Plant Height Components and Gibberellic Acid Response of Oat Dwarf Lines

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ABSTRACT

The use of oat (*Avena sativa* L.) dwarfing genes in breeding programs to improve lodging resistance has been limited, mainly because of decreases in yield and grain quality in many environments. The objectives of this study were to evaluate the effects of the dominant *Dw6*, *Dw7*, and *Dw8* dwarfing genes on plant height components and the gibberellic acid (GA) response of the dwarf lines. Plant height components including internode length, panicle length, and panicle exertion were measured in plants of three nondwarf and nine dwarf lines grown in the field at St. Paul in 1992 and 1993. Five experiments were performed in growth chambers to assay the response of nondwarf and dwarf oat genotypes to exogenous GA applied at the seedling stage. The *Dw6* gene in line OT207 caused a 34 to 37% reduction in plant height due to the reduction in the length of the three uppermost internodes but not internode number. The 46% reduction in height caused by the *Dw7* gene in line NC2469-3 resulted from decreases in both internode number and elongation. The *Dw8* gene present in derivatives of seven Japanese lines shortened all internodes but did not affect internode number, reducing plant height by about 50%. The dwarf lines that carry these dwarfing loci are responsive to exogenously added GA_3, GA_1 and GA_{20}, and thus the mutations appear not to involve disruptions of the conversion of GA_{20} to GA_1. The results indicate that different strategies may be needed to adjust for the different plant height component effects of each of the three dwarfing genes for their use in oat cultivar development.

The development of shorter cultivars with increased resistance to lodging is of interest in oat breeding. Major dwarfing genes have been used extensively in developing semidwarf wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.) high yielding cultivars (Gale and Youssefian, 1985). Until recently, seven major oat dwarfing mutants had been described officially and classified in *Avena* species (Marshall and Shaner, 1992); however, only two of them, the dominant *Dw6* and the semidominant *Dw7*, are readily available, and only *Dw6* is being used currently for cultivar development (Marshall et al., 1987; Anderson and McLean, 1989; D.D. Stuthman, personal communication, 1998). Potential new sources of dwarfism for oat improvement were reported in the hexaploid wild oat *A. fatua* L. by Morikawa (1989a). Among backcross derivatives of these dwarfs into the *A. sativa* cultivar Kanota, a new dominant dwarfing gene, *Dw8*, was identified and demonstrated to be distinct genetically from *Dw6* and *Dw7* (Milach et al., 1998). This gene has not been used yet for cultivar development.

Yield advantages in oat lines with the *Dw6* dwarfing gene have been obtained in some Australian environments (Anderson and McLean, 1989). However, the dwarf phenotype generally limits the use of dwarfing genes in oat breeding because of decreases in seed size, quality, and yield (Brown et al., 1980; Marshall and Murphy, 1981; Meyers et al., 1985; Kibite and Clayton, 2000; Milach and Federizzi, 2001).

Comparison of plant height components of the *Dw6* semidwarf line OT207 with its nondwarf progenitor line OT184 revealed a significant shortening in the *Dw6* mutant in the length of its three uppermost internodes, particularly the peduncle (Brown et al., 1980). This reduction in peduncle elongation has been associated with the failure of the panicle to emerge fully from the leaf sheath, particularly under nonideal growth conditions. Panicle exertion genes introduced or selected for in lines carrying the *Dw6* gene have been found to help ameliorate this problem (Farnham et al., 1990). The internode constitution of the dwarf lines with the *Dw7* gene (in line NC2469-3) or the *Dw8* gene in Kanota backcross derivatives has not been previously reported. This information is important in understanding how these genes affect plant height and in attempting to adjust for any undesirable effects they might have for cultivar development.

The gibberellic acid (GA) biosynthetic pathway can be dissected by means of dwarfing genes (Hedden and Proebsting, 1999). Dwarf mutants that are defective for different steps in the GA pathway and which respond to the exogenous application of GA have been described in maize (*Zea mays* L.), rice, and pea (*Pisum sativum* L.) (Phinney, 1984). The early 13-hydroxylation GA biosynthetic pathway leading to active GA_1 occurs in maize, rice and pea (Phinney, 1984). Intermediates of this pathway have been identified in oat by gas-chromatographic spectrometry, indicating that the pathway also occurs in oat (Kaufman et al., 1976). However, other dwarf mutants have been identified in wheat, rye (*Secale cereale* L.), maize, and rice that are insensitive to the application of GA (Gale and Youssefian, 1985; Borner, 1991; Harberd and Freeling, 1989; Mitsunaga et al., 1994). In these mutants dwarfism appears to be due to causes other than blocks in GA biosynthesis.

