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Changes in the activity of Sucrose Metabolizing Enzymes During the Germination of Wheat Seeds

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Abstract: Changes in the activities of acid invertase and sucrose synthase during germination of wheat (*Triticum aestivum* L. cv. Kanchan) seeds were investigated. The activities of both acid invertase and sucrose synthase were changed during growth of seedlings. Until third day of germination their activities increased and thereafter decreased. The activity of acid invertase in the soluble fraction was higher than that of cell wall- bound fraction and for soluble fraction coleoptiles showed a significantly higher acid invertase activity than roots. But in the cell wall-bound fraction a higher activity was found in roots. Sucrose synthase activity was also higher in roots than in coleoptiles. Sucrose content was negatively correlated with the acid invertase activity in both coleoptiles and roots accounting well for the relation between substrate and the activity. Fructose content was significantly higher than the glucose and sucrose content and the amount of sugar was higher in roots than in coleoptiles.

Key words: Germination, invertase, light, sucrose synthase, wheat seeds

Introduction

Non-germination and poor emergence of wheat seeds are regulated by different factors. Among them plant hormones have been widely recognized of crucial importance (Arai *et al.*, 1991). Unavailability of necessary utilizable substrate for the germination and growth of the embryonic axis is one of the important reasons for non-germination of wheat seeds (Bhattacharyya and Sen-Mondi, 1985). However, growth of an organ must also be supported by incoming nutrients from source organs and, in many plants, sucrose is a major form of carbon that is transported from source organs to growing organs (Hawker, 1985). The initial step in the metabolism of sucrose is therefore, important to continue the growth, and a number of authors have reported correlations between seed germinations and the activities of sucrose metabolizing enzymes, namely, invertase (ß-fructofuranosidase, EC 3.2.1.26) and sucrose synthase (UDP-glucose: D-fructose 2 glucosyltransferase, EC 2.4.1.13). In plant tissues, cleavage of the glycocidic bond in sucrose is done either by sucrose synthase or invertase. Sucrose synthase requires UDP as co-substrate and produces fructose and UDP-glucose, whereas invertase simply splits sucrose into glucose and fructose (Copeland, 1990). Sucrose synthase is a cytoplasmic enzyme that catalyzes a reversible reaction and under normal conditions acts only in the breakdown of sucrose. There are two types of invertase in plants. Acid invertase, which has optimum activity at pH 5.0, is present in vacuoles and in the apoplast, bound to cell walls. Neutral (alkaline) invertase having optimum activity at pH 7.0-7.5 and is found in cytoplasm. Invertases catalyze an irreversible reaction.

Earlier studies have shown that high activity of invertase is associated with actively growing tissues, such as young seedlings (Masuda and Sugawara, 1980; Faye and Ghorbel, 1983; Krishnan *et al.*, 1985), leaves (Pollock and Lloyd, 1977; Pressy and Avants, 1980) and roots (Ricardo and ApRees, 1970). The results of several studies have suggested that sucrose metabolizing enzymes are essential for growth by making sugars available for cell expansion (ApRees, 1974; Kaufman *et al.*, 1973; Seitz *et al.*, 1968). They make available of the utilizable substrate from the endosperm to the embryonic axis during seed germination (Bhattacharyya and Sen-Mondi, 1985).

As the first step towards understanding the biochemical events in embryonic cells during wheat seed germination we studied the activities of sucrose metabolizing enzymes with the aim of defining the role of enzymes.

Materials and Methods

Plant materials: Wheat (*Triticum aestivum* L.) seeds (cv. Kanchan) were obtained from Wheat Research Center, Bangladesh Agricultural Research Institute, Joydebpur, Bangladesh. Seeds were sterilized with a dilute solution (100 ppm) of sodium hypochlorite and sown on two layers of filter paper moistened with distilled water on petri dishes. The coleoptiles and roots were harvested at 24 h intervals upto 4 days and analyzed for enzyme activity.

