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Effects of Ring D-Modified Gibberellins on Gibberellin Levels and Development in Selected *Sorghum bicolor* Maturity Genotypes

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Abstract. Plants of early flowering mutant and wild type genotypes of *Sorghum bicolor* were treated with ring D-modified gibberellins (GAs), and the effects on endogenous GA levels were determined. The growth and timing of floral initiation in 58M plants grown under 18-h days (which significantly delays floral initiation in this short day plant) following treatment with these compounds, relative to GA_3 and GA_5 treatments, were also investigated. Application of the endo-isomer of C16,17-dihydro-GA_5 (endo-DiHGA_5), the exo-isomer of C16,17-dihydro-GA_5 (exo-DiHGA_5), and C16α,17-dichloromethanodihydro-GA_5 (DMDGA_5) altered GA levels in both genotypes. Each ring D-modified GA significantly inhibited shoot growth while significantly decreasing levels of GA_1 and increasing levels of its immediate precursor, GA_{20}. Gibberellin A_8 levels also decreased. Tillering was not affected by any treatment. For the early flowering genotype 58M, grown under noninductive long days, both dihydro-GA_5 isomers promoted floral initiation while shoot growth was strongly inhibited, and floral development was strongly advanced beyond floral stage 4. Gibberellin A_3 and GA_5, applied under the same conditions, promoted shoot growth slightly and gave ''floral-like'' apical meristems that did not develop past floral stage 1. These results suggest that the reduced shoot growth of sorghum, which follows application of those ring D-modified GAs, is due to their inhibiting the 3β hydroxylation of GA_{20} to GA_1, thereby reducing the GA_1 content. That floral initiation was hastened and floral development promoted in genotype 58M by application of both isomers of DiHGA_5 are in contrast to the effects of other GA biosynthesis inhibitors, which act earlier in the GA biosynthesis pathway, but are consistent with results seen for long day grasses. This suggests that endo-DiHGA_5 and exo-DiHGA_5 may be acting directly in promoting floral initiation and subsequent floral apex development of this short day plant under long day conditions.

Key Words. Flowering—Growth—Tillering—Gibberellin metabolism—Growth retardants

Sorghum bicolor (L.) Moench is a C-4 tropical monocot that is a SD plant with regard to floral induction (Doggett 1988, Garner and Allard 1923). Four genes that regulate the critical night length necessary to promote floral initiation have been discovered in sorghum because of mutation, and three of them now exist in near isogenic lines in the milo cultivar (Quinby 1967, 1973). These genes are termed maturity genes. Maturity gene three (Ma_3) exists as three alleles, one of which (ma_3^R) results in plants that are deficient in a light-stable phytochrome (Childs et al. 1991, 1992, Foster et al. 1994). In recent work (Childs et al. 1997), Ma_3 mapped to the same locus as the *PHYB* gene, and sequencing of the phytochrome B gene from 100M (Ma_3/Ma_3) and 58M (ma_3^R/ma_3^R) revealed a single base pair deletion near the 3′ end of the 58M gene, which results in a frameshift followed shortly by a stop codon. The deletion occurs just before the putative second dimerization site in *PHYB* (Wagner and Quail 1995). Although 58M flowers earlier than wild type, floral development is otherwise normal.

Abbreviations: DAP, days after planting; DMDGA_5, C16α,17-C-dichloromethanodihydro-GA_5; GA, gibberellin(s) or gibberellin-like substance(s); GC-MS-SIM, gas chromatography-mass spectrometry-selected ion monitoring; endo-DiHGA_5, the endo-isomer of C16,17-dihydro-GA_5; PHYB, phytochrome B apoprotein; *PHYB*, phytochrome B wild type gene; *phyB*, phytochrome B mutant gene; LD, long day; SD, short day; exo-DiHGA_5, exo-isomer of C16,17-dihydro GA_5; HPLC, high performance liquid chromatography.

