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Genetic, Environmental and Hormonal Control of Maize Development

by

Stewart Boone Rood

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The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Genetic, Environmental and Hormonal Control of Maize Development" submitted by Stewart B. Rood in partial fulfillment of the requirements for the degree of Doctor of Philosphy.

Supervisor, R. P. Pharis, Department of Biology

D. J. Major, Canada Department of Agriculture, Lethbridge

D. M. Reid, Department of Biology

M. Kapoor, Department of Biology

F. W. Bachelor, Department of Chemistry

External examiner, B. O. Phinney, University of California at Los Angeles

(Date) NOV. 24, 1981

Abstract

The influence of genotype, photoperiod and temperature on "time to flowering" and on the level of endogenous gibberellin-like substances (GAs) was examined in northerly-adapted maize (Zea mays L.).

Eight early maturing inbreds were selected for inclusion in a diallel cross to determine the inheritance of quantitative characters relating to flowering-time. Dominance for early flowering was almost complete while incomplete dominance for increased tillering and increased leaf number were observed. In seven of eight populations tillering and flowering-time were correlated and a pleiotropic relationship obtained. Apparent overdomiance for decreased plastochron (leaf development rate) explains how dominance for decreased flowering-time and increasing leaf number may act simultaneously.

Increasing the photoperiod delayed flowering for 10 of 12 early maturing inbreds. The response to photoperiod was adequately described by a three-line response model. A diallel analysis of the photoperiodic response indicated that day neutrality was recessive and that basic vegetative phase (i.e. time to flowering under non-delaying photoperiods) accurately represented flowering-time in field trials. Thus, the photoperiod induced delay in flowering of the early maturing maize inbreds and hybrids was slight.

Decreasing temperature delayed development in two early maturing maize inbreds and their F_1 hybrid. Growth rate across temperature conditions and genotypes was correlated with endogenous GA levels. Under favorable conditions the hybrid displayed heterosis for growth rate and GA level. Coupled with knowledge about (i) rsponse to

exogenous application of GA_3 and (ii) dwarf maize phenotypes, the possible role of GAs as a phytohormonal basis for heterosis is discussed. Although abscisic acid (ABA) level was determined at various developmental stages, correlations between endogenous ABA content and growth rate were not observed.

By reducing the ambient light intensity a feminization of the apical inflorescence was elicited in an extremely early maturing maize inbred. Accompanying this sex reversal was an increase in endogenous GA level in the developing apical meristems. Coupled with previous knowledge, the possible causal association between GA content and sexuality of maize is examined.

When applied exogenously to northerly adapted maize genotypes, GA_{20} or GA_4 were metabolized to GA_1 and GA_8. Numerous conjugate-like metabolites were also observed. The rate and direction of metabolism was dependent on developmental stage.

When $[^3H]$-GA_{20} or $[^3H]$-GA_4 were applied during rapid grain filling, the $[^3H]$ GAs and $[^3H]$ metabolites were found in the mature seed. Levels of conjugates were high in the dry seed and decreased during imbibition and germination, the converse being true for the acidic precursors. Thus, reversible conjugation of GAs was correlated with seed maturation and subsequent germination in northerly adapted maize.

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List of Abbreviations

ABA	Abscisic Acid
ABA-Me	ABA Methyl Ester
AMO (-1618)	2'-isopropyl-4'-(trimethlammonium chloride)-5-methyl phenyl piperidine carboxylate
BuOH	Butanol
BVP	Basic Vegetative Phase
CCC	(2-chloroethyl)-trimethylammonium chloride
C/D R GA_{20}	C/D ring rearranged GA_{20}
CHU	Corn Heat Unit
EtOAc	Ethyl Acetate
EtOH	Ethanol
GA	Gibberellin
GA_3	Gibberellic Acid
GA_x-G	GA_x-Glucoside
GA_x-GE	GA_x-Glucosyl Ester
GA_x-Me	GA_x-Methyl Ester
GCA	General Combining Ability
GC-MS	Gas-Liquid Chromatography - Mass Spectrometry
GLC	Gas-Liquid Chromatography
GLC-ECD	GLC- Electron Capture Detection
GLC-RC	GLC-Radio Counting
HPLC	High Performance/Pressure Liquid Chromatography
HPLC-RC	HPLC-Radio Counting
MeOH	Methanol
MeTMSi	Methyl Ester Trimethylsilyl Ether Derivative
MOP	Maximum Optimal Photoperiod
NAR	Net Assimilation Rate
PR	Potence Ratio
PVPP	Polyvinylpolyprrolidone (polyclar AT)
RGR	Relative Growth Rate

