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Genetic Control of Endosperm Amylase Activity and Gibberellic Acid Responses in Standard-Height and Short-Statured Wheats

(α-amylase/enzyme induction/wheat grain/*Triticum aestivum* L.)

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ABSTRACT **In contrast to standard-height wheat genotypes, short-statured wheats having major genes for dwarfness do not show increased seedling growth after treatment with gibberellic acid. Endogenous gibberellic acid induces synthesis of amylase in the endosperm of germinating seeds, but the amount of amylase synthesized is greatly increased by exogenous gibberellic acid treatment in standard-height and in short-statured wheats that have dwarfing genes from the variety "Norin 10." "D6899," which has the "Tom Thumb" gene for height reduction, had about one-fourth of the amylase activity of standard-height and Norin 10-derived, short-statured wheats. This genotype showed little or no increased amylase activity after gibberellic acid treatment. Genetic analyses showed that the amount of amylase synthesized was controlled by a single gene and was dependent on the number of copies of the structural gene present in the endosperm. Dwarfism in wheat may be related to a blockage in gibberellic acid utilization because other workers have found that the amount of endogenous amylase synthesized in Norin 10-derived, short-statured wheats is not growth-limiting, but it is not known if low amylase synthesis is related to dwarfism in the Tom Thumb derivative. No recombinants were recovered in a small population, suggesting that the Tom Thumb gene may pleiotropically affect plant height and the lack of response to gibberellic acid in amylase synthesis and seedling growth.**

Gibberellic acid (GA) plays an important role in determining the differential growth patterns of plants with dwarf and normal phenotypes. Dwarfs of several species have been induced to grow normally in response to GA (1), although certain dwarfs show only a low response (1, 2). The ability of wheat plants to respond to GA differs greatly among tall and short-statured wheats. Tall genotypes show marked increases in growth, whereas short-statured or dwarf types show very limited response (3–5). Wheats showing little GA response are termed GA-insensitive.

Hydrolytic enzymes, such as α-amylase (EC 3.2.1.1) in cereal endosperm, are induced by endogenous GA, which occurs in the scutellum of the embryo. Many factors influence the level of amylase activity attained in response to GA (6), including little-known genetic effects (4, 7), and it is not known if plant height and GA response are controlled by pleiotropic genes or if the two characteristics are controlled by independent or linked genes. Varieties or genotypes have not been identified that have specific alleles that condition amylase activity or the gibberellin response. We report here the GA effects on seedling growth and amylase activity for 6 genotypes of wheat, *Triticum aestivum* L., that differ widely in mature plant height and the identification of a single gene that has a very large effect on amylase synthesis in germinating grains.

Abbreviation: GA, gibberellic acid.

MATERIALS AND METHODS

The six genotypes studied have been described and the genes affecting plant height were identified (8). The genotypes can be placed in three groups on the basis of mature plant height (Table 1). "Ramona 50" and "Nainari 60" have standard height; "D6301" and "Norin 10" are short-statured; and "Olesen" and "D6899" are also called short-statured, but are significantly shorter than the previous two genotypes. Norin 10 is a Japanese variety that was used to develop short-statured cultivated wheat varieties. Genetic tests showed it to have two genes for dwarfness (8). D6301 has the two Norin 10 dwarfing genes. Olesen is a Rhodesian variety that has the two Norin 10 genes and a third gene of uncertain origin. D6899 has the Tom Thumb gene on chromosome 4A (9) and neither of the Norin 10 dwarfing genes. Norin 10 has semi-winter growth habit, while the rest of the genotypes have spring growth habit.

Coleoptile and first leaf length and endosperm amylase activity of the germinating seeds were compared for the six genotypes. The seeds were germinated in a darkened growth chamber at 21°, using the slant-board techniques described by Jones and Cobb (10), with 0 or 1 mg/liter of GA (potassium salt, 20% inert ingredients, Calbiochem, Los Angeles) added to the water reservoir. Coleoptile lengths were measured after 7 days. Lengths of the first seedling leaves were determined after growth for an additional 10 days under a 16-hr day-length in a nutrient solution with or without the GA. Amylase activity in the endosperm of germinating seeds was determined after germination in the dark at 21° for 5 days in petri dishes containing filter paper and distilled water. Seeds treated with GA were allowed to germinate the initial 24 hr in distilled water containing 100 ppm of GA, a concentration previously determined as being more effective than 1, 10, or 1000 ppm in affecting germination and amylase activity. The endosperm was removed from five uniform seedlings of each variety per treatment and ground in a mortar containing 5 ml of 0.2% $CaCl_2$ solution. The homogenate was filtered through Whatman no. 1 filter paper, and the filtrate obtained was used as the amylase source. Heating the filtrate at 70° for 10 min, which differentially inactivates β-amylase and leaves at least 93% of the α-amylase activity (11), indicated that approximately 75% of the total activity was due to α-amylase and that this pro-

