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Genetic Regulation of Development in *Sorghum bicolor*[1]

II. EFFECT OF THE ma_3^R ALLELE MIMICKED BY GA_3

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ABSTRACT

The class III, ma_3^R-containing maturity genotypes of *Sorghum bicolor* (L.) Moench differ from all of the other maturity genotypes in that they flower earlier, exhibit rapid shoot elongation sooner after floral differentiation, and exhibit several morphological differences. In the present study, a class III, ma_3^R-containing genotype (44M) was compared to two other genotypes (60M, 80M) that differed only at the third maturity locus. GA_3 treatment caused 60M and 80M to undergo floral differentiation earlier, to exhibit an increase in shoot elongation rate sooner after floral initiation, and to exhibit morphological changes including: increased leaf blade and leaf sheath length, decreased leaf width and leaf area, as well as increased dry weight, especially in the culm weight. Since every major morphological character which is different in class III genotypes was changed by GA_3 treatment of the class II genotypes (60M, 80M), thereby causing them to be more like the class III genotypes, it is clear that application of GA_3 makes maturity genotypes with Ma_3 or ma_3 behave as if they contained ma_3^R. All of these results support the hypothesis that the ma_3^R allele increases gibberellin activity in the maturity genotypes with that allele.

Sorghum (*Sorghum bicolor*) is a quantitative SD plant (5, 10) in which allelic variation is known for several genes which modify photoperiod requirements (7). In the milo group of cultivars seven different photoperiod-responsive alleles have been assigned to three loci. This has allowed the construction of 11 genotypes which differ at maturity loci Ma_1, Ma_2, and Ma_3 and are homozygous for each independently inherited allele (7, 8). Three alleles (Ma_3, ma_3, ma_3^R) occur at the third maturity locus, and simple dominants and recessives at the first two loci. No recessive has occurred at the fourth locus in the milo-type cultivars; thus, all of the maturity genotypes are homozygous for Ma_4.

A systematic study of the photoperiodic behavior and other developmental or morphological characteristics of the maturity genotypes revealed that they fall into three groups or classes (5). Genotypes containing the allele ma_3^R differed from the other eight in that they are relatively photoperiod insensitive, have longer leaf sheaths, longer but narrower leaf blades, less leaf area, and they initiate rapid shoot elongation sooner after floral initiation (5). In contrast, the two genotypes which contain Ma_1Ma_2 and either Ma_3 or ma_3 but not ma_3^R have extreme photoperiod sensitivity but no obvious morphological or other development differences from the other six non-ma_3^R genotypes.

Since application of GA_3 hastens floral differentiation in the sorghum maturity genotypes (10) and since promotion of leaf sheath elongation is the basis of the well known d_5 dwarf maize bioassay for gibberellins (6), we hypothesized that the class III, ma_3^R-containing genotypes may express higher levels of gibberellin activity than the non-ma_3^R genotypes (5). A similar proposal was made earlier (8) based on the 'grassy' appearance (9) of the ma_3^R genotypes. One approach to testing the hypothesis was to treat non-ma_3^R genotypes with gibberellins and determine whether their photoperiodism, development, or morphology were altered to make them more like the ma_3^R genotypes. Since our focus was on ma_3^R, we simplified the experiment by eliminating the other major photoperiod influencing gene combination (Ma_1Ma_2; [5]) and selected for comparisons, genotypes without Ma_1Ma_2 or ma_3^R (*i.e.* genotypes in class II) *versus* a genotype without Ma_1Ma_2 but with ma_3^R (44M).

We report here the effects of relatively small amounts of GA_3 on 60M ($Ma_1ma_2ma_3Ma_4$) and 80M ($Ma_1ma_2Ma_3Ma_4$) compared to 44M ($Ma_1ma_2ma_3^RMa_4$). Since 44M is assumed to be higher in expressed gibberellin activity than 60M or 80M, 44M was not treated with GA_3, but its usual growth pattern was used as a control. The objective of the GA_3 application was to determine whether or not it is possible to discreetly change the features unique to the class III, ma_3^R genotypes without distorting the plants so as to produce 'abnormal' morphology or behavior. Thus, treatment levels were kept low and more extreme symptom expression presumably could be achieved with larger doses of GA_3.

