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ENZYME ACTIVITIES AND MOBILIZATION OF NUTRIENTS IN BRASSICA (*BRASSICA* SPP.) AND WHEAT (*TRITICUM AESTIVUM* L.) SEEDS DURING GERMINATION

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Abstract

Context: Determination of the activities of hydrolytic enzymes from germinating wheat seeds and investigation of degraded nutrients from brassica and wheat seeds at different periods of germination are important factors for identification of richest sources of hydrolytic enzymes and nutrients.

Objectives: To study the activities of hydrolytic enzymes and degradation of seed storage substances of brassica (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) seeds during germination.

Materials and Methods: Three varieties of brassica (*Brassica napus* L., *B. juncea* L. *and B. campestris* L.) and three varieties of wheat (Akbar, Kanchan and Agrani) seeds were analyzed. Amylase, invertase, protease and lipase activities were assayed. Degradation of seed storage nutrients during germination were determined by conventional biochemical methods.

Results: The activities of amylase, invertase, protease and lipase varied from 8.02 – 48.69, 2.45 – 15.32, 20.96 – 45.45 and 2.72 – 12.76 units/ ml respectively. Degradation of nutrients in the three species of brassica and wheat seeds was also studied at different periods of germination. The amount of free sugar in brassica and wheat seeds ranged from 0.93 – 4.27% and 3.82 – 4.88%; reducing sugar content from 0.012 - 0.093% and 0.032 - 0.078%; starch from 1.42 – 4.70% and 10.26 – 69.65%; total protein from 3.1 – 25.37% and 2.3 – 18.37%; water-soluble protein from 1.5 – 14.24% and 1.0 – 6.50%; and oil content from 2.49 – 43.6% and 1.04 – 1.92% respectively.

Conclusion: The results suggest that the extracts from brassica and wheat seeds can be good sources of nutrients and hydrolytic enzymes which are applicable in food industry to improve food quality.

Key words: Brassica, Wheat, Carbohydrate, Protein, Lipid, Hydrolytic Enzyme.

Introduction

In plant seeds the storage nutrient substances are protein, fat and carbohydrate. During germination these storage nutrients used up for seedling growth. Seeds are classified into two distinct types according to the main compounds stored: those accumulate mostly lipids and proteins and those accumulate mostly carbohydrates and proteins (Mayer and Poljakoff-Mayber 1989). These reserve substances are degraded during germination and early plantlet growth and translocated to the growing parts. In plants a large proportion of carbon reserves as triacylglycerols (TAG), such as rapeseed (*Brassica napus* L.) and Arabidopsis, the activation of the β-oxidation and glyoxylate cycle during germination ensures conversion of fatty acid to carbohydrates necessary for the growth of the seedling before establishment of photosynthesis (Dieuaid *et al.* 1992, Pistelli *et al*. 1996). This is the first step of TAGs conversion to sugars required for growth of the germinating embryo (Ben-Miled *et al.* 2000). The germination of lipid-rich seeds such as rapeseed has been involved among other processes, the rapid mobilization of storage TAGs) in the cotyledons of seedlings. Such hydrolysis of TAGs is catalyzed by highly active lipases. Enzymes involved in

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the process of lipid mobilization, such as malate synthase and isocitrate lyase, are detectable towards the late stages of embryo development (Chia and Rawsthorne 2000).

Starch is the major component of most of the world's crop yield and the degradation of starch is essential in the germination of these plants (Yamasaki 2003). Starch degradation in the cereal grain requires the concerted action of several enzymes (MacGregor 1987) including limit dextrinase, β-amylase, α-glucosidase (Sun and Henson 1990), and α-amylase. Plants accumulate and store proteins in protein storage vacuole during seed development. Upon seed germination, storage proteins are degraded by hydrolytic enzymes to provide nutrients for embryo/seedling growth (Bing *et al.* 2003). Some metabolic changes in reserve compounds can be detected histologically in germinating seeds with the use of appropriate and specific histochemical procedures. There have been very few reports on the degradation of seed storage substances particularly on oil and cereal seeds. Therefore, the aim of the present work was to study the activities of hydrolytic enzymes and degradation of seed storage substances of brassica (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) during germination.

