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Gibberellins: A Phytohormonal Basis for Heterosis in Maize

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Four commercially important maize parental inbreds and their 12 F_1 hybrids were studied to investigate the role of the phytohormone gibberellin (GA) in the regulation of heterosis (hybrid vigor). All hybrids grew faster than any inbred. In contrast, all inbreds showed a greater promotion of shoot growth after the exogenous application of GA_3. Concentrations of endogenous GA_1, the biological effector for shoot growth in maize, and GA_{19}, a precursor of GA_1, were measured in apical meristematic shoot cylinders for three of the inbreds and their hybrids by gas chromatography–mass spectrometry with selected ion monitoring; deuterated GAs were used as quantitative internal standards. In 34 of 36 comparisons, hybrids contained higher concentrations of endogenous GAs than their parental inbreds. Preferential growth acceleration of the inbreds by exogenous GA_3 indicates that a deficiency of endogenous GA limits the growth of the inbreds and is thus a cause of inbreeding depression. Conversely, the increased endogenous concentration of GA in the hybrids could provide a phytohormonal basis for heterosis for shoot growth.

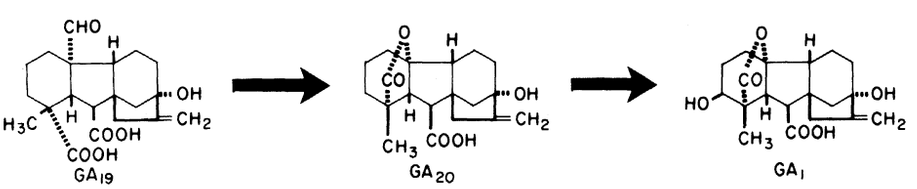
IN ANIMALS AND PARTICULARLY IN plants, it is commonly observed that hybrids—that is, genotypes resulting from the crossing of dissimilar parents—outperform either of their parental genotypes. This phenomenon is referred to as heterosis, or hybrid vigor, and underlies much of the improvement in crop yields achieved in the 20th century. Although heterosis is economically important for agriculture, horticulture, and silviculture, its physiological basis is unclear.

At least three lines of evidence support a role for endogenous gibberellins (GAs) in the regulation of heterosis in maize: (i) Inbreds, homozygous lines resulting from repeated self-pollinations, are particularly responsive to the exogenous application of GA_3 (*1, 2*). GA_3 can accelerate shoot growth in many maize genotypes, but inbreds are most responsive. The correlation between inbreeding and the responsiveness to exogenous GA_3 suggests that the growth of inbreds is limited by a deficiency of endogenous GAs. (ii) The endogenous GA-like substances have been quantified by bioassay from two inbreds and their heterotic hybrid (*3*), and significantly higher concentrations of GA-like activity were observed in the hybrid over a sequence of harvests (*3*). (iii) Heterosis for early seedling growth of maize is frequently observed (*4, 5*), and the rapid production of hydrolytic enzymes such as α-amylase and protease is essential to provide respiratory substrates for this heterosis of early seedling growth (*4, 6*). GAs participate in regulating the levels of hydrolytic enzymes, in germinating cereal seeds and young seedlings (*7*), and this argument for the relation between GAs, hydrolytic enzymes, and heterosis for seedling growth was independently proposed by Paleg (*8*) and by Sarkissian *et al.* (*4*).

A definitive experiment to evaluate the role of GAs in regulating heterosis in the growth of the maize shoot must include more than a single hybrid and its parents, and the assessment of endogenous GA concentration must be unequivocal.

Although maize shoots contain at least eight endogenous GAs (*9, 10*), only GA_1 is the biologically active "effector" in the regulation of maize shoot elongation (*10*). Other GAs serve as precursors to GA_1 (for example, $GA_{19} \rightarrow GA_{20} \rightarrow GA_1$) (Fig. 1), are metabolites of GA_1, or are the results of branch points in the GA metabolic pathway (*10*). Thus, the analysis of GA_1 is particularly relevant.