MATERIALS AND METHODS

Sorghum bicolor (L.) Moench genotypes 60M ($Ma_1ma_2Ma_3Ma_4$), 80M ($Ma_1ma_2ma_3Ma_4$), and 44M ($Ma_1ma_2ma_3^RMa_4$) (single maturity gene symbols indicate homozygosity) were grown in a growth room as previously described (5). Briefly, lighting was a mixture of fluorescent and incandescent sources which gave a light intensity of 250 to 300 $\mu E\ m^{-2}\ s^{-1}$ (PAR) at the soil surface. Lights were on 12 h with the temperature at 30°C; the night temperature was 21°C with 60% RH continuous. Seed were supplied by J. R. Quinby, Pioneer Hi-Bred International, Inc., Plainview, TX.

Aqueous solutions of GA_3 (Abbott Laboratories) with 0.05% Tween 20 (v/v) as a surfactant were applied beginning 8 d after planting. Droplets were placed in the whorl of leaves at the shoot tip with a calibrated, micrometer-activated syringe; the volume was increased as plants grew but care was taken not to exceed the volume the whorl could contain. Since previous experience revealed that levels of GA_3 which would modify growth of sorghum seedlings were without effect on older plants (4), we progressively increased the volume and concentration of GA_3 solution, which was applied at 3 d intervals as follows: d 8 and 11, 10 μM, 15 μl; d 14 and 17, 10 μM, 25 μl; d 20 and 23, 20 μM,

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50 μl; d 26 and 29, 20 μM, 100 μl; d 32 and 35, 50 μM, 150 μl; d 37 and 40, 100 μM, 250 μl. These levels modified morphology without the excessive leaf sheath and leaf blade elongation and the accompanying lodging that can occur with excessive concentrations of GA_3 (4).

The date of floral initiation was determined by harvesting plants from a population of 30 to 45 plants per genotype and examining the dissected apical meristem under a microscope. Stage 2 (3) was accepted as the criteria for floral differentiation. This experiment was repeated and average results from the two experiments are reported (Table I).

Morphological data were collected in two experiments. In the first, 15 plants per genotype per treatment were examined for leaf number, total height, and culm height each 5 d from d 10 to d 50 (after planting) (Figs. 1 and 2); at d 50 fresh weight, dry weight, leaf blade area, and leaf blade width were recorded (Tables I and II). Since the last collared leaf is always the longest one thus determining total height, leaf blade length was calculated by subtracting culm height (soil surface to youngest collared leaf) from total height (soil surface to tip of the tallest leaf). A second experiment involved sets of 10 plants per genotype per treatment each harvested at 30, 40, or 50 d for determination of leaf blade area and dry weight (Figs. 3 and 4). Other morphological measurements were repeated on these plants but are not reported since they did not differ noticeably from the previous experiment. Morphological data reported as averages of 15 or 10 plants plus or minus the standard deviation. Plants were grown in pots, thinned to either 2 or 3 per pot, and pots with uniform plants randomly assigned to treatments (GA_3 or control) on d 8. Leaf area for detached leaf blades was measured with a leaf area meter (Lambda Instrument Corp., Inc.).

RESULTS AND DISCUSSION

Application of GA_3 hastened the differentiation of the apical meristem into an inflorescence 8 to 10 d earlier than untreated, control plants for both 60M and 80M (Table I). The floral initiation date of GA_3-treated 60M and 80M was only 2 or 3 d later than untreated 44M; thus, GA_3 made the two non-ma_3^R genotypes undergo floral initiation at approximately the same time as the ma_3^R genotype. Floral initiation dates in this experiment for untreated, control plants are essentially the same as those for the same genotypes in a previous study (5). The capacity of GA_3 or GA_3 plus FR at the end of the d to hasten the floral initiation of some of the sorghum maturity genotypes was observed earlier (10).

The effect of GA_3 application on height of 80M contrasted to untreated 80M and 44M is shown in Figure 1. The data for 60M were essentially identical to those for 80M and were omitted for clarity. The GA_3 treatment regime used increased the total plant

Table I. *Effect of GA_3 on the Number of Days from Planting to Differentiation of a Terminal, Floral Meristem (above) and on Leaf Blade Area at 50 Days (below) in Sorghum Maturity Genotypes 60M and 80M*

Genotype 44M was included as a control.

Treatments	Genotypes		
	60M	80M	44M
	days to initiation		
Control	33	35	22
+GA_3	25	24	
	leaf blade area[a] cm^2/plant		
Control	968 ± 250	840 ± 82	324 ± 48
+GA_3	752 ± 162	534 ± 93	

[a] Average value ± 1 SD.

