Alpha-Amylase Activity in Wheat Kernels Matured and Germinated Under Different Temperature Conditions[1]

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ABSTRACT

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Wheat cultivars Hyslop, Yamhill, PI 178211, Brevor, and Tom Thumb were grown in growth chambers at 15.5 and 26.6° C, and in the field, where temperatures ranged from 15.5 to 20.5° C during the grain-filling period. Mature kernels harvested from these environments were germinated at 15.5, 20, and 29.5° C. Endosperm α-amylase activity during maturation and germination was analyzed. α-Amylase activity during maturation varied with temperature. At 15.5° C, the activity peaked 20 days after anthesis, then gradually decreased. Activity in the field-grown material was intermediate and gradually decreased with maturity. At 26° C, the activity was lowest. Cultivars varied quantitatively, but a general trend persisted. An inverse relationship between α-amylase activity and kernel weight was observed within and among cultivars. During germination in the growth chambers, activity was highest at 15.5° C, but in field-grown kernels, activity was highest at 20° C. Kernels of cultivars PI 178211, Brevor, and Tom Thumb grown at 15.5° C were consistently low in α-amylase activity during maturation and germination.

Alpha-amylase is an important starch-degrading enzyme in the endosperm of cereal grains. It degrades starch by hydrolyzing the α-1,4-glucosidic linkages that produce dextrins and very small amounts of maltose and glucose. The maltose and dextrins are further degraded by glucosidases, β-amylase, and phosphorylase to glucose and glucose-6-phosphate (Briggs 1972). The reaction products provide substrates and an energy source for the embryo during germination.

Wheat kernel α-amylase is found during development and germination (Kruger 1972a, 1972b), and different isozymes are found during each stage of growth. Kruger (1972b) also observed a correlation between the decline of starch and the presence of germination isozymes. A quantitative study was not conducted, however.

Environmental factors influence the synthesis and activity of all enzymes. Alpha-amylase synthesis, in particular, has been found to be temperature dependent (Groat and Briggs 1969). The stimulated synthesis of α-amylase by exogenous gibberellin in whole wheat kernels and in endosperm halves was found to increase with increasing temperature until 30° C. When temperatures were higher than 30° C, however, the enzyme activity decreased sharply (Moro et al 1963). Lowered α-amylase activity was observed in germinating crimson clover seeds at sub- or super-optimal temperatures of germination (Ching 1975). A greater amino acid incorporation for protein synthesis was reported at higher temperatures (Marcus 1969, Ching 1975). In addition to affecting the synthesis and activity of enzymes in cells, temperature changes also cause changes in nutrient uptake, translocation, respiration, and photosynthesis in plants and seeds.

Alpha-amylase activity is a major cause of damage to cereal starch quality that occurs during presprouting (Strand 1980, McCrate et al 1981). The enzyme in mature wheat kernels is either the residue of enzyme that formed during maturation (Kruger 1972a) or is synthesized during germination (Kruger 1972b, Briggs 1972). The extent to which the residual enzyme degrades starch upon wetting of mature kernels depends on the enzyme content, which is specific to cultivars (Derera et al 1977), and on the enzyme activity, which is related to the degree and length of wetting. A high residual α-amylase activity was associated with starch degradation in preharvest-sprouted wheat kernels (Bingham and Whitmore 1966, Gale 1976, Kruger 1976). No such association was reported in rye (Stoy and Sundin 1976). In rye, preharvest sprouting is highly inversely correlated with seed dormancy, and the formation and expression of seed dormancy are generally controlled by temperature (Vegis 1964, Reiner and Loch 1976, Strand 1980).

Information on α-amylase activity in kernels of wheat cultivars grown at different temperatures is limited. Because α-amylase activity is related to presprout damage and flour quality, the effects of temperature on α-amylase activity during maturation and

TABLE I
Characteristics of Cultivars Used as Experimental Material

Cultivar	Letter Designation	Maturity	Kernels	Seed Dormancy[a]
Hyslop	H	Intermediate	White, soft	Low
Yamhill	Y	Early	White, soft	Low
PI 178211	P	Intermediate	Red, hard	High
Brevor	B	Intermediate	White, soft	Intermediate
Tom Thumb	T	Late	Amber, hard	High

[a] Reddy et al. 1984. Unpublished data.

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germination of kernels of several wheat cultivars grown in the Pacific Northwest states were investigated.

