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Ageing and Anatomical Influence on Seed Storability in Rice (*Oryza sativa* L.) Hybrids and Parental Lines

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ABSTRACT. *A study on effect of morphological variation and anatomy of seeds on storability of two rice hybrids (ADTRH 1 and CORH 2) and parental lines (A, B and R lines) was carried out by conducting natural and accelerated ageing methods. The results revealed that the seed storability of R lines (C 20 R and IR 66 R) was superior to that of the hybrids (ADTRH 1 and CORH 2), female parent (IR 58025 A) and maintainer line (IR 58025 B). Between hybrids, ADTRH 1 performed better than CORH 2 in both natural and accelerated ageing conditions. Physiological parameters of seed quality such as germination, root length, vigor index and electrical conductivity of seed leachate showed similar changes in both natural and accelerated ageing conditions. The histological study on stored seeds of rice hybrids and parental lines revealed that C 20 R and IR 66 R exhibited anatomically more favorable features for better storability than all the other lines. B line (IR 58025 B) also showed a considerable level of storability. The favorable features observed in those lines were thick cuticle, intact and well preserved scleroids of husk along with well developed aleurone layer with prominent nuclei and dense cytoplasm. It could be concluded that R lines and B line have high storability compared to A line and their respective hybrids.*

INTRODUCTION

Rice (*Oryza sativa* L.) is a major dietary staple food for high percentage of the world's population particularly in Asia, where more than 90% of rice is grown. It is consumed by about 60% of the people in the Globe (Kumar and Prasanna, 2001). In India, rice ranks the first in area under cultivation with 44.97 million ha and also in production of 89.48 million tonnes (Anon, 2000). Among the many genetic approaches being explored to break the yield barrier in rice, hybrid rice technology appears to be the most feasible and readily adoptable one (Janaiah and Hossain, 2000). China has successfully demonstrated the usefulness of hybrid rice to meet increased demands for rice. On an average, hybrid rice in China yields about 27% (1.5 million t/ha) more than the inbred high-yielding varieties (Yuan, 1996). India has emerged as the second largest hybrid rice-growing country in the world (Yuan, 1997). However, the success in hybrid rice in India could be visualized only if there is adequate quantity of quality hybrid seeds made available for farmers (Ponnuswamy *et al.*, 2000).

Maintenance of seed vigor and viability during storage is a matter of prime concern in India. Owing to the prevailing sub-tropical climate in the major parts of the country, seeds of most crop species show rapid deterioration and hybrid rice is no exception. In general, there are differences among species (Agrawal, 1976) and also among varieties within a species (Agrawal, 1978) with respect to loss of viability during storage of rice. Differential storability of rice hybrids and parental lines was reported by several authors (Kalavathi *et al.*, 1999; Kamaraj and Krishnasamy, 2002). However,

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several authors (Kalavathi *et al.*, 1999; Kamaraj and Krishnasamy, 2002). However, information on the effect of anatomical features on seed storability, particularly of hybrid rice is lacking. Hence an attempt was made to study the anatomical effect on seed storability by a histological study in rice hybrids ADTRH 1 and CORH 2 and their parental lines IR 58025 A, IR 58025 B, IR 66 R and C 20 R.

MATERIALS AND METHODS

Freshly harvested and graded seeds of rice hybrids (ADTRH 1: IR 58025 A x IR 66 R), and (CORH 2: IR 58025 A x C 20 R) and their parental lines (A line: IR 58025 A; B line: IR 58025 B; and R lines: IR 66 R and C 20 R) were tested for initial germination and those showed the initial germination more than 90% were dried to 11% moisture content, cleaned, treated with Thiram (2 g/kg of seed) and used for the study. Experiment was conducted in a factorial arrangement using Complete Randomized Design.