In wheat, the presence of GA-insensitive dwarfing genes can be identified by a GA response test conducted at the seedling stage (Yamada, 1990). The genetics of the *rht1* and *rht2* recessive dwarfing genes were not clearly resolved until the GA-insensitive response assay

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Abbreviations: GA, gibberellic acid; RFLP, restriction fragment length polymorphism.

was used in the genetic analysis (Gale and Youssefian, 1985). Oat counterparts of the wheat Norin 10 genes have not yet been identified, probably because in oat fewer studies have been conducted to develop assays to identify and manipulate these type of genes. Searching for such mutants in oat will likely be a challenge, especially if they are recessive with a moderate effect on plant height. The use of GA assays to identify GA-insensitive oat mutants would enable distinguishing among such mutants and plants that have reduced height because of other mutations or environmental effects.

The *Dw6* and *Dw7* dwarf mutants in oat have been identified as responsive to GA_3 at the seedling stage (Federizzi, 1986). Farnham et al. (1990) compared the response of the *Dw6* line OT207 and its progenitor line OT184 with applied GA_3 at the boot stage and concluded that OT207 is also GA-sensitive at this stage. Comparisons have not been made between *Dw6*, *Dw7*, and *Dw8* dwarf mutants and their respective nondwarf counterpart lines for their relative responses to applied GA at the seedling stage.

The objectives of this study were to determine the effect of the *Dw6*, *Dw7*, and *Dw8* dwarfing genes on plant height components and to investigate the response of these three sources of dwarfism in oat to GA applied at the seedling stage.

MATERIALS AND METHODS

Plant Materials

The dwarf genotypes used in our studies represent three distinct sources of dominant dwarfing genes (*Dw6*, *Dw7*, and *Dw8*). The semidwarf OT207 line was derived from genotype OT184 by fast-neutron irradiation and carries the *Dw6* gene (Brown et al., 1980). The NC2469-3 dwarf was selected as a spontaneous mutation from the line NC2469 and carries the *Dw7* gene (Marshall and Murphy, 1981). The lines AV13/6/7, AV16/8/4, AV21/5, AV14/5/1, AV17/3/10, AV12/9/9, and AV18/2/4 are sources of dwarfism isolated in Japan from accessions of *A. fatua* and introgressed into the hexaploid oat cultivar Kanota by three backcrosses (Morikawa, 1989b). On the basis of allelism tests, these lines all carry a newly described dominant dwarfing gene, *Dw8* (Milach et al., 1998). Dwarf genotypes were compared with their respective nondwarf counterpart lines.

Plant Height Components of Dwarf and Nondwarf Lines

Two rows for each of the nondwarf lines, OT184 and Kanota, and the dwarf lines, OT207, AV13/6/7, AV16/8/4, AV-21/5, AV14/5/1, AV17/3/10, AV12/9/9, and AV18/2/4, were planted in field nurseries at St. Paul, MN, in 1992 in a split plot design. Genotypes were the main plots with 10 replications per plot, which consisted of two rows 1.5 m long. The tall NC2469 and the dwarf line NC2469-3 were not included in the first year experiment because seed of the NC2469 line was not available in 1992. At maturity, plants of each genotype were pulled intact from the field plots. Plant height, internode lengths, panicle length, and panicle exertion were measured on the main tiller of each individual plant. The distance between the bottom of the panicle to the flag-leaf ligule was measured as panicle exertion (negative numbers indicating the portion of the panicle inside the leaf sheaf). A similar experiment was conducted in the field in 1993 at St. Paul. The main plots in 1993 had five 1.5-m-long rows and consisted of 40 replications. Plants of each of the nondwarf lines OT184, NC2469, and Kanota and the nine dwarf genotypes included in this study were pulled in the field and measured for the same traits. Means and standard deviations were calculated for each trait in both years. Plant height components of the dwarf lines were compared with their respective nondwarf counterpart lines by paired *t*-test.

Response to Gibberellic Acid

Five experiments were performed in growth chambers to assay the response of nondwarf and dwarf oat genotypes to exogenous GA applied at the seedling stage. The growth chamber conditions were similar for all experiments with the temperature at 20°C, 95% relative humidity. A 12-h photoperiod and a photon flux density of 300 to 400 $\mu mol\ m^{-2}\ s^{-1}$ was supplied at canopy height from an adjustable height fixture containing a mixture of incandescent and cool white fluorescent lamps. Seed germination of nondwarf and dwarf genotypes in all experiments was carried out in 10-cm diam petri dishes with wetted Whatman No. 2 filter paper (Fisher Scientific, Pittsburgh, PA) for 5 d at 4°C, followed by 2 d at room temperature, before transfer to pots or trays for GA seedling treatments. Gibberellic acid in the GA_3 form (Sigma, St. Louis, MO) was used in the first two experiments and in the GA_1 and GA_{20} forms (cordially provided by Dr. B.O. Phinney, University of California, Los Angeles, CA) in the last three experiments. Regression analysis was performed for each genotype by PROC REG of SAS 6.03 (SAS Inst., 1990) for all experiments.