MATERIALS AND METHODS

Five cultivars of winter wheat (Table I) varying in maturity date, kernel color, and seed dormancy were planted in October, 1976, at the Hyslop Experimental Farm, Corvallis, OR. Before anthesis, four plants of each cultivar were transplanted into large pots. After three days of acclimation, two plants of each cultivar were moved to growth chambers that had an 18-hr photoperiod, temperatures of 15.5 and 26.6°C, and a light intensity of 12,000 lx. Kernels of each cultivar were therefore grown under three different temperature conditions: in a growth chamber at 15.5 and 26.6°C, and in a field in which night temperatures varied from 8.2 to 11.7°C and day temperatures varied from 22.1 to 29.2°C during the grain-filling period. Individual spikes on each plant were tagged with the date of anthesis. For α-amylase analysis, two kernels were taken from the middle of one spike of each wheat plant at 10-day intervals from anthesis until full maturity.

When mature, the kernels from the individual tagged spikes of each cultivar grown under the three maturation temperatures were harvested and stored in glass jars in a freezer. Uniform kernels of each cultivar, ripened at the three different temperatures, were placed for germination at 15.5, 20, and 29.5°C. The α-amylase assay was conducted on the first, second, third, and sixth days of germination on a sample of two kernels selected to represent the majority of the kernels.

TABLE II
Total α-Amylase Activity[a] at Various Growth Stages of Kernels Ripened at Different Temperature Conditions

Days After Anthesis	Cultivars	Temperature Conditions[b] 15.5°C	26.6°C	Field (15.5–20.5°C)
	H	26.5	16.5	35.5
	Y	22.5	15.0	36.0
	P	12.0	13.5	46.5
10	B	7.5	26.5	20.0
	T	31.0	8.0	16.0
	H	42.5	20.0	15.0
	Y	72.0	11.0	12.0
20	P	52.5	18.5	20.5
	B	45.5	23.0	34.5
	T	19.0	22.5	21.0
	H	39.0	16.0	25.0
	Y	35.0	20.0	17.5
30	P	26.0	13.5	6.0
	B	36.5	10.5	4.5
	T	26.0	7.5	6.5
	H	24.0	5.0	18.5
	Y	23.5	9.0	20.5
40	P	10.0	7.5	24.5
	B	24.5	7.5	21.0
	T	15.0	12.5	3.5
	H	19.0	···	11.5
	Y	16.5	···	5.5
50	P	10.0	Mature	4.0
	B	26.0	···	3.7
	T	12.5	···	7.5
	H	11.0	13.5	5.2
	Y	10.0	21.0	8.5
Dry	P	8.5	7.5	8.5
	B	4.0	8.5	13.0
	T	9.0	2.5	8.5

[a] Micrograms of starch hydrolyzed per minute per kernel.
[b] At $P = 0.05$, the shortest significant ranges (Duncan's multiple range test) for comparing cultivars and temperatures are 8.2 and 7.8, respectively.

Determination of Alpha-Amylase Activity

A sample of the endosperm of two kernels was ground to a smooth paste with a mortar and pestle at room temperature in 5 ml of calcium-acetate buffer containing 10 m*M* each of sodium acetate and calcium chloride (pH 6.0). To inactivate β-amylase and other labile hydrolytic and proteolytic enzymes, the extract was heated in a thermo-block at 70°C for 20 min with occasional stirring (Briggs 1967). The slurry was centrifuged at 10,000 × *g* for 5 min, and the supernatant was decanted and used as the enzyme preparation.

Each reaction mixture contained 1 ml of reaction buffer (same composition as the grinding buffer but adjusted to pH 4.8) and 1 ml of substrate starch solution (1% w/v in the reaction buffer). The enzyme preparation in various volumes plus reaction buffer to 0.5 ml was incubated at room temperature (22–24°C) for 2–10 min, depending on the enzyme activity. The enzyme reaction was stopped with 1 ml of color reagent (60 mg each of KI and I_2 in 100 ml of 0.05*N* HCl). The color changes over time were read against the zero-time mixture at 620 nm on a Beckman DB spectrophotometer. Total α-amylase activity was calculated as micrograms of starch hydrolyzed per kernel per minute (total activity).

The data for total α-amylase activity obtained during maturation and germination of kernels were subjected to statistical analysis in a split-plot factorial design. For comparisons of the enzyme activity among cultivars, temperatures, and days of germination, the shortest significant ranges (Rp) were calculated according to Duncan's multiple range test (Duncan 1955).

RESULTS

Alpha-Amylase Activity During Maturation

In kernels developed at 15.5°C, the enzyme activity peaked at 20 days after anthesis and decreased thereafter in all cultivars except Tom Thumb (Table II). At 26.6°C, however, the enzyme activity was relatively low throughout, and no pattern emerged (Table II). In the field-grown material, an initial high enzyme activity at 10 or 20 days was followed by fluctuations during the development of kernels (Table II).