Natural ageing

Seed samples (250 g) from each genotype were packed in fresh gada cloth bag and kept under ambient storage condition (room temperature ranged from 29-38°C and relative humidity varied from 81-93%) at Department of Seed Science and Technology, Coimbatore for six months. At monthly intervals, seed samples were drawn from each genotype and evaluated for germination based on ISTA (1999) guidelines.

Accelerated ageing

Seed samples (100 g) from each genotype were packed in perforated butter paper cover and kept under accelerated ageing condition (40°C and 100% relative humidity) maintained at B.O.D. incubator. Samples were drawn at three days intervals upto nine days from each genotype and evaluated for germination.

Seed germination

Four replicates of 100 randomly selected seeds were used for germination in roll towel in the germination room maintained at a temperature of 25 ± 1°C and 95 ± 3% relative humidity and evaluated after 14 days. All normal seedlings (seedlings with well-developed root and shoot systems) were counted and expressed as germination in percentage. Seed germination experiments were carried out based on ISTA (1999) guidelines.

Root length

At final count, ten normal seedlings in each replication were taken at random and the length of root was measured from collar region to tip of the primary root and the mean values were calculated.

Shoot length

Ten normal seedlings selected for root measurement were again measured from collar region to tip of the primary leaf and the mean values were calculated.

Vigour index

The vigour index of the seedling was calculated using the following formula suggested by Abdul-Baki and Anderson (1973):

Vigour index = Germination (%) x Total seedling length (cm)

where, Total seedling length = shoot length + root length.

Electrical conductivity of seed leachate

Fifty seeds were randomly taken and soaked in 50 ml of de-ionized water for 16 h at room temperature and decanted to obtain the leachate (Presley, 1958). The electrical conductivity of seed leachate was measured using a Digital Conductivity Meter (Type MCD 287) with an electrode possessing cell constant of 1.0 and expressed in decisimens (dSm^{-1}).

Histological studies

Histological studies were made with seeds of rice hybrids and parental lines stored for six months. The materials were processed and sectioned by rotary microtome adopting the method suggested by Johansen (1940) and magnified upto 100 µm and viewed under binocular microscope.

RESULTS AND DISCUSSION

Storage potential of hybrids and parental line seeds

Natural ageing

Seeds, like any other living organisms are subjected to deterioration with age. The seed deterioration proceeds at varying rates depending on the kind and type of seed, environmental and pathological factors, and storage conditions. The deterioration process continues until the seed is dead (Roberts and Ellis, 1980). The initial germination of fresh seeds of both ADTRH 1 and CORH 2 rice hybrids and their parental lines (IR 58025 A, IR 58025 B, IR 66 R and C 20 R) was more than 90%. After six months of natural ageing under ambient storage conditions, the germination of both the R lines (IR 66 R and C 20 R) was the highest (90%) by IR 58025 B line (Table 1).

The rate of reduction in germination was minimum in R lines, while maximum in IR 58025 A line. Though A line recorded considerable initial germination, it deteriorated faster than other genotypes under storage as reported by Cheng (1993). Superiority of male parent over the female observed in the present study had also been reported earlier by Prabagaran (1996) in hybrid rice. According to Kalavathi *et al.* (1999) also, R lines were better storers than A lines of rice hybrids. Between hybrids tested in the present study, ADTRH 1 maintained higher germination than CORH 2. Kamaraj (2001) observed the superiority of hybrids (ADTRH 1 and CORH 2) over the seeds of male sterile line IR 58025 A in maintaining higher germination and vigour. As in germination, vigour components studied (root length and vigour index) also exhibited similar trend as reported by Cheng (1993) and Prabagaran (1996) (Tables 2-4).