TABLE 1. *Mature plant height and comparisons of coleoptile length, first seedling leaf length, and amylase activity in endosperm of germinating seeds of six wheat varieties untreated and treated with gibberellic acid (GA)*

Variety	Plant height (cm)	Coleoptile length (mm)		First seedling leaf length (mm)		Amylase activity $(\Delta A \cdot T_v)/t \cdot v$	
		Untreated	GA effect	Untreated	GA effect	Untreated	GA effect
Ramona 50	108	83.6	15.9†	125.8	19.0*	214	56†
Nainari 60	107	67.0	14.5†	124.5	28.3†	224	67†
D6301‡	80	51.8	2.5	100.8	6.7	194	18
Norin 10	71	38.2	−1.1	78.5	−6.0	176	29*
Olesen	50	38.8	0.6	81.6	4.8	196	22*
D6899§	49	38.0	0.7	72.0	−4.5	48	7
Standard error	1	1.7		5.0		12	

Height data were obtained from a field experiment. Measurements of coleoptile length and first seedling leaf length are means of 10 seedlings in each of two replicates per treatment. Amylase measurements are means of duplicate determinations of the activity in two samples of three seeds each.

*† GA effects significantly different from the untreated controls at the 0.05 and 0.01 probability levels, respectively.

‡ Selection from the hybrid Mayo 54 × Norin 10-Brevor.

§ Selection from the hybrid Tom Thumb-Sonora 64 × Tacuari.

portion did not vary significantly among the genotypes studied. Amylase activity (α- and β-amylase combined) was assayed by the starch iodine method (12). Activities are expressed as $(\Delta A \cdot T_v)/t \cdot v$, where A is the absorbance of zero time control minus the absorbance of sample, T_v is the total volume of filtrate, t is the time (min) of incubation with starch, and v is the volume of filtrate taken for incubation.

RESULTS AND DISCUSSION

The two standard-height genotypes, Ramona 50 and Nainari 60, had long coleoptiles and first leaves and high amylase activity when compared to the four short-statured genotypes. D6301, Norin 10, Olesen, and D6899 had coleoptiles and first leaves reduced in length to about the same degree as mature height in comparison with the standard-height varieties. Endosperm amylase activity was lower for D6301, Norin 10, and Olesen than for Ramona 50 and Nainari 60, but the differences were not all significant ($P > 0.05$). Most striking, however, was the approximately 4-fold lower amylase activity of D6899 when compared to the short-statured and standard height genotypes. Thus, in this group of genotypes, there is not a direct relationship of plant height to endosperm amylase activity during seed germination.

Table 1 also shows the mean differences between GA-treated and untreated wheat genotypes. Coleoptile and first leaf lengths showed a positive GA effect for the standard height varieties and no significant effects for the short-statured varieties. The results for amylase activity show a positive response for all genotypes, being significant for the standard height varieties and for two of the four short-statured varieties. The enhancement of enzyme activity for the short wheats was lower than for tall wheats, in contrast to results of Radley (4), where the short wheats behaved the same as tall wheats. The increases in amylase activity due to GA were about the same for the three short wheats that have dwarfing genes from Norin 10, but D6899 showed a small and nonsignificant response to GA. Thus, these short-statured wheats differ in their sensitivity to GA during enzyme induction as the germination process starts, but are similar in terms of early seedling growth. Norin 10-derived short wheats and Tom Thumb have very high levels of endogenous GA-like activity (4, 13), and since they fail to show a growth response to GA, it is suggested that normal growth is inhibited in the short-statured wheats because of a blockage in the use of GA rather than because of inadequate biosynthesis.

Because of the extremely low amount of amylase synthesized during germination of D6899 and because a single major gene controls the dwarfing effect (8), it was believed that genetic control of the amount of aymlase produced might also be simply inherited. The inheritance of amylase activity was examined in segregating and nonsegregating generations of Ramona 50 (R) × D6899 (D). Ten seeds of each parent; 10 seeds each of the reciprocal F_1s, R × D and D × R; 50 seeds of the backcrosses D^2 × R and R^2 × D; and 100 seeds of the F_2 (R × D) were prepared individually for determination of amylase activity in the manner described previously. Although the absolute values are lower because the analyses were made on single seeds, the 4-fold difference in amylase activity between the parents (Table 1) was confirmed (Table 2). The mean values of amylase activity in F_1 seeds indicated partial dominance for low activity of D6899 over the high activity of Ramona 50. The F_2 distribution appeared bimodal and with a suggested break between 20 and 24 units of amylase activity. With the hypothesis that amylase activity is governed by a single gene with partial dominance for low activity, the F_2 is expected to segregate in a ratio of three seeds with low activity to one seed with high activity. Some deviation from a 3 to 1 ratio was found ($\chi^2 = 4.32$; $0.02 < P < 0.05$), with an excess of seeds in the range of the high-amylase parent. The segregation in the backcross to Ramona 50 separates nicely into two groups in a 1:1 ratio, as expected for a single gene. The distribution for the backcross to D6899 was inclusive of the ranges in activity for D6899 and the F_1 D6899 × Ramona 50. There is a suggestion of bimodality in this distribution, which is expected with the segregation of a single incompletely dominant gene, but the number of seeds analyzed was too small to determine if the 1:1 segregation was realized.