Materials and Methods

Materials: Three species of brassica seeds (*Brassica napus* L., *B. juncea* L. and *B. campestris* L.) and three varieties of wheat (Akbar, Kanchan and Agrani) seeds were collected from Bangladesh Agriculture Research Institute Regional Centres of Pabna, Dinajpur, Rajshahi and Jessore. After collection, the seeds were cleaned, dried in the sunlight and kept in a polyethylene bag and stored in the deep freeze (-10°C) for further biochemical analysis. Glucose, BSA, Dinitrosalicylic acid (DNS) and Sodium tungstate were purchased from Sigma Chemicals Ltd., USA. Chloroform, Ethanol and Trichloro acetic acid (TCA) were purchased from Pharmacia Fine Chemicals Ltd., Sweden. All other chemicals used were commercially available and of high purity.

Germination of seed: Quality and mature seeds of brassica and wheat were soaked in distilled water within a glass beaker with potassium permanganate for six hours for avoiding the growth of microorganism on seed surfaces during germination. The seeds were taken out from water and scattered in 20 cm petri dishes on two sheets of Whatman No. 1 filter paper moistened with distilled water. The dishes were then placed in the light room at 25 ºC for 120 h including soaking time. The germinating seeds at different hours (0, 24, 32, 48, 96 and 120 h) were collected and stored separately in the deep freeze for further experimental purposes.

Preparation of the crude extract: Germinated seeds (100g) were crushed in a mortar and suspended in 40 ml of 30% acetone. After occasional gentle stirring for 3 h at 4°C the suspension was filtered through double layer of muslin cloth. Filtrate was centrifuged in a refrigerated centrifuge at 5500 rpm for 15 min at 4ºC. The supernatant was used as "crude extract". The crude extract was saturated to 30 - 50% by the addition of solid ammonium sulphate under constant and gentle stirring at 4 ºC. The resulting precipitate was collected by centrifugation, dissolved in minimum volume of pre-cooled distilled water and dialyzed against distilled water for 24 h at 4 ºC. The dialyzed solution was then centrifuged in a refrigerated centrifuge machine at 5500 rpm for 15 min. The clear supernatant thus obtained was designated as "crude enzyme solution".

Measurement of enzyme activity: Amylase activity was assayed following the method as described by Jayaraman (1985). Starch solution (1%) was used as substrate (1 g in 100 ml of 0.1 M phosphate buffer, pH 6.7). Invertase activity was assayed following the method of Mahadevan and Sridhar (1982). Sucrose solution (2.5%) was used as a substrate. The protease activity was measured following the method of Kunitz (1947). The milk protein casein was used as substrate. Lipase activity was assayed by the method described by Sugihara *et al.* (1990). Olive oil was used as substrate.

Degradation of nutrients during germination: Total protein content of brassica and wheat seeds was determined by the method of Micro-Kjeldahl (Wong 1923) and the water-soluble protein content by the method of Lowry *et al.* (1951). BSA was used as substrate and protein content was calculated from a standard curve constructed with bovine serum albumin. Free sugar content was determined calorimetrically by the anthrone method (Morse 1947). Glucose was used as standard substrate. Reducing sugar content was estimated by DNS (Dinitrosalicylic acid) method (Miller 1972). Extraction of sugar from brassica and wheat seeds was done following the method of Loomis and Shull (1937) and the amount of reducing sugar was calculated from a standard curve constructed with glucose. The starch content was determined by the anthrone method (Morse 1947, Jayaraman 1985) and the amounts of starch were also calculated from a standard curve constructed with glucose.The oil was extracted from the brassica and wheat seeds of previously cleaned, dried and stored seeds by the solvent extraction process. For this purpose, the seeds were crushed in a glass mortar and the oil was extracted with petroleum ether (40-60^{0}C): acetone (1:1 v/v) in a Soxhlet apparatus. The amount of oil recorded. Each analysis was performed in triplicates and the averages were taken.