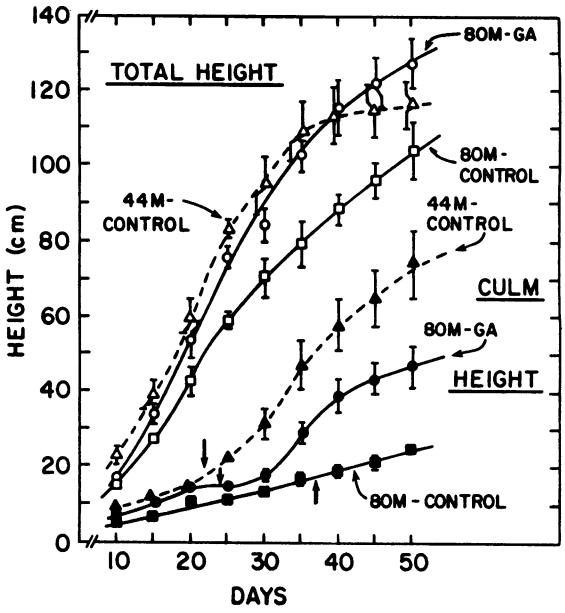

FIG. 1. Effect of GA_3 on the total height (upper curves) and culm (lower curves) height in genotype 80M from d 10 to 50 after planting. Bold vertical arrows indicate floral differentiation (initiation) dates. Genotype 44M included as a control. Standard deviations shown except where smaller than data point symbols.

height and the culm height of 60M and 80M. Thus, the treated plants had longer leaf blades and longer leaf sheaths than untreated (control) plants. The leaf morphology of the treated 60M and 80M plants became more like untreated 44M plants than the untreated (control) 60M and 80M plants. The application of GA_3 began on d 8 and the standard errors of the means of GA_3-treated and control plants did not overlap from d 15 onward. The most obvious difference between GA_3-treated 80M (or 60M) and untreated 44M is less elongation of the leaf sheaths in the former than in the latter, but the capacity of GA_3 to promote leaf sheath elongation was clearly demonstrated. In preliminary work for a previous study (4) excessive levels of GA_3 were observed to promote leaf sheath and leaf blade elongation so excessively that the plants fell over (PW Morgan, FR Miller, JR Quinby, unpublished data).

Another way to compare leaf and shoot morphology in this experiment is the length of the upmost, collared leaf, and the proportion (percentage) of the total plant height due to the culm. GA_3 promoted leaf blade elongation of 80M (data from 60M are indistinguishable and are omitted for the sake of clarity) so that it was similar to untreated 44M for the first 30 d (Fig. 2). In addition, GA_3 treatment increased the proportion of the total height due to the culm; thus, the summed effect on all the leaf sheaths was greater than the effect on the last fully expanded leaf blade. Figure 2 reveals, in agreement with Figure 1, that in 44M the shoot elongation that begins at floral initiation is similar qualitatively but is more rapid than that induced by the levels of GA_3 employed here. In 44M we determined that at 50 d the increase in proportion of the total height due to the culm results from elongation of both internodes and leaf sheaths. Previously, GA_3 has been shown to promote internode elongation, but the amount needed increases markedly with age (4). In 44M the rapid shoot or culm elongation after floral initiation coincides with a marked reduction in leaf blade length as the final leaves emerge (Fig. 2). In GA_3-treated 80M this presumed competition phenomenon is also expressed, though less obviously, as the relative length of leaf blades decreases more rapidly in treated 80M than control 80M and they become equal on d 50 after expressing the greatest divergence on d 25. The relatively smaller

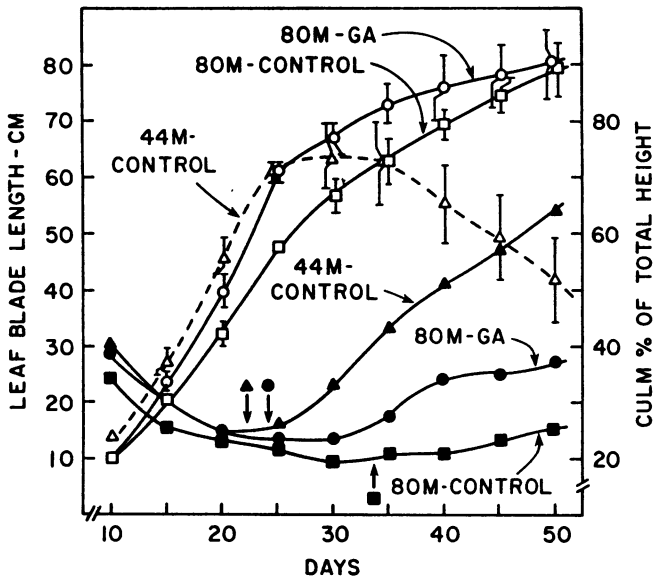

FIG. 2. Effect of GA_3 on the length of the topmost, collared leaf (upper curves) and percentage of the total height composed of the culm (shoot from ground level to youngest leaf collar) (lower curves) for genotypes 80M from d 10 to 50 after planting. Bold vertical arrows indicate floral differentiation (initiation) dates. Genotype 44M included as a control. Standard deviations shown except where smaller than data point symbols.