Table 1. **Influence of natural ageing on seed germination (%) in rice hybrids and parental lines.**

Genotypes (G)	Period of storage (months) (P)				
	Initial	3	4	5	6
IR 58025 A	93	90	86	80	74
IR 58025 B	93	92	92	90	88
IR 66 R	95	94	92	92	90
C 20 R	98	95	92	92	90
ADTRH 1	94	92	90	87	80
CORH 2	92	90	84	82	75
Mean	94	92	89	87	83
	G		P		GxP
SE	1.43		1.56		3.50
CD (p=0.05)	2.92		3.19		7.14

CD = Critical difference

Table 2. **Influence of natural ageing on root length (cm) of seedling in rice hybrids and parental lines.**

Genotypes (G)	Period of storage (months) (P)				
	Initial	3	4	5	6
IR 58025 A	22.8	22.0	21.4	20.4	19.1
IR 58025 B	21.2	21.0	20.7	20.2	19.3
IR 66 R	23.5	23.0	22.6	21.9	21.5
C 20 R	25.2	24.7	23.9	22.8	22.5
ADTRH 1	21.8	21.4	21.2	20.4	19.4
CORH 2	23.9	23.5	22.8	21.2	20.0
Mean	23.1	22.6	22.1	21.2	20.3
	G		P		GxP
SE	0.48		0.53		NS
CD (p=0.05)	0.98		1.08		NS

CD = Critical difference; NS = Not significant

Accelerated ageing

By artificial ageing the seed, the rate of deteriorative process is rapidly increased. The two most important factors influence the rate of deterioration are temperature and relative humidity (Delouche and Baskin, 1973). It is assumed that the process of deterioration of accelerated ageing condition is same as that in natural ageing but only the rate of deterioration is rapid. It increases catabolic changes at the cellular level beyond the threshold of tolerance leading to the lethality of such seeds (Balraj *et al.*, 2001).

Deshpande and Mahadevappa (1994) reported that four days of accelerated ageing (43±1°C and 98±1% RH) period was equivalent to six months of natural ageing and eight days of accelerated ageing equivalent to one year of natural ageing in rice.

Table 3. Influence of natural ageing on vigour index in rice hybrids and parental lines.

Genotypes (G)	Period of storage (months) (P)				
	Initial	3	4	5	6
IR 58025 A	3274	3024	2812	2480	2109
IR 58025 B	3143	3008	2953	2718	2490
IR 66 R	3525	3365	3183	3100	2934
C 20 R	3969	3705	3450	3284	3051
ADTRH 1	3309	3156	2952	2732	2288
CORH 2	3496	3285	2974	2706	2288
Mean	3450	3255	3053	2833	2518
CD (p=0.05)	G		P		GxP
	132.52		145.17		NS

CD = Critical difference; NS = Not significant

Table 4. Influence of natural ageing on electrical conductivity of seed leachate (dSm^{-1}) in rice hybrids and parental lines.

Genotypes (G)	Period of storage (months) (P)				
	Initial	3	4	5	6
IR 58025 A	0.082	0.090	0.098	0.106	0.118
IR 58025 B	0.091	0.094	0.097	0.100	0.106
IR 66 R	0.096	0.100	0.103	0.105	0.110
C 20 R	0.109	0.112	0.115	0.119	0.125
ADTRH 1	0.086	0.089	0.092	0.095	0.102
CORH 2	0.101	0.105	0.111	0.115	0.122
Mean	0.094	0.098	0.103	0.107	0.114
	G		P		GxP
SE	0.005		0.002		0.010
CD (p=0.05)	0.011		0.005		0.022

CD - Critical difference

In the present investigation, comparison of accelerated ageing among hybrids and parental lines also revealed the superiority of R lines and B line. Among the genotypes, hybrids deteriorated faster than parents (Table 5). Deshpande and Mahadevappa (1994) also observed the better performance of R lines than that of hybrid rice after accelerated ageing. Accelerated ageing induced the deteriorative process rapidly accompanied by loss in germination due to high levels of temperature and relative humidity employed for accelerated ageing. Similar observations were made earlier by Ramanadane (1995) and Singh *et al.* (2002). Physiological parameters in hybrids and parental line seeds showed similar changes in both natural and accelerated aging conditions. However, the deteriorative process was highly amplified as a result of artificial ageing due to high temperature and relative humidity (Tables 6-8). Similar observations were made earlier by Ramanadane (1995) and Singh *et al.* (2002).