Results

The amylase activities of the wheat variety Akbar, Kanchan and Agrani were estimated during different germinating periods. Among the three varieties, the amylase activities were found to be highest in Akbar and lowest in Agrani at 48 h of germination. Among the varieties the maximum activity of invertase was found in Akbar and minimum in Kanchan at 72 hours of germination. The highest protease activity was found in Kanchan and lowest in Agrani at 40 hours of germination while Akbar contained the value between them. Of the varieties Agrani represents the highest lipase activity and Akbar represents the lowest activity at 40 hours of germination. From the above result it was clear that Akbar is a suitable source of amylase and invertase, Kanchan is of protease and Agrani is of lipase for purification and characterization (Table 1).

Amount of degraded nutrients in three species of *Brassica* and wheat seeds is shown in Table 2. From the table, it is found that wheat seeds contain a larger amount of free sugar than that of *Brassica* seeds. *B. juncia* contains a little higher amount of free sugar (4.27%) than that of *B. napus* (4.20%) and *B. campestries* (4.15%) variety. While in wheat seeds, Akbar variety contains higher amount of free sugar (4.88%) than that of Kanchan (4.87%) and Agrani (4.76%) varieties. During germination, the degradation of free sugar in Brassica seeds is rapid while the degradation in wheat seeds is comparatively slow. Degradation of reducing sugar in different periods of germination in the *Brassica* and wheat seeds is significant. In *Brassica* seeds reducing sugar is converted from 0.093 to 0.012%, whereas in wheat seeds the conversion is from 0.078 to 0.032%. The result indicates that degradation of reducing sugar is very faster in *Brassica* seeds than that of wheat seeds (Table 2).

It is found that, wheat seeds contain a larger amount of starch (69.65 - 67.35%) than that of *Brassica* seeds (4.70 - 4.57%). In wheat seeds, Akbar variety contains the highest amount of starch (69.65%), followed by Kanchan (68.25%) and Agrani (67.35%). Similarly, in *Brassica* seeds, *B. juncia* contains the highest amount of starch (4.70%), followed by *B. napus* (4.67%) and *B. campestries* (4.57%). *Brassica* seeds contain a significant amount of protein (total 25.37 - 25.17% and water-soluble 18.37 - 17.80%). In wheat seeds, Akbar variety contain the highest amount of protein (total 14.24%, water-soluble 6.50%), while Agrani contain the lowest amount of protein (total 12.35%, water-soluble 6.14%) (Table 2).

The oil content in *Brassica* seeds is much higher than that of wheat seeds as usual. In *Brassica* seeds, *B. napus* contains the highest amount of oil (43.6%), followed by *B. campestries* (42.4%) and *B. juncia* (41.3%). While in wheat seeds, Akbar variety contains slightly higher amount of lipid than that of other two varieties.

Table 1. Activities of amylase, invertase, protease and lipase in the three different varieties of wheat seed

Variety	Hour after	Activity of enzymes (Units/ml)			
		Amylase	Invertase	Protease	Lipase
Akbar	36	10.95±0.02	02.55±0.02	25.04±0.01	06.35±0.03
	40	35.52±0.01	07.85±0.01	38.95±0.02	10.55±0.03
	48	48.69±0.02	11.08±0.01	34.51±0.02	05.19 ±0.01
	72	18.82±0.04	15.32±0.02	26.45±0.03	04.35±0.02
	96	12.45±0.03	09.15±0.03	22.35±0.02	03.85±0.03
	120	10.23±0.01	03.30±0.02	21.02±0.01	02.72±0.02
Kanchan	36	09.04±0.02	02.45±0.01	27.15±0.01	06.92±0.02
	40	31.85±0.03	06.15±0.02	45.45±0.04	11.32±0.02
	48	42.25±0.01	10.10±0.01	36.00±0.03	06.94±0.01
	72	15.05±0.01	13.21±0.02	28.25±0.02	05.75±0.03
	96	11.35±0.02	08.45±0.03	23.35±0.03	04.85±0.01
	120	10.11±0.05	03.01±0.01	21.71±0.01	03.02±0.02
Agrani	36	09.03±0.01	02.52±0.01	24.01±0.02	08.45±0.01
	40	32.65±0.02	06.95±0.03	38.01±0.02	12.76±0.02
	48	38.11±0.03	10.29±0.02	34.86±0.02	10.27±0.03
	72	19.18±0.02	13.59±0.02	26.35±0.03	07.65±0.01
	96	10.95±0.04	08.55±0.03	22.25±0.01	05.01±0.02
	120	08.02±0.01	03.19±0.01	20.96±0.02	04.35±0.03