Rt	Retention Time
SCA	Specific Combining Ability
SiO_2	Silicic Acid
V_r	Variance (in diallel analyses)
W_r	Covariance (in diallel analyses)

General Introduction

Maize (Zea mays L.), a C4 cereal, is a principal crop plant throughout the Western Hemisphere. As no wild form of maize has been reported despite rather intensive searches its botanical origin is presently unknown (and a constant subject of controversy) (46,88).

The original range of "wild" maize can be partially reconstructed based on the distribution of ancient cobs as well as pollen grains (46,88). The plant is thought to have originated in Central America and was also cultivated in equatorial regions of South America long before the European immigration to the Americas (46). Throughout the recorded history of North America, the range of maize cultivation has progressed northward to its present status in which certain maize genotypes are grown for grain at about 51°N. The northward progression of maize "adaptation" has principally resulted from the breeding of genotypes with progressively shorter durations to flowering and grain maturation. However, the reduction in the length of the frost-free season is not the only climatic change accompanying a northward progression. Temperatures during the growing season are generally reduced with a northward movement and additionally, days are lengthened. Thus, in order for a given genotype to produce mature seed at northerly latitudes, the plant must: i) flower after a shorter time interval, ii) grow and develop under cool temperature conditions (particularly during the spring and fall), and iii) flower despite long daylengths. During the course of this investigation these three aspects of developmental adaptation have been investigated.

Development of a number of genotypes differing in degree of northerly adaptation has been compared and the specific responses of these genotypes to photoperiod and temperature have been examined. The possible role of the group of phytohormones, gibberellins (GAs), in the mediation of a number of processes related to maize development and developmental adaptation have been considered and finally, certain aspects of gibberellin metabolism have been examined during maize development.

I. Quantitative genetics of developmental traits of northerly-adapted maize

Simple Mendelian inheritance, to which most undergraduate biology students are exposed, is rare. Very few natural processes are simply controlled in an "all or nothing" fashion by a limited number of independent factors (genes). Rather, growth and development in the natural setting are regulated by a myriad of genetic and environmental factors which interact to produce a given phenotype. The total phenotype may be dissected into component characters, but each of these is also probably regulated by a number of genes. Analyses of such polygenic characters are achieved through statistical procedures which form the field of quantitative genetics. In this field, the researcher is unable to determine the degree of dominance of a specific allele but rather is forced to describe overall dominance of all alleles of the polygenes affecting a specific trait (i.e. the potence ratio). The oversimplification is further revealed when we recognize that even in the relatively few cases where the relevant polygenes have been described, complete dominance is seldom observed. Nor is arithmetic codominance generally the case, but rather, incomplete dominance is observed. Thus, a hybrid will often display a phenotype which is not predicted simply on the basis of parental performance.

Having recognized the probable complexities of dissecting characters of maize into specific alleles of specific genes, maize breeders and geneticists often remain at the level to which quantitative genetics is addressed. Such an approach has been utilized during this research

project.

Specific characters which have been examined in this study share a common thread: all relate to flowering time. Flowering (anthesis) is a readily observable event which, in maize, occurs approximately one-half way through the plant life cycle. It is not to be confused with tassel initiation, an earlier event which marks the onset of the reproductive phase of the life cycle. A shortening of the duration to tassel initiation and then to flowering has been a principal means of shortening the life cycle of maize genotypes, thus increasing their fitness at northerly-latitudes. Early flowering genotypes tend to be early maturing.