Table 5. Influence of accelerated ageing on seed germination (%) in rice hybrids and parental lines.

Genotypes (G)	Days after accelerated ageing (D)			
	Initial	3	6	9
IR 58025 A	93	80	74	2
IR 58025 B	93	88	76	0
IR 66 R	95	86	73	2
C 20 R	98	94	82	4
ADTRH 1	94	74	62	4
CORH 2	92	46	42	0
Mean	94	78	68	2
	G	D	GxD	
	2.10	1.48	3.64	
CD (p=0.05)	4.41	3.12	7.64	

CD = Critical difference

Table 6. Influence of accelerated ageing on root length (cm) in rice hybrids and parental lines.

Genotypes (G)	Days after accelerated ageing (D)		
	Initial	3	6
IR 58025 A	22.8	22.6	22.0
IR 58025 B	21.2	22.7	20.4
IR 66 R	23.5	22.6	22.3
C 20 R	25.2	24.1	20.4
ADTRH 1	21.8	21.5	21.2
CORH 2	23.9	21.8	21.2
Mean	23.1	22.6	21.3
	G	D	GxD
SE	0.69	0.49	NS
CD (p=0.05)	1.44	1.02	NS

CD = Critical difference; NS = Not significant

In the present investigation, irrespective of hybrids and parental lines, the electrical conductivity of seed leachate was significantly increased with ageing period. Younis *et al.* (1990) found decreased germination potential and increased membrane leakage in seeds of different rice cultivars under accelerated ageing conditions. Percentage of leaching of rice seeds aged for four and eight days exhibited significant positive association with germination (Krishnasamy and Seshu, 1990). Aswathaiah *et al.* (1987) also reported that the loss of germinability was accompanied by increased leaching of organic and inorganic constituents from the seeds during imbibition. Physiological parameters of seed quality like germination, root length, vigour index and membrane integrity as reflected by electrical conductivity of seed leachate showed similar changes in seeds under both natural and accelerated ageing conditions.

Table 7. Influence of accelerated ageing on vigour index in rice hybrids and parental lines.

Genotypes (G)	Days after accelerated ageing (D)		
	Initial	3	6
IR 58025 A	3266	2566	2295
IR 58025 B	3146	2737	2158
IR 66 R	3525	2818	2343
C 20 R	4159	3408	2408
ADTRH 1	2950	2345	1814
CORH 2	3488	1553	1295
Mean	3422	2571	2052
	G	D	GxD
SE	150.04	106.1	259.88
CD (p=0.05)	315.23	222.90	545.99

CD = Critical difference

Histology of rice hybrids and parental lines seeds

The anatomical variations observed in husk and aleurone layer of all the genotypes are illustrated in Plates 1-6 and described in Table 9. Of the six genotypes studied in the present investigation, seeds of C 20 R and IR 66 R exhibited anatomically more favourable features for better storability than the others. Such features includes thick cuticle, intact and well preserved scleroids of the husk, well developed aleurone layers with prominent nuclei and dense cytoplasm (Table 9, Plates 4a and 4b). The aleuron layer secretes α-amylase and proteolytic enzymes, which hydrolyse food reserves of endosperm. The remaining genotypes showed either disintegration of the cells of husk or poor differentiation of aleurone layer. However, ADTRH 1 had physiologically active aleurone layer with dense protoplast and also showed a soft husk compared to R lines. When IR 58025 A and IR 58025 B lines were compared, the former showed tendency for disintegration of sclerotic zones of husk and degeneration of the aleurone layer. IR 58025 B line had intact cellular organization of the husk and active aleurone layer showing in better storability than A line.

