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CHANGES OF RICE AMYLASES DURING MATURING AND GERMINATION*

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Abstract

Using a floating rice (CO. 64) and a non-floating rice (Poik Reour) of *Indica* type collected in Cambodia and a *Japonica* type rice (Norin No. 22), changes of amylase contents in the shoots and the grains during maturing, dormant and germinating periods were investigated. One of the results obtained was that α-amylase of the grains, which was not detected at dormant period, appeared at earlier stage of maturing and disappeared soon later. Another was that there was a strong possibility of *de novo* synthesis of β-amylase during germination. Compared with other rice plants, the floating rice was found to contain higher levels of both α- and β-amylase in the grains and the shoots.

Introduction

Recent studies have demonstrated that rice amylase is composed of multiple forms[3,5,11]. In our previous papers[6,7], ungerminated rice grains of *Japonica* type have been shown to contain active β-amylase and four types of zymogen β-amylases but no measurable α-amylase. It has also been found that the active β-amylase is widespread in the grain, relatively up to the inner layers of the endosperm, whereas the zymogen β-amylases are located rather in the outer layers of the grain.

Although rice varieties of *Japonica* type are generally assumed to contain amylases mainly in the grains, those of *Indica* type, especially floating rices are believed to contain amylases not only in the grains but also in the leaves and stems. This is probaly due to the fact that a high amount of reducing sugars distribute widely in the plant, which makes it possible to increase rapidly the total internode length under submerged conditions[12,13].

The present paper deals with the investigation of distribution of α- and β-amylases both in the grains and plants among rice varieties, and their changes during maturing, dormant and germinating periods using *Japonica* and *Indica* types of rices.

Materials and Methods

Rice samples

Rice samples used in the experiments were three varieties; CO. 64, a floating rice and Poik Reour, a non-floating rice, of *Indica* type which were both collected in Combodia by Prof. Dr. T. SATO, Kobe University in 1960, and a Japanese rice cultivar of Norin No. 22 of *Japonica* type. These samples were grown in pots in 1973 as described previously[14].

Enzyme assay

α-Amylase and β-amylase activities were measured as described previously[6]. The methods to measure total β-amylase, zymogen β-amylases were also described in the previous paper[6]. The activities were expressed in terms of starch or maltose mg per one grain of the samples, and in the measurement of β-amylase activity corrections were made for the effect of co-existent α-amylase in the enzyme preparations.

Results

Amylase activities in rice plants

Fig. 1 shows three rice samples at heading

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time. The average height of CO. 64 plant was 170 cm, and those of Poik Reour and Norin No. 22 were 120 cm and 80 cm, respectively.

As shown in Table 1, 20 seedlings of each sample at the 5th leaf stage were taken on the 14th day after sowing, and 20 plants of each sample at the 8-9th leaf stage, on the 36th day after sowing. After removal of the roots, each sample was cut into as small pieces as possible with scissors, ground in homogenizer and extracted with 5% potassium sulfate and 0.1% papain solutions. The activities measured are shown in Table 1. α-Amylase activity from the seedlings of CO. 64 was about three times that of Norin No. 22 and about seven times that of Poik Reour.

Fig. 1. *Indica* and *Japonica* types of rice samples at heading time.
From left to right: CO. 64, Poik Reour and Norin No. 22.

These differences in the activities, however, were not seen in the samples at the 8-9th leaf stage.

On the other hand, β-amylase activities of *Indica* type at the 5th leaf stage were small but increased about 10 to 17 times at the 8-9th leaf stage. No β-amylase activity of Norin No. 22 was detected both in the samples at the 5th and 8-9th leaf stages.

Amylase activities during maturing and dormant periods

One hundred grains were taken from several ears of each sample at suitable intervals during maturing, ground in a mortar with 25ml of extracting reagents and held at room temperature for 5 hrs with occasional shaking. After centrifugation and filtration through Toyo No. 2 filter paper, the filtrates obtained were used for enzyme assay.