A difference was found for amylase activity of the F_1, depending on whether Ramona 50 or D6899 was used as the maternal parent. This result would be expected if the enzymatic activity depends on the number of copies of a structural gene present in the endosperm, as Carlson (14) demonstrated in trisomic barley. Because the endosperm is triploid tissue, endosperm genotypes were postulated as $a_1a_1a_1$ for Ramona 50,

TABLE 2. *Frequency distributions and mean amylase activity in germinating seeds of parent, F_1, F_2, and backcross populations of Ramona 50 × D6899 (maternal parent listed first)*

Parent or population	Amylase activity, $(\Delta A \cdot T_v)/t \cdot v$ (upper class limit)										No. of seeds	Mean
	4	8	12	16	20	24	28	32	36	40		
Ramona 50						1	4	3	2		10	30.4
D6899	2	8									10	7.1
Ramona 50 × D6899, F_1		1	1	5	2	1					10	15.4
D6899 × Ramona 50, F_1		4	4	1	1						10	11.8
Ramona 50 × D6899, F_2	1	10	20	19	7	9	12	11	10	1	100	17.7
D6899[2] × Ramona 50, BC	17	10	14	7	2						50	9.2
Ramona 50[2] × D6899, BC	2	12	9	1	1		3	5	12	5	50	20.5

$a_2a_2a_2$ for D6899, $a_1a_1a_2$ for the F_1 when Ramona 50 was the maternal parent, and $a_1a_2a_2$ when D6899 was the maternal parent. The mean amylase activities observed for these four genotypes were: $a_1a_1a_1$, 30.4; $a_1a_1a_2$, 15.4; $a_1a_2a_2$, 11.8; and $a_2a_2a_2$, 7.1. From these values, the mean activities of the backcross and F_2 populations were predicted and compared with the observed generation means (in parentheses): F_2, 16.2 (17.7); backcross to Ramona 50, 21.1 (20.5); and backcross to D6899, 11.2 (9.2). The observed and predicted values agree reasonably well. Thus, the results from analyses of frequency distributions and generation means both support the single-gene hypothesis.

Each F_2 seedling, from which the endosperm had been removed for amylase determinations, was measured for coleoptile length, transplanted to soil, and grown to maturity in the greenhouse for determination of mature plant height. Correlations of amylase activity with coleoptile length and plant height for the F_2 generation were $r = 0.82$ and 0.75 ($P < 0.01$), respectively.

Amylase activity and seedling response to GA treatment were studied in the F_3 generation using seeds from 21 random F_2 plants. Table 3 shows the data for the parents and 21 random F_3 lines, grouped according to their genotype as assumed from F_2 plant heights. Levels of amylase activity for the seven F_3 lines assumed to have the Ramona 50 genotype and for the four F_3 lines assumed to have the D6899 genotype are similar to the parental values. Mean amylase activity of the ten F_3 lines derived from heterozygous F_2 plants was intermediate to the activities of the high- and low-amylase lines. Similar patterns can be noted for the three groups of genotypes with respect to response to GA; that is, the tall genotypes characterized by high amylase activity responded to GA, whereas the short, low-amylase genotypes did not. Although the populations were small, these results show close associations of GA response, amylase activity, seedling growth, and plant height in this cross. Further studies are necessary on this point, but it is clear that not all wheats have the same physiological bases for dwarfism. Hu *et al.* (15) indicated that GA insensitivity and plant height were controlled by separate, but linked, genes in Norin 10-derived materials, and Gale and Marshall (13) stated that possibly only one of the Norin 10 genes is associated with GA insensitivity.

The limited GA response shown by D6899, predominantly controlled by a single gene, offers a genetic approach to study biosynthetic pathways and gene action of growth processes in higher plants. Except for its short stature, D6899 grows and reproduces well in the field or growth chamber. This genotype should be useful for further investigations involving the induction of hydrolytic enzymes in response to GA.

TABLE 3. *Amylase activity, coleoptile length, response in coleoptile length from treatment with gibberellic acid (GA), and plant height for parents and F_3 lines from the cross Ramona 50 × D6899*

Identity	F_2 genotype	No. of F_3 lines*	Amylase activity	Coleoptile length (mm)		F_2 plant height (cm)
				Untreated	GA effect	
Ramona 50	a_1a_1		275	96.1	8.1†	102
F_2 tall	a_1a_1	7	262	87.0	10.2†	101
F_2 intermediate	a_1a_2	10	165	67.7	5.3†	53
F_2 short	a_2a_2	4	54	48.0	1.3	32
D6899	a_2a_2		40	41.6	1.2	30

* Amylase activity of each F_3 line was determined from duplicate determinations using 10 seeds per assay.

† GA effect significantly different from the untreated populations at the 0.01 probability level.

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