Although significant cultivar differences in enzyme activity were observed during most of the stages of kernel development at all temperatures, the rank order of cultivars at different stages of maturation was not the same within and across different maturation temperatures (Table II). In general, the residual

TABLE III
Total α-Amylase Activity[a] in Kernels of Different Cultivars Ripened at 15.5°C and Germinated at 15.5, 20, and 29.5°C

Germination Temperature (°C)	Cultivars	Days of Germination 1	2	3	6
	H	90[b]	115	202[c]	699[c]
	Y	158	161	237[c]	955[c]
15.5	P	70	79	64	100
	B	86	101	109	187
	T	134	124	99	133
	H	65	74	166	171
	Y	122	150	186	186
20.0	P	30	44	53	94
	B	42	75	93	101
	T	103	124	130	191
	H	52	31	91	125
	Y	82	84	108	158
29.5	P	19	32	38	111
	B	57	56	94	127
	T	60	65	50	62

[a] Micrograms of starch hydrolyzed per minute per kernel.
[b] At $P = 0.05$, the shortest significant ranges (Duncan's multiple range test) for comparing cultivars and days of germination are 68 and 74, respectively.
[c] Enzyme activity in germinated kernels.

enzyme activity in dry kernels dropped to very low levels, with a few exceptions. In Hyslop and Yamhill kernels developed at 26.6° C, the residual enzyme activity is relatively higher.

Alpha-Amylase Activity During Germination

In most cases, the enzyme activity increased during the six-day germination period in all cultivars, regardless of ripening and germination temperatures (Tables III, IV, and V). However, significant increases in enzyme activity occurred only in germinated kernels on the third and sixth days of germination. The increase in activity with germination time varied with the different combinations of ripening and germination temperatures. All five cultivars showed germination-associated high enzyme activity when kernels were developed at 26.6° C and germinated at 15.5° C (Table IV). Only Hyslop and Yamhill, however, had such patterns when the kernels were developed either in the growth chamber at 15.5° C or in the field and germinated at 15.5° C (Tables III and V). None of the cultivars showed a rapid increase of enzyme activity when kernels were developed at 15.5° C and germinated at 20 or at 29.5° C (Table III). Differences in enzyme activity among cultivars were evident in all combinations of maturation and germination (Tables III, IV, and V).

TABLE IV
Total α-Amylase Activity[a] in Kernels of Different Cultivars Ripened at 26.6°C and Germinated at 15.5, 20, and 29.5°C

Germination Temperature (°C)	Cultivars	Days of Germination[a] 1	2	3	6
15.5	H	134[b]	130	252[c]	1,053[c]
	Y	165	175	308[c]	894[c]
	P	137	191	242[c]	934[c]
	B	87	111	117[c]	215[c]
	T	88	92	145[c]	302[c]
20.0	H	100	129	175	197
	Y	177	197	366[c]	1,267[c]
	P	117	142	161	200
	B	69	118	131	138
	T	42	55	138	131
29.5	H	68	48	100	103
	Y	64	74	134	1,044[c]
	P	118	73	97	115
	B	59	85	96	120
	T	22	56	69	64

[a] Micrograms of starch hydrolyzed per minute per kernel.
[b] At $P = 0.05$, the shortest significant ranges (Duncan's multiple range test) for comparing cultivars and days of germination are 68 and 74, respectively.
[c] Enzyme activity in germinated kernels.

TABLE V
Total α-Amylase Activity[a] in Kernels of Different Cultivars Ripened in Field and Germinated at 15.5, 20, and 29.5°C

Germination Temperature (°C)	Cultivars	Days of Germination[a] 1	2	3	6
15.5	H	79	119	212[c]	1,070[c]
	Y	85	157	246[c]	960[c]
	P	100	96	98	102
	B	52	46	61	59
	T	39	80	74	97
20.0	H	94	71	256[c]	1,274[c]
	Y	137	148	264[c]	1,858[c]
	P	47	54	85	152
	B	31	46	64	102
	T	35	35	136	112
29.5	H	64	48	98	130[d]
	Y	76	129	139	175[d]
	P	19	52	79	102
	B	27	42	54	56
	T	24	42	64	80

[a] Micrograms of starch hydrolyzed per minute per kernel.
[b] At $P = 0.05$, the shortest significant ranges (Duncan's multiple range test) for comparing cultivars and days of germination are 68 and 74, respectively.
[c] Enzyme activity in germinated kernels.
[d] Enzyme activity in barely germinated kernels.