As shown in Figs. 2 to 4, α-amylase activity of CO. 64 increased to reach the peak on the 11 th day, that of Poik Reour on the 3rd day, and that of Norin No. 22 on the 13th day after flowering, respectively. The maximum activity of Norin No. 22 (1.97) was higher than that of Poik Reour (1.64) and that of CO. 64 (1.25). After reaching each peak, α-amylase activity rapidly decreased and became undetected at later periods of maturing.

Active β-amylase in each case, as shown in Figs. 2 to 4, increased almost in parallel with α-amylase and reached each peak nearly at the same period with that of α-amylase. Then it decreased gradually as the maturing day progressed. The rates of increase and decrease in the case of Norin No. 22 were much higher than those of other samples.

Table 1. Amylase activities in rice shoots

Growing stage	Variety	Overall length	α-Amylase activity	β-Amylase activity
5 th Leaf stage	CO. 64	33.5 cm	80.0	0.430
	Poik Reour	27.0	11.3	0.330
	Norin No. 22	24.4	26.4	—
8-9 th Leaf stage	CO. 64	60.1	15.2	7.020
	Poik Reour	48.5	13.1	3.240
	Norin No. 22	42.2	12.3	—

Notes: α-amylase activity was expressed in terms of starch mg per each stem, 60 min. and β-amylase activity, in terms of maltose mg per each stem, 1 min.

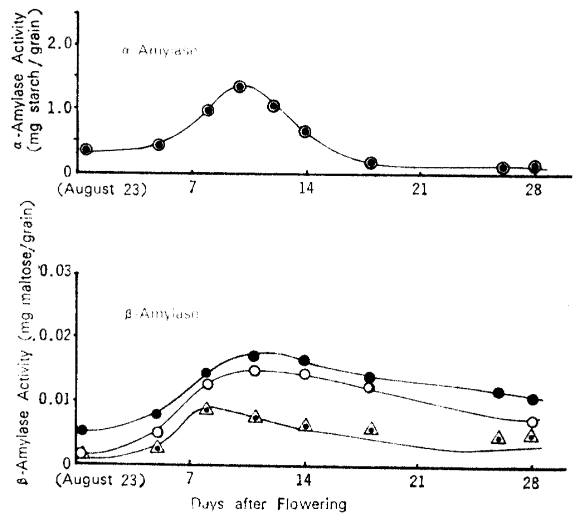

Fig. 2. Changes of amylases during maturing of CO. 64.

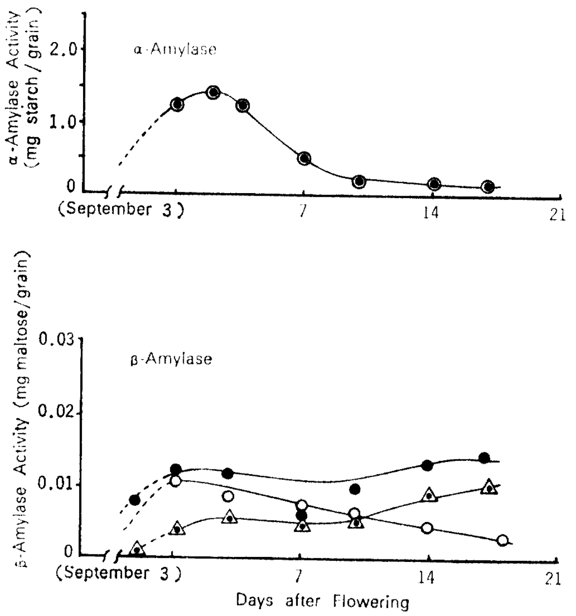

Fig. 3. Changes of amylases during maturing of Poik Reour.

The maximum activity (0.03) of Norin No. 22 was the highest of the three samples. The salt-soluble zymogen β-amylase activities of Norin No. 22 and Poik Reour, which were not detected in the flowering periods, increased with the advancement of maturing day, but in the case of CO. 64 no marked changes were seen. Corresponding to these changes of active and zymogen β-amylases, total β-amylase in each case increased to reach each peak, then remained nearly constant except that of CO. 64 which showed a gradual decrease.