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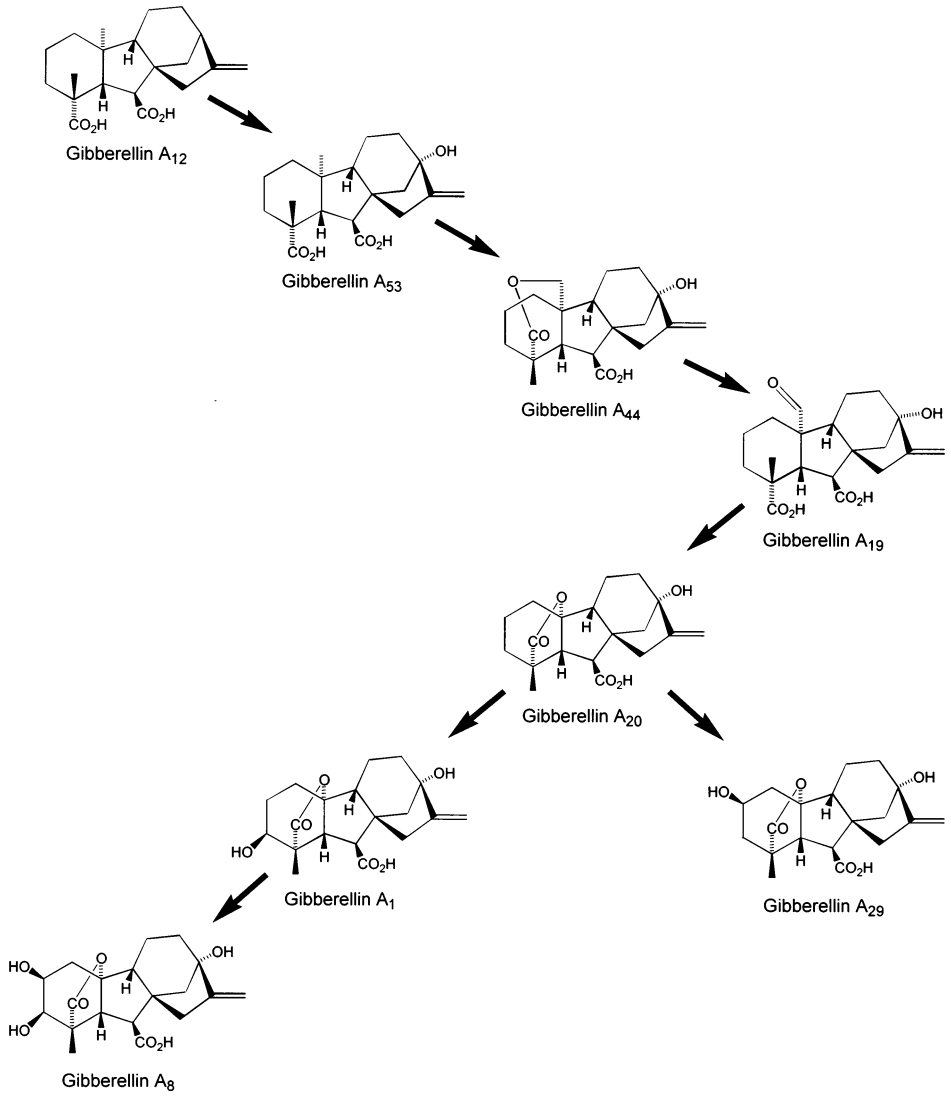

Fig. 1. Early 13 hydroxylation pathway of gibberellin biosynthesis (adapted from Phinney 1984). All of the individual GAs in this pathway occur in sorghum.

Under SD conditions (10 h), most sorghums undergo floral initiation on about the same day (Miller et al. 1968, Quinby and Karper 1945), but when the photoperiod is extended to 12–14 h, flowering of most genotypes is progressively delayed (Lane 1963, Pao and Morgan 1986a). Although genotypes homozygous for ma_3^R initially appeared to be insensitive to photoperiod (Pao and Morgan 1986a), it was shown recently that very long photoperiods (14 h up to continuous light) would progressively delay their floral initiation (Childs et al. 1995a, 1995b).

Because treatment with GA_3 hastened floral initiation of many of the maturity genotypes (Pao and Morgan 1986b, Williams and Morgan 1979) and treatment with an early step GA biosynthesis inhibitor delayed floral initiation while inhibiting shoot growth (Beall et al. 1991), the ma_3^R lesion was suspected to affect GA biosynthesis or action (Beall et al. 1991, Pao and Morgan 1986b). This hypothesis was also consistent with the tall, apically dominant, pale green phenotype of seedlings with the ma_3^R/ma_3^R genotype (Pao and Morgan 1986a). Vegetative tissue of *S. bicolor* seedlings possesses the early C-13 hydroxylation pathway of GA biosynthesis (Fig. 1) (Beall et al. 1991, Rood et al. 1986), and in tissues harvested in the morning the ma_3^R-containing genotype 58M has two- to fivefold higher levels of several GAs in this pathway than the two otherwise identical genotypes homozygous for Ma_3 or ma_3 (Beall et al. 1991). Subsequently, in 58M, the endogenous levels of GA_{12}, GA_{53}, GA_{20}, and GA_1 were found to exhibit diurnal rhythms, and the latter two were either disrupted or shifted so that they peaked at a different time of day relative to similar rhythms in the wild type genotypes 90M (ma_3/ma_3) and 100M (Ma_3/Ma_3) (Foster and Morgan 1995). We thus speculated that the difference in the timing of maxima and minima GA levels may be in-

volved in the differences in flowering behavior between wild type and mutant sorghum genotypes.

Investigations using another grass species (*Lolium temulentum* cv. Ceres, a LD plant) have shown that exposure to a single LD, which is sufficient to induce flowering, transiently increases the levels of endogenous GAs, especially the putative polyhydroxylated GAs (Pharis et al. 1987). Subsequent studies (Evans et al. 1990, 1994a, 1994b) eventually led to recognition that a highly florigenic GA structure for *Lolium*, under either noninductive SD or with a partially inductive single LD, had to include a double bond in ring A (e.g. C-1,2 or C-2,3-didehydro) and at least a single hydroxyl at C-13 (additional hydroxyl groups at C-12β and C-15 enhanced the florigenic effect). Further, the presence of the C-3β hydroxyl was not required for high florigenic qualities and could be, in fact, somewhat detrimental. Conversely, stem elongation in this species required C-3β hydroxylation. Thus, GA_4 and GA_1, both of which are C-3β hydroxylated and without a double bond in ring A, were low or neglible in florigenic activity but highly promotive of stem elongation in *Lolium.*