Experiments I and II

The methodology used in these experiments was based on Gale and Youssefian (1985). Seedlings of two nondwarf (OT-184 and Kanota) and eight dwarf genotypes (OT207, AV13/6/7, AV12/9/9, AV16/8/4, AV21/5, AV14/5/1, AV17/3/10, and AV18/2/4) were transplanted to pots containing only vermiculite. A completely randomized design with two replications (pots) was used in both experiments. Five seedlings per pot were planted for each genotype. Treatments were applied seven days after transplanting to vermiculite by watering the pots with the GA solution. Treatments consisted of 50 mL of MS (Murashige and Skoog, 1962) salt solution or water supplemented with GA_3 at concentrations of 0 (control), 50, 100 and 150 mg L^{-1}. In Exp. I, MS solution with GA_3 was applied for 5 d, followed by 4 d of water with GA_3. In Exp. II, MS solution with GA_3 was alternated with water with GA_3. Nine days after the treatments were first applied, seedlings were measured for height to the second leaf insertion with a 30-cm-length ruler.

Experiments III, IV, and V

The methodology used in these experiments was based on the modified microdrop assay (Nishijima et al., 1990). The nondwarf genotypes OT184 and Kanota and the dwarfs OT207 and AV13/6/7 were tested in Exp. III and IV. The tall NC2469 and the dwarf NC2469-3 were only tested in Exp. III. The genotypes tested in Exp. V included Kanota and the *Dw8* backcross derivatives AV12/9/9, AV16/8/4, AV14/5/1, AV17/3/10, and AV18/2/4. Germinated seedlings were transplanted to 20- by 40-cm trays filled with a mixture of 1 vermiculite: 1 soil. Three trays were treated with GA_1 and three with GA_{20}. Each tray had 10 rows with 10 seedlings of the same genotype per row. Treatments consisted of 0 (control), 1, 10, 100, and

1000 mg L^{-1} of GA_1 or GA_{20}. Genotypes and treatments were randomized within GA_1 or GA_{20}, so that each tray would be treated with only one type of GA. Treatments were first applied 3 d after transplanting. By means of a calibrated micropipet, a 10-mL drop of water mixed with a wetting agent, Tween 80 (Fisher Scientific, Pittsburgh, PA), and supplemented with GA_1 or GA_{20} was applied to the surface of the first leaf sheath. This procedure was repeated for 2 d and the height to the first-leaf insertion was measured 5 d later.

RESULTS AND DISCUSSION

Plant Height Components of Dwarf and Nondwarf Lines

The *Dw6* dwarf line OT207 when compared with its counterpart (nondwarf line OT184) from which it was derived was 34% shorter in 1992 field plots and 37% shorter in 1993 (Table 1). The variation between measurements taken in 1992 and 1993 appeared to result from environmental differences between the two years. The 1993 oat growing season was unusually rainy and humid with 452 mm rainfall in May–July 1993 compared with 308 mm rainfall in May–July 1992. This difference likely resulted in both the dwarf and nondwarf lines being taller than normal in 1993. OT207 had the same number of internodes as OT184 in both years, since both had the internodes p-4 to p in 1992 and p-5 to p in 1993. This component did not contribute to the height difference between these lines. The three uppermost internodes of OT207 were 30 to 50% shorter than those of OT184 in both 1992 and 1993, and together accounted for about 75% of the difference in height between OT207 and OT184. This resulted in a lack of panicle exertion from the leaf sheath in the dwarf line. The panicle length itself was reduced only 16 to 18%. The two lower internodes of plants of the dwarf line did not differ statistically from those of the progenitor line in 1992 (Table 1). These results are in agreement with those obtained by Brown et al. (1980). The extended height of the dwarf and nondwarf lines in 1993 resulted from the increased internode number and increased elongation of the bottom internodes. These changes maximized the differences between dwarf and nondwarf for the bottom internodes in 1993 (Table 1).

In oat, as is typical in grasses, the lower internodes are the first to differentiate and elongate with the internodes elongating basipetally from the intercalary meristem of each internode (Kaufman et al., 1965). The major effect of the *Dw6* gene appears to be late in development, although it does influence the lower, first-to-elongate internodes to some extent. Kaufman and Brock (1992) described oat stem elongation as occurring in a sequential fashion in which, as the previous internode ceases elongating, the next internode enters its burst of growth. Thus, the effect of *Dw6* may be specific to the elongation of the upper internodes. Farnham et al. (1990) reported being able to reverse the effect of the *Dw6* mutant on peduncle extension by applying GA_3 to the dwarf plants at the boot stage.