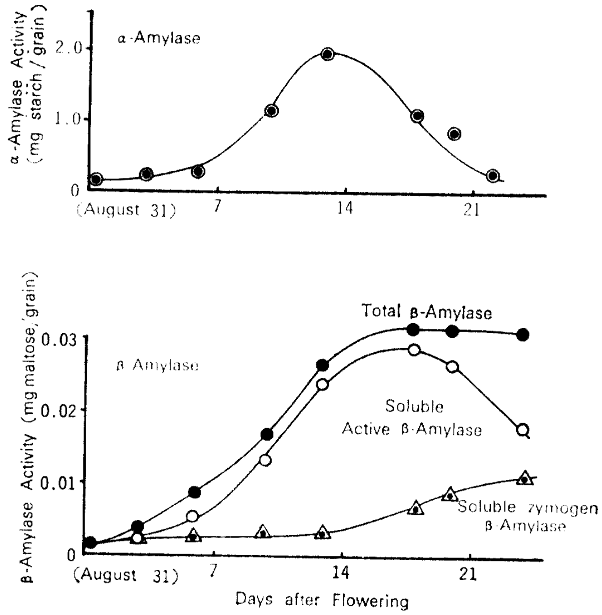

Fig. 4. Changes of amylases during maturing of Norin No. 22.

During the dormant period, α-amylase activity of each sample was too small to be measured. β-Amylase activities were also very small, remaining nearly constant up to germination.

Amylase activities during germination

Fifteen grains of each sample were taken to a mortar, ground with quarz sands and extracted with 20ml of extracting reagents. After filtration with Hyfro Super-Cel as filter aid, the filtrates obtained were used for enzyme assay in the same way above.

As shown in Figs. 5 to 7, α-amylase activity of each sample remarkably increased during germination and reached each peak around the 12th day of germination. The rate of increase of Norin No. 22 was the highest among the samples tested. After reaching the peak, the activity of Norin No. 22 showed a rapid decrease, but those of CO. 64 and Poik Reour held nearly the same values.

Active β-amylase activity of each sample showed a rapid increase during germination and reached each peak around the 12th day of germination. The salt-soluble zymogen β-amylase of CO. 64 increased in parallel with the active β-amylase to reach the peak on the 10th day, while those of Poik Reour and Norin No. 22 changed rather slowly. Corresponding to these changes, the total β-

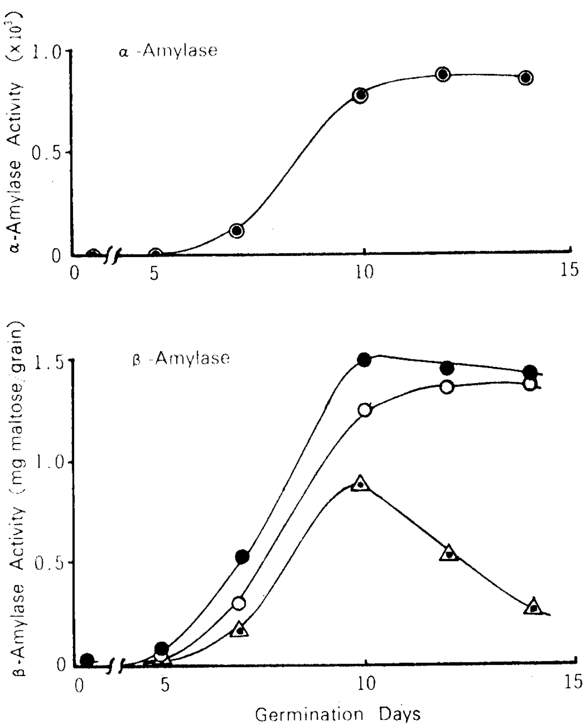

Fig. 5. Changes of amylases during germination of CO. 64.