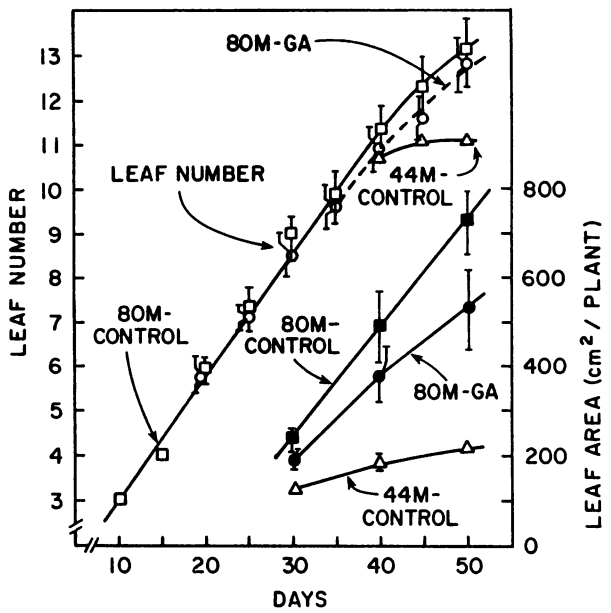

FIG. 3. Effect of GA_3 on leaf number (upper curves) and leaf area (lower curves) of 80M with d after planting. Genotype 44M included as a control. Standard deviations shown except where smaller than data point symbols.

expression of the apparent competitive reduction in leaf size after rapid shoot elongation in 80M is consistent with the observation that culm or shoot elongation was less obviously promoted by the level of GA_3 employed in 80M than occurred naturally in 44M following floral initiation. As noted earlier (5), maturity genotypes without ma_3^R (either class I or class II), are much slower to begin rapid internode and leaf sheath elongation following floral initiation than the class III, ma_3^R containing genotypes. This is again illustrated by comparing the lag between floral initiation and culm elongation for 80M and 44M (Figs. 1 and 2).

Application of GA_3 reduced the leaf area of 60M and 80M (Table I). The reduction in leaf area amounted to 22% in 60M and 36% in 80M; however, the area of the GA_3-treated genotypes was still greater than 44M. This was undoubtedly due to the rapid decline in leaf length that occurs in ma_3^R-containing genotypes such as 44M after floral initiation (Fig. 2) and which was not fully duplicated in 60M or 80M by the level of GA_3 applied in this study.

Application of GA_3 reduced leaf width for both 60M and 80M (Table II) thus making the treated plants from these genotypes more like untreated, control 44M plants than the untreated 60M and 80M plants are. With the treatment regime used, the highest concentration was applied on the last two dates and the reduction in leaf width was greater for some of the youngest leaves (L_2, L_3) than for the oldest leaf measured (L_5). In most of these examples (L_2, L_3) the control and GA_3-treated means plus or minus one standard deviation did not overlap and in every case the GA_3-treated leaf was narrower than the equivalent control leaf. Earlier we observed that the ma_3^R genotypes had longer leaf blades but less leaf area (5). Consequently, the ma_3^R genotypes should have narrower leaves than others, and GA_3 should reduce the width of leaves of non-ma_3^R genotypes. These conclusions were confirmed here (Table II).

To confirm that differences in leaf area observed at 50 d (Table I) persisted during the experiment, leaf area was observed at 30, 40, and 50 d in a second experiment (Fig. 3). GA_3 reduced the leaf area of 80M at all three dates. Although there was some overlap of the standard errors at the first two dates, the trend for reduction in leaf area with GA_3 treatment was consistent with all three dates for both genotypes (data for 60M were indistinguishable and are omitted for clarity). That this reduction in leaf area is not due to differences in leaf number is indicated by the very similar leaf emergence rate for GA_3-treated and control 80M plants (Fig. 3) which contrasts with the GA_3-mediated reduction in leaf width (Table II).