The 46% reduction in height of NC2469-3 compared with its nondwarf counterpart NC2469 resulted from decreases in both internode number, as the dwarf line did not exhibit internode p-5, and internode elongation (Table 2). Marshall and Murphy (1981) similarly found a 42.5% reduction in the height of NC2469-3 compared with NC2469. However, the authors did not investigate plant height components of these lines. All internodes were significantly reduced in the NC2469-3 line, but the two internodes below the peduncle were the least affected (Table 2). Although NC2469-3 had one fewer internodes compared with its nondwarf counterpart, the reductions in internode lengths accounted for most of the decrease in plant height with the length reduction being most marked in the lower internodes. The compact panicle type of the dwarf line was 38% shorter than the normal and also contributed to reduction in plant height. According to Federizzi and Qualset (1989), the compact panicle of NC2469-3 is due to one additive gene (*C*) linked (0.08 ± 0.01 recombination frequency) to *Dw7* and not to a pleiotropic effect of the dwarfing gene. The lack of panicle exertion was not as intense in this dwarf as in the OT207 line. The *Dw7* gene acted differently from *Dw6* and appeared to exert an effect on internodes elongated both early and later in plant development.

The *Dw8* gene, shown by allelism tests to be present in a series of Japanese lines generated from dwarf *A*.

Table 1. Plant height components of the *Dw6* dwarf OT207 line compared to its nondwarf progenitor OT184 when grown in St. Paul in 1992 and 1993.

Year/genotype	Mean length of internode†						Mean panicle exertion	Mean panicle length	Mean plant height
	p-5	p-4	p-3	p-2	p-1	p			
	cm								
1992‡									
OT184	–§	4.0	6.8	10.6	19.3	46.5	22.0	26.0	112.8
OT207	–	3.6	6.3	7.4**	9.2**	22.8**	−1.0**	21.7**	74.6**
%Reduction	–	10	7	30	52	51	>100	16	34
1993¶									
OT184	2.6	8.5	12.9	16.0	22.0	44.4	18.5	28.9	135.4
OT207	2.6	6.8**	9.7**	10.2**	10.7**	22.5**	−1.9**	23.6**	84.7**
%Reduction	0	20	25	36	51	49	>100	18	37

** Significantly different from OT184 at the 0.01 probability level.
† Internode designation according to the nomenclature used in oat, where p or peduncle is the uppermost internode and p-1, p-2, p-3, p-4, and p-5 are below p, respectively, from the top to the bottom of the plant.
‡ Mean values in 1992 were calculated with n = 10 plants.
§ p-5 internode elongation was not observed in 1992.
¶ Mean values in 1993 were calculated with n = 40 plants.

Table 2. Plant height components of the *Dw7* dwarf NC2469-3 line compared to its nondwarf progenitor NC2469 when grown at St. Paul in 1993.

Genotype	Mean length of internode†						Mean panicle exertion	Mean panicle length	Mean plant height
	p-5	p-4	p-3	p-2	p-1	p			
	cm‡								
NC2469	3.9	9.3	11.5	12.9	17.4	37.0	15.6	22.6	113.4
NC2469-3	–§**	1.9**	4.9**	9.0**	14.2**	18.0**	−0.1**	14.0**	61.5**
% Reduction	>100	80	57	30	18	51	>100	38	46

** Significantly different from NC2469 at the 0.01 probability level.
† Internode designation according to the nomenclature used in oat, where p or peduncle is the uppermost internode and p-1, p-2, p-3, p-4, and p-5 are below p, respectively, from the top to the bottom of the plant.
‡ Mean values were calculated with n = 40 plants.
§ p-5 internode elongation was not observed for NC2469-3.

fatua accessions backcrossed three times into Kanota (Milach et al. 1998), differed from the *Dw6* and *Dw7* genes in its effect on plant height. Because the results obtained for plant height components of Kanota and the Japanese lines in 1992 were similar to those in 1993, only the 1993 data are presented here to allow the effects of the *Dw8* gene to be compared directly with those of the *Dw6* and *Dw7* genes by means of data from the same year (Table 3). Our results with *Dw8* are in agreement with those of Morikawa (1989b), who measured plant height components on the original *A. fatua* dwarf source lines and in F_2 progeny of crosses of these lines with Kanota. The measurements reported here are based on F_6 dwarf progeny following three backcrosses to Kanota. The internode number of the Japanese lines was not different from that of their nondwarf counterpart Kanota, as both had internodes p-4 to p. Plant height in all Japanese lines was reduced by about 50% compared with Kanota in both 1992 and 1993. All internodes contributed similarly to plant height reduction in all Japanese lines. Although the panicle exertion was affected in all lines, only AV21/5, AV14/5/1, and AV17/3/10 did not completely exert the panicle. There were differences among the Japanese lines for panicle length; two had panicles the same length as those of Kanota, four had larger panicles, and one had shorter panicles, indicating that there are additional genes influencing this trait or that different alleles are present among the various lines (Table 3). The overall similarities in plant height components among the Japanese lines indicate that they are determined by the same *Dw8* locus, which is in agreement with a lack of segregation of tall plants when the various Japanese lines were intercrossed (Milach et al. 1998). The *Dw8* gene differed from *Dw7* in not affecting the internode number and from *Dw6* in significantly affecting all internode lengths.

