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Role of Bran during the Germination of Rice.

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Introduction. The rice grain during the resting stage shows very little of active enzymes. However, during the germination of seeds, the increase in enzymes is rapid. The seat of production of these enzymes, especially that of diastase, was investigated by scientists both by directly observing the histological changes in the cell contents during the germination of the seeds and also by examining the vitality possessed by different parts. Three parts of the seeds are essentially concerned in the production of enzymes during their germination viz. embryo, endosperm and aleurone layer. Evidences are strong to show that the epithelial layer of the scutellum secretes diastase; but the results of the previous investigators are contradictory with regard to the enzyme secreting capacity of the endosperm and the aleurone layer.

Historical. The consideration of the possession of vitality by different parts of the seeds is essential, since the secretion can take place only in living and growing parts and not in those parts which do not possess cellular organisation. From the investigations of Bonnet (1754), Sachs (1859), Gris (1864) and many others, it is difficult to judge whether the endosperm possesses vitality or not.

Haberlandt (1894) expressed that aleurone layer is secretory in function. Brown and Morris (1890) are of opinion that the proteid reserve of aleurone layer is used in the late stages of germination. They do not find an appreciable quantity of enzyme in the aleurone layer and its enzymic activity later in germination is due to the accumulation of the same formed during the germination. Stoward (1911) observed that the cytological changes in the aleurone layer were closely similar to those of columnar epithelium in barley. Nuclear and cytoplasmic changes synchronised with these phenomena in the epithelium. This justifies the view that the aleurone layer is secretory in function. Schander Helmet (1935) studied the effects of removing some or all of the aleurone layer in wheat, barley, oat and rice. In rice the removal of aleurone layer proportionately checked the growth so long as there was a connection between this layer and the scutellum, and the breakage of this connection was as good as the removal of the whole aleurone layer. The conclusion of the author is that the endosperm or aleurone layer furnishes a growth-activating substance that passes to the embryo during the swelling stage of germination, and that thereby the embryo is enabled to provide the endosperm with starch-digesting enzymes. From the foregoing literature it is seen that the diastase secretory powers

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of the epithelium of the scutellum is unquestioned whereas those of aleurone cells and endosperm are doubtful.

Material and Method. The previous investigators have been studying this question by following cytological changes in that layer or by noting the changes in the starch grain underlying this layer. In the experiments of Stoward (1911) the different parts were removed and grown in cultures and so these do not represent the conditions *in vivo*. In the following pages, the effect of the removal of the bran on the quantity of diastase secreted is discussed.

The paddy strain, GEB 24 of the Coimbatore Paddy Breeding Station was taken up for study. Husked rice was kept for germination removed at intervals, and kept for drying at 30°C for 24 hours. The seeds were separated into bran, embryo and endosperm and the diastase contents of the different parts were estimated. A blank was run without the starch and the reducing sugars in the material was accounted for before the quantity of diastase was calculated. Twenty-five seeds were taken for each experiment and the separated parts were well pulverised and added to 10 cc. of 1 % starch solution and the reaction was allowed to proceed for one hour at 40°C. The reducing sugars formed were estimated by Schaffer and Hartmann method as modified by Fred, Peterson and Stiles (1926). The quantity of diastase in 100 seeds in terms of mg. of glucose was then calculated.

Experiment 1. In this experiment, husked rice was germinated and then separated into different parts viz , embryo, pure endosperm and bran, and the quantities of diastase present in these three parts were estimated. The data are presented below.

TABLE I. Diastase in different parts of germinating rice seeds.
(Quantity for 100 seeds.)

Hours after soaking.	Embryo.	Endosperm.	Bran.
48	45·68	69·36	16·80
72	42·08	85·92	24·08
96	104·48	139·04	29·68
120	89·28	111·76	49·76

The above results show that with the increase in the period of soaking there is a steady increase in the diastase of all the parts. Embryo and endosperm show a set-back on the fifth day, whereas the bran shows an increase through-out. The bran contains the least quantity.

Experiment 2. In this experiment the bran on the seed was completely removed before keeping them for germination. The seeds show signs of germination and the radicle emerges only to one or two mm.

TABLE II. Quantity of diastase in rice seeds when they germinate without bran.
(Quantity for 100 seeds.)

Hours after soaking.	Embryo.	Endosperm.
48	6·88	13·52
72	3·52	12·56
96	5·36	10·00

This experiment shows that the absence of bran has caused a great decrease in the amount of diastase secreted during the germination of rice.

Experiment 3. In this experiment bran was ringed out near the embryo leaving a large portion at the distal end of the seed but unconnected with the embryo. The analysis for diastase content on the second and third days showed that the embryo contained 3·52 and 3·50 and the endosperm contained 5·64 and 5·36 respectively, in terms of glucose for 100 seeds.

Experiment 4. In this experiment bran was ringed out at the distal end (i. e., away from the embryo) leaving a large portion in touch with the embryo. In this case the seeds germinated well. The diastase contents on the second and third days were : embryo, 44·64 and 53·76 respectively; endosperm, 61·76 and 82·88 respectively. Since the diastase content is not reduced, it is evident that the contact of the embryo with the bran is essential for the diastase secretion.

Experiment 5. In this experiment, the embryo was connected to the bran through the dorsal and ventral veins only. The bran on the lateral sides of the proximal half of the seed was removed. Thus the contact between the bran situated at the distal half of the seed and the embryo was maintained through the dorsal and ventral veins. The seeds germinated well.

TABLE. 3. Quantity of diastase in germinating rice seeds with bran connected to the embryo through the dorsal and ventral veins only.

(Quantity for 100 seeds.)