Table 8. **Influence of accelerated ageing on electrical conductivity of seed leachate (dSm^{-1}) in rice hybrids and parental lines.**

Genotypes (G)	Days after accelerated ageing (D)		
	Initial	3	6
IR 58025 A	0.082	0.097	0.123
IR 58025 B	0.091	0.103	0.113
IR 66 R	0.096	0.112	0.119
C 20 R	0.109	0.115	0.122
ADTRH 1	0.086	0.091	0.093
CORH 2	0.101	0.120	0.131
Mean	0.094	0.106	0.117
	G	D	GxD
SE	0.006	0.004	0.011
CD (p=0.05)	0.013	0.009	0.023

CD = Critical difference

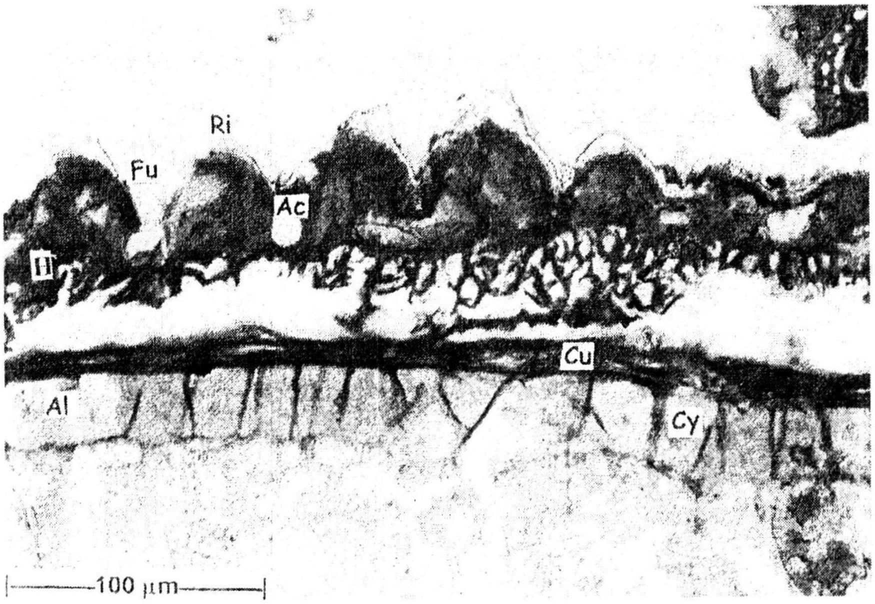

Plate 1. **T.S. of IR 58025 A rice grain – husk, aleurone layer and endosperm.** H = Husk; Fu = Furrow; Ri = Ridges; Ac = Air cavity; Cu = Cuticle; Al = Aleurone layer; Cy = Cytoplasm.

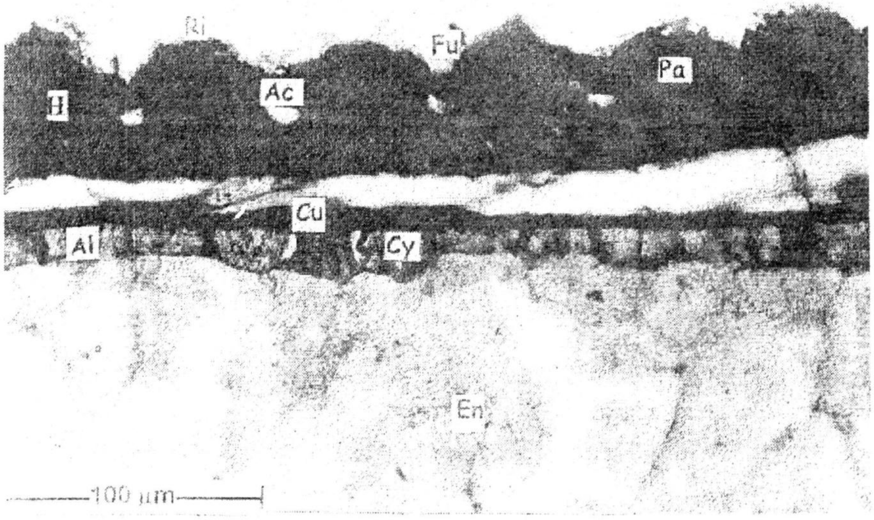

Plate 2. **T.S. of IR 58025 B rice grain – husk, aleurone layer and endosperm.**
H = Husk; Fu = Furrow; Ri = Ridges; Ac – Air cavity; Pa = Parenchyma; Cu = Cuticle; Al = Aleurone layer; Cy = Cytoplasm; En – Endosperm.

