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STARCH, SUGAR, AMYLASE AND INVERTASE ACTIVITY IN THE GERMINATING SEEDS OF MODERN WHEAT VARIETIES

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Abstract: Amylase and invertase activity was studied in germinating seeds of eight modern wheat varieties (Akbar, Aghrani, Ananda, Balaka, Barkat, Kanchan, Pavon and Sonalika). Activities of the enzymes were low at the initial stages and thereafter significantly increased and further decreased at later stages of germination. The peak amylase and invertase activity was observed at 60 and 72 h of incubation for germination respectively. Protein content was significantly increased with the period of germination. Starch depletion was slow initially and thereafter fast. The degradation rate of starch was about 60% and accumulation of sugar was 4 times higher in seeds at 96 h of germination over the ungerminated seeds.

Keywords: Amylase activity, germinating seeds, invertase activity, wheat.

INTRODUCTION

Stored starch plays an important role in the development of embryo during germination of seeds. The increase in metabolic activity in germinating seeds is due to the induction of some of the hydrolytic enzymes.[1,2] Amylase and invertase are two important hydrolytic enzymes that increase sugars in germinating seeds of rice.[3] Starch is hydrolysed by the amylolytic enzymes to provide substrate and energy sources for the embryo during germination. The amylase activity was slow up to 48 h after germination.[4] The simultaneous increase in soluble sugars and amylase activity and decrease in starch in *Vicia faba* could be due to faster breakdown of starch.[5] The amylolytic breakdown of storage carbohydrates is also important in the brewery industry.[6]

The present study reports the activities of two hydrolytic enzymes and the degradation of starch during a 4 d period of germination of seeds of modern wheat varieties.

METHODS AND MATERIALS

Seeds of eight Bangladesh wheat varieties viz. Aghrani, Akbar, Ananda, Balaka, Barkat, Kanchan, Pavon and Sonalika were obtained from the Agronomy Department of the Bangladesh Agricultural University (BAU). The experiment was conducted in a split plot design with varieties in the main plots and varying periods of incubation (0, 12, 24, 36, 48, 60, 72, 84 and 96 h) in subplots. Seeds were soaked in distilled water and allowed to germinate from 0 h (dry seed) to 96 h.

Samples were taken every 12 h. Two grams of seeds were ground in 10 ml of 0.2M acetate buffer (pH 4.8). The suspension was centrifuged at 2000 rpm for 5 min to remove cell debris. The supernatent was again centrifuged at 10478g at 0°C for 55 min and made up to 20 ml with buffer. Protein was estimated by absorption at 260 and 280 nm.[7]

For reducing sugars, two grams of seeds were boiled with 80% ethanol. From the alcohol-insoluble residue, starch was estimated colorimetrically, with anthrone reagent.[8]

The specific activities of amylase and invertase were assayed according to the method of Mahadevan and Sridhar[9] and reducing sugar contents were estimated by Nelson's modification of Somogyi's method.[10]

The data of the experiment were analyzed statistically and the mean differences were adjudged by the LSD test at 5% level of significance.

RESULTS AND DISCUSSION

Germination rate of eight varieties was variable (Table 1). Germination started at 36 h and gradually increased up to 96 h. At 36 h of incubation, Balaka, Barkat and Sonalika showed the higher percentage of germination followed by other varieties. At 96 h of incubation, the varieties completed about 95% germination.

Table 1: Germination percent of eight wheat varieties at different hours of incubation.

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96
Aghrani	0	0	0	8.3	15	60	90	94	99
Akbar	0	0	0	3	24	55	73	80	90
Ananda	0	0	0	6	20	30	40	58	90
Balaka	0	0	0	15	16	48	74	90	97
Barkat	0	0	0	12	14	18	38	67	89
Kanchan	0	0	0	6	15	20	32	57	80
Pavon	0	0	0	8	25	34	45	50	84
Sonalika	0	0	0	10	45	70	87	96	98

Table 2 shows the variation in amylase activity between wheat strains during germination. Amylase activity was the highest in Balaka which was similar to Barkat, Pavon and Aghrani and lowest in Akbar. Mean amylase

activity was significantly increased up to 60 h of incubation and declined thereafter. The activity was about 5 times higher at the peak and 3 times higher at 96 h compared to ungerminated seeds. Maximal amylase activity in Aghrani occurred at 48-60 h. In Pavon, maximal amylase activity was achieved in the period 36-72 h. The results are in agreement with the finding of Thimmaiah[11] who found that amylase activity was variable among the wheat cultivars and the activity was low at initial stages and increased steeply and further decreased at later stages of germination. Amylase activity increased up to 120 h of germination in cotton seeds.[4]

Table 2: Amylase activity of eight wheat varieties at different hours of germination (units of reducing sugar/h/mg of protein).

