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Gibberellins and Heterosis in Maize[1]

I. ENDOGENOUS GIBBERELLIN-LIKE SUBSTANCES

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ABSTRACT

Under controlled environment and/or field conditions, vegetative growth (height, internode length, leaf area, shoot dry weight, grain yield) was greater in an F_1 maize hybrid than in either parental inbred. Endogenous gibberellin (GA)-like substances in apical meristem cylinders were also higher in the hybrid than in either inbred, both on a per plant and per gram dry weight basis. There were no apparent qualitative differences in GA-like substances, however. Levels of GA-like substances in all genotypes were highest prior to tassel initiation. Chromatographic comparisons of the GA-like substances and authentic standards of GA native to maize on gradient-eluted SiO_2 partition and reverse-phase C_{18} high-pressure liquid chromatography columns are described. No consistent differences in abscisic acid levels of the three genotypes were observed. This correlation of heterosis for endogenous GA-like substances with heterosis for growth suggests that amounts of endogenous GA may be related to hybrid vigor in maize.

Hybrid vigor, the phenotype of heterosis, exists when hybrid performance exceeds that of the parental genotypes. Hybrid vigor is repeatedly observed for growth, yield, and even certain developmental characters of maize (19, 20, 23). Indeed, the impressive increases in yield of maize during the 20th century have largely resulted from the increased use of hybrid seed (6, 7). However, while plant breeders and agronomists have been utilizing heterosis as a means of improving crop productivity, the physiological basis of heterosis is not presently understood (23).

The involvement of plant hormones in heterosis is both an attractive and logical possibility. Changes in endogenous hormone balance could amplify genotypic differences, thus leading to major phenotypic effects. Although the possibility of hormonal involvement in heterosis has been suggested previously (14), Sinha and Khanna (23) note that no correlative relationships between endogenous hormone levels and hybrid vigor had been described prior to 1975. We are unaware of any subsequent reports of such correlative relationships.

In a previous study (17), it was observed that growth rate and content of endogenous GA[4]-like substances were correlated for two maize inbreds and their F_1 hybrid grown under two low-temperature regimes with dominance for increased growth rate being observed in the hybrid grown under the low-temperature conditions (17). Apparent 'overdominance' (inasmuch as it is difficult to separate overdominance *per se* from complementation, overdominance will be used in this paper) had been repeatedly observed when the same maize genotypes were grown under warmer growth cabinet or field conditions (20). Thus, the next logical question to be addressed was: under favorable temperature conditions are growth rates and levels of endogenous GA-like substances still correlated; does heterosis for increased levels of GA-like substances accompany heterosis for growth rate? The present study was initiated to investigate this question. Additionally, because ABA is sometimes assigned an inhibitory role in the regulation of plant growth, we also examined the levels of endogenous ABA in maize inbreds and their hybrid.

MATERIALS AND METHODS

Plant Materials. Two previously described (19) maize inbreds, CM7 and CM49, and their F_1 hybrid, CM7 × CM49, were included in this study. Ten seeds of each genotype were planted in each plastic pot (22 × 22 cm) filled with 'Cornell mix' (2). Pots were placed in a walk-in growth room (Controlled Environments Ltd., Winnipeg, Manitoba) under 25/20°C (day/night) temperatures; rises and falls in temperature were 5°C h^{-1}. Day thermoperiod and photoperiod were 14 h with PAR at 808 $\mu E\ m^{-2}\ s^{-1}$. Pots were watered twice daily.

Six, 15, 21, 28, and 38 d after seedling emergence (day 3 after planting), the shoots of five plants of each genotype were cut from the roots at the soil surface. Heights to the tallest extended leaf tip, total leaf areas of all exerted blades (measured with a Wescor LI 3000 area meter), and shoot dry weights were measured. From these data, mean RGR and mean NAR were calculated where:

$$\mathrm{RGR} = \frac{(\log_e W_2 - \log_e W_1)}{(t_2 - t_1)}$$

$$\mathrm{NAR} = \frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{(A_2 - A_1)(t_2 - t_1)}$$

and W, A, and t represent shoot dry weight, leaf area, and day of harvest, respectively (16). Final heights of these three genotypes had previously been measured under these growth room as well as under field conditions (20). Average final internode lengths were calculated by dividing final plant height by leaf number (20). Grain yield was measured under field conditions (19) over two seasons.