Other GA structural types were also found which were highly florigenic when applied to *Lolium.* For example, 2,2-dimethyl-GA_4 was exceptionally florigenic and was highly growth promotive (Evans et al. 1990, 1994a, 1994b). However, the most interesting florigenic structures involved modifications to ring D, and one of the more effective of these structures was C-16,17-dihydro-GA_5 (mixed isomers), which promoted flowering while inhibiting stem elongation (Evans et al. 1990, 1994a, 1994b). Flowering could also be accelerated by C-16,17-dihydro-GA_5 when applied to a SD plant, *Xanthium pennsylvanicum*, under marginally inductive conditions, and the combination of C-16,17-dihydro-GA_5 + GA_3 was especially promotive (Evans et al. 1993). This is very unlike the effect seen with other GAs, where promotion of stem elongation accompanies enhanced flowering. In fact, the florigenic effect of all of the tested GAs could be enhanced by converting the C-16-exocyclic to the C-16,17-dihydro form (Evans et al. 1994a, 1994b). C-16,17-dihydro-GA_5 and 2,2-dimethyl-GA_4 were also highly promotive of flowering under LD warm conditions in a cold-requiring cultivar of *Brassica napus*, whereas GA_3 only promoted stem elongation (Evans et al. 1993). A more complete survey of ring D modification effects on flowering vs stem elongation of *Lolium* is given in Mander et al. (1995).

The effects of the two isomers of C-16,17-dihydro-GA_5 (endo-DiHGA_5 and exo-DiHGA_5) on shoot growth and GA biosynthesis in the early flowering, PHYB-deficient sorghum genotype 58M (ma_3^R/ma_3^R) are presented in this report. Additionally, the effects of a related derivative C-16α,17-dihydromethanodihydro-GA_5 (DMDGA_5) on shoot growth and GA biosynthesis in both 58M and a wild type genotype, 90M (ma_3/ma_3), are

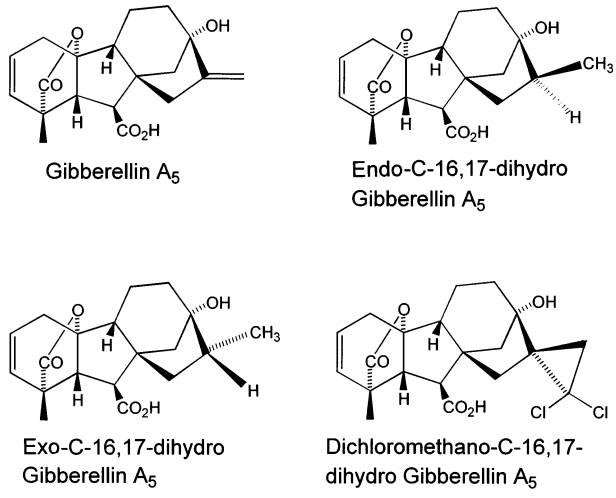

Fig. 2. Structures of GAs and GA derivatives applied in this study.

compared with effects induced by endo-DiHGA_5 and exo-DiHGA_5. The flowering and growth behavior in 58M were also investigated following treatment with the two C-16,17-dihydro-GA_5 isomers compared with the effects of GA_3 and GA_5 application.

Materials and Methods

Plants

Plants of *S. bicolor* (L.) Moench maturity genotypes 58M (ma_3^R/ma_3^R) and 90M (ma_3/ma_3) (Quinby 1967, 1973) were grown from seed supplied by Dr. Fred Miller in growth chambers (EGC, Chagrin Falls, OH) under conditions described previously (Beall et al. 1991, Foster and Morgan 1995).

Gibberellins

Gibberellin A_5, endo-DiHGA_5 (97% endo-DiHGA_5 and 3% exo-DiHGA_5), exo-DiHGA_5 (91% exo-DiHGA_5 and 9% endo-DiHGA_5), and DMDGA_5 were provided by L. N. Mander. Structures are shown in Fig. 2, and details of their synthesis are presented in Mander et al. (1995). Gibberellin A_3, purchased from Eli Lilly (lot 9TH39), contained 60.9% GA_3, 17.2% GA_1, 18.3% isolactone GA_3, and 3.6% gibberellenic acid. The [17,17-$^2H_2$]gibberellins A_1, A_3, A_5, A_6, A_8, A_9, A_{12}, A_{19}, A_{20}, A_{29}, A_{44}, A_{53}, and A_{81} for use as GC-MS-SIM quantitative internal standards were provided by L. N. Mander.

Gibberellin and Gibberellin Derivative Applications

Gibberellins and GA derivatives were dissolved in 95% ethanol and applied with Hamilton microsyringes so that the droplets spread over most of the surface of the youngest leaf blade (which was at least 25% of its final leaf blade length) exerted from the whorl. Preliminary experiments had determined that the small amounts of ethanol we used did not alter development of sorghum plants, and thus control plants received no ethanol. The GA doses were varied because of differences

in biologic activity, lower solubility of the endo-DiHGA$_5$ and exo-DiHGA$_5$ isomers, and results from preliminary experiments which showed that over time a single dose at these levels proved ineffective. Accordingly, volumes of the solutions applied were increased, dependent upon plant responsiveness (e.g. larger plants required higher doses).

Gibberellin Analysis

The endogenous sorghum GAs were extracted and analyzed by GC-MS-SIM using [17,17-^{2}H$_2$]GA internal standards for quantification by isotope dilution analyses as described previously (Foster and Morgan 1995), except that ethyl acetate was used in place of ether for the acid buffer/organic phase partitioning step.

