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Correlation between β-amylase activity and starch content in different cultivars of radish (*Raphanus sativus* L.)

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ABSTRACT

Radish (*Raphanus sativus* L.) taproot shows amylolytic activity. However, data available on the differences in these amylolytic activities and starch content among radish cultivars in Bangladesh are quiet scanty. We analyzed the β-amylase activities and starch contents of 5 kinds of radish cultivar. The Tasaki cultivar showed the highest amylase activity, with a level about 2 times higher than that of the Red Bombay cultivar, which had the lowest. Cultivars with higher β-amylase activities showed higher starch contents. These results suggest the existence of intraspecies variations in amylolytic activities in radishes, and positive correlations between amylase activity and starch content. A positive correlation ($R^2 \approx 0.74$) was found in regression analysis.

KEYWORDS

Amylolytic activity;
Brassicaceae;
β-amylase;
Radish;
Raphanus sativus;
Starch.

INTRODUCTION

Radish (*Raphanus sativus* L.) belongs to the Brassicaceae family and is an important vegetable crop worldwide, especially in Asia and Europe, due to its wide adaptation, high yield and abundant nutritional content. Significantly, high nutritive value and carbohydrate content of roots and tuber crops attribute to a remarkable position in the food security of the developing world. Radish is a popular vegetable in Bangladesh and is widely cultivated for their edible roots. Many varieties are cultivated differing greatly in size, shape and color of the root on commercial scale[1]. Commonly, Radish is a winter crop but can be grown to some extent in summer. It has a hot, sharp, bitter taste and plays a vital role in providing a substantial amount of vitamins, minerals and other nutrients in our diet. It is consumed fresh as salad and after cooking with fish or other vegetables. Sometimes radish is used as an ingredient of pickles. Most plant cells synthesize and degrade starch at some point in their development-as a major function in storage organs such as tubers, roots and embryos, as a transient phase in meristem and organ development, and on a diurnal basis in leaves[2]. Higher plants play an important role as a source of many useful enzymes[3]. Indeed, amylolytic activity has been recorded in the radish taproot. The existence of potent diastase activity in the radish was first described in the early 19th century[4]. In general, plant starch-hydrolyzing enzymes include α-amylase, β-amylase, isoamylase, and limit dextrinase[5]. β-amylase was previously purified from radish[6], indicating its involvement in the amylolytic activity in radish taproots. Radishes have long been grown as a food crop, but they also have various medicinal actions. The roots stimulate the appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile[7]. The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints[8]. The plant contains raphanin, which is antibacterial and antifungal[8,9]. Methanolic extract of the radish sprout has a very high antioxidative activity owing to the different sinapic acid esters and flavonoids with very high radical-capturing capacity as the basis of their biological activity[10]. Dichloromethanic fraction of the sprouts obtained from the methanolic extract contains nicotinamide adenine dinucleotide and chinon-reductase that are playing a significant role in the defense of the liver cells against chemically carcinogenic and other compounds. These results indicate that the radish sprouts can be considered a safe, useful, new source of food that reduces the risk of cancer development[11]. Since radish is believed to show amylolytic activity, it is added to starch-containing foods, such as boiled rice, rice cakes and noodles, to aid digestion. Hara *et al*.,[12] recently reported that the amylolytic activity of the radish (Comet cultivar) is due to the accumulation of β-amylase in the taproot. There are many radish cultivars; however, little is known about differences in their amylase activities. In our present work, we report that 5 radish cultivars show different levels of β-amylase activity. Correlations between β-amylase activity and starch content in the different cultivars were also explored.

EXPERIMENTAL

Materials

The different varieties of radish were collected directly from the cultivation field of the local area in the winter season. Maltose, Glucose, BSA and Dinitrosalicylic acid (DNS) were purchased from Sigma Chemicals Ltd., USA. All other chemicals were of analytical grade and were used without further purification.

Preparation of the crude enzyme extract

Fresh, healthy radish roots were washed thoroughly with distilled water, cut into small pieces and ground with distilled water and sand in a mortar at 4ºC. The extracts were filtered with few layers of cheese cloth and further clarified by centrifugation at 5500 r.p.m. for 15 min at 4ºC. The clear supernatant was collected and used as crude enzyme extract.

Ammonium sulphate fractionation

The crude extract was saturated to 30-50% by the addition of solid ammonium sulphate under constant and gentle stirring at 4°C. The resulting precipitate was collected by centrifugation, dissolved in minimum volume of pre-cold distilled water and dialyzed against distilled water for 24 h at 4°C. The dialyzed solution was then centrifuged in a refrigerated centrifuge machine at 5500 r.p.m. for 15 min to remove the insoluble materials. The clear supernatant thus obtained was designated as "crude enzyme solution".

Measurement of amylase activity

Amylase activity was assayed following the method as described by Jayaraman[13]. One milliliter of crude enzyme solution was added to 1 ml of 1% soluble starch containing 0.1 M phosphate buffer pH 6.7 and the mixture was incubated at 37^0 C for 15 min. The amount of reducing sugar produced was determined by Somogyi[14] and Nelson[15] methods. One unit of enzyme activity was defined as the amount; which catalyzed the formation of 1 μmol of maltose under the assay conditions.