At 15, 21, and 28 d after emergence, shoot cylinders were excised by cutting the shoots at the root crown, and 5 (day 15 and 21 harvests) or 10 cm (day 28 harvest) above the root crown. These shoot cylinders which contained the apical meristems and

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[4] Abbreviations: GA, gibberellin(s); RGR, relative growth rate; NAR, net assimilation rate; ABA-Me, ABA methyl ester; ECD, electron capture detector; PR, potence ratio; C/D R GA_{20}, C/D ring rearranged GA_{20}.

FIG. 1. Inbred and hybrid maize seedlings 21 d after emergence. Left to right: inbred CM7, hybrid CM7 × CM49, inbred CM49.

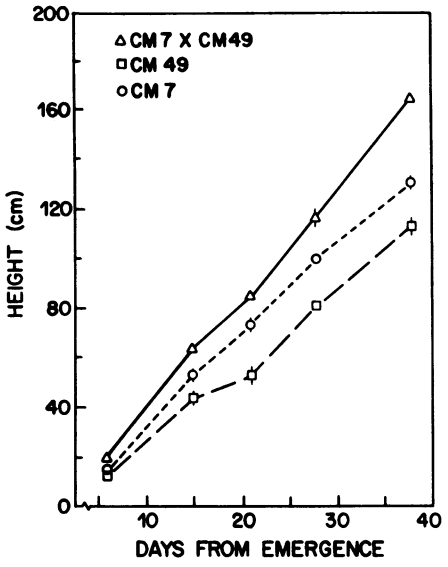

FIG. 2. Total height of two maize inbreds, CM7 and CM49, and their F_1 hybrid, CM7 × CM49, at five dates after seedling emergence in a controlled environment room with day/night temperature at 25/20°C and 14 h thermo- and photoperiod. Vertical bars represent SE.

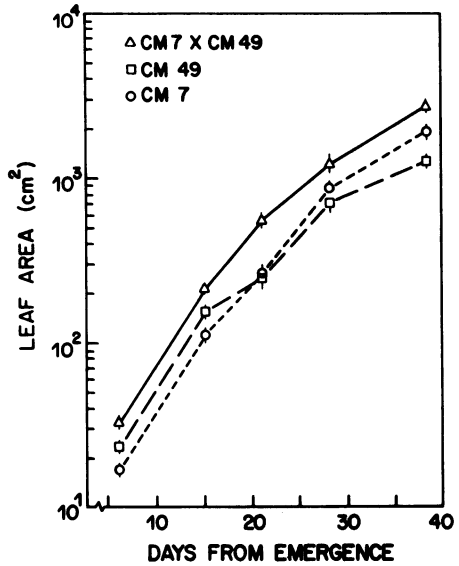

FIG. 3. Leaf area (cm^2/plant) of inbred and hybrid maize seedlings 21 d after emergence. Conditions as in Figure 2.

surrounding leaf sheath tissue were immediately frozen in dry ice and then lyophilized.

Analysis of GA-Like Substances. Four replicate samples of 25, 10, or 5 (day 15, 21, and 28 harvests, respectively) cylinders for each inbred and its hybrid (36 samples in total) were extracted for GA-like substances as previously described (18, 21). After separation by solvent partitioning (5, 21) and purification of the acidic, ethyl acetate soluble fraction by polyvinylpolypyrrolidone (8) and charcoal:celite (4) columns, gradient-elution SiO_2 partition chromatography (3, 15) was performed. Detection and quantification of GA-like substances were achieved using a modified (11) 'Tanginbozu' dwarf-rice micro-drop assay (13), with serial dilution application of 1/200, 1/400, and 1/800 aliquots of each SiO_2 partition column fraction. Paired *t* tests were used to determine possible significance of differences in levels of GA-like substances.

Biologically active SiO_2 partition column fractions were bulked and redeveloped on a second SiO_2 partition column. Biologically active peaks from this second SiO_2 partition column were further chromatographed using gradient-eluted reverse-phase C_{18} HPLC,[5] as previously described (18). The resultant HPLC fractions were bioassayed as above. Authentic GA were also chromatographed on SiO_2 partition and reverse-phase C_{18} HPLC columns (3, 18).

