Relationship between Endogenous Germination Inhibitors and Dormancy in Rice Seeds

By MITSURU HAYASHI

Faculty of Agriculture, Kagoshima University
(Korimoto, Kagoshima, 890 Japan)

There are two theories concerning seed dormany of rice[4]. One is that the dormancy may result from endogenous inhibitors existing in the hull of seeds since the dormancy is effectively removed by dehulling. The other is that the dormancy may result from the limited entry of oxygen into embryos due to covering structures because the dormancy is removed not only by dehulling, but also by an injury of covering structures, and high temperature treatments applied to seeds. As for the removal of the dormancy, the former concerns the mechanical removal of the inhibitors contained in the hull, while the latter concerns some chemical reactions occurring in the seeds.

The present investigation was carried out with an aim of clarifying the relationship between the dormancy-breaking in rice seeds and corresponding changes in the level of endogenous germination inhibitors.

Determination of endogenous germination inhibitors in rice seeds

A technique of paper chromatography followed by a bioassay has been used in studies of seed dormancy of various plants[1,5,15] and endogenous inhibitors have been shown to play a part in seed dormancy[4,9,10,14,16,18]. Concerning rice seed dormancy, however, it has not been proved conclusively whether the inhibitors regulate the dormancy or not, although several workers have suggested that inhibitors present in seed coats may play a role in causing dormancy[6,18]. Therefore, this investigation was carried out by means of chromatography and bioassays, to prove the existence of inhibitors, and to correlate biological and chemical properties of the inhibitors to rice seed dormancy.

1) *Biological determination of germination inhibitors*

The inhibitors of dormant seeds and that of non-dormant seeds were assayed, using paper chromatography, *Avena* straight growth test, and germination test of excised embryos. The germination test is based on measuring percent germination of embryos, excised from non-dormant rice seeds and sown on culture media containing germination inhibitors extracted from dormant seeds.

Activities of the growth inhibitors which were detected only in the dormant seeds, were found only in the acidic ethyl ether soluble fraction of the extract, and their Rf zones on chromatogram developed with ammoniacal isopropanol (8:1:1 v/v) were Rf 0.6–0.8 and Rf. 0.9–1.0. These were hereafter referred to as inhibitor-A and inhibitor-B, respectively (Figs. 1 and 2). Both of the inhibitors were obviously proved to be genuine germination inhibitors by the germination test of excised embryos. Furthermore, the inhibitor-A showed stronger inhibitory action on seed germination than the inhibitor-B (Table 1).

2) *Chemical determination of germination inhibitors*

The inhibitors of dormant seeds were identified by means of chromatography.

Inhibitor-A: Using the strongly dormant

Table 1. Effects of endogenous inhibitors extracted from dormant seeds (var. Ketaktara) on the germination of excised embryos of non-dormant seeds (var. Hadsaduri)

Days after sowing	No inhibitor (cont.)	Inhibitor-A*			Inhibitor-B*		
		25	50	100	25	50	100
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
3	58	29	17	15	60	36	33
7	100	100	100	96	96	96	96

* Inhibitor-A and Inhibitor-B were eluted from Rf 0.6—0.8 and 0.9—1.0, respectively, on paper chromatograms of acidic fraction of extract.
Relative concentration (100, 50, and 25): The elution was diluted with water 1, 2, and 4 times, respectively.