A diallel has been utilized as a tool allowing for the analysis of quantitative genetics of maize development. A diallel is a breeding program whereby n parental inbreds are crossed in all possible combinations producing n^2 experimental genotypes. N of the genotypes are inbreds while the other $(n^2 - n)$ are single-cross hybrids. The hybrids can be further divided into $\frac{1}{2}(n^2 - n)$ F_1's and the $\frac{1}{2}(n^2 - n)$ reciprocal F_1's.

Hayman (63), Griffing (55), and a number of others have developed and described statistical analysis and interpretation of diallel data. Indeed, a literature search of diallel publications may reveal as many publications addressed to problems of statistical analysis as publications actually presenting new diallel data. Rather than weighing the pros and cons of a number of analyses, only Griffing's (55) and Hayman's (63) methods will be applied to these data, as these methods are most commonly used by other researchers. As data from inbreds, F_1's, and

reciprocals was generally available during this study, Griffing's (55) Method I is appropriate. Interpretation of diallel data must reflect the sampling nature (fixed or random) of the inbred parents. In accordance with the view of Baker (6) a fixed model must be used in this and in most agronomic research as the inbreds have already been selected for one or more characters. Inbreds included in this study have already been selected for early maturity and reasonable growth cabinet vigor. Consequently, extrapolation of findings from these diallel data to the overall maize population may not necessarily be valid.

I.1 Diallel analysis of flowering-time in maize using a corn heat unit transformation.

Abstract

A set of eight corn (Zea mays L.) inbreds was studied in a diallel cross over 2 years and in a growth room to investigate general and specific combining ability for flowering-time. Diallel analysis of days from emergence to flowering revealed a failure of the joint Wr/Vr regression in one of the years, indicating a lack of agreement with the simple additive-dominance model of inheritance. The array position changed across the years and environments, complicating genetic interpretation. Transforming data of flowering-time to cumulative corn heat units (CHU) to flowering gave a better fit. Joint regression was satisfactory and array position was more consistent across years and environments. Dominance was incomplete for low CHU to flowering. Positive (increasing CHU) and negative, as well as dominant and recessive, alleles were in about equal frequencies. Heritability estimates in the broad - and narrow - sense were high. The estimates of general combining ability were higher than the estimates of specific combining ability in all studies.

Introduction

The diallel cross technique has been used extensively to investgate the inheritance of flowering-time in Nicotiana rustica (63), Zea mays L. (12), and other plant species (71, 116). Epistatic interactions have been required to explain some diallel data, leading to the rejection of the simple additive-dominance model of inheritance (1, 71, 72, 116). Relative performance of genotypes has also varied across years, leading Jana (71) to propose that seasonal differences in both epistatic effects and dominance relationships exist. Thus, flowering-time has not been found to be a genetically simple system.

Maize development is a function of heat accumulation rather than time (22, 52, 70, 92). Consequently, maize hybrid maturity classification is based on cumulative heat units rather than calendar days. The improvement of fit of the simple additive-dominance model of inheritance, basing flowering-time on cumulative heat units rather than calendar days, has not been considered previously. This study was undertaken to investigate the inheritance of flowering-time in early maturing maize and to compare analysis using calendar days with analysis using cumulative corn heat units (CHU).

Materials and Methods

Eight early-maturing maize inbreds (Zea mays L.) were included in the study. The inbreds were developed at five locations: CL3 and CL5 at Lethbridge, Alberta; CM7 and CM49 at Morden, Manitoba; CG8 at Guelph, Ontario; W103 at Madison, Wisconsin; and 66A4-2 and 66D34-1 at Ottawa, Ontario -- 66A4-2 from Howe's Early Alberta, a very early open-pollinated variety, and 66D34-1 from INRA 260, a European variety.