ABA Analysis. After removal of aliquots for GA bioassay, the SiO_2 partition column fractions in which ABA would elute (*e.g.* fractions 5–12) were bulked, dissolved in methanol, filtered (0.5 μm Millipore FH), and a spike of 5 nCi [^{3}H]ABA (24 Ci $mmol^{-1}$, purchased from Amersham and purified by us prior to use by isocratic reverse-phase HPLC-radioactivity counting) was added to the bulked fractions from each extract. Spiked extracts were chromatographed using reverse-phase C_{18} HPLC (11). The HPLC column was a semipreparative 50 cm × 9.4 mm (i.d.) Whatman Partisil-10 ODS-2, M9, and the eluant was 100% methanol at 1.6 ml min^{-1}. In this isocratic separation mode, authentic ABA eluted at 10.6 min. The [^{3}H]ABA-containing fraction was taken to dryness *in vacuo* and derivatized with ethereal diazomethane. The efficiency of derivatization was determined by liquid scintillation spectrometry of aliquots after separating ABA from [^{3}H]ABA-Me on a second reverse-phase C_{18} HPLC column (25).

The HPLC fractions containing [^{3}H]ABA-Me were solubilized in ethyl acetate and injected onto a 1.4 m × 0.4 cm column packed with 2% SE 30 in a Packard model 430 GLC fitted with a model

[5] Waters Scientific Ltd., Mississauga, Ontario.

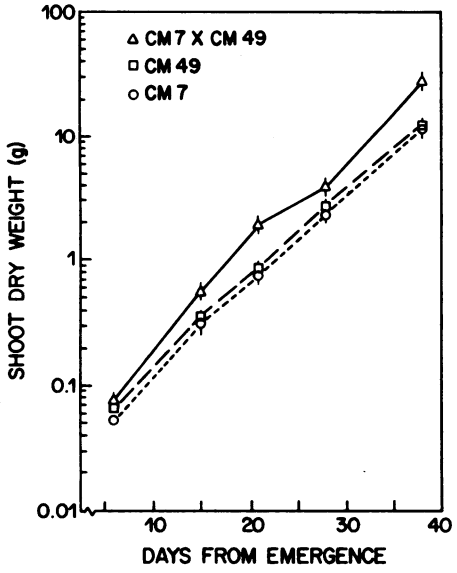

FIG. 4. Shoot dry weight (g/plant) of inbred and hybrid maize seedlings 21 d after emergence. Conditions as in Figure 2.

Table I. *Relative Shoot Growth Rates of Two Maize Inbreds and Their F_1 Hybrid*

	Relative Shoot Growth Rates at Following Days from Emergence				
	6–15	15–21	21–28	28–38	6–38
	$mg/g \cdot d^{-1}$				
Inbred					
CM7	193	145	165	161	168
CM49	192	138	152	160	163
Hybrid					
CM7 × CM49	226	203	95	201	184

Table II. *NAR of Two Maize Inbreds and Their F_1 Hybrid*

	NAR at Following Days from Emergence			
	6–15	15–21	21–28	28–38
	$mg/cm^2 \cdot d^{-1}$			
Inbred				
CM7	0.532	0.402	0.507	0.677
CM49	0.378	0.451	0.459	1.051
Hybrid				
CM7 × CM49	0.585	0.641	0.510	1.315

902 ^{63}Ni ECD (22). Injector, oven, and detector temperatures were 210, 180, and 225°C, respectively, and isothermal chromatography was carried out using 30 ml min^{-1} N_2. Retention times of ABA-Me and *t*-ABA-Me were 7.92 and 11.72 min, respectively. In eight randomly assigned extracts, UV irradiation was carried out after initial GLC-ECD, the sample then being rechromatographed on the GLC-ECD.

Inheritance Analysis. Significant differences in phytohormone levels were detected with the Kruskal-Wallis test (24). Specific differences between phytohormone levels in the different genotypes were revealed using the Wilcoxon-Mann-Whitney statistic (24).

Inheritance analysis was conducted as described by Mather and Jinks (12). The additive component of variation (*d*) was calculated as the absolute value of CM7 minus the mean of CM7 and CM49 (midparent value). The dominance component of variation (*h*) was calculated as the hybrid value minus the midparent value, and PR was taken as *h/d*.