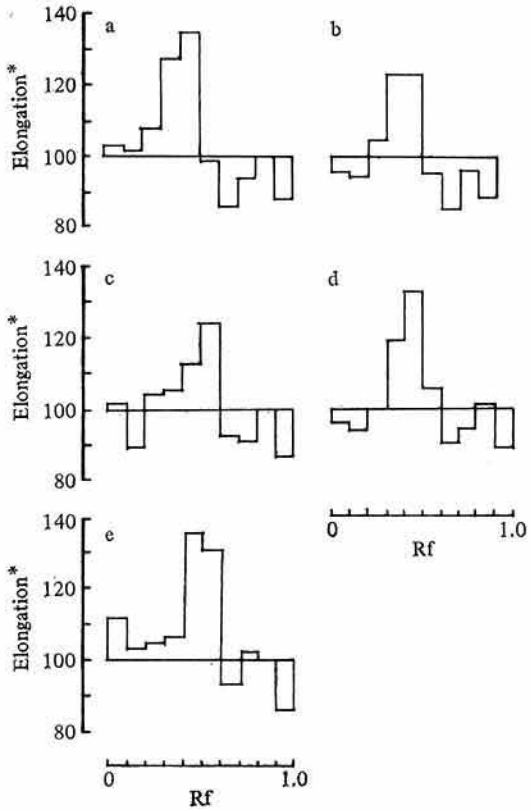

Fig. 1. Histograms representing the *Avena* straight growth test of acidic fraction of extract obtained from dormant seeds of five varieties
a; Ketaktara, b; Hadsaduri,
c; Norin No. 48, d; Kumari,
e; Gendjah.
* Relative length of *Avena* coleoptile (control=100).

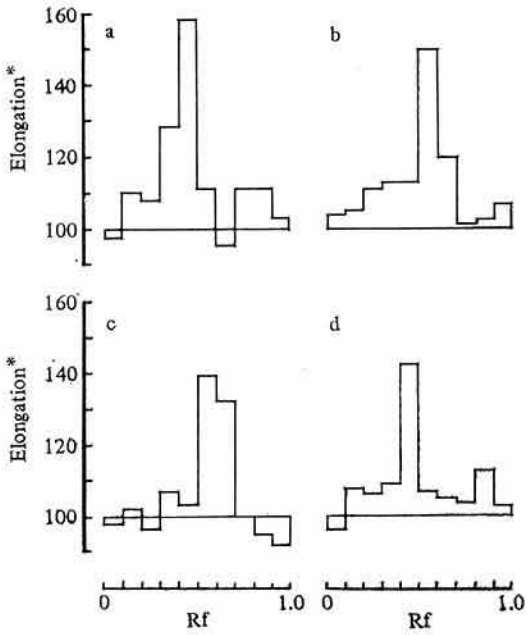

Fig. 2. Histograms representing the *Avena* straight growth test of acidic fraction of extract obtained from husked seeds of non-dormant varieties
a; Akebono, b; Sendai,
c; Russian 120, d; Peta 23.
* Relative length of *Avena* coleoptile (control=100).

seeds, the acidic ethyl acetate soluble fraction of the extract was fractionated by two kinds of column chromatography, i.e., firstly by charcoal adsorption chromatography and secondly by silicic acid partition chromatography. Then, all of the samples fractionated were bioassayed. The sample of a fraction which showed the strongest inhibitory activity

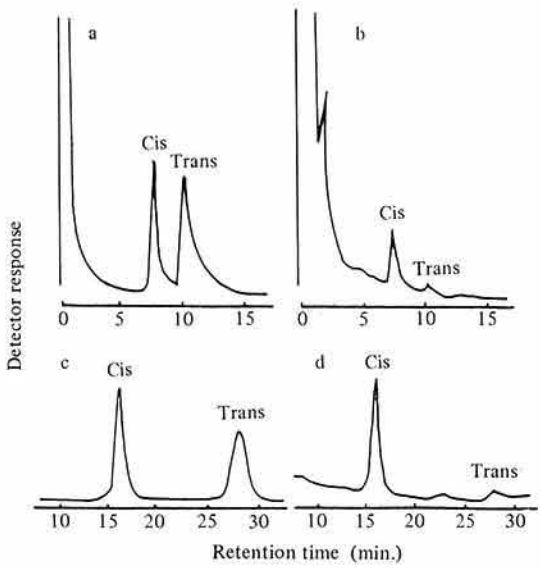

Fig. 3. Gas chromatograms of the methylated sample eluted with 30% ethyl acetate in n-hexane solution on a partition chromatography (b and d) and of the methylated authentic ABA (a and c) on the column of silicon SE-30 (a and b) and silicon XE-60 (c and d)

in the bioassay was methylated with diazomethane and thereafter was analyzed by gas chromatography with two kinds of glass columns packed with different silicons at 210°C[3]. This substance (inhibitor-A) showed the same retention time as authentic abscisic acid (±ABA), and the detected ABA of the extract was composed almost entirely of cis-ABA with small quantity of trans-ABA (Fig. 3).