Dry weights of GA_3-treated and control 80M plants were similar at d 30, but GA_3-treated plants were heavier on d 40 and d 50, by approximately the increase in weight of the culm portion of the plant (Fig. 4). This probably should be viewed as an effect on development rather than on assimilation because the GA_3 treatment promoted culm or leaf sheath elongation (Figs. 1 and 2) and probably by d 50 some internode elongation had begun. Previously, the class III, ma_3^R-containing genotypes were shown to contain a greater proportion of their dry weight in the culm than do the class I and II, non-ma_3^R containing genotypes (5). In Figure 4 the GA_3-treatment is illustrated to increase the culm-proportion of the total dry weight of class II genotypes (data for 60M were similar to those for 80M and are omitted for clarity). Genotype 44M similarly had a greater proportion of its dry weight in the culm than did nontreated 80M or 60M (data not given). Thus, the GA_3-treated class II genotypes (60M and 80M) have a greater proportion of their dry weight in the culm, and they are advanced in development due to 8 to 10 d earlier floral initiation than in control plants. On this basis the treated plants have both altered morphology and more advanced development than the control plants. The same differences were previously noted between ma_3^R-containing and non-ma_3^R-containing genotypes (5).

Several investigators have noted effects of GA_3 on the morphology of other plants similar to effects on sorghum observed in this study. For example, maize treated with GA_3 at weekly intervals beginning in the seedling stage exhibited reduced leaf area (1). GA_3 application increased height, internode length, leaf blade, and leaf sheath length and decreased tiller number in rice (2). These effects were observed for both the dwarf, thus presumably gibberellin-deficient, and the nondwarf, presumably gibberellin-adequate, lines. Neither leaf blade area nor widths were

Table II. *Effect of GA_3 on the Width of Leaf Blades of 80M, 60M, and 44M at 50 Days*

The widest portion of the leaf blade was measured; leaves numbered from the topmost, newly formed leaf downward. Values shown plus or minus one standard deviation.

Genotype and Treatment	Leaf Position				
	1	2	3	4	5
			mm		
80M control	37.2 ± 4.1	37.8 ± 5.0	33.2 ± 2.8	30.4 ± 2.4	26.2 ± 0.8
80M GA	32.2 ± 3.2	29.8 ± 3.3	24.0 ± 3.9	23.4 ± 3.0	23.0 ± 3.8
60M control	34.2 ± 2.6	39.4 ± 4.5	34.2 ± 5.2	31.8 ± 3.4	28.8 ± 5.1
60M GA	32.4 ± 6.1	29.4 ± 5.0	28.4 ± 4.2	28.6 ± 3.8	27.4 ± 2.3
44M control	22.8 ± 1.8	24.8 ± 1.1	26.6 ± 1.5	23.4 ± 0.9	22.2 ± 1.3

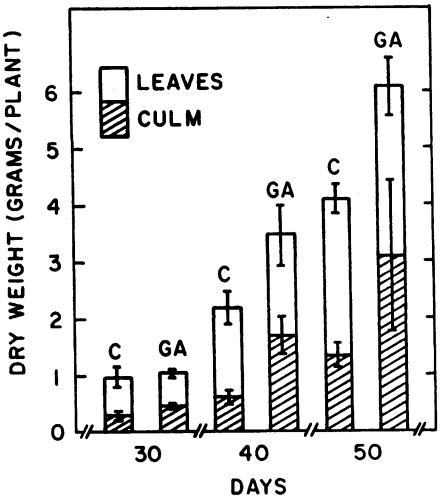

FIG. 4. Effect of GA_3 on dry weight of culm (lower, shaded portion of bars) and leaves (upper, open portion of bars) of 80M at 30, 40, and 50 d after planting. Standard deviation bars shown.

published (2). In a greenhouse experiment dealing with root development of sorghum, GA_3 reduced leaf area, apparently by damaging leaves and disrupting normal development (11, 12).

All of the characteristics which are uniquely different in the ma_3^R genotypes were modified by application of GA_3 to genotypes that differed in identified maturity genes only at the third locus. The result was that floral initiation was hastened, culm (leaf sheath and internode) elongation after floral initiation was promoted, leaf blade and leaf sheath length were increased, and leaf area and leaf width were decreased by GA_3 treatment. Thus, GA_3 induced several developmental and morphological changes in the non-ma_3^R containing genotypes (60M and 80M), and for every basis of comparison GA_3 caused them to be more similar to the ma_3^R-containing genotype (44M) than they were to untreated, control 60M and 80M plants. Since these lines are near isogenic (7, 8) except for the maturity genes and since their maturity behavior is inherited simply at loci which have been identified and designated (7, 8), it seems certain that the third maturity locus and GA_3 were the variables in this study. We conclude that the third maturity loci appears to regulate the gibberellin economy and that the ma_3^R allele increases the expression of gibberellin activity. Whether this action of ma_3^R is on gibberellin identity or amount, sensitivity to gibberellins or on activity of an anti-gibberellin compound(s) is the subject of current investigations in our laboratory.

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