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96	Mean
Aghrani	8.49	14.34	20.70	21.57	31.38	29.52	19.4	17.20	14.39	19.67
Akbar	6.68	10.23	15.07	17.58	23.76	29.68	17.80	17.02	15.90	17.08
Ananda	6.08	8.14	12.61	16.45	19.18	35.39	30.90	23.03	19.95	19.08
Balaka	6.73	12.51	18.74	24.83	27.45	32.95	25.91	21.83	16.74	20.85
Barkat	6.21	12.90	15.97	21.44	24.90	31.92	26.48	25.13	19.69	20.52
Kanchan	5.53	10.20	17.30	20.83	24.49	28.83	23.87	19.95	16.05	18.56
Pavon	5.05	7.25	20.43	34.18	33.45	33.73	36.73	23.62	18.77	20.36
Sonalika	6.86	10.58	12.66	13.52	16.47	29.65	35.48	23.76	20.09	18.79
Mean	6.45	10.77	16.69	21.30	26.78	31.46	27.07	21.44	17.70	
		h				hxv				v
LSD (0.05)		1.48				4.51				1.65

Invertase activity differed between varieties and with time after germination (Table 3). The mean invertase activity was high in Barkat , Akbar, Kanchan, Pavon, Sonalika and Ananda and lowest in Balaka. Invertase activity increased up to 72 h and thereafter declined. There were varietal differences in the time required to achieve peak invertase activity. Takeo Nomura *et al.*[12] found that invertase activity in germinating rice seed also showed a similar peak in activity. The soluble protein contents of the eight varieties were not similar (Table 4). The protein content was highest in Kanchan (52 mg protein per gram seeds) and in Balaka (up to 45 mg protein per gram of seeds). The rest of the varieties ranked in intermediate position. Soluble protein contents increased with germinating time. The soluble protein contents were found to be different in cotton varieties[13] and to increase during germination of rice seeds.[2]

Table 3: Invertase activity of eight wheat varieties at different hours of germination (units of reducing sugar/h/mg of protein).

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96	Mean
Aghrani	4.59	5.94	6.57	7.54	8.55	9.57	9.78	10.70	10.34	8.18
Akbar	4.92	5.92	6.40	8.25	12.36	12.90	14.18	13.38	8.54	9.65
Ananda	5.24	6.48	7.73	8.65	8.83	12.90	13.31	11.50	8.26	9.21
Balaka	4.47	5.30	6.56	6.83	8.22	10.15	9.41	8.68	7.36	4.44
Barkat	4.90	6.41	8.74	9.77	11.30	13.09	14.56	12.04	10.34	10.13
Kanchan	4.36	5.38	7.38	7.86	10.61	13.31	15.01	12.66	9.59	9.57
Pavon	4.97	6.16	6.71	9.09	10.60	12.34	15.15	10.70	8.56	9.36
Sonalika	3.72	6.31	6.98	7.64	10.42	11.14	12.25	10.39	8.94	8.64
Mean	4.65	5.99	7.13	8.20	10.1	11.93	12.96	11.26	8.99	
			h			hxv			v	
LSD (0.05)			1.12			3.11			0.99	

Table 4: Soluble protein of eight wheat varieties at different hours of germination (mg of protein/gram of wheat).