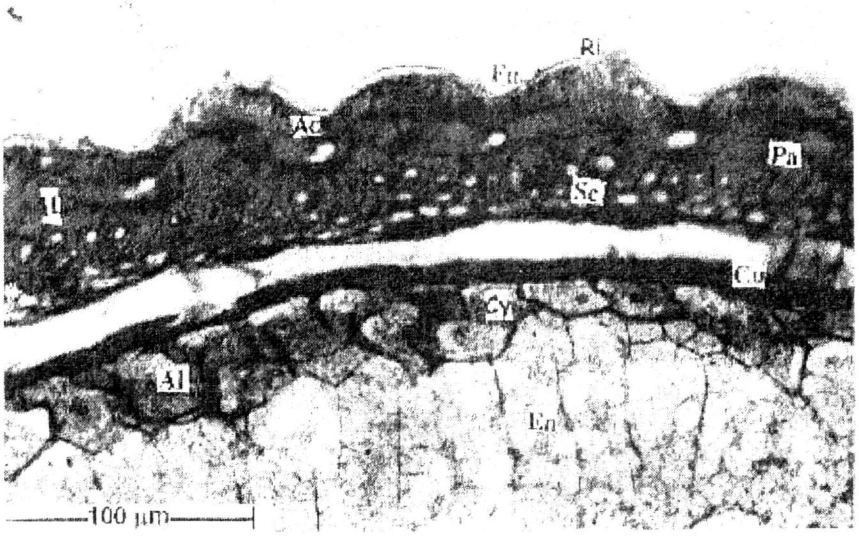

Plate 3. **T.S. of IR 66 rice grain – husk, aleurone layer and endosperm.**
H = Husk; Fu = Furrow; Ri = Ridges; Ac – Air cavity; Pa = Parenchyma; Sc – Scleroids; Cu = Cuticle; Al = Aleurone layer; Cy = Cytoplasm; En = Endosperm.

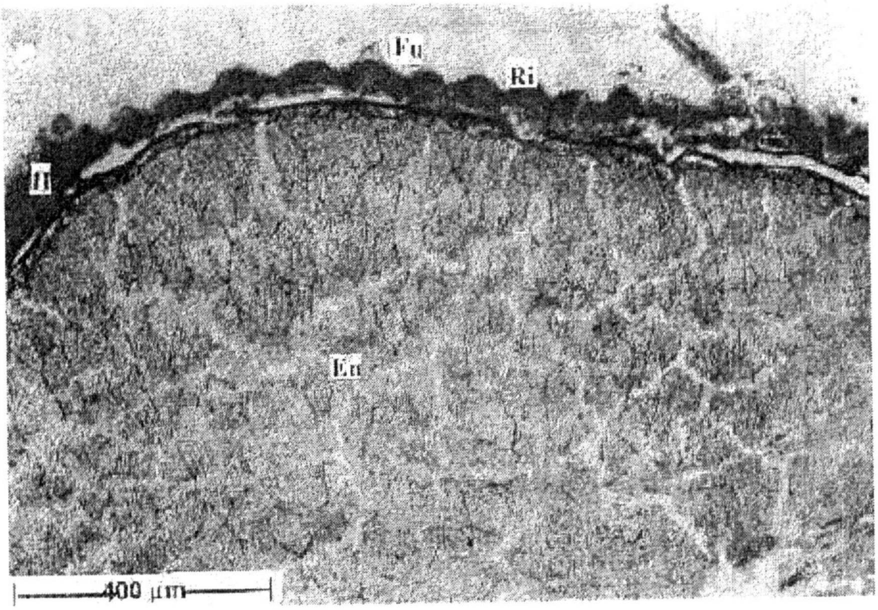

Plate 4a. **T.S. of CR 20 rice grain – husk, aleurone layer and endosperm.**
H = Husk; Fu = Furrow; Ri = Ridges; En = Endosperm.