Extraction buffers: A 0.2 M citrate-phosphate (C-P) buffer at pH 5.0 for soluble acid invertase and 0.2 M NaCl C-P buffer at the same pH for cell wall-bound acid invertase were used. On the other hand, 0.2 M potassium-phosphate (K-P) buffer at pH 7.8 containing 10.0 mM ascorbate, 15.0 mM $MgCl_2$, 1.0 mM EDTA and 1.0 mM dithiothreitol (DTT) was used for the extraction of sucrose synthase (SS).

Invertase extraction: Five g of tissues from coleoptiles and roots were mixed with 1.0% of polyvinylpolypyrrolidone (PVPP) and 1.0 g sea sand. The sample was then homogenized using a cooled mortar and pestle with 10.0 ml of 0.2 M C-P buffer (pH 5.0). The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 11000 x g for 10 min. The total supernatant was dialyzed with 40 times diluted 0.2 M C-P buffer (pH 5.0) for 12 h and the inner solution was designated as the soluble fraction. The residual tissues were re-extracted in 10 ml of 0.2 M NaCl C-P buffer for about 24 h with occasional stirring. The supernatant was dialyzed as described above and the dialyzed solution was designated as cell wall-bound fraction (Arai, 1991). All extraction procedures were carried out at 4 °C and followed immediately by the enzyme assays.

Invertase assay: The standard assay medium for acid invertase consisted of 0.2 ml of 0.2 M C-P buffer (pH 5.0), 0.1 ml of 0.5 M sucrose, 0.1 ml of water and 0.1 ml of crude enzyme solution. The blank experiment contained distilled water instead of sucrose. The assay mixture was incubated at 45 °C for 15 min. After the reaction, assay mixture was neutralized with 0.1 N NaOH or 0.1 N HCl, a coloring Somogyi's copper reagent was added and the mixture was heated for 10 min in boiling water. The amount of reducing sugars was estimated by the method of Somogyi (Somogyi, 1952). Soluble protein content was determined by the method of Lowry (Lowry *et al.*, 1951) using bovine serum albumin as a standard. The enzyme activity was expressed as the amount of glucose

produced per min per mg of protein.

Sucrose synthase extraction: Five g of tissues from coleoptiles and roots were mixed with 1% of polyvinylpolypyrrolidone (PVPP) and 1.0 g sea sand. The sample was then homogenized with 10.0 ml of 0.2 M K-P buffer (pH 7.8) containing 10 mM ascorbate, 15.0 mM $MgCl_2$, 1.0 mM EDTA and 1 mM dithiothreitol (DTT), using a cooled mortar and pestle. The resulting homogenate was then filtered through four layers of cotton cloth and the filtrate was centrifuged at 11000 xg for 20 min. The total supernatant was dialyzed with 40 times diluted 0.2 M K-P buffer (pH 7.8) for 16 h and the inner solution was used as the crude enzyme. All extraction procedures were carried out at 4 °C.

Sucrose synthase assay: Sucrose synthase activity was assayed at 37°C by the method of Hubbard (Hubbard *et al.*, 1989) with slight modifications. Reaction mixtures (70 μl) contained 50.0 mM Hepes-NaOH buffer (pH 7.5), 15.0 mM $MgCl_2$, 25.0 mM fructose, and 25.0 mM UDP glucose. The mixtures were incubated for 30 min at 37 °C and the reaction was terminated with the addition of 70.0 μl of 30.0% KOH. Enzyme blanks were terminated with KOH at 0 min. Tubes were kept at 100 °C for 10 min to destroy any unreacted fructose or fructose-6-phosphate. After cooling, 2.0 ml of anthrone reagent (150.0 mg anthrone with 100.0 ml of 15.0% H_2SO_4) was added and incubated in a 40°C water bath for 15 min. After cooling colour development was measured at 620 nm. The soluble protein content was determined by the method of Lowry (Lowry *et al.*, 1951) using bovine serum albumin as the standard. The enzyme activity was measured as μmole of sucrose produced per min per mg protein.