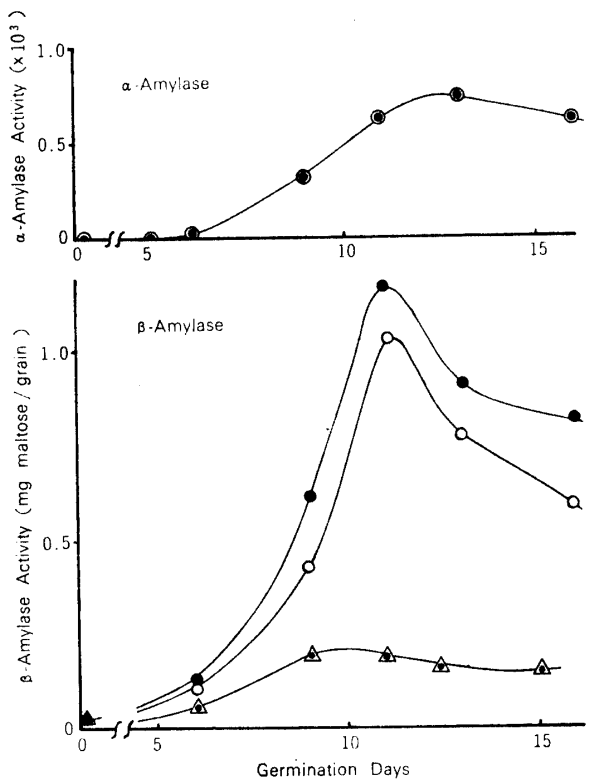

Fig. 6. Changes of amylases during germination of Poik Reour.

amylase activities fluctuated similarly during germination. The maximum value of CO. 64, as shown in Table 3, was 1.48, that of Poik

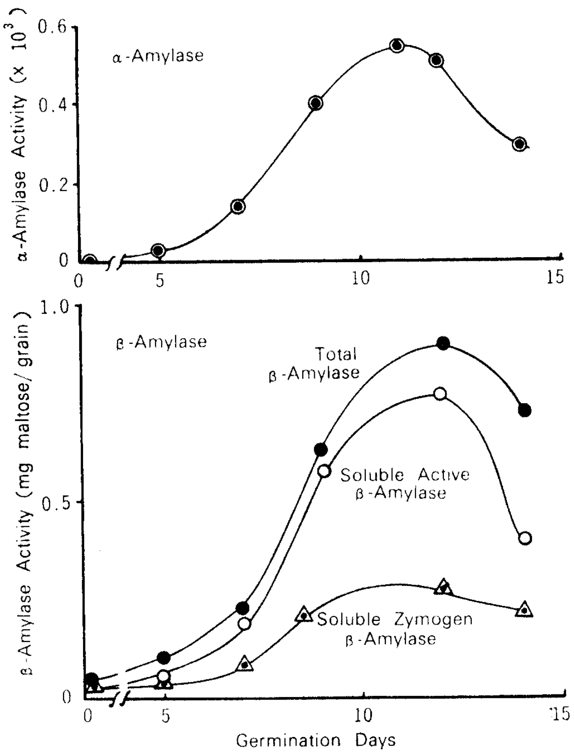

Fig. 7. Changes of amylases during germination of Norin No. 22.

Reour, 1.18 and that of Norin No. 22, 0.89, respectively.