Inhibitor-B: On the basis of the fact that the inhibitor-B on paper chromatograms gave reddish color in the color reaction to Ehrlich's reagent, it was estimated to be an indolic compound[4].

3) Distribution of the inhibitors in seed organs

It has been considered that a sort of inhibitor which might be able to induce seed dormancy may be contained in the hulls of rice seeds because the dormancy is effectively removed by dehulling. However, in some genetic studies concerning rice seed dormancy, it has been shown that a genetic factor in the embryo may play a part in dormancy[7,17]. Therefore, the distribution of the inhibitor-A and the inhibitor-B in each of the three organs of seeds, i.e., embryo, endosperm, and hull, was examined by means of the biological determination.

It was clearly demonstrated that the two inhibitors existed in all the organs, and that they showed nearly equal biological activities among the three different organs (Fig. 4). This result indicates that, when the activity is expressed per seed, almost equal quantities of the inhibitors are contained in each organ.

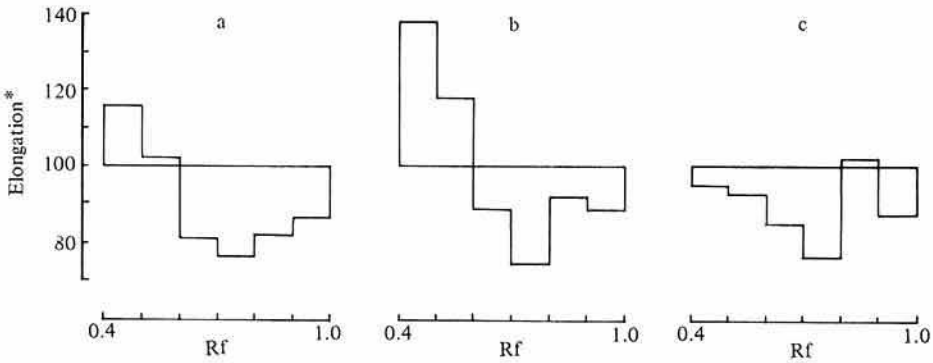

Fig. 4. Histograms representing the *Avena* straight growth test of acidic fraction of extract obtained from embryo, endosperm, and hulls (var. Ketaktara)
a; embryo, b; endosperm, c; hull.
* Relative length of *Avena* coleoptile (control=100).

Thus, it should be noted that the concentration of the inhibitors is the highest in the embryo because of the smallest volume of the embryo.

Quantitative changes of the inhibitors and the dormancy-breaking

1) Under natural condition

A correlation has been shown to exist between seasonal changes in the state of dormancy of buds of certain species and corresponding changes in the level of endogenous inhibitors in the buds. A positive correlation has also been shown between the content of inhibitors and the depth of dormancy of seeds in various plants[14,16]. Thus, the disappearance of the inhibitors is correlated with the release from the dormancy. To see whether such a correlation as found in seeds of other plants could be demonstrated or not between the level of endogenous inhibitors and the rice seed dormancy, the following experiment was carried out.

Using *Avena* straight growth test, levels of the inhibitors in husked seeds and hulls of Hadsaduri (a strongly dormant variety) were assayed several times during a period from the 20th day after flowering to the termination of dormancy. The results showed that both inhibitors (A and B) in the husked seeds and hulls were detected most strongly on the 20th day after flowering and that their levels lowered in proportion to the progress of the dormancy-breaking (Figs. 5 and 6). Then, content of inhibitor-A in seeds of 4 varieties, differing in the length of the dormant period was assayed by the same method. In non-dormant or weakly dormant seeds, the content of the inhibitor decreased suddenly in an early stage of ripening, and germinability of the seeds was promoted in response to the decrease of the inhibitor. On the other hand, the decrease of the inhibitor in strongly dormant seeds was hardly recognized during the ripening period, but the inhibitor was gradually decreased during the period after complete maturity. Thus, a parallel relationship between the dormancy-breaking and the

