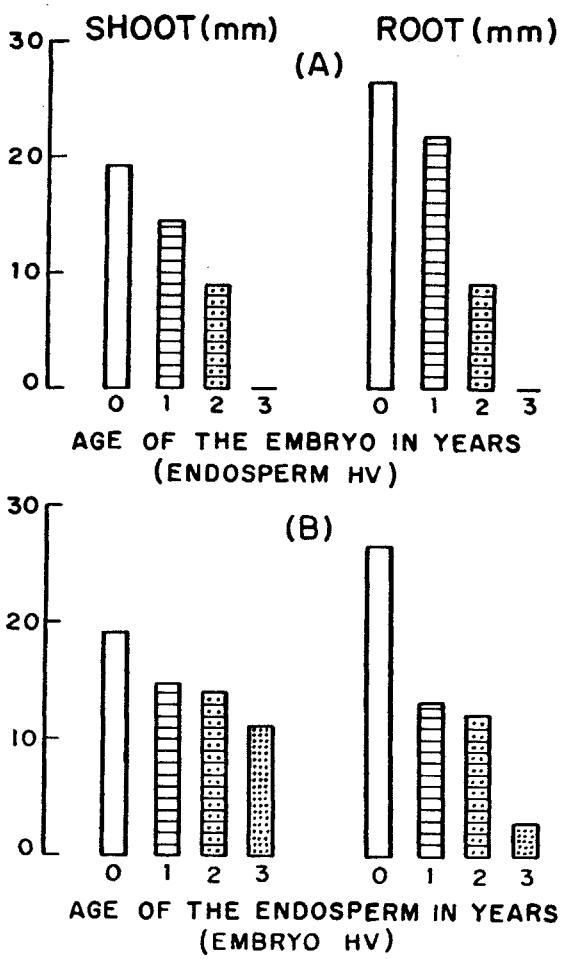

Figure 1 (A) Shoot and root growth of differentially deteriorated embryos of 0-3-year-old seeds transplanted on high-vigour endosperms, and (B) growth of high-vigour embryos transplanted on differentially deteriorated endosperms of 0-3-year-old seeds

HV-high-vigour embryo or endosperm of harvest-fresh seed; 0, 1, 2, 3-age of the seed in years

aleurone of high-vigour endosperm altogether failed to support the growth of high-vigour embryos transplanted on to them (table 2). Even in whole seeds, removal of aleurone cells gave identical results.

We have studied the question of α-amylase induction in aged endosperms (isolated from 0–3-year-old seeds) as well as in endosperms having decreasing amount of aleurone

Table 2 *Role of aleurone on the germinability of high-vigour embryo of rice seeds**

Treatments	Germination (%)	Mean shoot length (mm)	Mean root length (mm)
Whole seed (endosperm with aleurone)	100	30.9 ±1.0	56.0 ±2.36
Whole seed (endosperm without aleurone)	100	1.5 ±0.17	1.2 ±0.11
Isolated embryo (without endosperm)	100	1.4 ±0.5	0
Transplanted embryo on endosperm with aleurone	100	19.0 ±1.84	26.3 ±1.04
Transplanted embryo on endosperm without aleurone	100	1.4 ±0.21	1.0 ±0.11

*Data computed from three replications of 10 seeds/transplants each

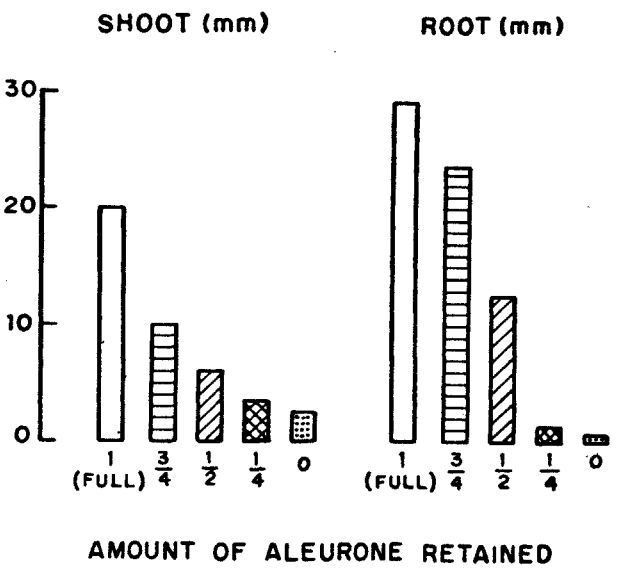

Figure 2 Shoot and root growth of harvest-fresh (high-vigour) rice seeds having different amount (full, 3/4, 1/2, 1/4 and nil) of aleurone tissue on the endosperm

tissue. Seeds stored for longer durations under warm-humid conditions showed a gradual fall in germinability. This was accompanied by a corresponding reduction in the synthesis of α-amylase in whole seeds as well as in embryo-less half seeds (table 3). A similar trend in GA-induced α-amylase synthesis was observed in the scraped endosperms (containing 3/4, 1/2, 1/4, 0 aleurone tissue). Synthesis of α-amylase was proportional to the amount of aleurone retained on the high-vigour endosperms (table 4). The data would, however, show that GA-induced α-amylase induction could be noted to a certain extent in seeds which had just lost viability. In those seeds, in spite of the failure of the embryo to sprout, appreciable α-amylase induction could be noted when the half seeds were incubated with GA.

Table 3 *α-Amylase activity of differentialy aged rice seeds*

(Starch hydrolyzed/hr in μg)

Age of seed (Year)	Full seed+	Half-seed incubation++	
		Without GA	With GA
0 (harvest-fresh)	159.0	4.4	18.6
1	152.1	3.1	16.5
2	106.3	2.3	12.5
3	19 1	2.0	8.5

* Values expressed as μg of starch hydrolyzed by enzyme equivalent of one half-seed

\+ Full seed was allowed to sprout for 48 hr, then one-half of the seed (embryo-less half) was cut off and enzyme extracted for assay

++Embryo-less half seeds were incubated for 48 hr with or without GA (5×10^{-6}M) followed by extraction and assay of enzyme

Table 4 *α-Amylase activity of harvest-fresh (high-vigour) rice seeds having different amounts of aleurone tissue on the endosperm*

Starch hydrolyzed/hr (in μg)

Amount of aleurone on endosperm	Full seed+	Half-seed incubation++	
		Without GA	With GA
0 (full)	178.1	4.0	21.2
3/4	149.5	3.9	17.7
1/2	134.2	3.6	15.7
1/4	95.0	3.1	10.1
0 (nil)	45.1	2.1	4.0

Other details same as in Table 3

Whether any mutagenic or inhibitory substance is translocated from the deteriorated endosperm to the embryo resulting in a reduction of growth (D'Amato 1964, Avanzi et al. 1967, Melleti et al. 1968, Floris 1970) requires further investigation. We have however, noted that keeping a large number of non-viable or highly irradiated (120 Kr-X-ray) seeds in the germination medium or addition of extracts of non-viable and irradiated seeds to the medium would not noticeably reduce the growth of high-vigour seedlings or high-vigour embryos transplanted on high-vigour endosperms. Any leaching of inhibitors or mutagenic substances should have adversely affected embryo growth.

It would therefore appear that the failure of the aleurone cells of deteriorated endosperm to induce the synthesis of enzymes necessary for hydrolysis and mobilization of reserve materials is a basic reason of poor growth of high-vigour embryos transplanted on them.

Acknowledgements

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