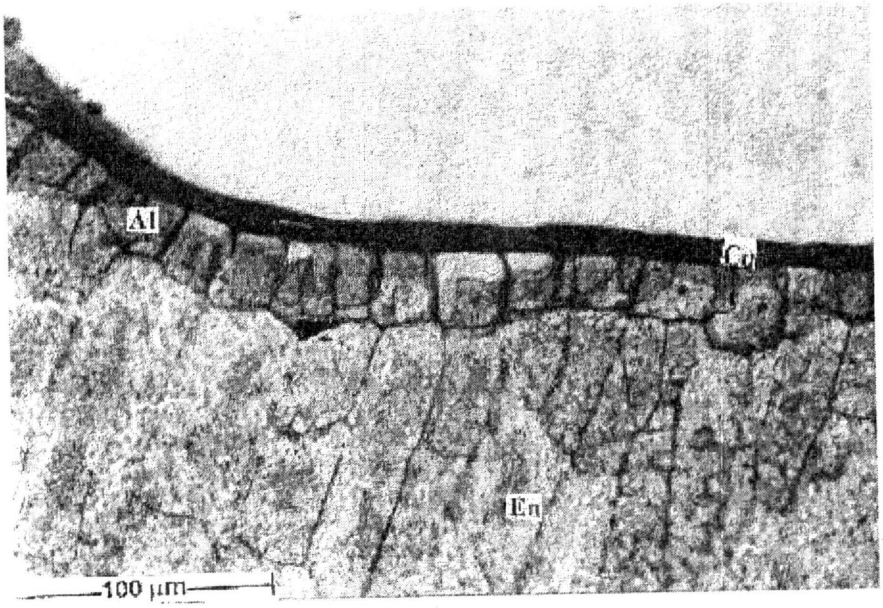

Plate 4b. **T.S. of C 20 R rice grain – husk, aleurone layer and endosperm.**
Cu = Cuticle; Al = Aleurone layer; Cy = Cytoplasm.

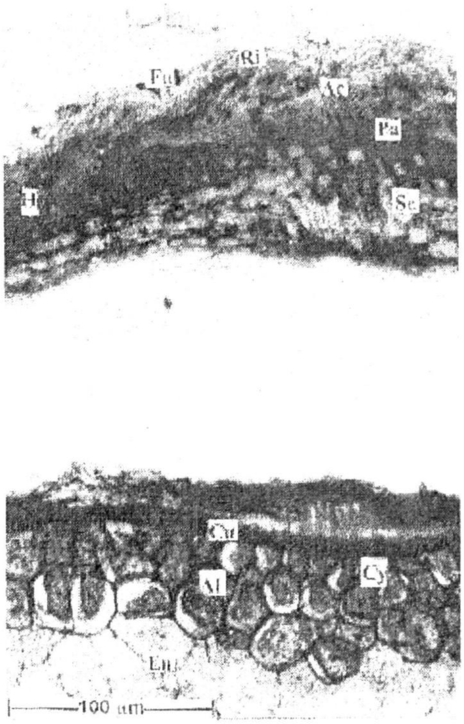

Plate 5. **T.S. of ADTRH 1 rice grain – husk, aleurone layer and endosperm.**
H = Husk; Fu = Furrow; Ri = Ridges; Ac = Air cavity; Pa = Parenchyma; Sc = Scleroids; Cu = Cuticle; Al = Aleurone layer; Cy = Cytoplasm; En = Endosperm.