In the present study, we crossed four maize inbreds in diallel combinations to obtain a matrix of all possible single-cross hybrids and their inbred parents (Table 1). The selected inbreds represent some of the economically most important maize inbreds available; the inbreds, A632, B73, and



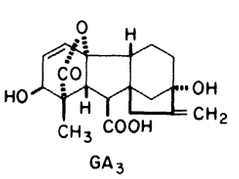


Fig. 1. Chemical structures and metabolic relationships of the GAs included in the present study. GA_3 is a commercially available analog of GA_1, which is the biological effector for shoot growth in maize (*10*).

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Mo17, from which CH807 was derived, are the most important public inbreds in North American breeding programs and are used in more than 60% of the commercial hybrids developed from public inbreds (*11*).

As repeatedly observed in the field as well as in controlled environments (*12*), the hybrids outperformed any of the inbreds. The hybrids were taller as a result of longer leaf sheaths (Table 1) and leaf blades, and had greater shoot dry weights and leaf areas than the inbreds. Thus, heterosis was substantial in the present study.

Endogenous GAs in the apical meristem and surrounding leaf sheath tissue were extracted and purified from a three-parent diallel (that is, three inbreds and six hybrids). The concentrations of GA_1, the biological effector for shoot growth, and GA_{19}, an abundant precursor to GA_1, were determined by adding 100 ng each of [17,17-^{2}H]GA_1 and [17,17-^{2}H]GA_{19} to each extract as internal standards for quantitation by gas chromatography–mass spectrometry with selected ion monitoring (Fig. 2) (*13*).

Statistically significant ($P < 0.05$) genotypic differences in GA concentration were observed for each GA (for example, for GA_1, day 26: $\chi^2 = 18.5$ by Kruskal-Wallis nonparametric analysis). In 11 of 12 comparisons, mean concentrations of GA_1 were higher in hybrids than in their inbred parents on day 26 (Table 2). The same pattern was observed for GA_1 concentration determined at day 14 and for GA_{19} concentration at day 26 (Table 2). For these two GAs over two harvest dates, the GA concentration of the hybrids was greater than that of the corresponding parental inbreds for 34 of 36 comparisons [6 hybrids × 2 inbred comparisons × (2 dates for GA_1 + 1 date for GA_{19}) = 36 comparisons (Table 2)].

If the heterosis for GA_1 level is responsible for heterosis of shoot growth and not simply correlated with heterosis of shoot growth, the exogenous application of GA_1, or its C-1,2 dehydro analog, GA_3 (Fig. 1) (used in lieu of GA_1 because of the limited availability of GA_1), must preferentially accelerate the growth of inbreds relative to hybrids. This has been shown for a few inbreds (*1, 2*) and is now confirmed for the genotypes in our study (Table 1). In all cases, inbreds were more responsive than the hybrids to the exogenous GA_3; after the GA_3 application the inbreds approached hybrids in terms of shoot height (Table 1).

Maize inbreds thus contain lower concentrations of endogenous GA_{19} and GA_1 than their heterotic hybrids. The consequence of an endogenous GA_1 deficiency in maize is well known (*10, 14*); GA_1-deficient mutants are phenotypically dwarfed, with short leaves and very short internodes, and they produce small quantities of grain. These same characteristics of short height, smaller leaf areas, and reduced grain yields are expressed by maize inbreds. Thus, at least certain maize inbreds may be viewed as being metabolically similar to GA_1-deficient

Table 1. Shoot heights to the uppermost ligule (leaf sheath lengths) of four maize inbred (italicized) and all possible single-cross F_1 hybrid combinations in controlled environment conditions (day, 25°C; night, 15°C). The GA_3 was dissolved in 1 ml of 1% ethanol and pipetted into the leaf whorl, a funnel of leaf sheaths surrounding the shoot apical meristem. The upper values indicate ligule heights of 26-day-old control (no GA_3) plants, and the lower values (in parentheses) represent ligule height growth increments 12 days after the exogenous application of 0.5 mg of GA_3. Data represent mean values of 16 measurements. Standard errors for shoot heights ranged from 0.7 to 1.3 and averaged 0.8 cm. Standard errors for growth increments ranged from 0.0 to 0.4 and averaged 0.2 cm.