In our first investigation of the effects of endo-DiHGA$_5$ and exo-DiHGA$_5$ on GA levels we found that the above procedure did not adequately separate GA$_{20}$ and the applied endo-DiHGA$_5$ and exo-DiHGA$_5$ isomers. Hence, in the second experiment, the GA$_{20}$-containing fractions were, after C18 reversed phase HPLC separation at Texas A&M University, analyzed by GC-MS-SIM at the University of Calgary. The samples were derivatized, and GC-MS-SIM analysis was accomplished as described in Takagi et al. (1994), with the following exceptions. To separate GA$_{20}$ and [^{2}H$_2$]GA$_{20}$ from the contaminating applied dihydro-GA$_5$ compounds, a 15-meter DB1701 column (J&W Scientific) was used. Temperature conditions were initially 60°C (0.1 min) to 180°C at 20°C/min, then 2°C/min from 180 to 200°C (held 4 min at 200°C), and finally 10°C/min from 200 to 280°C (held 4 min at 280°C), with monitoring from 13 to 21 min. GA$_5$, GA$_6$, and GA$_{81}$ were analyzed because they were assumed to be possible metabolities of the modified GA$_5$ applied or possible metabolities of GA$_{20}$ when its conversion to GA$_1$ is inhibited. These compounds were not detected (see Tables 2 and 4), and they are not discussed further.

Effect of Endo-DiHGA$_5$ and Exo-DiHGA$_5$ Isomers on Endogenous GA Levels

This experiment was conducted to determine the effects of endo-DiHGA$_5$ and exo-DiHGA$_5$ on endogenous GA content and shoot growth using genotype 58M grown in a 12-h photoperiod with a 32°C/22°C day/night temperature regime. There were three treatments (endo-DiHGA$_5$, exo-DiHGA$_5$, and control) with three replicates for each treatment. Each replicate consisted of the plants harvested from two pots, and each pot contained 16–22 plants. The dihydro-GA$_5$ isomers were applied at 5, 8, and 12 DAP. Thus, at five DAP plants received 18.9 μg/plant of one of the two isomers of dihydro-GA$_5$ in 2 μL, with second (8 DAP) and third (12 DAP) applications utilizing 5-μL drops and 47.3 μg/plant for each isomer. At 14 DAP, plant shoots were cut after measuring culm height (the height from the soil surface to the tallest leaf sheath), frozen in liquid N$_2$, and lyophilized prior to extraction for analysis of endogenous GA levels.

Effect of DMDGA$_5$ on Endogenous GA Levels

This experiment was conducted using genotypes 58M and 90M grown as above to compare the effects of endo-DiHGA$_5$ and exo-DiHGA$_5$ on GA levels and shoot growth with DMDGA$_5$, a related but generally more potent inhibitor of plant growth (Mander et al. 1995) (Fig. 2). There were four treatments, including an untreated control, with three replicates of each treatment. A replicate consisted of three pots; each pot contained about 12 plants. The dihydro-GA$_5$ isomers were applied initially at 5 DAP as described above. In preliminary experiments it was determined that DMDGA$_5$ was five to ten times more effective than the dihydro-GA$_5$ isomers in inhibiting shoot growth of sorghum seedlings; therefore, it was applied initially as 2 μl of 1.5 μg/μL solution (3 μg/plant). The second (8 DAP) and third (12 DAP) applications involved 5 μL/plant (e.g. 47.3 μg/plant for endo-DiHGA$_5$ and exo-DiHGA$_5$, and 7.5 μg/plant for DMDGA$_5$). Plants were measured and harvested 14 DAP as above.

Table 1. Amount of each GA and GA$_5$ derivative applied in 95% ethanol on specific dates to sorghum seedlings to determine the effects of these compounds on growth and timing of floral initiation.

	GA$_3$ (μg)		GA$_5$ (μg)		Endo-DiHGA$_5$ (μg)		Exo-DiHGA$_5$ (μg)	
DAP	Low (3.3)[a]	High (33)	Low (3.3)	High (33)	Low (1.9)	High (9.4)	Low (1.9)	High (9.4)
9	33	330	33	330	19	94	19	94
15	66	660	66	660	19	94	19	94
21	66	660	165	1,650	19	94	19	94
27	66	660	165	1,650	19	94	19	94
33	66	660	165	1,650	19	94	19	94
40	66	660	165	1,650	38	186	38	186
44	66	660	165	1,650	38	186	38	186

[a] Concentrations, in μg/μL.

Effect of Endo-DiHGA$_5$ and Exo-DiHGA$_5$ on Growth and Floral Initiation in Sorghum Genotype 58M

This investigation of the effects of the GA$_5$ derivatives on floral initiation was conducted with genotype 58M grown under 18-h photperiods with a 33°C/20°C day/night temperature regime. Gibberellins A$_3$ and A$_5$ were applied for comparison of effects. Each was applied at three rates (Table 1): high, low, and a control (no treatment). DMDGA$_5$ was not available for use in this experiment. Thus, there were 12 treatments, and each was applied to five to six pots containing four to five plants each.

Growth was monitored at regular intervals by measuring the height from the soil surface to the tallest leaf sheath (culm height). At intervals, apices of two to four plants, selected at random from each treatment population, were dissected and examined microscopically for evidence of floral initiation. The stage of floral bud differentiation was assigned according to Lane (1963). At the conclusion of the experiment on flowering, all remaining plants were measured (50 DAP) and floral stage determined (51 DAP).