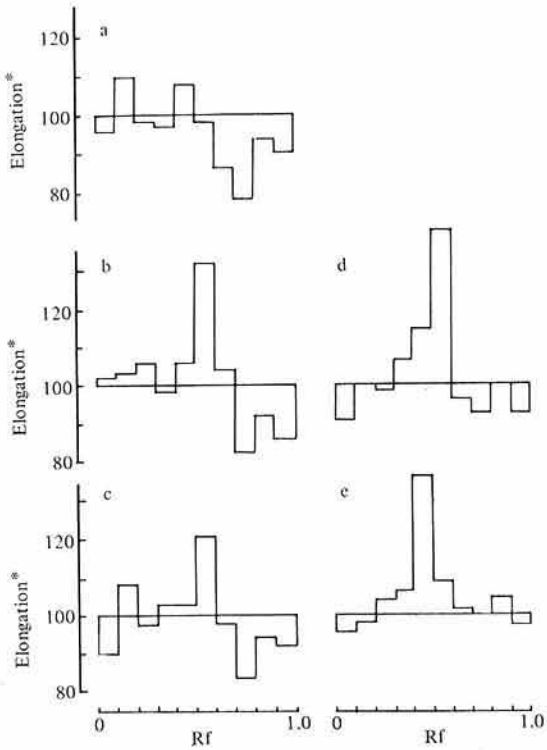

Fig. 5. Histograms representing the *Avena* straight growth test of the acidic fraction of extract obtained from the husked seeds (var. Hadsaduri)
a ; 20 days, b ; 30 days, c ; 45 days, d ; 52 days, and e ; 68 days after flowering.
* Relative length of *Avena* coleoptile (control=100).

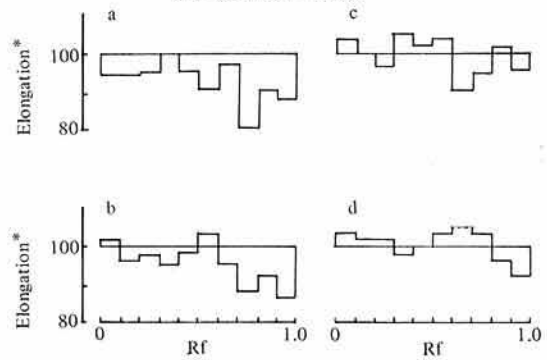

Fig. 6. Histograms representing the *Avena* straight growth test of the acidic fraction of extracts obtained from hulls of seeds (var. Hadsaduri)
a ; 30 days, b ; 45 days, c ; 52 days, and d ; 87 days after flowering.
* Relative length of *Avena* coleoptile (control=100).

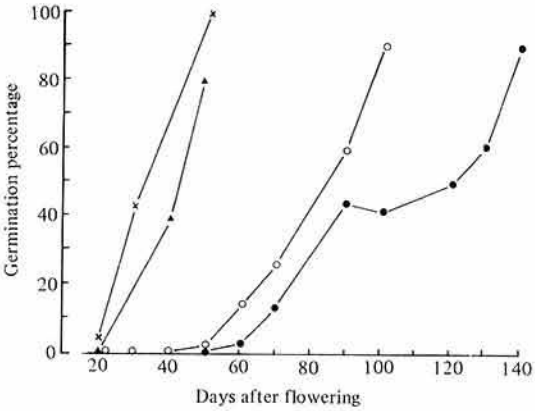

Fig. 7. Relation between transition of seed dormancy and aging of seeds after flowering in 4 varieties
—×—; Norin No. 48,
—▲—; Gendjah, —○—; Hadsaduri,
—●—; Ketaktara.