Determination of sucrose, glucose and fructose contents by high performance liquid chromatography(HPLC): About 5.0 g of sample for coleoptile and roots was mixed with 1.0 g sea sand and homogenized in a cooled mortar and pestle. Ten ml of distilled water was added to the homogenate and centrifuged at 11000 x g for 10 min. The mixture was filtered through a cellulose nitrate membrane filter (0.45 μm pore size). Soluble sugars were analyzed by HPLC, using a stainless steel column (10.7 mm ID x 30.0 cm) packed with silica gel (gel pack C610). The mobile phase (filtered water) was pumped through the column at a flow rate of 1.0 ml/min. The pressure was adjusted to 14-15 kg/cm² and the temperature at 60 °C. ARI monitor (Hitachi L-3300) was used. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

Statistics: A randomized complete block design was adopted with three replications. The level of significance was calculated from F value of ANOVA. The relationship between sugars and enzyme activities were described with linear correlation analysis.

Results

Acid invertase activity in soluble fraction: The acid invertase activity in soluble fraction was higher in coleoptile than in root, as shown in Fig. 1. In both the coleoptile and root, the activity was increased until third day of germination and thereafter it was decreased.

Acid invertase activity in cell wall-bound fraction: Acid invertase activity in cell wall- bound fraction is shown in Fig. 2. The activity was significantly higher in root than in coleoptile. However, in both coleoptile and root the cell wall-bound acid invertase activity increased remarkably until third day of germination and then decreased.

Sucrose synthase activity: Sucrose synthase activity was found higher in root than in coleoptile (Fig. 3). Like invertase activity sucrose synthase was increased until third day of germination and then started to decrease. A highly significant negative correlation was also found between sucrose synthase activity and sucrose content.

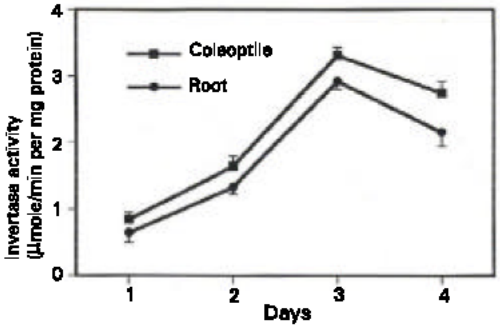

Fig. 1: Change in invertase activity in the soluble fraction of coleoptile and root of germinating wheat seeds. Each point represents the mean of three replications. Vertical bars indicate SE.

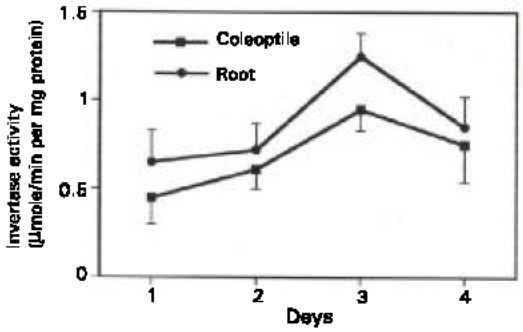

Fig. 2: Changes in acid invertase activity in the cell wall bound fraction of coleoptile and root of germinating wheat seeds. Each point represents the mean of three replications. Vertical bars indicate SE.

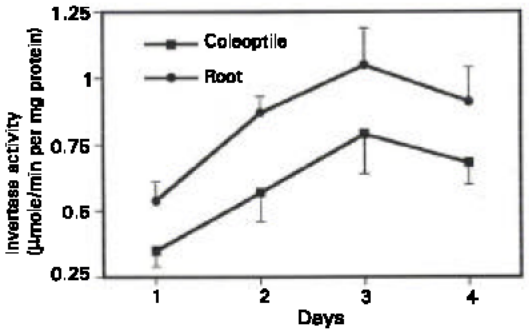

Fig. 3: Changes in sucrose synthase activity in coleoptile and root of germinating wheat seeds. Each point represents the mean of three replications. Vertical bars indicate SE.