The F_1's, including reciprocals and eight inbreds, were grown at the Agriculture Canada Research Station, Lethbridge, Alberta. Four seeds of each F_1 and inbred were planted May 15, 1978, and May 15, 1979 in each of 14 hills, 36 cm apart, in one-row plots, 75 cm apart. After emergence, each hill was thinned to two plants, resulting in a population density of 75,000 plants/ha. There were two replications in 1978 and three in 1979. A split-plot design was used separating inbreds and F_1's. Data were recorded on flowering time when 10% anthesis (anthers first extruding from the glumes) occurred.

In 1978, two replicates of the complete diallel were grown in a growth room (Controlled Environments Ltd., Winnipeg, Manitoba) at 25/20°C alternating day and night temperatures (5°C/h rise/fall). The 18-h photoperiod consisted of a 14-h high intensity (808 u Einsteins sec^{-1} m^{-2}) photosynthetic period and a 4-h low intensity (68 u Einsteins sec^{-1} m^{-2}) photoperiod extension. This photoperiod was similar in length to the maximum effective natural photoperiod in Lethbridge during the pre-flowering period (40,121). Dates to seedling emergence and flowering of each plant were recorded. Daily temperatures were recorded to calculate the daily corn heat unit (CHU) value according to the

metric version (87) of the CHU equation developed by Brown (14).

The genetic analysis using flowering-time and CHU to anthesis was based on the diallel cross technique of Hayman (63) as outlined by Mather and Jinks (89). The definition and method of calculation of variance (Vr), covariance (Wr), second degree statistics, and components of variation used in this study have been described in detail by Hayman (1954). Narrow- and broad-sense heritabilities were calculated, using the formula of Mather and Jinks (89). Griffing's (55) fixed model method 1 analysis with the modification suggested by Thompson (139) was used to estimate general combining ability (GCA) and specific combining ability (SCA) effects. The relative importance of GCA was calculated with the formula suggested by Baker (6):

$$\text{Importance of GCA} = [2\sigma_{GCA}^2]/[2\sigma_{GCA}^2 + \sigma_{SCA}^2].$$

For this analysis, σ_{GCA}^2 and σ_{SCA}^2 were calculated using Griffing's (55) method 3 analysis.

On the basis of mean field performance in 1978 and 1979, the inbred parents (66A4-2, CG8, CM7, CM49, CL3, W103, and 66D34-1) are referred to as array members 1 to 8 respectively. In the growth room, daily temperature regime was constant and hence daily CHU accumulation was constant. Inheritance analyses of flowering time and CHU in the growth room were identical and for this reason only CHU analysis is presented.

Results and Discussion

The ranges for flowering-time were from 47 to 63, 46 to 65, and 34 to 56 days for the 1978 and 1979 field studies and growth room studies, respectively. The CHU ranges were from 895 to 1207, 841 to 1188, and 998 to 1643. All genotypes required more CHU to anthesis in 1978 than in 1979. Either the CHU equation requries modification for this maize population or, alternatively, factors that affected the rate of development other than temperature varied between the years. Nonetheless, the decreased CHU requirement in 1979 was relatively constant across the F_1 and the inbreds (Table 1). Heterosis values for the CHU analysis were similar in both years and environments (6% difference) (Table 1). The heterosis value reflects dominance of alleles for early flowering (low CHU requirement), not overdominance. Heterosis for early flowering in maize has previously been reported by Yang (150), Warner (145), Chase and Nanda (18), and Bonaparte (12).

Analysis of variance revealed highly significant genotype effects ($P < 0.01$) for flowering-time and CHU. Reciprocal effects were non-significant. Differences in the magnitude of (Wr + Vr) over arrays were significant for flowering time in 1979 (VR = 16.1, $P < 0.01$) and in the growth room (VR = 4.77, $P < 0.05$), and for CHU in 1978 (VR = 25.9, $P < 0.01$), 1979 (VR = 21.0, $P < 0.01$), and in the growth room (VR = 4.77, $P < 0.05$). Thus, non-additive genetic variation existed for flowering-time and CHU. Differences in the magnitude of (Wr - Vr) over arrays were not significant in all three studies for flowering-time or CHU, implying that non-additive genetic variation occurred in the form of independently distributed dominance effects (89).