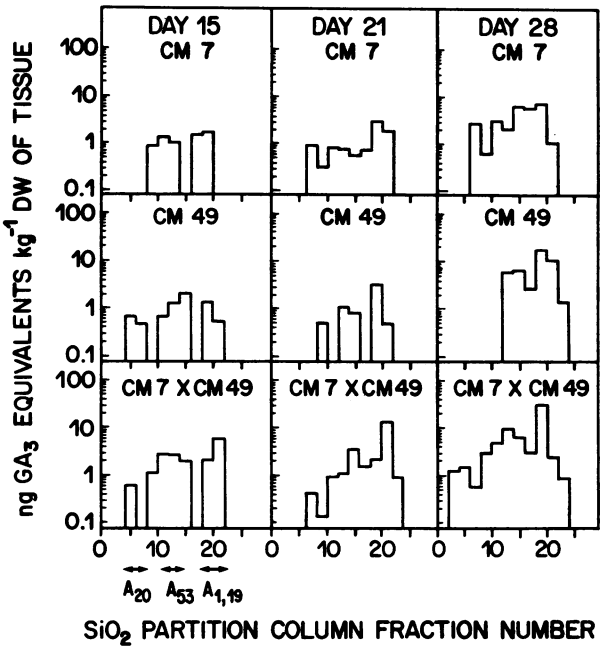

FIG. 5. Elution profile of GA-like substances, as determined by bioassay in serial dilution on dwarf rice cv 'Tan-ginbozu' from SiO_2 partition columns loaded with the acidic, ethyl acetate-soluble fraction of extracts from apical meristem cylinders of two maize inbreds, CM7 and CM49, and their F_1 hybrid, CM7 × CM49, harvested at three dates after seedling emergence. Individual replicates were picked to represent a 'typical' qualitative elution profile. Average values of total GA-like substances for each genotype at each date of harvest are shown in Tables III and IV, along with an indication of their precision and statistical significance (inbred *versus* hybrid).

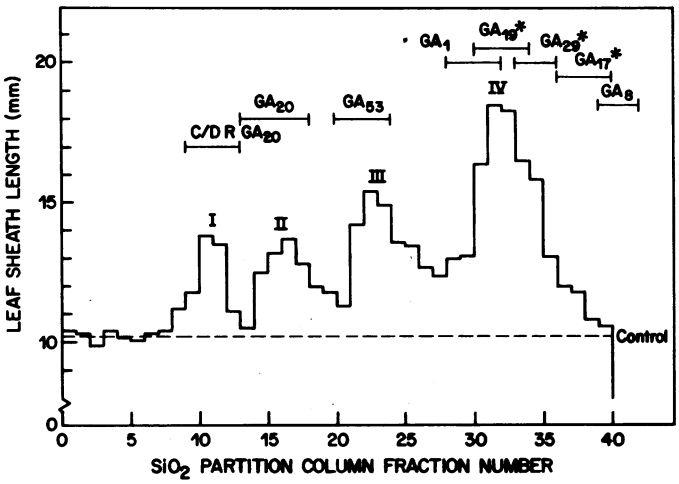

FIG. 6. Elution profile of GA-like substances from a SiO_2 partition column loaded with extracts from apical meristems of maize hybrid CM7 × CM49, as determined by bioassay in serial dilution on dwarf rice cv 'Tan-ginbozu.' Elution regions of some standard GA that are native to maize are shown above the elution profile.

RESULTS AND DISCUSSION

Growth. The hybrid grew far more rapidly than either parental inbred under the controlled environment conditions (Figs. 1–4). The hybrid was taller and produced greater leaf areas at all

harvests (Figs. 2 and 3). Shoot dry weight of the hybrid was not significantly greater than that of CM49 on day 6, although on all subsequent harvests hybrid shoots were significantly heavier (Fig. 4).

It had previously been suggested that heterosis for maize growth is the result of an initial advantage of larger hybrid embryos (1). However, in our study, RGR and NAR of the hybrid were greater than those of parental inbreds, and we think it very unlikely that initially larger embryos could account for the increased RGR and NAR observed. Others have also concluded that superior hybrid growth cannot be explained entirely in terms of an initial advantage of the embryo (23).