DISCUSSION

At early stages of kernel development, α-amylase is located primarily in the pericarp (Kruger 1972a). In the present study, a higher α-amylase activity at 10 or 20 days after anthesis was attributed to pericarp development. However, the enzyme activity varied with ripening temperatures. The enzyme activity is higher at 15.5° C than that at 26.6° C or under field conditions (Table II). The lower enzyme activity at 26.6° C and field conditions may result either from a lower synthesis, a higher rate of inactivation or degradation of the enzyme, or both. Since α-amylase degrades starch, a possible consequence of lower enzyme activity could be a greater net starch deposition, resulting in higher kernel weight when kernels are grown at higher temperatures. The data in Table VI support this suggestion.

A general decrease in enzyme activity with maturation at all ripening temperatures (Table II) supports the earlier findings of Kruger (1972a) and Duffus (1969). The residual enzyme activity in dry kernels of all cultivars is very low compared with the de novo synthesized enzyme activity in germinating kernels (Table II, dry kernels; Tables III, IV, and V). The residual enzyme activity may, however, contribute to the degradation of starch in preharvested rain-damaged kernels.

TABLE VI
Average Fresh Kernel Weight (mg) During Maturation at Three Different Temperature Conditions

Days After Anthesis	Cultivars	Temperature Conditions[a] 15.5°C	26.6°C	Field (15.5–20.5°C)
10	H	25.0	45.5	39.0
	Y	23.5	46.5	37.0
	P	14.5	35.0	29.5
	B	18.5	37.5	23.5
	T	33.5	38.0	28.0
20	H	52.5	71.0	69.5
	Y	50.5	92.0	65.0
	P	36.5	67.5	60.0
	B	45.0	75.0	57.0
	T	36.5	52.0	47.0
30	H	57.5	87.5	86.0
	Y	57.0	95.0	102.0
	P	44.5	75.5	72.5
	B	51.5	92.0	80.5
	T	45.0	49.0	51.5
40	H	63.5	55.0	70.5
	Y	54.5	57.5	81.0
	P	42.5	46.5	65.5
	B	60.5	55.5	65.0
	T	34.5	33.5	30.0
50	H	37.0	...	57.0
	Y	39.5	...	64.0
	P	27.5	Mature	39.0
	B	43.0	...	49.5
	T	27.0	...	32.0
Dry	H	37.5	50.5	48.0
	Y	31.5	56.6	56.3
	P	22.0	43.0	38.0
	B	33.5	59.0	47.5
	T	23.0	32.5	33.5

[a] At $P = 0.05$, the shortest significant ranges (Duncan's multiple range test) for comparing cultivars and temperatures are 6.4 and 6.1, respectively.

During the germination of kernels, the increase in enzyme activity on the third and sixth days of germination was significantly different among cultivars. For example, in kernels developed at 26.6°C and germinated at 15.5°C, the enzyme activity on the sixth day of germination was significantly lower in Brevor and Tom Thumb than in other cultivars (Table IV).

Based on the α-amylase activity in imbibed (mostly in first-day germinating kernels) and germinated kernels at different temperature conditions, the cultivars can be ranked from high to low as follows: Yamhill, Hyslop, PI 178211, Brevor, and Tom Thumb. Differences in α-amylase activity due to cultivars is important in breeding wheat cultivars resistant to preharvest sprout damage. Since preharvest sprouting damage is caused by a lack of dormancy and a high level of α-amylase activity in sprouted kernels, the cultivars that produce kernels that are dormant and low in enzyme activity can be used as parents, and this characteristic can be selected for in a breeding program. Among the cultivars used in the present study, kernels of PI 178211, Brevor, and Tom Thumb were relatively dormant[5] and generally lower in α-amylase activity than Yamhill and Hyslop. PI 178211 has a red pericarp and intermediately high α-amylase activity in germinating kernels that will limit its value as germ plasm in regions in which white wheats are preferred. Similarly, Tom Thumb may be of limited value because low α-amylase activity and gibberellin insensitivity are closely associated with a gene that controls dwarfism (Gale 1976). The association has never been broken, although many attempts to do so have been made. If the close association can be broken through recombination, Tom Thumb may become a valuable source of germ plasm for low α-amylase activity in rain-damaged and in germinating kernels. In the meantime, Brevor, which also produces white kernels that are intermediate in their level of dormancy[5] and in α-amylase activity when germinated, may represent the best source of germ plasm for wheat breeders in developing cultivars for states in the Pacific Northwest.

[5]Reddy et al. 1984. Unpublished data.

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