Response to Gibberellic Acid

All the dwarf genotypes and their nondwarf counterparts studied in GA-response Exp. I and II responded significantly to GA_3 applied in solution to the vermiculite at the seedling stage. The intercepts and slopes for the linear response of nondwarf and dwarf genotypes to increased levels of applied GA_3 were significantly different from zero in Exp. I and II (Table 4). The predictor GA_3 level explained from 51% (for OT207 in Exp. II) to 83% (for OT184 in Exp. I) of the variation in height to the second leaf insertion.

The intercept was on average one centimeter greater

Table 3. Plant height components of the *Dw8* dwarf Japanese lines compared to their nondwarf counterpart Kanota when grown at St. Paul in 1993.

Genotype	Mean length of internode†					Mean panicle exertion	Mean panicle length	Mean plant height
	p-4	p-3	p-2	p-1	p			
	cm‡							
KANOTA	3.7	10.8	14.5	18.4	37.6	16.7	22.6	108
AV13/6/7	1.2**	2.2**	2.7**	8.0**	18.3**	4.8**	22.6	54.2**
% Reduction	68	80	82	57	51	71	0	50
AV12/9/9	1.4**	2.3**	3.2**	9.2**	19.2**	4.2**	26.8**	62.4**
% Reduction	61	79	78	50	49	75	−18	42
AV16/8/4	0.9**	2.1**	2.2**	6.1**	17.9**	4.4**	22.3	52.0**
% Reduction	75	81	84	67	52	73	1	52
AV21/5	0.6**	1.3**	1.5**	4.7**	10.9**	−1.9**	24.9**	44.6**
% Reduction	84	88	90	74	71	>100	−10	59
AV14/5/1	1.7**	2.2**	2.7**	6.9**	12.5**	−0.9**	25.7**	52.6**
% Reduction	53	80	82	62	66	>100	−13	51
AV17/3/10	1.3**	2.5**	2.8**	6.3**	14.3**	−1.2**	25.4**	52.5**
% Reduction	65	76	81	66	62	>100	−12	51
AV18/2/4	0.8**	2.1**	2.4**	7.2**	14.5**	2.0**	19.0**	46.5**
% Reduction	78	80	83	61	61	88	16	57

** Significantly different from Kanota at the 0.01 probability level.
† Internode designation according to the nomenclature used in oat, where p or peduncle is the uppermost internode and p-1, p-2, p-3, and p-4 are below p, respectively, from the top to the bottom of the plant.
‡ Mean values were calculated with n = 40 plants.

Table 4. Intercepts and slopes for the linear response of a *Dw6* dwarf (OT207) and its nondwarf progenitor (OT184) and seven independent *Dw7* dwarf lines (AV lines) and their nondwarf recurrent parent Kanota to applications of 0, 50, 100 and 150 mg L^{-1} of GA_3 in duplicated Experiments I and II.

		Intercept		Slope		
Genotype	Experiment	a ± SE	95% C.I.†	b ± SE**	95% C.I.‡	r^2**
OT184	I	6.2 ± 0.3	(5.7, 6.7)	1.3 ± 0.1	(1.1, 1.5)	0.83
	II	3.9 ± 0.4	(3.0, 4.8)	1.5 ± 0.1	(1.2, 1.8)	0.71
OT207	I	5.1 ± 0.4	(4.3, 5.9)	0.9 ± 0.1	(0.7, 1.2)	0.53
	II	4.2 ± 0.5	(3.3, 5.2)	1.1 ± 0.2	(0.7, 1.4)	0.51
KANOTA	I	5.0 ± 0.4	(4.2, 5.8)	1.9 ± 0.1	(1.6, 2.2)	0.80
	II	4.6 ± 0.3	(4.0, 5.3)	1.1 ± 0.1	(0.8, 1.3)	0.69
AV13/6/7	I	4.5 ± 0.2	(4.0, 4.9)	0.9 ± 0.1	(0.7, 1.1)	0.75
	II	2.5 ± 0.2	(2.0, 3.1)	0.8 ± 0.1	(0.6, 1.0)	0.65
AV12/9/9	I	5.2 ± 0.3	(4.5, 5.9)	1.0 ± 0.1	(0.7, 1.2)	0.60
	II	2.9 ± 0.3	(2.3, 3.5)	1.1 ± 0.1	(0.9, 1.3)	0.73
AV16/8/4	I	4.7 ± 0.5	(3.7, 5.7)	1.3 ± 0.2	(0.9, 1.7)	0.56
	II	2.6 ± 0.3	(2.0, 3.2)	1.1 ± 0.1	(0.7, 1.3)	0.76
AV21/5	I	3.7 ± 0.3	(2.9, 4.4)	1.2 ± 0.1	(0.9, 1.4)	0.65
	II	2.4 ± 0.3	(1.8, 3.0)	1.1 ± 0.1	(0.9, 1.3)	0.71
AV14/5/1	I	3.3 ± 0.2	(2.8, 3.7)	1.3 ± 0.1	(1.1, 1.4)	0.79
	II	2.8 ± 0.3	(2.2, 3.4)	0.9 ± 0.1	(0.7, 1.1)	0.62
AV17/3/10	I	3.8 ± 0.3	(3.3, 4.4)	1.3 ± 0.1	(1.1, 1.5)	0.81
	II	2.7 ± 0.3	(2.0, 3.4)	0.9 ± 0.1	(0.7, 1.2)	0.59
AV18/2/4	I	4.1 ± 0.3	(3.5, 4.7)	0.8 ± 0.1	(0.6, 1.0)	0.58
	II	2.4 ± 0.3	(1.8, 3.0)	0.9 ± 0.1	(0.6, 1.1)	0.61