Determination of total and water soluble protein content

Total protein content of radish was determined by the method of Micro-Kjeldahl[16], while water soluble protein content was determined following the method as described by Lowry *et al*.,[17].

Determination of starch content

The starch content in radish root was determined by the anthrone method as described in Laboratory

Manual in Biochemistry[13]. About 5 gms of radish root were cut into small pieces and homogenized well with 20 ml of water and filtered through double layer of muslin cloth. To the filtrate, twice the volume of ethanol was added to precipitate the polysaccharide (starch). After keeping overnight in cold, the precipitate was collected by centrifugation at 3000 r.p.m. for 15 min. The precipitate was then dried over a steam bath. 40 ml of 1 M HCl was added to the dried precipitate and heated to about 70ºC. 2 ml of the diluted solution was taken in another 100 ml volumetric flask and diluted to 100 ml 1 M HCl. 1 ml aliquot of the extract of each variety was pipetted in to test tubes and 4 ml of anthrone reagent was added to the solution of each tube and mix well. Glass marbles were placed on top of each tube to prevent loss of water by evaporation. The tubes were placed in a boiling water bath for 10 min. A reagent blank was prepared by taking 1ml of water and 4 ml of anthrone reagent in a test tube and treated similarly. The absorbance of the blue-green solution was measured by colorimeter at 680 nm. The amount of starch present in radish root was calculated from the standard curve of glucose.

RESULTS AND DISCUSSION

Different varieties of radish were collected from the cultivation field during the winter season. TABLE shows the amylase activities, protein and starch contents of the 5 radish cultivars. The 5 cultivars exhibited different amylase activities, with the Tasaki cultivar showing the highest activity (2.75 U/ml), and the Red Bombay showing the lowest (1.35 U/ml). The order of averages of amylase activities was Tasaki > Pinky > Japanese Mino early type > Druti > Red Bombay. When amylase activities were plotted against proteins for the 5 cultivars, no correlation was found (data not shown). However, when amylase activities were plotted against starch contents, there was a positive correlation (Figure 1 $R^2 \approx 0.74$). In our present work, we demonstrate variation in the amylase activities of radish cultivars in Bangladesh. It is known that there are intraspecies variations in β-amylase activities in some crops, such as barley[18], rice[19] and soybeans[20]. Because amylase is a starch-degrading enzyme, it is prying that starch contents are positively correlated with amylase activities in radish cultivars (Figure 1). Hence, β-amylase may be considered nonphysiologically significant for germination, at least in soybean seeds[20] or alfalfa taproots[21]. It has been suggested that the enzyme might be a storage protein[22] or could play an unknown regulatory role in the starch synthesis pathway[23]. It is needless to say that consumers have a propensity to prefer sweet radishes. The plausible reason behind this favor is that amylase breaks down insoluble complex starches from vegetable materials in the food to simple sugars like glucose that can then be absorbed. Moreover, many reports have been demonstrated that β-amylase has a great commercial value in food and beverage industries. The practical interest of β-amylase was concentrated on its capacity to produce maltose syrups from starch[24]. Cultivars showing both high amylase activity and high starch content are therefore attractive to consumers and researchers of agro based industries as well. The present study thus gives information that may be used to select cultivars that increase sweetness and β-amylase activity for industrial application. We found considerable intraspecies variations in radish amylase activities. This may have different aspects. Effective use of radishes lies in the exploration of the intraspecies variations in

TABLE : β-amylase activities, protein and starch contents in different verities of radish root

Cultivar	β-amylase Activity (U/ml)	Amount (gm %)		
		Total Protein	Soluble Protein	Starch
Tasaki	2.75±0.01	0.69±0.03	0.16±0.02	0.50±0.01
Pinky	2.23±0.03	0.64±0.01	0.17±0.02	0.45±0.01
Druti	1.77±0.03	0.51±0.04	0.37±0.01	0.22±0.02
Japanese Mino early type	2.02±0.04	0.70±0.01	0.15±0.01	0.30±0.05
Red Bombay	1.35±0.02	0.77±0.02	0.13±0.03	0.27±0.02

Values represent the means ± standard deviations of triplicate determinations.

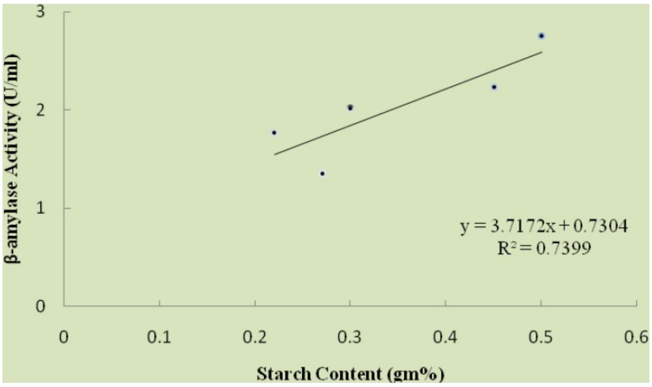

Figure 1 : Relationship between β-amylase activities and starch contents in 5 radish cultivars. The solid line is the regression line.

valuable components in radish cultivars.

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