Soluble sugar content: Among the three sugars, fructose content was always higher than glucose and sucrose in both coleoptile and root (Fig. 4). Fructose and glucose content were increased upto third day of germination and then started to decrease. On the other hand sucrose content decreased upto third day and then increased slightly. Both the coleoptile and root followed the same pattern.

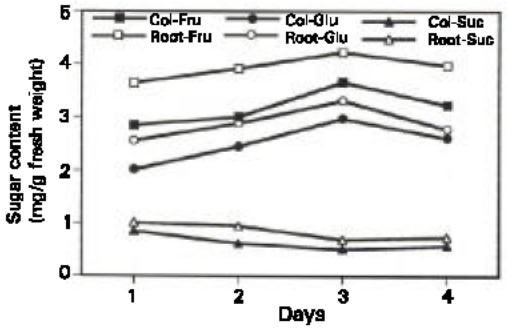

Fig. 4: Changes in sugar in coleoptile and roots of germinating wheat seeds. Each point represents the mean of three replications.

Table 1: Correlation coefficients (r) among enzyme activities and sugar contents in coleoptile and root of germinating wheat seeds

Sugar contents	Portions	Enzymes activity		
		Acid invertase		SS
		(SF)	(CWBF)	
Sucrose	Coleoptile	-0.796**	-0.706**	-0.604**
	Root	-0.700**	-0.743**	-0.607**
Glucose	Coleoptile	0.473**	0.516**	0.186
	Root	0.634**	0.673**	0.336
Fructose	Coleoptile	0.509*	0.628**	0.478*
	Root	0.570**	0.584**	0.438*

SF = Soluble fraction, CWBF = Cell Wall-bound fraction, SS = Sucrose synthase, * and ** denote significant at $p<0.05$ and $p<0.01$ respectively, n = 12.

Correlation coefficients (r) between enzyme activity and sugar content: Table 1 shows the correlation coefficients (r) between enzyme activities and sugar contents. Both of the acid invertases and sucrose synthase activities correlated negatively with sucrose content. The correlation was highly significant in both coleoptile and root. Significant positive correlation was found between glucose and fructose content and enzyme activities in all cases except glucose content and sucrose synthase activity. There was a positive correlation but non

Discussion

The results of present study indicate that both the sucrose metabolizing enzymes acid invertase and sucrose synthase are present in germinating wheat seeds and play a significant role in supporting the growth of plant organs by converting sucrose to monosaccharides. The activities of the enzymes increased upto third day of germination with the rapid increase of coleoptile and root. Thereafter, the growth of the coleoptile and root slowed down decreasing the enzyme activities along with it. This characteristic of sucrose metabolizing enzymes has also been described by other authors (Arai *et al.*, 1991; Krishnan *et al.*, 1985) and it has been suggested that rapidly growing tissues always have elevated levels of acid invertase and sucrose synthase as compared to levels in non slow d invertase activity in the soluble fraction was higher in coleoptile than in root. Similar observation has been recorded for mungbean (Arai *et al.*, 1991) due to comparatively slower growth of root than coleoptile. Both the acid invertase and sucrose synthase activity gradually increased upto third day in both coleoptile and root (Fig. 3) with a concomitant decrease in sucrose content (Fig. 4). There was thus a significant negative correlations between enzyme activities and sucrose content (Table 1). Other soluble sugars, glucose and fructose were positively correlated with enzyme activities. The correlation results indicate that like other germinating seeds, broad bean seeds (Nakamura, 1959), maize endosperm (Tsai, 1974) and rice grains (Nomura and Akazawa, 1973), the activities of acid invertase along with the sucrose synthase were directed to catalyze the sucrose hydrolysis in germinating wheat seeds. Therefore, it is likely that invertase and sucrose synthase are closely correlated with germinating wheat seeds, and suggest that they are important for growth, making sugars available for cell expansion.