Discussion

Amylase and invertase play a major role in carbohydrate metabolism in several plant tissues. Starch is the major component of most of the world's crop yield and the degradation of starch is essential in the germination of these plants (Yamasaki 2003). From the results, it was found that amylase activity increased considerably during germination. The finding is in good agreement with those reported by Evans *et al.* (1997) and Sopanen and Lauriere (1989). The expression and distribution of plant invertases has been especially well documented, because these are considered to play an important role in sugar metabolism (Kastle and Clark 1903). Upon seed germination, storage proteins are degraded by protease enzymes to provide nutrients for embryo/seedling growth (Bing *et al.* 2003). Lipases are lipolytic enzymes catalyze the hydrolysis of fats as well as esters of fatty acids with alcohol's (Sarda and Desnuelle 1957, Wills 1965).

The present results clearly demonstrate that the percentage of both types of proteins present in different species/ varities of *Brassica* and wheat seeds decrease gradually up to 48 h and then sharply decline up to 120 h of germination. This indicates that after 48 h of germination, the proteolytic enzymes may vigorously involve for hydrolysis on seed storage proteins. The degradation of nutrient components namely sugar, protein, starch and lipid were found to be much different and the results are in good agreement with earlier results reported by Gad *et al.* (1965). The seed storage substances gradually decrease with the increase of germination time. The decrease in different types of nutrient content in the germinating seeds probably caused by the involvement of the hydrolytic enzymes, which hydrolyses seed storage nutrient, a process that generates amino acids and sugars for the development of embryo and seedling growth. The decrease in oil content in the germinating seeds probably caused by the involvement of a lypolytic enzyme, which is responsible for hydrolysis of triacylglycerol that ultimately generate sugars for the growth of germinating embryo. This finding is in good agreement with those reported by Miled Ben *et al.* (2000).

Table 2. Amounts of degraded nutrients in the three species of *Brassica* and three varieties of wheat seeds at different periods of germination