** Significant at the 0.01 probability level.
† and ‡ Lower and upper endpoints for a 95% confidence interval for intercept and slope, respectively.

in Exp. I than in II for all genotypes (Table 4). We observed that the 2-d-old seedlings transplanted to vermiculite in Exp. I appeared to be slightly taller than the ones in Exp. II. This likely resulted from faster germination in Exp. I and is probably the cause of the differences in intercept.

The regression coefficients (*b*) obtained in Exp. I were similar to those from Exp. II for most genotypes, except Kanota (Table 4). OT184 had slightly larger regression coefficients than its dwarf derivative OT207 in Exp. I and II. However, the 95% confidence intervals for the OT184 and OT207 coefficients overlap, indicating that these differences were not significant. Kanota had a regression coefficient significantly larger than the Japanese lines in Exp. I. These differences were not detected in any of the subsequent experiments and resulted from the higher response of Kanota in Exp. I. Even though Kanota and the Japanese lines might differ in the magnitude of response to applied GA, such differences are small when detected in only one out of the five experiments.

These results demonstrated that both nondwarf and dwarf genotypes responded significantly to applied GA_3 at the seedling stage. This finding led us to further investigate the possible role of the dwarfing genes in the GA biosynthetic pathway. In the early 13-hydroxylation pathway, the 3 beta-hydroxylation of GA_{20} leads to GA_1, which is the active endogenous GA form in maize, rice, and pea (Phinney, 1984). A mutation in the gene that controls the GA_{20} to GA_1 step results in accumulation of GA_{20} in the dwarf plant. Such mutants are responsive to GA_1 but not GA_{20}, and have been described in species including pea (Ingram et al., 1984), rice, and maize (Phinney, 1984).

Dwarf lines for each of the three dwarfing genes were compared with their corresponding nondwarf progenitor lines for response to applied GA_1 and GA_{20}. All genotypes responded to both GA_1 and GA_{20} with similar results obtained for each genotype across two experiments (Table 5). When the other *Dw8* lines were tested in a similar experiment, each of them also responded to both GA_1 and GA_{20} (Exp. V, data not shown). These results indicate that none of the oat dwarf lines studied in this paper result from a modification in the step of conversion of GA_{20} to GA_1 although regression coefficients (*b*) for the response to GA_{20} were smaller than the ones for GA_1 for tall and dwarf genotypes. This result was expected since GA_{20} is less biologically active than GA_1 (Phinney, 1984). Higher amounts of GA_{20} would need to be applied to obtain a similar response as with GA_1.

GA-sensitive dwarfs from several species, including maize (Phinney, 1984), *Arabidopsis* (Koornneef et al., 1985), and rice (Murakami, 1968), respond more to GA applications than their wild-type relatives. Dwarfs of pea (Ross et al., 1989), tomato (*Lycopersicon esculentum* Mill.) (Koornneef et al., 1990), and lettuce (*Lactuca sativa* L.) (Waycott and Taiz, 1991) respond less than their normal counterparts. Farnham et al. (1990) observed that OT207 responded substantially more to applied GA_3 at the boot stage than did its tall mother-line OT184. The authors classified the *Dw6* gene as GA-sensitive and suggested that the action of *Dw6* is involved with GA metabolism.

In the GA experiments, OT207 responded about the same as its tall counterpart OT184 to applied GA_3 at the seedling stage (Table 4). The differences in the magnitude of the OT207 response to GA at the seedling and at the boot stage may result from a differential expression of the *Dw6* gene in these distinct developmental stages. Indeed, as we described previously, the *Dw6* gene appears to have a major effect in the elongation of the three uppermost oat internodes.