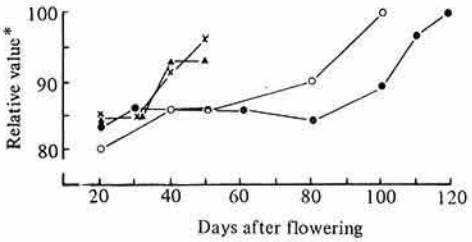

Fig. 8. Relative amount of inhibitor-A eluted from Rf 0.6—0.8 on paper chromatogram of the acidic fraction of extracts of seeds in 4 varieties
—×—; Norin No. 48,
—▲—; Gendjah, —○—; Hadsaduri,
—●—; Ketaktara.
* Relative length of *Avena* coleoptile (control=100).

decrease of the inhibitor was recognized (Figs. 7 and 8). Furthermore, the level of the inhibitors in seeds differing in the degree of dormancy, i.e., seeds showing the germination of 0%, 33%, or 98% was assayed quantitatively by means of germination test of the excised embryos. Again, the level of the two inhibitors showed a close relationship with the degree of the intensity of dormancy of the seeds. Inhibitor-A (ABA) was ascertained to have stronger inhibitory action on seed germination than inhibitor-B, confirming that ABA was the main factor in the rice seed dormancy (Table 2).

2) Under artificial condition

It is already known that the dormancy of rice seeds is broken by some artificial treatments on dry seeds such as high temperature, removal or puncture of covering structures, application of oxygen, etc.[8,12,13]. However, the effect of these treatments on water-soaked seeds is still unknown.

As to this problem, the author[2,4] ascertained that the dormancy of seeds saturated with water was effectively broken by high temperature treatments of a very short period, as compared with that of dry seeds. When soaked seeds were stored at 40°C for 1 to 2 days in air with saturated humidity or when seeds were soaked in 40°C hot-water, which was aerated continuously, for only 1 day, the dormancy of seeds of any variety was completely broken. Furthermore, it was also ascertained that the dormancy-breaking of the

Table 2. Levels of endogenous germination inhibitors (as expressed by percent germination* of excised embryos) in seeds (var. Hadsaduri) with different degrees of dormancy**

Days after sowing	Germination inhibitor-A in seeds with			Germination inhibitor-B in seeds with		
	Strong dormancy	Weak dormancy	No dormancy	Strong dormancy	Weak dormancy	No dormancy
	(%)	(%)	(%)	(%)	(%)	(%)
2	0[b]	1[b]	8[a]	10[c]	18[b]	26[a]
3	25[c]	51[b]	85[a]	90[a]	85[a]	88[a]
4	87[a]	93[a]	91[a]	97[a]	94[a]	93[a]

* The means of 6 replications. The means not bearing the same superscript within a line under each inhibitor are significantly different at the 5% level.
** Germinability of seeds with strong, weak, and no dormancy was 0, 33, and 98%, respectively.

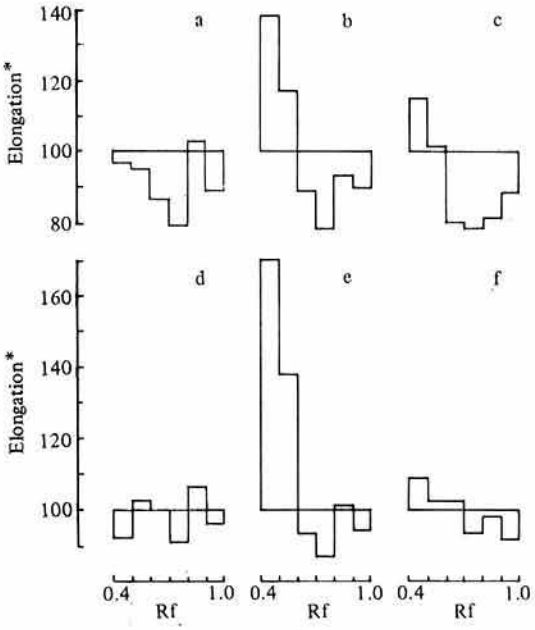

Fig. 9. Histograms representing the *Avena* straight growth test of the acidic fraction of extract obtained from hulls (a, d), endosperms (b, e), and embryos (c, f)
a, b, c: from untreated seeds.
d, e, f: from seeds soaked in water (30°C) for 24 hr, and treated with high temperature (40°C, 2 days).
* See Fig. 8.

soaked seeds treated or not treated with high temperatures was promoted under high oxygen tension, but was inhibited under low oxygen tension.