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References

ApRees, T., 1974. Pathways of carbohydrate breakdown in higher plants. In: Biochemistry. Series One, vol II, Plant Biochemistry. Edited by Northcote, D.H. Butterworth, London.

Arai, M., H. Mori and H. Imaseki, 1991. Roles of sucrose - metabolizing enzymes in growth of seedlings: Purification of acid invertase from growing hypocotyles of mung bean seedlings. Pl. Cell Physiol., 32: 1291-1298.

Bhattacharyya, S. and S. Sen-Mandi, 1985. Studies into causes of non-germination of aged wheat seeds. An. of Bot., 56: 475-479.

Copeland, L., 1990. Enzymes of sucrose metabolism. In: Methods in Plant Biochemistry. Vol III. Enzymes of primary metabolism. Lea, P.J. (Edi.) Academic Press, London, pp:73-86.

Faye, L. and A. Ghorbel, 1983. Studies on b-fructosidase from radish seedlings. II. Biochemical and immunocytochemical for cell-wall-bound forms *In vitro*. Pl. Sci. Lett., 29: 33-48.

Hawker, J. S., 1985. Sucrose. In: Biochemistry of storage carbohydrates in green plants. Dey, P. M.(Edi.) and Dixon, R.A. pp. 1-51. Academic Press, London.

Hubbard, N.L., S.C. Huber and D.M. Pharr, 1989. Sucrose phosphate synthase and acid invertase as determinats of sucrose concentration in developing muskmelon (*Cucumis melo* L.) Fruits. Pl. Physiol., 91: 1527-1536.

Kaufman, P. B., N.S. Ghoshek, J.D. Lacroix, S.L. Sony and H. Ikuma, 1973. Regulation of invertase levels in *Avena* stem segments by gibberellic acid, sucrose, glucose and fructose. Pl. Physiol., 52: 221-228.

Krishnan, H. B., J.T. Blanchette and T.W. Okita, 1985. Wheat invertases. Characterization of cell wall-bound and soluble forms. Pl. Physiol., 78: 241-245.

Lowry, O. H., N.J. Rosebrough, A.L. Farr and R. Rendal, 1951. Protein measurement with folin-phenol reagent. J. Biol. Chem., 193: 265-275.

Masuda, H. and S. Sugawara, 1980. Purification and some properties of cell wall-bound invertases from sugar beet seedlings and slices of mature roots. Pl. Physiol., 66: 93-96.

Nelson, 1944. A photometric adaption of the Somogyi for the determination of glucose. J. Biol. Chem., 153: 375-380.

Nakamura, M., 1959. The sucrose synthesizing enzyme in the Beans. Bull. Agric. Chem. Soc. Jpn., 23: 398-405.

Nomura, T. And T. Akazawa, 1973. Enzymic mechanism of starc synthesis in ripening rice grains. 7 Purification and enzymic properties of sucrose synthetase. Arch. Biochem. Biophys. 156: 644-652.

Pollock, C. J. and E.J. Lloyd, 1977. The distribution of acid invertase in developing leaves of *Lolium temulentum* L. Planta, 133: 197-200.

Pressy, R. and J.K. Avants, 1980. Invertases in oat seedlings. Separation, properties and changes in activities in seedlings segments. Pl. Physiol., 65: 136-140.

Ricardo, C. P. P. and T. ApRees, 1970. Invertase activity during the development of carrot roots. Phytochem., 9: 239-247.

Seitz, K. and A. Lang, 1968. Invertase activity and cell growth in lentil epicotyls. Pl. Physiol., 43: 1075-1082.

Somogyi, M., 1952. Notes on sugar determination. J. Biol. Chem., 195: 19-23.

Tsai, S.Y., 1974. Sucrose-UDP Glucosyltransferase of *Zea mays* endosperm. Phytochem., 885-891.