NOTE

Comparison of Amylase Activity and Carbohydrate Profile in Germinating Seeds of *Setaria italica, Echinochloa frumentacea*, and *Panicum miliaceum*

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The evaluation of amylases during germination of common cereals such as wheat, rice, and barley has been widely discussed (Frydenberg and Nielsen 1965, Kruger 1972, Palmiano and Juliano 1972). However, little information is available concerning the enzyme activities in millets. Chandrasekara and Swaminathan (1953) described the amylase activity in ragi (finger millet, *Eleusine coracana*) and in ragimalt. A comparison of β-amylase activity between bajra (pearl millet, *Pennisetum americanum* L. Leeke) and barley was reported by Sheorain and Wagle (1976). The biochemical changes occurring during germination of the minor millets *Setaria italica* L., *Panicum miliaceum* Lamk., and *Echinochloa frumentacea* Roxb. have not been reported. These millets are used for human consumption in India and northern China. In this work, an attempt was made to study the changes in carbohydrate and in amylase activities during germination of these three millets.

MATERIALS AND METHODS

Seeds of *Panicum miliaceum* (var. Co. 1), *Setaria italica* (var. Co. 5), and *Echinochloa frumentacea* (var. Co. 1) were obtained from Millet Breeding Section of Tamil Nadu Agricultural University, Coimbatore. The seeds were germinated by being placed on a double layer of moistened filter paper in petri dishes and kept at room temperature ($\pm$ 29° C) in the dark from one to eight days. The filter papers were moistened at 24-hr intervals. Seedlings of various stages (from one to eight days) at one-day intervals were used for the determination of α- (E.C. 3.2.1.1) and β-amylase (E.C. 3.2.1.2) activities. The extraction and assays were done as described by Meredith and Jenkins (1973). Protein was estimated in the extract by the method of Lowry et al (1951). The enzyme activity unit is expressed as milligrams of maltose produced per minute per milligram of protein. More seedlings at these stages were dried and powdered. Carbohydrate was estimated by the anthrone method (Hodge and Hofreiter 1962).

Powdered samples were extracted by boiling 80% alcohol and determining the reducing sugars in these extracts (Nelson 1944). Starch and amylose were estimated in the residue after ethanol extraction according to the method of McCready et al (1950).

RESULTS AND DISCUSSION

The changes in the activity of amylases are shown in Fig. 1. In all three millets, the α-amylase activity was very low at the initial stages of germination and increased steeply after the lag period. Maximal α-amylase activity was obtained in a sample from the fifth day of germination for both *Echinochloa frumentacea* and *Panicum miliaceum*. The activity of *Setaria italica* was maximal on the fourth day; however, it was lower in activity than the other samples.

The β-amylase activities in all three millets increased gradually until the sixth day of germination, reached maximum levels on the seventh day, and thereafter showed a decreasing trend. *Echinochloa frumentacea* had the lowest initial β-amylase activity but maintained the highest activity during the first seven days of germination.

Starch content decreased progressively during germination (Table I). The decrease in the starch content was accompanied by an increase in the level of reducing sugars. The total carbohydrate also decreased considerably in all three millets. Amylose content increased during the earlier stages of germination, then gradually