		Species/ variety	Free Sugar (g %) at different hour (h) (Mean ± SD)					
			(0 h)	24 h	48 h	72h	96 h	120 h
Free sugar	Brassica	*B. napus*	4.20 ± 0.02	2.92 ± 0.05	2.58 ± 0.07	1.12 ± 0.04	0.99 ± 0.03	0.95 ± 0.03
		B. campestries	4.15 ± 0.02	2.87 ± 0.07	2.55 ± 0.05	1.11 ± 0.03	0.95 ± 0.06	0.93 ± 0.06
		B. juncia	4.27 ± 0.03	2.94 ± 0.04	2.58 ± 0.05	1.18 ± 0.02	1.05 ± 0.05	0.99 ± 0.09
	Wheat	Akbar	4.88 ± 0.07	4.79 ± 0.07	4.72 ± 0.04	4.66 ± 0.04	4.03 ± 0.04	3.82 ± 0.04
		Kanchan	4.87 ± 0.07	4.78 ± 0.03	4.75 ± 0.05	4.67 ± 0.07	4.22 ± 0.05	3.96 ± 0.06
		Agrani	4.76 ± 0.06	4.65 ± 0.05	4.63 ± 0.04	4.59 ± 0.04	4.20 ± 0.07	3.83 ± 0.05
Reducing sugar	Brassica	*B. napus*	0.093± 0.06	0.070± 0.06	0.042± 0.06	0.037± 0.07	0.022± 0.06	0.012± 0.04
		B. campestries	0.091± 0.04	0.070± 0.04	0.042± 0.05	0.037±0.05	0.022± 0.04	0.012± 0.07
		B. juncia	0.089± 0.07	0.069± 0.04	0.042± 0.07	0.037± 0.06	0.022± 0.05	0.012± 0.05
	Wheat	Akbar	0.078± 0.05	0.068± 0.05	0.057± 0.06	0.054± 0.05	0.049 ± 0.07	0.042± 0.06
		Kanchan	0.067± 0.07	0.056± 0.07	0.047± 0.06	0.044± 0.07	0.039± 0.05	0.033± 0.06
		Agrani	0.064± 0.04	0.054± 0.07	0.042± 0.07	0.041± 0.05	0.037± 0.06	0.032± 0.05
Starch	Brassica	*B. napus*	4.67± 006	4.32± 0.06	2.57± 0.06	1.79± 0.07	1.58± 0.07	1.44± 0.05
		B. campestries	4.57± 006	4.30± 0.05	2.52± 0.06	1.75± 0.06	1.55± 0.06	1.42± 0.08
		B. juncia	4.70± 005	4.40± 0.05	2.67± 0.06	1.85± 0.07	1.65± 0.05	1.52± 0.06
	Wheat	Akbar	69.65± 005	58.49± 0.08	38.29± 0.07	28.25± 0.05	18.36± 0.05	10.26± 0.05
		Kanchan	68.25± 005	58.15± 0.05	38.12± 0.06	28.14± 0.05	18.32± 0.06	10.28± 0.07
		Agrani	67.35± 0.06	56.39± 0.09	38.11± 0.05	27.34± 0.06	17.28± 0.06	9.38± 0.07
Total protein	Brassica	*B. napus*	25.37± 0.06	22.62± 0.07	18.87± 0.06	9.98± 0.07	7.9± 0.05	4.5± 0.05
		B. campestries	25.27± 0.06	21.62± 0.05	17.82± 0.06	8.95± 0.05	6.8± 0.07	3.4± 0.05
		B. juncia	25.17± 0.06	21.32± 0.07	17.52± 0.04	8.55± 0.05	6.3± 0.03	3.1± 0.02
	Wheat	Akbar	14.24±0.03	9.88± 0.03	8.32± 0.03	5.87± 0.04	3.90± 0.02	1.5± 0.02
		Kanchan	13.57± 0.03	8.92± 0.03	7.85± 0.02	5.12± 0.03	3.11± 0.03	1.6± 0.02
		Agrani	12.35± 0.04	7.95± 0.05	6.98± 0.05	4.82± 0.04	3.88± 0.04	1.5± 0.03
Water soluble protein	Brassica	*B. napus*	18.37± 0.06	15.62± 0.06	11.87± 0.02	08.98± 0.05	5.91± 0.04	3.2± 0.03
		B. campestries	17.85± 0.05	14.60± 0.04	10.85± 0.04	07.96± 0.02	4.90± 0.03	2.4± 0.04
		B. juncia	17.80± 0.03	14.55± 0.02	10.82± 0.02	07.91± 0.03	4.85± 0.05	2.3± 0.02
	Wheat	Akbar	6.50± 0.03	6.42± 0.03	5.35± 0.05	3.57± 0.02	1.98± 0.03	1.1± 0.03
		Kanchan	6.48± 0.04	6.52± 0.03	5.27± 0.04	3.20± 0.03	1.99± 0.04	1.2± 0.04
		Agrani	6.14± 0.04	6.02± 0.04	5.29± 0.05	3.85± 0.04	1.62± 0.04	1.0± 0.02
Oil	Brassica	*B. napus*	43.6± 0.04	38.33± 0.03	15.43± 0.05	5.79± 0.05	3.34± 0.03	2.92± 0.04
		B. campestries	42.4± 0.05	38.30± 0.02	15.40± 0.04	5.78± 0.04	3.32± 0.03	2.90± 0.05
		B. juncia	41.3± 0.02	38.28± 0.04	15.40± 0.03	5.76± 0.02	3.30± 0.02	2.89± 0.05
	Wheat	Akbar	1.92± 0.04	1.61± 0.03	1.52± 0.05	1.43± 0.04	1.15± 0.04	1.08± 0.03
		Kanchan	1.73± 0.04	1.54± 0.03	1.35± 0.05	1.29± 0.04	1.20± 0.04	1.10± 0.03
		Agrani	1.65± 0.05	1.46± 0.02	1.40± 0.05	1.33± 0.04	1.27± 0.02	1.13± 0.04

Conclusion

The results suggest that the extracts from brassica and wheat seeds can be good sources of nutrients and hydrolytic enzymes which are applicable in food industry to improve food quality.

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