Hours after soaking.	Embryo.	Endosperm.
48	6·88	14·76
72	11·36	44·16
96	12·56	28·56

The establishment of contact between the embryo and bran has resulted in a liberal secretion of diastase. The reduction in the extent of contact has reduced the diastase content.

Experiment 6. This experiment was planned to find out whether the contact of the bran is essential through-out the course of germination. In the germinating seeds, the bran was ringed out near the embryo, 6, 12, 24 and 48 hours after first soaking and the germination was allowed to proceed further. The analyses for the diastase contents showed, that, the longer the contact of the embryo with the bran, the greater the secretion. Even if the contact is broken 6 hours after soaking, the secretion is fairly large. The diastase content of the bran itself was very small. This shows that the secretion is not essentially in the bran.

Experiment 7. To find out if the bran secretes diastase in the absence of the embryo, the seeds were degermed and germinated. There was practically no increase in diastase.

Experiment 8. Embryos of rice seeds were carefully separated twelve hours after soaking. In one case a small portion of the endosperm was left attached to the embryo, and in another case the endosperm was removed as completely as possible.

TABLE. 4. Quantity of diastase in germinating excised embryos.

(Quantity for 100 embryos)

Hours after soaking.	Embryo only kept for germination.	Embryo with a bit of endosperm kept for germination.
48	30·08	32·64
72	54 80	72·88
96	59 76	90·40

There is a progressive increase in the diastase content of the embryo even when it is kept alone for germination. When a small portion of the endosperm is in contact with the embryo the secretion is greater.

Discussion. From the foregoing experiments it is clear that the bran plays an important role during the germination of rice, not by the secretion of diastase, but by enabling the embryo to secrete the same. The investigations of Schander Helmet and Brown and Morris show that there is a flow of some material from the aleurone layer to the embryo during the early stages of germination. The above experiments show that the contact of the embryo with the bran is essential only in the early stages and that the bran does not contribute diastase by way of secretion. How the bran enables the embryo to secrete more diastase is not clear.

Diastase is a complex enzyme possessing two components viz., α amylase and β amylase. Nordh and Ohlson (1933) are of opinion that the dormant seeds contain only α amylase while β amylase appears only during the sprouting of the seeds. Recent investigations of Giri and Sreenivasan (1936) show that α and β amylases are both present in the rice seed in an insoluble form in the dormant stage and that they become soluble only during the germination process. They contend that Ohlsson's view that α amylase arises only during the germination of seeds is untenable. Waldtschmidt-Leitz *et al* consider that the increase in diastase during the germination is due to 'amylokinase' or to an increase in the soluble part as a result of proteolytic decomposition.

To test whether the bran layer contained any substance which is capable of rendering the inactive amylases of rice active, the following experiments were conducted :

Since the diastase present in small quantity in ungerminated seed is not soluble in water, it is clear that the bean of the ungerminated seed does not possess any activating substance. Such an activating substance may arise there during the process of germination. Hence the bran from seeds germinated for 3 days was taken. The diastase content of the seeds increases during the first 24 hours of germination but it is not evident in the extract

i. e., the enzyme is not soluble. Seeds germinated for one day were taken powdered, water added and also 0·2 gm. of the bran from the three-day germinated seeds. The diastasic activity was tested after 24 hours. There was no diastase in the extract. This shows that the bran does not play the role of activating the inactive α and β amylase in rice. Probably it supplies some important ingredient to the embryo which enables the latter to do that function.

The solubility of the two amylases in the seeds germinating with and without bran was tested by Venkata Giri's (1934) iodine colour test using agar-starch as substrate. The colours of the rings formed at different stages of germination were tested both by using the materials direct and by taking their water extract.

TABLE V. Giri's colour tests for α and β amylases.

Hours after soaking.	Normal seeds.		Branless seed.	
	Material	extract.	Material	extract.
0	V. W.	...	V. W.	...
24	V. W.	V.	V. W.	...
48	V. W.	V.	V. W.	...
72	V. W.	V. W.	V. W.	...

V. = Violet colour.
V. W. = Violet ring with white centre.

The foregoing table shows that both α and β amylases are present in the seeds even from the beginning, whereas they are not present in the extracts in the initial stages of germination. β amylase becomes active before the α does. Both the amylases are absent in the extracts from the seeds germinated without bran portion. Presence of bran during extraction showed no difference in colour tests. This shows that the bran does not play the role of rendering the insoluble α and β amylases soluble. From Table 5 it is evident that β amylase is first rendered active.

Conclusion. The absence of bran causes a considerable decrease in the quantity of diastase secreted during the germination of rice. The presence of bran on the seed, without there being any contact between this part and the embryo shows the same effect as the complete removal of bran from all over the seed. This shows that the bran sends some important substances to the embryo which enables the latter to secrete diastase abundantly. The contact between the embryo and the bran is not essential through-out the germination period, but if the bran is removed a few hours after the soaking of the seed, the scretion is not interfered with. The longer the contact in the early stages of germination, the larger was the quantity of secretion. The diastase present in the bran is very little and it does not secrete eny significant amount in the absence of embryo. Therefore it may be concluded that the bran translocates some important substance to the embryo, which enables the latter to secrete large quantities of diastase. The iodine colour tests showed that the bran did not play any,

part in rendering the amylases soluble ; and also that β amylase was rendered soluble first.

Summary. The experiments have definitely proved the importance of bran during the early stages of germination. The break in the contact between the embryo and bran has the same effect as the complete removal of bran. The translocation of substance from the bran to the embryo takes place within six hours after soaking of the seed. β amylase is rendered soluble in water first and later only the β amylase is rendered soluble.

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