Except for the slight depression in the hybrid growth curve (Fig. 3) which led to a very low RGR value for the interval day 21 to 28, RGR of the hybrid exceeded that of either inbred (Table I). Thus, shoot dry weight increases per g dry weight were greater in the hybrid, and growth rate was more rapid. A similar trend was observed with respect to shoot dry weight per unit leaf area (Table II, NAR). In all three genotypes, NAR values were highest during rapid internodal elongation which began at about day 28 (Table II).

Endogenous GA-Like Substances. Three principal regions containing GA-like substances eluted from the initial SiO_2 partition columns (Fig. 5). After bulking biologically active SiO_2 partition column fractions and performing an additional SiO_2 partition column separation, a fourth less-polar peak of GA-like activity was also present (Fig. 6, peak I). Peak I (Fig. 6) cochromatographed with C/D R GA_{20} (Figs. 6 and 7). As GA_{20} may have undergone C/D ring rearrangement during our work-up procedures (18), it is possible that peak I (Fig. 6) and IIB (Fig. 7) may represent C/D R GA_{20}, which could have been GA_{20} in the original plant tissue. Biological activity within peaks IIA, IIIA, and IV cochromatographed with GA_{20}, GA_{53}, and GA_{19}, respectively, on sequential SiO_2 → HPLC columns (Figs. 6 and 7; Table III). These three GA are native in maize (9). Peak IIIB (Fig. 7) did not cochromatograph with any GA known to be native in

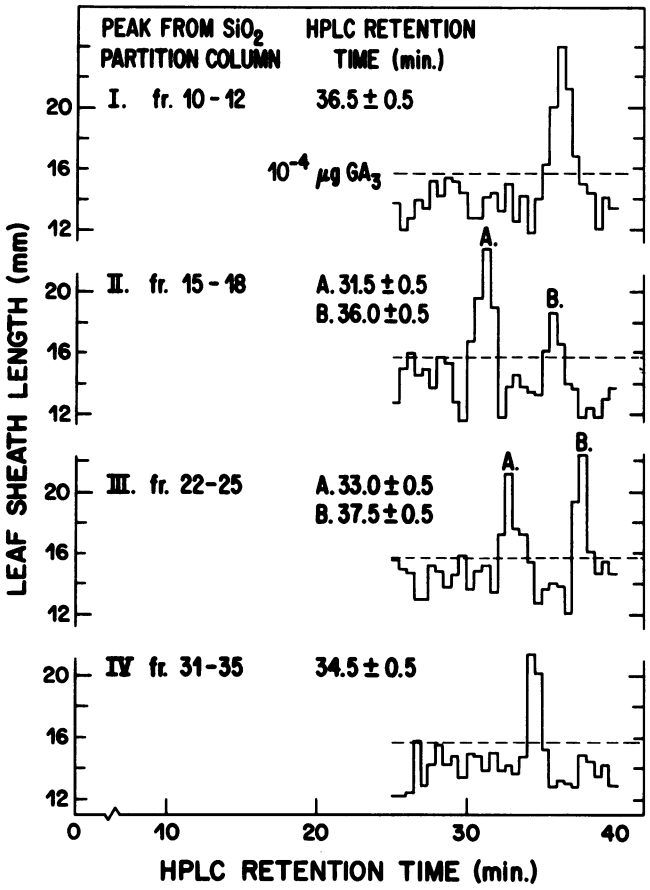

FIG. 7. Elution profile of GA-like substances, as determined by bioassay in serial dilution on dwarf rice cv 'Tan-ginbozu,' from a C_{18} reverse-phase HPLC column loaded with SiO_2 partition column peaks I to IV (Fig. 6) from apical meristems of maize hybrid CM7 × CM49. Retention times of GA known to be native to maize are shown in Table III.

Table III. *Retention Times (Rt) of Standard Gibberellins on Reverse-Phase C_{18} HPLC*

All but GA_4 and C/D rearranged GA_{20} are known to be native to maize (Hedden *et al.* 1981). Standards were coinjected or injected immediately after [^{3}H]GA_{20}. Retention times from the various procedures were corrected so that Rt of the internal standard [^{3}H]GA_{20} was 31.5 min. The Rt of GA_{29}, GA_{44}, and GA_{17} are based on Jones *et al.* (10). The Rt of GA_{29} was also confirmed by P. Davies (Cornell University, personal communication). For GA_{19} and GA_{53}, 0.5-min fractions were collected for bioassay.