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96	Mean
Aghrani	31.30	39.52	40.41	40.97	42.45	42.50	42.85	43.59	44.85	40.94
Akbar	29.27	36.28	39.41	40.48	42.03	43.58	45.39	46.56	47.43	41.16
Ananda	27.13	34.63	34.98	39.39	40.63	44.74	45.95	46.56	48.07	40.23
Balaka	27.46	36.67	38.80	41.14	42.21	43.17	43.59	43.31	45.20	40.17
Barkat	31.30	36.32	37.36	39.13	41.70	42.56	45.11	44.86	54.29	41.63
Kanchan	30.03	37.37	41.97	42.40	48.34	46.83	46.91	49.61	51.98	43.94
Pavon	26.78	35.58	39.98	41.67	43.28	43.07	47.38	48.14	48.89	41.64
Sonalika	28.45	31.41	39.00	41.98	40.73	43.79	46.23	51.29	53.72	41.84
Mean	28.97	35.97	38.99	40.89	42.67	43.78	45.43	46.74	49.30	
			h			hxv			v	
LSD (0.05)			1.74			5.68			2.95	

The breakdown of starch in the varieties is shown in Table 5. The rate of degradation was variable. Starch depletion in the varieties was up to 60% at 96 h of incubation. The accumulation of reducing sugars was variable among varieties and period on incubation (Table 6). Sonalika showed highest amount of sugar which was identical with Kanchan and Balaka and the lowest was in Akbar. The sugar content was significantly increased with the period of germination. The rate of accumulation of sugars was higher in Balaka, Barkat at 96 h and it was 5 times higher than the ungerminated seeds. These results are in agreement with those observed by Dhanunjayanath[14] who found 74% depletion of starch and 4 times increase of sugar at 96 h over the zero period of germination in horse gram.

Table 5: Percent starch contents of eight wheat varieties at different hours of germination

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96	Mean
Aghrani	60.31	58.95	55.53	50.54	44.79	39.84	33.53	29.77	23.87	44.17
Akbar	56.24	53.88	51.22	47.50	43.93	38.37	34.51	28.11	23.65	41.99
Ananda	56.84	54.58	50.13	46.40	42.70	39.65	33.75	29.11	23.65	41.90
Balaka	57.79	55.65	51.52	47.55	44.19	38.49	34.10	28.12	23.22	42.29
Barkat	58.77	55.99	52.36	47.56	42.13	37.25	32.09	27.89	23.34	41.93
Kanchan	56.55	54.59	50.82	45.91	41.86	37.45	34.40	29.81	25.26	41.85
Pavon	58.56	57.09	51.66	46.62	41.63	37.89	33.59	29.49	24.33	42.32
Sonalika	61.86	59.23	53.85	48.73	44.15	37.86	32.86	28.19	23.16	43.32
Mean	58.36	56.24	52.21	47.60	43.17	38.41	33.60	28.81	23.81	

	h	hxv	v
LSD (0.05)	0.91	3.49	3.51

From the results, it seems that the cultivars showed variability with respect to the level of hydrolytic enzymes. This could be ascribed to their growth rate and varietal genetic difference. The enzyme activity was significantly low in dry seeds compared to seedling due to residual enzyme synthesis during maturation of seed before harvest and the *de novo* synthesis of the enzymes during germination. The accumulation of more sugars at later stage was due to the breakdown of starch by the activity of hydrolytic enzymes.

Table 6: Reducing sugar contents of eight wheat varieties at different hours of germination (mg/gm of wheat).

Hours (h)/ Varieties (v)	0	12	24	36	48	60	72	84	96	Mean
Aghrani	1.85	1.55	2.22	2.80	3.41	5.89	7.42	7.62	8.38	4.57
Akbar	1.61	1.79	1.98	2.51	2.93	3.27	3.73	5.93	7.28	3.45
Ananda	1.71	1.88	2.12	2.67	3.31	4.27	4.78	5.39	6.00	3.57
Balaka	1.69	2.92	3.37	3.93	4.82	5.92	6.52	7.62	8.29	5.01
Barkat	2.10	2.45	2.88	3.53	3.90	4.53	5.29	6.70	7.25	4.29
Kanchan	2.57	3.66	4.28	5.27	5.80	6.43	6.77	7.17	7.17	5.52
Pavon	2.48	2.99	3.28	3.53	3.70	4.13	4.82	5.96	7.37	4.25
Sonalika	3.03	3.88	4.43	5.28	6.05	6.41	6.86	7.19	7.84	5.66
Mean	2.13	2.64	3.07	3.70	4.24	5.11	5.76	6.70	7.51	

	h	hxv	v
LSD (0.05)	0.27	0.38	0.58

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