From these results, it was postulated that the dormancy-breaking in rice seeds was dependent on some oxidation reaction which might be related to inactivation or destruction of endogenous inhibitors. From this viewpoint, the relation between the treatments effective in removing dormancy and the level of the inhibitors was investigated by means of bioassays.

The levels of both inhibitors in hulls, endosperms, and embryos of soaked seeds treated with high temperature of 40°C for 2 days were obviously lowered at the same rate in all organs (Fig. 9).

When several artificial treatments such as high temperature, husking or injury, were given to soaked or dry seeds, the lowering in the level of the inhibitors occurred with all kinds of treatments, but the lowering was much more remarkable in soaked seeds than in dry seeds (Tables 3 and 4).

The level of the inhibitors in the soaked seeds treated with high temperature was lowered remarkably under the condition of 100% oxygen, but it stayed unchanged under the condition of 0% oxygen (Table 5).

From all these results, it was obviously proved that the artificial dormancy-breaking was also associated with the decrease of the inhibitors similar to the case of the natural dormancy-breaking and it was a common fea-

Table 3. Levels of the endogenous germination inhibitors (as expressed by percent germination* of excised embryos) in seeds (var. Hadsaduri) treated with high temperature**

Days after sowing	Germination inhibitor-A in seeds				Germination inhibitor-B in seeds			
	Not treated	Treatment			Not treated	Treatment		
		I	II	III		I	II	III
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
2	0[c]	9[b]	29[a]	18[a]	12[b]	45[a]	58[a]	30[a]
3	27[b]	85[a]	89[a]	75[a]	80[a]	94[a]	94[a]	87[a]
4	88[a]	93[a]	96[a]	89[a]	95[a]	97[a]	97[a]	97[a]

* See the note of Table 2.
** Treatment I: Seeds were placed at 50°C for 10 days.
II: After the treatment I, the seeds were soaked in water for 2 days.
III: Seeds were directly soaked in continuously aerated 40°C hot water for 2 days.

Table 4. Levels of the endogenous germination inhibitors (as assayed by *Avena* straight growth test) in dry or soaked dormant seeds (var. Ketaktara) with or without husking or husk-injuring treatment

Germination inhibitor	Dry seeds			Soaked seeds	
	Not treated	Husking	Injuring	Husking	injuring
A	78.4	86.3	86.5	96.3	95.2
B	76.8	84.9	85.5	96.4	93.1

Figures in the table show relative values to the control (not containing inhibitors) by taking the control=100.

Table 5. Effects of high temperature treatment (40°C, 2 days) with or without oxygen supply on the endogenous germination inhibitors as assayed by percent germination of excised embryos

Days after sowing	Germination inhibitor-A			Germination inhibitor-B		
	No temp. treatment	Treated at		No temp. treatment	Treated at	
		100%	0% oxygen		100%	0% oxygen
	(%)	(%)	(%)	(%)	(%)	(%)
2	0	10	3	10	39	6
3	25	76	27	85	84	87
4	94	96	98	95	99	97

Figures in the table are means of 5 replications.

ture that the decrease of the inhibitors was remarkably promoted by the application of moisture and oxygen to seeds.

It has been suggested that the covering structures of seeds resist the entry of oxygen, and hence the availability of oxygen to embryos in intact seeds is limited[11,13]. It was also suggested that some damage might occur on covering structures of dry seeds treated with high temperature because of the fact that the inhibitor-A (ABA) left in the high temperature-treated seeds disappeared rapidly when the seeds were soaked in water (see Table 3). In this connection, the covering structures (hull) of dry seeds whose dormancy was effectively removed by high temperature (50°C for 10 days) was observed using a scanning electron microscope. Many cracks were found on the hull of the treated seeds. Those cracks were very dense and irregular in shape, with the maximum width reaching 90 μm (Plates 1 and 2). No such cracks were observed on untreated hulls.