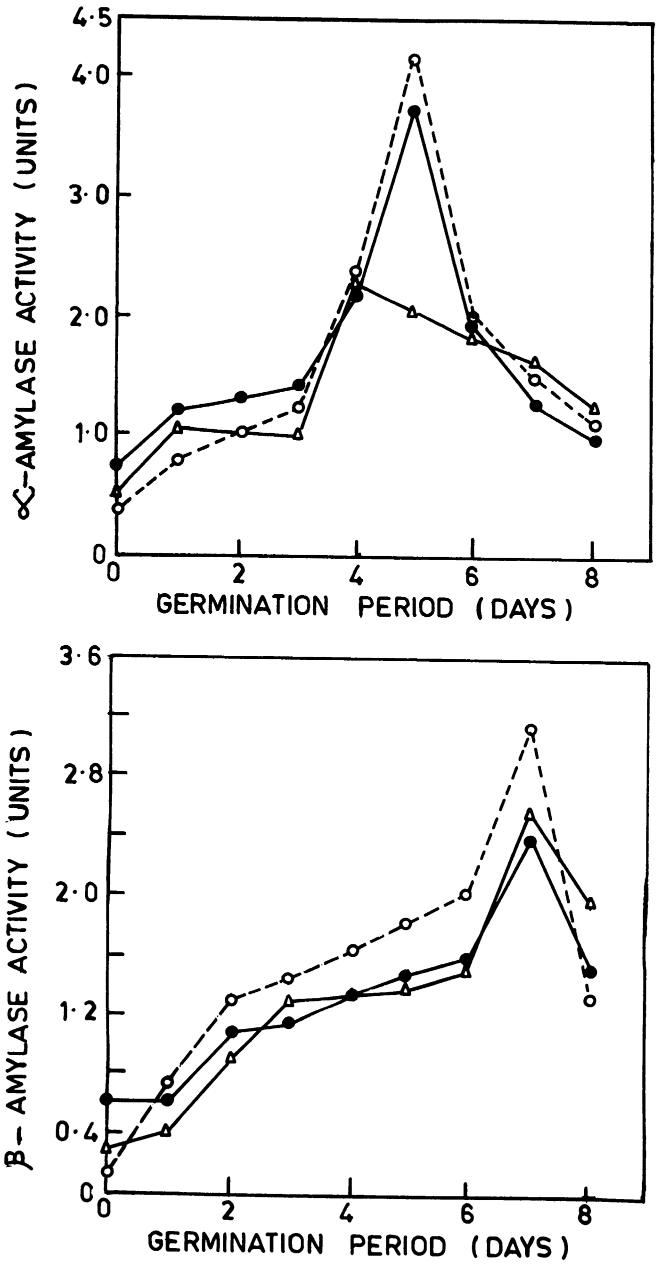

Fig. 1. Changes in α- and β-amylase activities during germination. ----△----△---- = *Setaria italica*, ----o----o---- = *Echinochloa frumentacea*, and ----●----●---- = *Panicum miliaceum*.

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TABLE I
Changes in the Carbohydrate Profile During Germination

Germination (days)	Total Carbo-hydrates (%)	Starch (%)	Amylose (%)	Reducing Sugars (%)
Setaria italica				
Ungerminated	67.9	51.0	11.5	0.30
2	60.0	46.1	13.5	0.50
4	57.2	36.3	10.5	6.30
6	56.3	33.6	9.0	10.30
8	54.3	13.4	6.7	10.10
Echinochloa frumentacea				
Ungerminated	67.6	58.6	13.5	0.15
2	65.7	51.6	12.0	0.60
4	60.4	30.5	10.2	8.70
6	56.3	22.2	8.7	10.70
8	50.4	13.8	7.0	12.30
Panicum miliaceum				
Ungerminated	69.8	58.0	12.0	0.16
2	66.0	53.3	13.3	0.70
4	62.4	32.0	11.0	6.80
6	54.3	26.1	7.0	12.80
8	54.3	15.5	5.3	11.30

decreased. Changes in the protein content were insignificant.

During early germination, a major portion of the soluble sugars in the dry seed may be utilized for respiratory activity (Nomura et al 1969). For further energy, the system depends on enzymic activity on starch. The major enzyme involved in starch degradation is α-amylase, which produces additional substrate for β-amylase to act upon (Juliano and Varner 1969). This may be the reason that α-amylase activity increases in the early stages of germination and decreases in latter stages. The increase of β-amylase activity until the seventh day of germination may be caused by the release of latent forms bound to storage protein, as shown by Rowsell and Goad (1962) for wheat and by Tronier and Ory (1970) for barley.

During the germination of cereal grains, several significant changes occur in the starch. These changes include a decrease in the starch content and an apparent increase in the amylose content. These changes were suggested to be caused by limited α-amylolysis of both components of starch. α-Amylase, therefore, was concluded to be the predominant enzyme involved during germination (Manners 1974). Similar results were observed in the three millets in the present study. Ungerminated seeds of *Setaria italica, Echinochloa frumentacea,* and *Panicum miliaceum* contained 0.32, 0.15, and 0.20% reducing sugars, respectively. On germination, the quantities of the reducing sugars increased dramatically, which can be attributed to the degradation of starch into sugars. The decrease in the starch content is not totally reflected in the total carbohydrate content. This may be because of the partial compensation by simple sugars derived from starch (Hough et al 1971).

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