Results and Discussion

The two dihydro-GA$_5$ isomers were known to reduce shoot height when applied to grass species (Evans et al. 1993, 1994b), implicating GAs in the response. We therefore assessed their effects on endogenous GA levels in sorghum. The two DiHGA$_5$ isomers inhibited shoot growth by an average of 56% by 14 DAP and also reduced the levels of several endogenous GAs (Table 2). Specifically, levels of GA$_{12}$, GA$_{53}$, GA$_{19}$, GA$_1$, and GA$_8$ were reduced; GA$_{53}$, GA$_{19}$, GA$_1$, and GA$_8$ are members

Table 2. Effect of endo-DiHGA$_5$ and exo-DiHGA$_5$ on endogenous GA levels and culm height 14 DAP for sorghum maturity genotype 58M (mean ± S.D.). Treatment dates and amounts are given in the text. DW, dry weight.

	Control (ng/g DW)	Endo-DiHGA$_5$ (ng/g DW)	Exo-DiHGA$_5$ (ng/g DW)
GA[a]			
GA_{12}	16.8 ± 3.5	13.3 ± 4.2	9.8 ± .02
GA_{53}	32.9 ± 1.7	17.2 ± 1.8	18.8 ± 2.4
GA_{44}	45.4 ± 0.3	50.4 ± 5.1	52.8 ± 1.6
GA_{19}	120.9 ± 5.8	96.2 ± 4.8	100.5 ± 5.4
GA_{20}	50.1 ± 2.5	—[b]	—[b]
GA_1	25.8 ± 2.6	5.2 ± 1.0	8.9 ± 1.6
GA_8	5.8 ± 0.1	N.D.[c]	N.D.[c]
	Culm height (mm) [mean ± S.D.]		
	128 ± 11	59 ± 10	54 ± 11

[a] GA_3, GA_5, and GA_6 were not present in any sample. GA_{29} was present at an undetermined level in one sample.
[b] GA_{20} levels could not be quantified for this trial because of contamination with ion m/z 418 from endo-DiHGA$_5$ and exo-DiHGA$_5$.
[c] N.D., not detected.

of the early C-13 hydroxylation pathway (Fig. 1). Unfortunately, tissue levels of GA_{20} could not be determined in this experiment because of contamination in the extract by ion *m/z* 418 from the applied DiHGA$_5$ isomers. Nonetheless, the very reduced levels of GA_1 and its C-2β hydroxylated metabolite, GA_8 (Table 2), are consistent with a reduced C-3β hydroxylation of GA_{20} to GA_1. Inhibition of conversion of $[^2H_2]GA_{20}$ to $[^2H_2]GA_1$ by exo-DiHGA$_5$ also has been shown recently in dwarf rice (Takagi et al. 1994) and *L. temulentum* (Junttilla et al. 1997). Since GA_1 is recognized to be the major GA that regulates shoot growth in *Zea mays* and many other species (Ingram et al. 1986, Kamiya et al. 1992, Phinney 1984), the dwarfing effect of the dihydro-GA$_5$ isomers together with reduced levels of GA_1 and GA_8 (Table 2) are consistent with inhibition of the $GA_{20} \rightarrow GA_1$ step. The tendency for endo-DiHGA$_5$ and exo-DiHGA$_5$ to reduce endogenous levels of the three C-20 GAs (GA_{12}, GA_{19}, GA_{53}) may be due to a feedback inhibition, presumably induced by elevated levels of GA_{20}. Feedback effects that are known include inhibition of expression of GA_{20}-oxidase genes in *Arabidopsis* after application of GA_3 (Phillips et al. 1995) and inhibition of GA_{20} levels by application of 2,2-dimethyl GA_4 (Hedden and Croker 1992). Maize dwarf-1 accumulates GA_{20} about 50-fold above wild type and contains twofold higher GA_{44} levels but lower GA_{12}, GA_{53}, and GA_{19} levels. Thus, the effects of GA_{20} pool size on concentrations of GAs upstream appear to be complex.

In a second experiment, we again examined endogenous GA levels in response to application of the two DiHGA$_5$ isomers and compared these effects with those induced by the application of DMDGA$_5$. Here, we also compared the growth responses of genotype 58M with the wild type 90M. The use of 90M allowed us to determine whether the effects of the DiHGA$_5$ isomers on early growth and endogenous GA levels are unique to PHYB-deficient 58M. Each of the three ring D-modified GAs was applied at a dose sufficient to inhibit shoot elongation of 58M by an average of 48% and of 90M by an average of 41% (Table 3). The effects of the DiHGA$_5$ isomers on endogenous GA levels (Table 4) were similar to those observed in the preceding experiment (Table 2), with significantly reduced levels of GA_1 and significantly elevated levels of GA_{20} (Table 4). There were differences, however, with regard to some of the C-20 GAs. For example, the DiHGA$_5$ isomers again reduced GA_{53} levels, but other C-20 GAs (e.g. GA_{12}, GA_{44}, GA_{19}) were not reduced (the variability in GA_{12} levels was particularly high). A similar trend (reduction of C-20 GAs by the DiHGA$_5$ isomers) also occurred for wild type genotype 90M for GA_{12}, GA_{53}, and GA_{19} (Table 4).

Table 3. Culm heights 14 DAP in sorghum genotypes 58M and 90M treated at 5, 8, and 12 DAP with endo-DiHGA$_5$, exo-DiHGA$_5$, and DMDGA$_5$ (mean ± S.D.). Doses/plant are given in the text.

	Culm height (mm)	
Treatment	58M	90M
Control	131 ± 12	84 ± 12
Endo-DiHGA$_5$	65 ± 9	52 ± 6
Exo-DiHGA$_5$	73 ± 12	51 ± 7
DMDGA$_5$	67 ± 18	45 ± 12

DMDGA$_5$ showed effects on C-20 GA levels in sorghum which were both similar to and different from the DiHGA$_5$ isomers. For example, in 90M, DMDGA$_5$ even though applied at a lower dose, was more effective than either endo-DiHGA$_5$ or exo-DiHGA$_5$ in lowering levels of GA_{53} and somewhat more effective in lowering levels of GA_{19} (a similar trend was evident for GA_{19} in 58M).