Considering that the access of oxygen to embryos in seeds was promoted by the occurrence of cracks in hulls of the treated seeds, it was assumed that an inactivation, or a decrease of the endogenous germination inhibitors in dormant seeds may be completed during a quite short period, as a result of enzymatic oxidation which might be brought about at the time when water and heat were given to the seeds, and this, in turn, resulted in marked reduction of the dormancy of the treated seeds. Thus, it was reasonably assumed that the rate of oxygen entrance into seeds may be proportional to the degree of damage of the covering structures, and hence the lowering of the level of endogenous germination inhibitors in seeds is influenced by the degree of damage of the covering structures.

Conclusive remarks

Concerning rice seed dormancy, a new interpretation was proposed as follows: the main factor inducing seed dormancy is endogenous abscisic acid (+ ABA), a germination in-

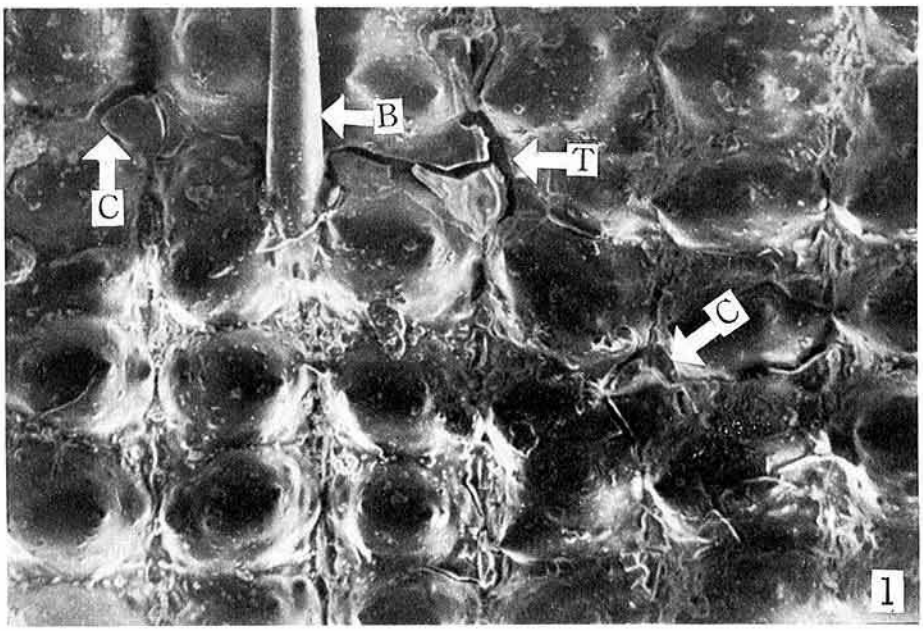

Plate 1. Observation of outer surface structure of a hull of seeds treated at 50°C for 10 days by SEM (×250)
T: longitudinal rows of tubercles, B: a bristle occurred among the rows of tubercles, c: cracks.

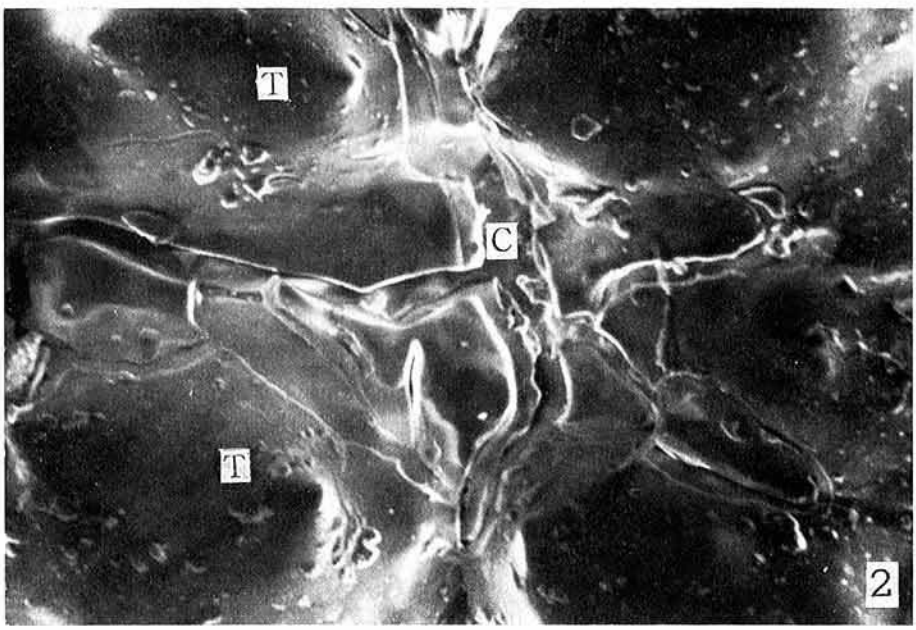

Plate 2. Very large cracks with maximum width reaching about 90μm (×750)