Discussion

Among the experimental results obtained it may first be noted that α-amylase appeared at earlier stages of development of rice grains as in the cases of other cereals[2]. α-Amylase appeared soon after flowering and reached a peak around the 12th or 13th day except that of Poik Reour and disappeared soon later. As shown in Table 2, the maximum values of α-amylase were different in rice varieties. Compared with those of other cereals, α-amylase activity of rice grains is much less than those of barley[2,8] and wheat[4], even in due consideration of different methods used for the measurement of the activity in them. On the other hand, α-amylase activity at these stages of maturing has been reported to be that of the husk or pericarp of grains[1,4]. The fate of α-amylase in the grain during maturing is one of the interesting subjects to be studied. The increase in α-amylase activity during germination, as shown in Figs. 2 to 4 and Table 2,

Table 2. Comparison of α-amylase activities during maturing and germination

Samples		Days after flowering	Maximum activity	Germination days	Maximum activity
Rice	CO. 94	11	1.25	12	800
	Poik Reour	(3)	1.64	13	704
	Norin No. 22	13	1.97	12	512
Barley	Conquest[9]	10	32.00	7	110
	Native[10]	10	38.00	6	115
Wheat	Raven[11]	10	15.00	7	150

Notes: the activities of rice samples were expressed in terms of mg starch/grain, 60 min. and others were expressed in terms of unit/grain by a modification of the method.

Table 3. Comparison of β-amylase activities during maturing and germination

Samples		Days after flowering	Maximum total activity	Germination days	Maximum total activity
Rice	CO. 64	14	0.016	10	1.48
	Poik Reour	17	0.014	11	1.18
	Norin No. 22	18	0.031	12	0.89
Barley	Conquest[9]	35	80	7	65
	Native[10]	30	180	7	105
Wheat	Raven[11]	40	30	7	23

Notes: the activities of rice samples and barley (native) were expressed in terms of maltose mg/grain, and others were expressed in terms of unit/grain by a modification of the method.

was remarkable. The maximum value of α-amylase of CO. 64 during germination was about 640 times that during maturing, and that even in the case of Norin No. 22 came to about 260 times. However, as seen from Table 2, the maximum values of α-amylase during germination of wheat and barley are calculated to be only 3 to 10 times those during maturing.

Secondly, it should be noted that total β-amylase in rice grains was found to increase greatly during germination. No β-amylase has been reported to be *de novo* synthesized during germination of barley, and the increase in β-amylase activity, to be only due to the activation of zymogen β-amylases synthesized during maturing[9]. Table 3 shows that the maximum values of total β-amylase during germination of CO. 64 and Poik Reour were about 90 times those during maturing. Even in the case of Norin No. 22 it came to about 30 times. These data indicate the possibility of *de novo* synthesis of β-amylase during germination of rice. This seems to be quite different from the case of barley β-amylase. Further detailed experiments to ascertain this, however, is necessary in future.

Thirdly, it is clear that a floating rice of CO. 64 contains the highest amount of both α- and β-amylase among the samples tested. Therefore, this high level of amylases may correspond to the high content of reducing sugars not only in the grains but in the plants[12]. This may also be related to one of the characteristics of floating rice plants which can rapidly increase total internode length not to be choked to death under submerged conditions. In this respect, the experimental results on the distribution of amylases in Norin No. 22 suggest that rice breeding in Japan may have resulted in selection of rice varieties containing amylases only in the grains. It is also assumed that, in addition to gibberellins, some factors must be contained in floating rices to hold such high levels of amylases both in the grains and in the plants[10,15]. To find out such factors in floating rices is necessary in future.

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米の成熟期及び発芽期中のアミラーゼの消長

新家　龍・山口　禎・西羅　寛

要　約

カンボジヤで採取されたインド型稲の浮稲（CO. 64）と非浮稲（Poik Reour）及び日本型稲の農林22号を用いて，それら生育中の茎葉部及び種子，並びに発芽期中の種子に含まれるアミラーゼの消長を調べた。その結果，いずれの場合にも，未発芽種子中に検出されなかったα-アミラーゼは成熟期初期の種子に出現して最高値を示したのち消失し，発芽期に再び急増することが判った。また，β-アミラーゼについては，その成熟期及び発芽期の消長から，発芽期中にも新たに合成される可能性の大きいことが示唆された。更に，実験に用いた上記3品種の中では，浮稲の種子と茎葉部に最も高いアミラーゼ含量が検出された。この高い酵素活性と浮稲の品種特性についても考察した。