Dwarf lines carrying the *Dw7* or *Dw8* genes also responded much like their nondwarf counterparts to applied GA (Table 4). Because of the sensitivity of these

Table 5. Intercepts and slopes for the log-linear response of nondwarf progenitor lines OT184, NC2469, and Kanota and their respective derived dwarf lines OT184 (*Dw6*), NC2469-3 (*Dw7*), and AV13/6/7 (*Dw8*), to 1, 10, 100, and 1000 mg L^{-1} of GA_1 and GA_{20} applied in a modified microdrop assay in Experiments III and IV.

			Intercept		Slope		
Genotype	GA	Expt.	a ± SE	95% C.I.†	b ± SE**	95% C.I.‡	r^2**
OT184	GA_1	III	4.2 ± 0.2	(3.8, 4.5)	1.0 ± 0.1	(0.8, 1.2)	0.82
		IV	4.7 ± 0.4	(3.9, 5.5)	0.8 ± 0.2	(0.4, 1.2)	0.38
	GA_{20}	III	4.6 ± 0.2	(4.3, 4.9)	0.5 ± 0.1	(0.3, 0.7)	0.59
		IV	4.7 ± 0.3	(4.0, 5.3)	0.6 ± 0.2	(0.3, 0.9)	0.33
OT207	GA_1	III	3.5 ± 0.2	(3.0, 3.9)	1.0 ± 0.1	(0.7, 1.3)	0.71
		IV	3.6 ± 0.2	(3.2, 4.1)	1.1 ± 0.1	(0.9, 1.4)	0.79
	GA_{20}	III	4.0 ± 0.3	(3.3, 4.7)	0.6 ± 0.2	(0.2, 1.0)	0.38
		IV	2.6 ± 0.3	(2.1, 3.2)	0.8 ± 0.1	(0.5, 1.1)	0.59
NC2469	GA_1	III	3.1 ± 0.2	(2.7, 3.5)	0.9 ± 0.1	(0.7, 1.1)	0.80
	GA_{20}	III	3.6 ± 0.2	(3.2, 3.9)	0.5 ± 0.1	(0.3, 0.7)	0.53
NC2469-3	GA_1	III	3.9 ± 0.2	(3.6, 4.3)	0.5 ± 0.1	(0.3, 0.7)	0.58
	GA_{20}	III	3.7 ± 0.2	(3.3, 4.1)	0.4 ± 0.1	(0.2, 0.8)	0.41
Kanota	GA_1	III	3.5 ± 0.3	(2.7, 4.2)	1.2 ± 0.2	(0.8, 1.6)	0.63
		IV	4.2 ± 0.3	(3.5, 4.9)	1.0 ± 0.2	(0.7, 1.4)	0.50
	GA_{20}	III	4.3 ± 0.2	(3.9, 4.6)	0.6 ± 0.1	(0.5, 0.8)	0.70
		IV	3.4 ± 0.3	(2.8, 4.0)	0.9 ± 0.1	(0.6, 1.2)	0.50
AV13/6/7	GA_1	III	2.6 ± 0.2	(2.3, 3.0)	1.0 ± 0.1	(0.8, 1.1)	0.86
		IV	3.1 ± 0.3	(2.5, 3.8)	1.1 ± 0.2	(0.7, 1.4)	0.56
	GA_{20}	III	2.3 ± 0.2	(1.9, 2.7)	0.6 ± 0.1	(0.4, 0.8)	0.67
		IV	2.7 ± 0.2	(2.3, 3.0)	0.6 ± 0.1	(0.4, 0.8)	0.53

** Significant at the 0.01 probability level.
† and ‡ Lower and upper endpoints for a 95% confidence interval for intercept and slope, respectively.

lines to GA, these genes are also likely to be involved in GA metabolism. However, it appears that different mechanisms are involved in the action of the *Dw7* and *Dw8* genes. The effect of the *Dw7* gene on plant height components was different from the effect of *Dw8*. Even though both dominant mutant genes similarly reduce the elongation of all plant internodes, only *Dw7* affected the number of internodes. It may be possible to adjust the effects of these genes by crossing the dwarf with tall lines that have more internodes and/or overall extended internodes.

A common feature of GA-sensitive mutants is that they are usually recessive and deficient for GA because of a block in the biosynthetic pathway (Phinney, 1984). Recessive genes involve the loss of wild-type function (Herskowitz, 1987). Interestingly, all the oat dwarf genotypes we studied were responsive to applied GA but controlled by dominant dwarfing genes. A lack of identified recessive mutants for GA biosynthesis in hexaploid oat is not surprising because any such mutation in a GA biosynthetic pathway would likely be masked by the action of homoeologous genes in the other progenitor genomes comprising hexaploid oat. A recessive dwarfing gene *dw5* has been identified in diploid oat derivatives (Nishiyama, 1957). Dominant or semidominant dwarfing mutations have been identified at the *Rht-B1* and *Rht-D1* loci of wheat and the *d8* locus of maize. These genes have recently been shown to be orthologs of the *Arabidopsis* gibberellic acid insensitive (*GAI*) gene (Peng et al., 1999). The *GAI* gene was proposed to be the basis of a signaling pathway that regulates negatively gibberellin responses (Peng et al., 1997). A deletion mutant in the GA-recognition portion of the GAI negative modulator (repressor) then acts in a dominant manner to produce GA-insensitive repression of growth genes, and thus reduced plant height. The three dominant dwarf oat mutants studied here, however, are GA-sensitive. To explain the dominance effect of these GA-sensitive dwarfing mutants we postulate that the genes affected may participate normally either directly or indirectly in negative regulation (repression) of genes for GA metabolism. GA biosynthesis is known to be regulated by numerous endogenous and environmental signals (Yamaguchi and Kamiya, 2000). Furthermore, individual steps in GA metabolism may be catalyzed by products of gene families with different tissue-specific and developmental patterns of expression (Hedden and Proebsting, 1999). Thus, the multiplicity in regulatory signals and in genes involved in GA metabolism provides several points at which dominant mutations could occur producing not only GA-deficiency but also resulting in various dwarfing phenotypes.