At the doses utilized, reductions in GA_1 were similar for each of DMDGA$_5$, endo-DiHGA$_5$, and exo-DiHGA$_5$ for both 90M and 58M (Table 4), as were reductions in height (Table 3), although DMDGA$_5$ tended to be more effective in reducing 90M growth (Table 3). However, DMDGA$_5$ elevated GA_{20} levels only fourfold, relative to a 5- to 6.5-fold increase caused by the two DiHGA$_5$ isomers across both genotypes (Table 4). This lowered efficacy by DMDGA$_5$ in elevating GA_{20} levels may simply be a function of dose (doses were sixfold lower for DMDGA$_5$ than for the DiHGA$_5$ isomers). Alternatively, it might be explained by an inability or reduced ability of DMDGA$_5$ to inhibit the C-2β hydroxylation (inactivation) of GA_{20}. For example, for 58M, the GA_{29}:GA_{20} ratio for control plants was 0.17, whereas the ratio for DMDGA$_5$-treated plants was 0.37. In 90M the GA_{29}:GA_{20} ratio was 0.63 for both control and DMDGA$_5$-treated plants. This contrasts markedly to the effects of the DiHGA$_5$ isomers, which reduced the

Table 4. Effect of endo-DiHGA$_5$ and exo-DiHGA$_5$, and DMDGA$_5$ on endogenous GA levels in sorghum maturity genotypes 58M and 90M 14 DAP. The ring D-modified GAs were applied at 5, 8, and 12 DAP at the doses listed in the text.

Gibberellin and treatment	58M (ng/g DW)	90M (ng/g DW)
GA_{12}		
Control	51.5 ± 22.7	14.6 ± 2.2
Endo-DiHGA$_5$	67.2 ± 55.7	9.3 ± 0.5
Exo-DiHGA$_5$	83.6 ± 50.5	10.6 ± 0.8
DMDGA$_5$	115.4 ± 43.3	9.0 ± 0.9
GA_{53}		
Control	12.8 ± 1.6	45.9 ± 7.3
Endo-DiHGA$_5$	5.8 ± 1.5	23.2 ± 1.9
Exo-DiGHA$_5$	5.3 ± 0.3	26.7 ± 2.6
DMDGA$_5$	4.9 ± 0.5	10.8 ± 0.4
GA_{44}		
Control	29.9 ± 0.6	19.1 ± 1.7
Endo-DiHGA$_5$	29.0 ± 2.3	27.3 ± 4.8
Exo-DiHGA$_5$	25.0 ± 1.3	25.9 ± 12.2
DMDGA$_5$	28.2 ± 1.1	18.4 ± 2.9
GA_{19}		
Control	85.0 ± 10.0	225.7 ± 23.3
Endo-DiHGA$_5$	95.6 ± 16.2	194.7 ± 21.0
Exo-DiHGA$_5$	92.2 ± 8.2	206.3 ± 21.7
DMDGA$_5$	74.2 ± 4.4	144.8 ± 14.2
GA_{20}		
Control	30.7 ± 5.0	18.6 ± 2.2
Endo-DiHGA$_5$	175.6 ± 15.4	112.1 ± 7.3
Exo-DiHGA$_5$	160.3 ± 3.3	121.7 ± 3.2
DMDGA$_5$	112.8 ± 6.7	88.6 ± 4.6
GA_1		
Control	15.6 ± 2.5	9.2 ± 2.4
Endo-DiHGA$_5$	2.1 ± 0.3	1.3 ± 0.3
Exo-DiHGA$_5$	3.5 ± 1.4	1.5 ± 0.9
DMDGA$_5$	1.7 ± 0.6	1.5 ± 0.1
GA_8		
Control	4.2 ± 0.2	2.5 ± 0.5
Endo-DiHGA$_5$	0.1 ± 0.1	0.2 ± 0.1
Exo-DiHGA$_5$	0.2 ± 0.2	0.2 ± 0.2
DMDGA$_5$	0.6 ± 0.1	0.3 ± 0.3
GA_{29}		
Control	5.2 ± 0.3	11.8 ± 0.9
Endo-DiHGA$_5$	10.8 ± 0.9	10.6 ± 0.7
Exo-DiHGA$_5$	15.4 ± 1.6	17.2 ± 1.0
DMDGA$_5$	41.4 ± 3.0	56.1 ± 3.3
GA_{81}	N.D.[a]	
GA_6	N.D.	

[a] N.D., not detected in any treatment or cultivar.