GA	Retention Time	Method of Detection
	min	
GA_8	12.4	HPLC-RC[a]
GA_{29}	16–20	HPLC-GC-MS
GA_1	24.6	HPLC-RC
GA_{20}	31.5	HPLC-RC
GA_{44}	31.5–33.0	HPLC-GC-MS
GA_{53}	33.0	HPLC-bioassay
GA_{19}	34.5	HPLC-bioassay
GA_{17}	34.5–35.1	HPLC-GC-MS
GA_4	35.1	HPLC-RC
C/D R GA_{20}	36.1	HPLC-RC

[a] RC, radioactivity counting.

Table IV. *Levels of GA-Like Substances in Shoot Apical Meristem Cylinders of Two Maize Inbreds and Their F_1 Hybrid*

	Days from Emergence											
	15				21				28			
	SiO_2 partition column fraction											
	5–10	11–16	17–22	Total[a]	5–10	11–16	17–22	Total	5–10	11–16	17–22	Total
	ng GA_3 eq/cylinder											
Inbred												
CM7	0.9	1.8	1.7	4.3 ± 1.4*a*	1.0	2.7	5.0	8.7 ± 1.7*a*	7.0	7.7	5.5	25.3 ± 6.0*a*
CM49	2.0	3.5	4.8	10.2 ± 1.7*b*	1.0	3.2	4.1	8.3 ± 1.4*a*	4.6	20.2	12.2	39.9 ± 2.9*a*
Hybrid												
CM7 × CM49	3.8	6.1	6.0	16.2 ± 2.4*c*	0.5	8.6	13.6	22.7 ± 1.8*b*	8.0	27.2	53.1	92.0 ± 19.4*b*

[a] Within a column, values followed by the same letter do not differ ($P \geq 0.05$).

maize (9).

Generally, similar elution patterns of GA-like substances from the initial SiO_2 partition columns were observed for all three genotypes (Fig. 5). Further, since repetitive differences of quantities of GA-like substances in each region were not apparent (Fig. 5), we have presented levels of total GA-like substances (Tables IV and V).

Table V. *Specific Activity of GA-Like Substances in Shoot Apical Meristem Cylinders of Two Maize Inbreds and Their F_1 Hybrid*

	GA-Like Substances at Following Days from Emergence		
	15	21	28
	μg GA_3 eq/kg tissue		
Inbred			
CM7	78*a*[a]	6.2*a*	19*a*
CM49	100*a*	8.2*a*	20*a*
Hybrid			
CM7 × CM49	109*a*	22.9*b*	29*b*

[a] Within a column, values followed by the same letter do not differ ($P \geq 0.05$).

Table VI. *ABA Levels in Shoot Apical Meristem Cylinders of Two Maize Inbreds and Their F_1 Hybrid*

	ABA Content at Following Days from Emergence		
	15	21	28
	ng ABA/cylinder		
Inbred			
CM7	0.86*a*[a]	2.22*a*	18.75*a*
CM49	2.39*b*	4.51*ab*	18.33*a*
Hybrid			
CM7 × CM49	1.26*a*	6.70*b*	22.86*a*

[a] Within a column, values followed by the same letter do not differ ($P \geq 0.05$).

Table VII. *Specific Activity of ABA of Two Maize Inbreds and Their F_1 Hybrid*

	ABA Content at Following Days from Emergence		
	15	21	28
	ng ABA/g tissue		
Inbred			
CM7	17.0*ab*[a]	23.4*bc*	14.3*ab*
CM49	24.8*bc*	39.1*c*	9.5*a*
Hybrid			
CM7 × CM49	8.4*a*	37.6*c*	7.8*a*

[a] Values followed by the same letter do not differ ($P \geq 0.05$).