One possibility on the nature of the oat dwarfing mutations was suggested on the basis of observations that seedlings of the *Dw7* line NC2469-3 grown in growth chamber and greenhouse environments often were dark green and accumulated anthocyanin (Milach and Federizzi, 2001). Similar coloration and dwarfing phenotypic effects have been observed in transgenic tomato and *Arabidopsis* plants transformed with an oat phytochrome A (*phyA*) gene (Boylan and Quail, 1989; 1991) and in tobacco (*Nicotiana tabacum* L.) plants transformed with a rice *phyA* gene (Nagatani et al., 1991). The dwarf phenotype also occurs in transgenic *Arabidopsis* plants overexpressing the *Arabidopsis* or the rice phytochrome B (*phyB*) genes (Wagner et al., 1991). Thus, overexpression of either *phyA* or *phyB* genes can cause dwarfism. The semidominant nature of the *Dw7* mutation would be in line with overexpression of a phytochrome gene. An oat *phyA* gene and a rice *phyB* gene were mapped on the hexaploid oat restriction fragment length polymorphism (RFLP) map (O'Do-

noughue et al., 1995) for comparison to the location of the previously mapped *Dw7* gene (Milach et al., 1997). The oat *phyA* gene mapped to linkage group 28 and the rice *phyB* gene to linkage group 30 of the oat map while *Dw7* was located on linkage group 22 (Milach and Federizzi, 2001). Independence of *Dw6*, *Dw7*, and *Dw8* from the mapped phytochrome loci indicates that they are not alleles of these phytochrome genes. However, because at least three phytochromes occur in oat (Wang et al., 1991), *Dw7* could be a mutation in a phytochrome structural gene not mapped or in a gene regulating phytochrome production.

The dominant oat dwarfing genes *Dw6*, *Dw7*, and *Dw8* appear to be quite distinct from the wheat *Rht1* and maize *Dw8* dominant dwarfing genes because of their difference in GA-sensitivity; however, these oat genes may be similar to GA-sensitive dominant genes which have been identified in wheat and rye. The *Rht12* mutant of wheat and the *Ddw1* mutant of rye are strong dominant GA-sensitive mutant genes mapping to homoeologous regions on the long arms of chromosomes 5A and 5R, respectively (Borner et al., 1998). The semidominant GA-sensitive dwarfing gene *Rht8* has been identified in many central European wheats and mapped to the short arm of 2DS (Korzun et al., 1998). Several additional semi- or partially dominant GA-sensitive dwarfing genes in wheat have been reported (Konzak, 1987) but have not been characterized or mapped. The segmental homoeology within hexaploid oat and a lack of common markers between wheat and the regions surrounding the mapped oat dwarfing genes precludes predictions at this time of possible orthology between various oat and wheat dwarfing mutants. Such relationships, however, should be discernible in the future, particularly as candidate genes for dwarfing and regulation of GA metabolism are identified in various plant species.

In summary, the three oat dwarfing genes—*Dw6*, *Dw7*, and *Dw8*—reduced plant height in different ways. The *Dw6* gene primarily reduced the length of the three uppermost internodes and did not affect internode number. The *Dw7* gene shortened all internodes but particularly the first and the last, and reduced internode number in the dwarf line. The *Dw8* gene significantly shortened all internodes but did not affect internode number. The three dwarfing loci investigated in this study are responsive to GA and are likely involved in GA metabolism. The oat dwarf mutants studied respond to both GA_1 and GA_{20} and, therefore, do not result from a modification in the conversion of GA_{20} to GA_1. Based on the GA-sensitivity and the dominant or semidominant inheritance pattern of the *Dw6*, *Dw7*, and *Dw8* mutants, we suggest that they may be variants in some type of negative regulation of GA metabolism in hexaploid oat. The results indicate that for use of the three dwarfing genes in oat cultivar development different strategies may be needed to adjust for their differing effects on various plant height components.

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