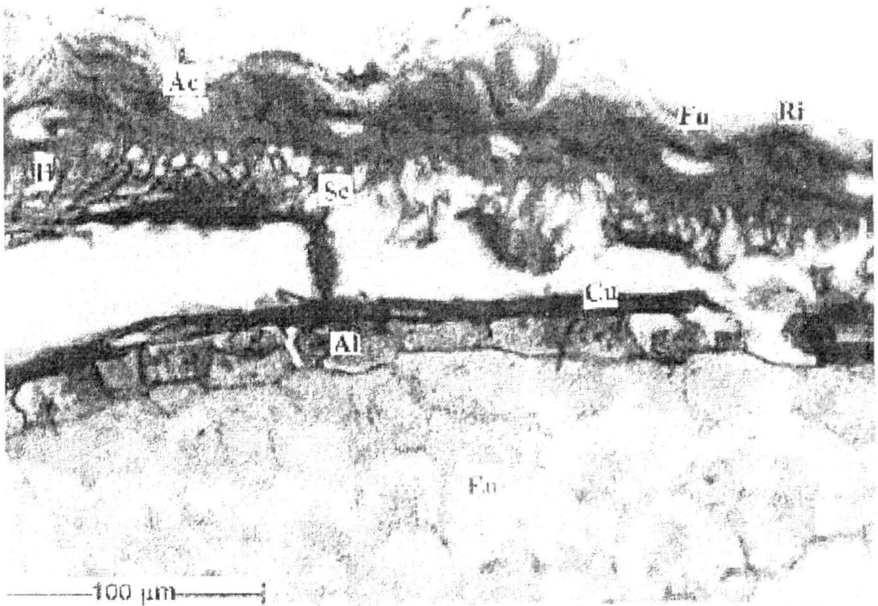

Plate 6. **T.S. of CORH 2 rice grain – husk, aleurone layer and endosperm.**
H = Husk; Fu = Furrow; Ri = Ridges; Ac = Air cavity; Pa = Parenchyma; Sc = Scleroids; Cu = Cuticle; Al = Aleurone layer; En = Endosperm.

Table 9. Anatomical variation of husk and aleurone layer of the caryopsis of rice hybrids and parental lines

Genotypes	Husk	Aleurone layer
IR 58025 A	Scleroid zone destroyed; ridges and furrows are more prominent and cuticle is less distinct (Plate 1).	Aleurone cells disintegrated; nuclei not evident; cytoplasmic contents are poor (Plate 1).
IR 58025 B	Slightly reduced in thickness. Furrows are small, air cavities are prominent than other genotypes. The cuticle is prominent (Plate 2).	Narrow, the cells are squarish; cytoplasm is dense, the nuclei is less evident (Plate 2).
IR 66 R	Thickest and consists of 4 or 5 layers of scleroids and outer ridges and furrows. The cuticle is prominent (Plate 3).	Aleurone cells have thick walls, prominent nuclei and dense cytoplasm. However, the cells are single layered (Plate 3).
C 20 R	Shows distinct ridges and furrows and prominent cuticle (Plates 4a and 4b).	Appears normal; cells are radially oblong, thick walled, prominently nucleated; the cytoplasm is fairly dense (Plates 4a and 4b).
ADTRH 1	Exhibits high degree of disintegration of scleroids; and the ridges and furrows also become less distinct (Plate 5).	Well developed; the nuclei are prominent and the cell contents are highly dense-dense protoplast (Plate 5).
CORH 2	Exhibits high degree of disintegration of scleroids (Plate 6).	Have narrow tangentially compressed cells. The cells have prominent nuclei and fairly dense cytoplasm. The cells are in single row (Plate 6).

CONCLUSIONS

It could be concluded that the storability of R lines (IR 66 R and C 20 R) were superior to A line and B line and their hybrids. Between hybrids, ADTRH 1 performed better than CORH 2. The high storability shown in R and B lines compared to A line and their hybrids evidenced that the anatomical variation observed in hybrid seeds have an influence on their storability.

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