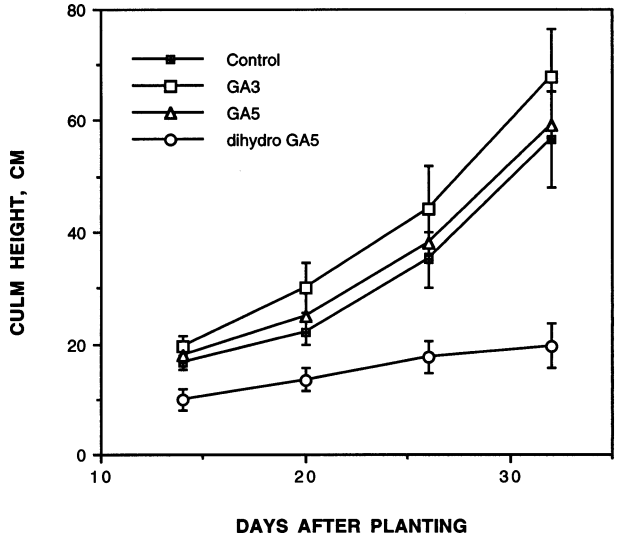

Fig. 3. Effect of GA_3, GA_5, endo-DiHGA$_5$, and exo-DiHGA$_5$ on height of 58M sorghum seedlings at various times after planting (mean ± SD). Treatment amount and times are shown in Table 1. Data from individual sets of control plants for each GA applied are presented as averages. Because of similar results, data for the two DiHGA$_5$ isomers at both concentrations and GA_3 and GA_5 at both concentrations are presented as averages. For clarity, standard deviations for GA_5 data are not plotted but are similar in magnitude to standard deviation for controls.

GA_{29}:GA_{20} ratio in both genotypes (average 0.08 in 58M and 0.12 in 90M) relative to the ratios in control plants (0.17 in 58M and 0.63 in 90M). Thus, at these relatively low doses, there is no evidence of DMDGA$_5$ inhibiting C-2β hydroxylation of $GA_{20} \rightarrow GA_{29}$, whereas it is highly effective at inhibiting C-3β hydroxylation of $GA_{20} \rightarrow GA_1$.

Effective growth retardation of a number of higher plant species can be gained by use of two of the acyclohexanedione class of inhibitors (CGA 163935 and BX-112). These compounds also effectively reduce C-3β hydroxylation of $GA_{20} \rightarrow GA_1$, resulting in an accumulation of GA_{20} (Griggs et al. 1991, Nakayama et al. 1990, Kamiya et al. 1992, Rademacher et al. 1992). The results presented here (Table 4) for sorghum (growth retardation, reduced GA_1, increased GA_{20}) suggest a similar mode of action for two of the ring D-modified GAs (Fig. 2).

In addition to reducing vegetative shoot growth, the ring D-modified GA_5 derivatives have been observed to enhance floral induction in *Lolium* (Evans et al. 1993, 1994b). Genotype 58M is delayed in floral initiation by very LD. For example, floral meristems are initiated at day 16 under 12-h light/12-h dark photoperiods and at day 28 under 18-h light/6-h dark photoperiods (Childs et al. 1995b). Therefore, we chose an 18-h daylength to provide a time frame within which the initiation date could be shifted forward or backward with the DiHGA$_5$ isomers. Under 18-h daylength conditions the effects on growth promotion were similar for the high and low GA_3 and GA_5 doses. This was also the case for the effects of the ring D-modified GA derivatives on height reduction; hence, heights were averaged across the several doses (Fig. 3). Both of the DiHGA$_5$ isomers inhibited shoot growth markedly (Fig. 3). In contrast, GA_3 and GA_5 treatments resulted in modest promotion of shoot growth. Consistent with the findings in the previous two experi-

Table 5. Effect of GA_3, GA_5, endo-$DiHGA_5$, and exo-$DiHGA_5$ on culm height of 58M sorghum at 50 DAP, which was 6 days after the last treatment (mean ± S.D.). Concentrations and amounts of GAs and GA derivatives applied and dates of application are given in Table 1.

	Culm height (cm)	
Treatment	High concentration	Low concentration
GA_3	118.4 ± 17.7	135.7 ± 16.0
GA_5	125.0 ± 18.8	136.5 ± 5.9
Endo-$DiHGA_5$	25.8 ± 7.3	22.1 ± 5.1
Exo-$DiHGA_5$	22.1 ± 3.2	18.4 ± 2.3
Control	117.0 ± 18.8	117.0 ± 18.8

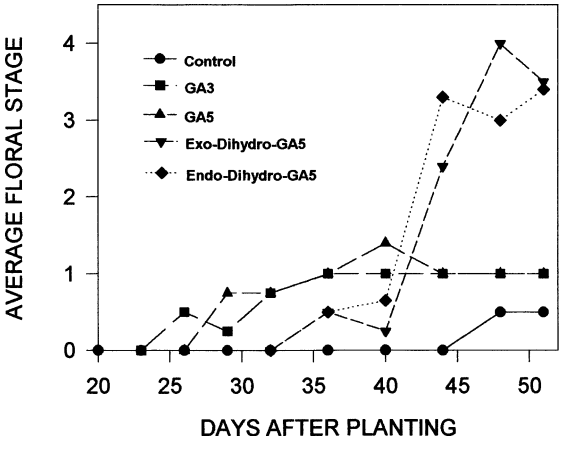

Fig. 4. Effect of GA_3, GA_5, endo-$DiHGA_5$, and exo-$DiHGA_5$ on date of floral initiation (DAP) and floral development (floral stage) in 58M. Data show average floral stage for two to three randomly selected plants except that on day 51 all remaining plants were dissected and examined with numbers of plants ranging from four to seven.

ments, both $DiHGA_5$ isomers again caused a very large inhibition of growth (Fig. 3 and Table 5). Also, GA_3, on a dose/plant basis, promoted growth appreciably more than GA_5.