hibitor, and either natural or artificial dormancy-breaking is induced in proportion to the inactivation of the endogenous germination inhibitor through the enzymatic oxidation of the germination inhibitor.

References

1) Eagles, C. F. & Wareing, P. F.: Dormancy regulators in woody plants. *Nature*, 199, 874–875 (1963).
2) Hayashi, M.: Studies on dormancy and germination of rice seed V. *Jpn. J. Trop. Agr.*, 20, 164–171 (1977) [In Japanese with English summary].
3) Hayashi, M.: Studies on dormancy and germination of rice seed VI. *Jpn. J. Trop. Agr.*, 23, 1–5 (1979) [In Japanese with English summary].
4) Hayashi, M.: Physiological studies on the relationship between levels of the endogenous germination inhibitors and the dormancy of rice seed. *Bull. Fac. Agr. Kagoshima Univ.*, 36, 1–43 (1986) [In Japanese with English summary].
5) Hemberg, T.: Growth-inhibiting substances in terminal buds of fraxinus. *Physiol. Plant.*, 2, 37 (1949).
6) Ikeda, M.: Studies on the viviparous germination of rice seed. *Bull. Fac. Agr. Kagoshima Univ.*, 13, 89–115 (1963) [In Japanese with English summary].
7) Ikehashi, H.: Studies on the environmental- and varietal differences of germination habits in rice seeds with special reference to plant breeding. *J. Centr. Agr. Exp. Sta.*, 19, 1–60 (1973) [In Japanese with English summary].
8) Jennings, P. R. & Jesue, de. J.: Effect of heat on breaking dormancy in rice seed. *Crop. Science*, 4, 530–553 (1964).
9) Khan, A. A.: Seed dormancy, changing concepts and theories. *In* The physiology and biochemistry of seed dormancy and germination. ed. Khan, A. A., North-Holland Pub. Co. Oxford, 29–50 (1977).
10) Lipe, W. N. & Orane, J. C.: Dormancy regulation in peach seeds. *Science*, 153, 541 (1966).
11) Porter, N. G. & Wareing, P. F.: The role of the oxygen permeability of the seed coat in the dormancy of seed of *Xanthium pennsylvanicum* Wallr. *J. Exp. Bot.*, 25, 583–594 (1974).
12) Roberts, E. H.: Dormancy in rice seed III. *J. Exp. Bot.*, 13, 75–94 (1962).
13) Roberts, E. H. & Smith, R. D.: Dormancy and pentose phosphate pathway. *In* The physiology and biochemistry of seed dormancy and germination. ed. Khan, A. A., North-Holland Pub. Co. Oxford, 385–406 (1977).
14) Rudnicki, R. M.: Studies on abscisic acid in apple seed. *Planta*, 86, 63–68 (1969).
15) Samish, R. M.: Dormancy in woody plant. *Ann. Rev. Plant Physiol.*, 5, 183–204 (1954).
16) Sondheimer, E., Tzon, D. S. & Galson, E. C.: Abscisic acid levels and seed dormancy. *Plant Physiol.*, 43, 1443–1447 (1968).
17) Takahashi, N.: Physicogenetical studies on germination of rice seeds with special reference to its genetical factors. *Bull. Inst. Agr. Res. Tohoku Univ.*, 14, 1–87 (1963) [In Japanese with English summary].
18) Takahashi, N.: Dormancy and germination of rice seeds with respect to germination inhibitors. *Bull. Inst. Agr. Res. Tohoku Univ.*, 18, 195–213 (1967) [In Japanese with English summary].

(Received for publication, October 8, 1986)