Male parent	Shoot height (cm) when female parent is			
	A632	B73	CH807	CO109
A632	*15.8* (4.7)	16.8 (1.1)	19.5 (1.9)	21.3 (0.6)
B73	18.5 (1.0)	*13.5* (*5.1*)	17.8 (0.6)	18.0 (0.0)
CH807	19.6 (2.2)	17.5 (1.1)	*15.3* (*5.5*)	20.4 (1.9)
CO109	20.4 (2.2)	16.9 (1.5)	20.6 (2.0)	*12.9* (*6.4*)

Table 2. Endogenous concentrations of GA_1 and GA_{19} from shoot cylinders containing apical meristems from three maize inbreds (italicized) and their three single-cross F_1 hybrids 14 and 26 days after seeding in growth room conditions. Extracts of about 1 g dry weight were purified by sequential solvent partitioning, polyvinylpolypyrrolidone columns, and silica gel partition column chromatography before C_{18} reversed-phase high-performance liquid chromatography and subsequent derivatization (*11*). Endogenous GA concentrations (nanograms per gram dry weight of tissue) were determined by gas chromatography–mass spectrometry with selected ion monitoring in which three ions (M^+ 508, mass-to-charge ratio 493 and 450) characteristic of the derivatized internal standard [17,17-$^2H_2$]GA and three corresponding ions (−2 atomic mass units) of the endogenous GA were monitored (*11*). Data represent mean values of four replicates, each containing two apical meristem shoot cylinders.

Female parent	GA and harvest	Endogenous GA concentration (ng/g) when male parent is		
		A632	B73	CH807
A632	GA_1 day 14	*17.3*	27.9	14.8
	GA_1 day 26	*30.2*	89.8	31.5
	GA_{19} day 26	*10.6*	17.6	20.9
B73	GA_1 day 14	23.6	*5.3*	24.9
	GA_1 day 26	54.7	*15.5*	34.1
	GA_{19} day 26	32.8	*4.4*	38.9
CH807	GA_1 day 14	47.7	45.8	*8.2*
	GA_1 day 26	29.1	95.6	*19.7*
	GA_{19} day 26	23.2	21.3	*10.9*

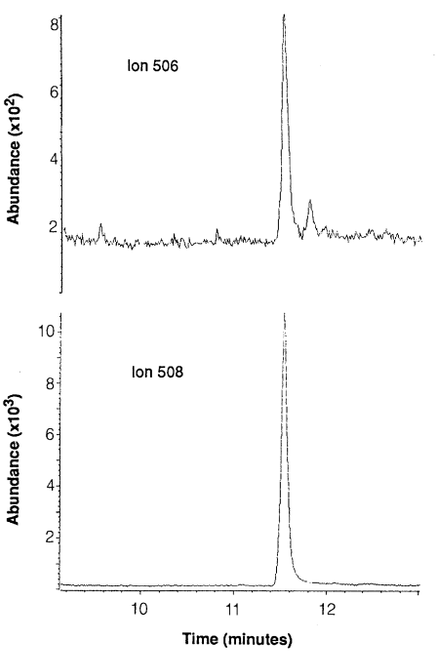

Fig. 2. Representative gas chromatograph–selected ion monitoring profiles for ion 506 (atomic mass units), the molecular ion (M^+) of derivatized GA_1 extracted and purified from the maize tissue, and for ion 508, the M^+ of a known quantity of internal standard derivatized [$^2H_2$]GA_1. A comparison of the relative abundances of these ions permits the accurate determination of endogenous GA_1 concentrations in the samples since the recoveries of endogenous GA_1 and [$^2H_2$]GA_1 are equivalent.

maize mutants. They both suffer from a deficiency of endogenous GA_1, and their poor shoot growth can be increased through the exogenous application of GA_3.

Thus, inbreeding depression in maize is due, in part, to a partial GA deficiency. Conversely, heterotic hybrids contain higher concentrations of GA_1 and hence their shoot growth is more vigorous. This model indicates that GAs play a major regulatory role in heterosis for maize shoot growth. Since shoot growth is positively correlated with grain yield (*15*), this model also implies a role for GAs in the regulation of heterosis for the economically important character of grain yield.