Pronounced stimulation of floral development by the $DiHGA_5$ isomers (Fig. 4) was the most remarkable result of these experiments (Fig. 4). The timing of floral initiation and subsequent flower development in plants treated with the two $DiHGA_5$ isomers is in marked contrast to the floral development of GA_3- and GA_5-treated plants, and, as well to the flowering behavior of the control plants. Flowering stage 1 (stage 0 is vegetative; Lane 1963) first appeared 26 and 29 DAP for the GA_3- and GA_5-treated plants, respectively, whereas the first control plants reached stage 1 only on DAP 48. In treatments with the $DiHGA_5$ isomers, the first plant reached floral stage 1 on DAP 36. Thus, all of the GA treatments, including those that inhibited growth, hastened floral initiation relative to controls. This has been seen previously for GA_3 (Pao and Morgan 1986b, Williams and Morgan

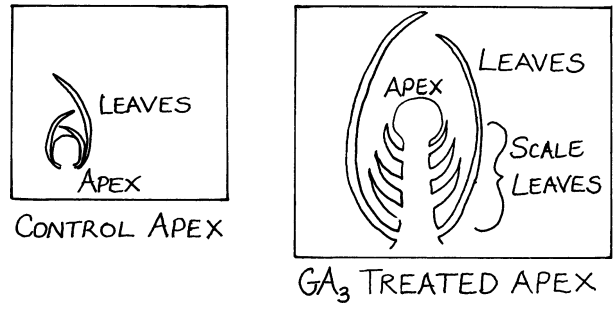

Fig. 5. Drawings of appearances and proportional sizes of shoot meristems of control plants and those treated with GA_3 after the treatments had altered meristem development (observations at 40 DAS, see Table 1 for treatment schedule). The appearance of GA_5-treated plant meristems was indistinguishable from that of GA_3-treated meristems.

1979). Subsequently, however, none of the GA_3- and GA_5-treated plants proceeded beyond floral stage 1, with the exception of two GA_5-treated plants judged to be between stages 1 and 2 on 40 DAP. In marked contrast, the floral development of the $DiHGA_5$ isomer-treated plants proceeded rapidly; many were beyond stage 4 with differentiated panicle branches by 51 DAP.

GA biosynthesis inhibitors known to block conversion of GA_{20} to GA_1 (BX-112 and CGA 163935) (Griggs et al. 1991, Kamiya et al. 1992, Nakayama et al. 1990, Rademacher et al. 1992), similar to the effects of the two $DiHGA_5$ isomers and $DMDGA_5$ observed here (Table 4), recently were shown to inhibit shoot growth but either not delay or possibly promote very slightly floral initiation in sorghum genotype 58M (Lee 1996). Further, these inhibitors reduced growth and delayed floral initiation of sorghum genotype 90M. Inhibitors influencing GA biosynthetic steps before GA_{12} inhibited both growth and date of floral initiation in both genotypes. Thus, the effect of the two $DiHGA_5$ isomers to inhibit conversion of GA_{20} to GA_1 and growth, while markedly promoting floral initiation and development, appears unique and suggests the possibility of a direct florigenic effect of these compounds.

Although plants treated with GA_3 and GA_5 reached floral stage 1, they then exhibited abnormal apex development (Fig. 5). The apical dome was expanded as is the case for a floral meristem, but it had a series of ''bract-like'' structures developing below it. These bract-like structures appeared more like leaves than like panicle branches. In fact, they looked similar to structures seen in field-grown sorghum treated with GA_3 under photoperiods unfavorable for floral initiation (Morgan and Quinby 1987). Nevertheless, we term these meristems ''floral'' because they appear more floral than vegetative (Lane 1963). That said, at the time of this experiment there was no way to eliminate the possibility that they are merely distorted vegetative meristems. Since the initial

appearance of the meristems on GA_3- and GA_5-treated plants is similar to meristems differentiating naturally under SD, we propose that these plants have initiated florally but that subsequent floral development is prevented by the very long photoperiod and/or the applied GA_3 or GA_4; that is, LD and/or high GA levels may prevent normal floral development in this SD plant.

There were other interesting effects of the two $DiHGA_5$ isomers. For example, shoots of plants treated with the two isomers were remarkably soft in consistency, suggesting an inhibition of synthesis of secondary cell wall components. Treated plants also had broadened leaf blades and darker green leaves than any of the control or GA_3- or GA_5-treated plants (leaf length was reduced by the $DiHGA_5$ isomers, and leaf area was not determined). GA biosynthesis inhibitor treatments normally increase tillering (Beall et al. 1991, Evans et al. 1993, Foster 1992, Isbell and Morgan 1982), and applied GAs reduce tillering of sorghum and other grasses during the period of treatment (Isbell and Morgan 1982, Morgan et al. 1977; for review see Cline 1991). However, in contrast to the effects seen for other grasses (Evans et al. 1993, Foster 1992), none of the plants treated with the two dihydro-GA_5 isomers exhibited increased tillering (observations on tillering were only made in the longer duration experiment in which floral initiation dates were determined; see Fig. 4 and Table 5).

Treatment of sorghum seedlings with the three ring D-modified GA_5 derivatives alters GA biosynthesis, most notably yielding a reduction in GA_1 level and increasing GA_{20} content (i.e. inhibition of 3β hydroxylation of GA_{20}). Significant retardation of shoot growth is associated with these reduced GA_1 levels. Additionally, the two C-16,17-dihydro-GA_5 isomers stimulated floral initiation and significantly enhanced floral meristem development when applied to sorghum under noninductive conditions. These effects on floral meristem development are in contrast to the effects of other GA biosynthesis inhibitors on sorghum where inhibition of vegatitive growth is usually accompanied by a delay in flowering (Lee 1996).

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