Harvests 1, 2, and 3 took place during the vegetative phase, just after tassel initiation, and at the onset of rapid internodal elongation, respectively. The hybrid contained higher levels of GA-like substances per cylinder at all three harvests than either parent (Table IV). The same trend occurred when data were analyzed on a per g dry weight basis, although differences were not statistically significant at day 15 (Table V). Although total activity of GA-like substances per cylinder was highest at the onset of shoot elongation, the activity per g was lower at this time (day 28) than during the vegetative phase (day 15) of growth (Table V). However, a 10-cm cylinder containing the apical meristem as well as basal shoot tissue was harvested on day 28, while a shorter segment, probably containing a different proportion of apical meristem tissue, was harvested at day 15. Consequently, direct comparison on a dry weight basis between the two samples is confounded.

Endogenous ABA. Since we did not add an internal standard of ABA at the initial extraction step, absolute levels of ABA cannot be estimated. However, relative differences between extracts can be noted if one assumes that losses during work up will not differ appreciably from sample to sample.

No consistent differences in ABA content of the three genotypes were observed (Tables VI and VII). In all genotypes, ABA levels per g dry weight were highest on day 21, right after tassel initiation (Table VII).

Correlation of Endogenous Hormones with Hybrid Vigor. Increased endogenous levels of GA-like substances were positively correlated with hybrid vigor while endogenous levels of ABA were neither positively nor negatively correlated with hybrid vigor. Apparent overdominance (performance of the hybrid outside of the performance range of parental inbreds) for growth rate and level of GA-like substances was thus observed.

This overdominance for seedling growth characteristics (Figs. 1–4) was carried over to maturity. Thus, overdominance for final plant height and mean final internode length was observed (Table VIII). Further, overdominance for increased grain yield occurred under field conditions (Table VIII).

The PR represents overall degree of dominance and can be used to quantify overdominance (12). For all traits studied, PR exceeded 1.0, a value indicating complete dominance (a value of 0.0 indicates codominance, while absolute values between 0.0 and 1.0 indicate incomplete dominance) (12). Thus, PR values indicate overdominance for increased level of GA-like substances per meristematic cylinder or per unit of tissue dry weight (Table IX).

Results of the present study show that heterosis for level of GA-like substances is well correlated with heterosis for growth rate in maize. This correlation between heterosis for content of GA-like substances and heterosis for growth rate suggests that GA may be involved in the regulation of hybrid vigor in maize. However, taken alone, this analysis does not address the question of a

Table VIII. *Inheritance Characteristics for Final Growth and Grain Yield of Early Maturing Maize*

Trait	Genotype			Components of Variation		PR
	CM7	CM49	CM7 × CM49	Additive (d)	Dominance (h)	
Final height (cm)						
Growth room	162	148	203	6.8	48.2	7.1
Field	165	133	212	16	63	3.9
Mean internode length (cm)						
Growth room	9.5	10.2	12.4	0.4	2.6	7.3
Field	12.3	9.3	14.7	1.5	3.9	2.6
Grain yield in field (g $plant^{-1}$)						
	26.5	34	90	4.1	60.6	14.7

Table IX. *Inheritance Characteristics for Growth and Level of GA-Like Substances in Early Maturing Maize Seedlings*

Trait	Midparent Value	Components of Variation		PR[a]
		Additive (d)	Dominance (h)	
Dry weight[b]	11.8 g	0.4	16.8	43.2
Leaf area[b]	1,698 cm^2	370	1,180	3.2
Height[b]	121.5 cm	8.5	43.5	5.1
RGR[c]	166 mg $g^{-1}d^{-1}$	2.5	18.5	7.3
NAR[c]	0.56 mg $cm^{-2}d^{-1}$	0.03	0.21	7.4
GA-like substances (ng GA_3 eq $cylinder^{-1}$)				
Day 15	7.2	3.0	9.0	3.4
Day 21	8.5	0.2	14.2	71.0
Day 28	32.6	7.3	59.4	8.1
Specific activity of GA-like substances (μg GA_3 eq kg^{-1} tissue)				
Day 15	89.0	11.0	20.0	1.8
Day 21	7.2	1.0	15.7	15.7
Day 28	19.5	0.5	9.5	19.0

[a] Potence ratio represents the overall degree of dominance and = h/d.
[b] Values for day 38.
[c] Mean values for days 6 to 38.

possible causal role for GA. In the second paper of this series, response of these maize genotypes to exogenous GA_3 and metabolism of [^{3}H]GA_{20} will be described. Within the context of the data presented herein, and data presented in the subsequent paper, a causal role for GA and heterosis appears possible.

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