The enhanced production of GA_1 in heterotic hybrids is probably the result of an enhancement of overall GA biosynthesis in the hybrids. Rates of GA_{20} and GA_1 metabolism, including glucosyl conjugation and 2β hydroxylation, are probably faster in at least one maize hybrid than in its parental inbreds (*2, 16*). Hence, to bring about increased GA_1 levels, biosynthetic rates must be very much faster in the hybrids. This enhanced biosynthesis could result from enzymic polymorphism, which is known to occur for a number of enzymes in maize and has been proposed as a general molecular explanation for heterosis (*17*). This model suggests that the existence of different isozymes allows the hybrid to perform better under a broader range of environments and developmental stages. Consequently, the heterozygous condition of the hybrid confers biosynthetic superiority.

Possibly as a result of enzymic polymorphism, GA concentration is enhanced and this enhanced GA level, in turn, induces enhanced biosynthesis of numerous other compounds; GAs are known to play a regulatory role in the induction of gene expression in higher plants (*7*). Thus, amplification of just a few genes responsible for GA biosynthesis could accelerate or activate numerous subsequent biosynthetic pathways. The consequent metabolic cascade could thereby amplify the advantages of the heterozygote. Although this specific mode of action is speculative, our results confirm that GAs are important in the regulation of heterosis, or conversely, inbreeding depression, in maize. Maize hybrids display hybrid vigor, at least in part, as a result of an enhanced GA concentration while the less productive parental inbreds are limited by a partial GA deficiency.

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Effect of Neuropeptides on Production of Inflammatory Cytokines by Human Monocytes

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Two groups of mediators, the neuropeptides substance P and K and the monocyte-derived cytokines, interact in the neural regulation of immunological and inflammatory responses. Substance P, substance K, and the carboxyl-terminal peptide SP(4–11) induce the release of interleukin-1, tumor necrosis factor–α, and interleukin-6 from human blood monocytes. The neuropeptide effects occur at low doses, are specific as shown by inhibition studies with a substance P antagonist, and require de novo protein synthesis. Since monocyte-derived cytokines regulate multiple cellular functions in inflammation and immunity and since neuropeptides can be released from peripheral nerve endings into surrounding tissues, these findings identify a potent mechanism for nervous system regulation of host defense responses.

THE CONCEPT THAT THE NERVOUS system modulates immunological and inflammatory responses has been supported by the identification of neuropeptide receptors on leukocytes and the demonstration that these peptides can regulate leukocyte functions (*1*). Mononuclear phagocytes, either as circulating blood monocytes or as tissue macrophages, influence host defense responses through their capacity to present antigens and to release several types of soluble mediators (*2*). These monocyte-derived cytokines act in a paracrine fashion in the local environment to stimulate immune responses, but also in an endocrine-like fashion on distant organs that participate in inflammatory responses. Systemic changes during inflammation such as increased acute-phase protein synthesis and fever probably represent action of these cytokines on liver and hypothalamus. Interleukin-1 (IL-1) and tumor necrosis factor–α (TNF-α) were the first monocyte-derived cytokines shown to have diverse regulatory properties in immunity and inflammation (*3, 4*).

Recently, a third molecule, termed B cell stimulatory factor–2 (BSF-2), interferon-β2, 26-kD protein, hybridoma-plasmacytoma growth factor, now known as interleukin-6 (IL-6), augments the activation of T lymphocytes, triggers acute-phase protein synthesis in hepatocytes, and probably induces febrile responses (*5–8*).

We examined the ability of substance P (SP) and related neuropeptides to regulate the production of inflammatory cytokines by human blood monocytes. We showed that the neuropeptides SP and substance K (SK) are potent and specific stimuli for the production of IL-1, TNF-α, and IL-6.

This interaction of neuropeptides that are released from unmyelinated sensory neurons in response to traumatic or inflammatory stimuli (*9*) with monokines that mediate localized and systemic host defense responses constitutes a potent mechanism for neural regulation of immunity and inflammation.

Blood monocytes cultured in the presence of SP released increased levels of IL-1 measured as thymocyte stimulatory activity. The effects of SP were detectable at doses as low

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