Table 1

Overall, parental, and F_1 means and heterosis for flowering-time and corn heat units (CHU) to flowering

	Flowering-time		CHU		
	1978	1979	1978	1979	Growth room
Overall mean	55.99	54.61	1042.5	996.6	1237.8
Parental mean	58.00	58.16	1133.9	1082.7	1328.6
F_1 mean	55.61	54.10	1029.5	984.4	1224.8
Heterosis $(\bar{X}_{F_1} - \bar{X}_P)$	-2.39**	-4.06**	-104.4**	-98.3**	-103.7**

**Statistically significant ($P < 0.01$).

The flowering-time data showed a poor fit with the simple additive-dominance model of inheritance. The slope of the joint regression for the 1978 data was different from unity (Fig. 1). Order of the members of the array also varied over the years (Fig. 1). Similar failures of the additive-dominance model and the reversal of position of array members over years have been reported previously in diallel analyses of flowering time (1, 71, 72). Hayman (63) suggested that scalar transformations may relieve apparent genetic disturbances. This method of scaling is commonly effective in studies involving segregating populations from two parent genotypes but is less often satisfactory in diallel analyses as different scalar transformations are requried for different genotypes within the array (89). As far as we know, no physiological transformation has been used in a diallel analysis of flowering-time.

The joint regression analysis of the transformed CHU data was highly significant ($P < 0.01$) and replicates within a test were in agreement with respect to member position. The slope was not different from unity in all studies (Fig. 2). As well, array order was more consistent after transformation to CHU (Fig. 2). Thus, the CHU data fit the simple additive-dominance model of inheritance.

Having satisfied (Wr-Vr) and joint regression analyses, inheritance analysis continued on the CHU data.

In all graphs, Wr intercepts were above the origin, indicating that dominance was incomplete (Fig. 2). Array members with the lowest CHU to flowering were closest to the Wr intercept. Hence, incomplete dominance of alleles for low CHU to flowering was observed. Additive (D)

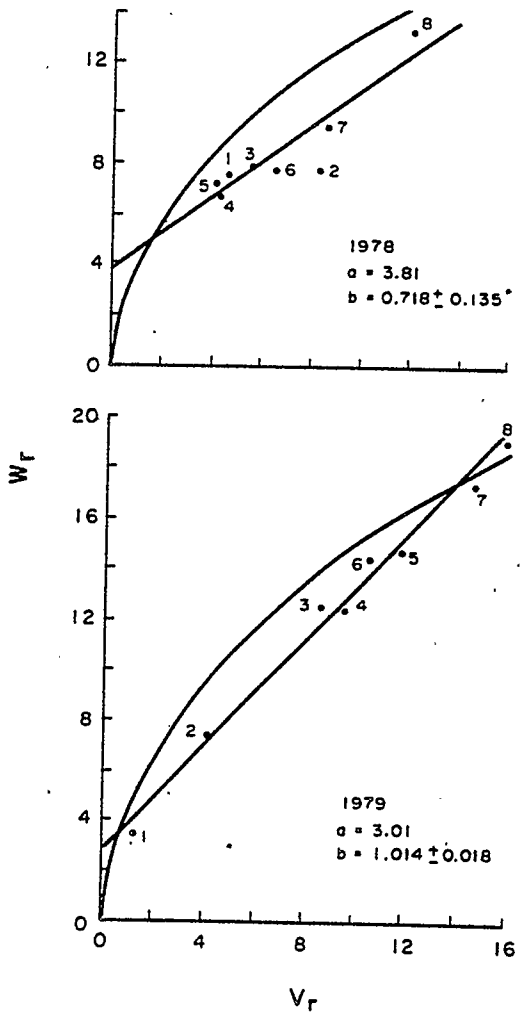


Fig. 1. Wr/Vr graphs for flowering-time, 1978 and 1979; * - slope (b) differs ($P < 0.05$) from unity.

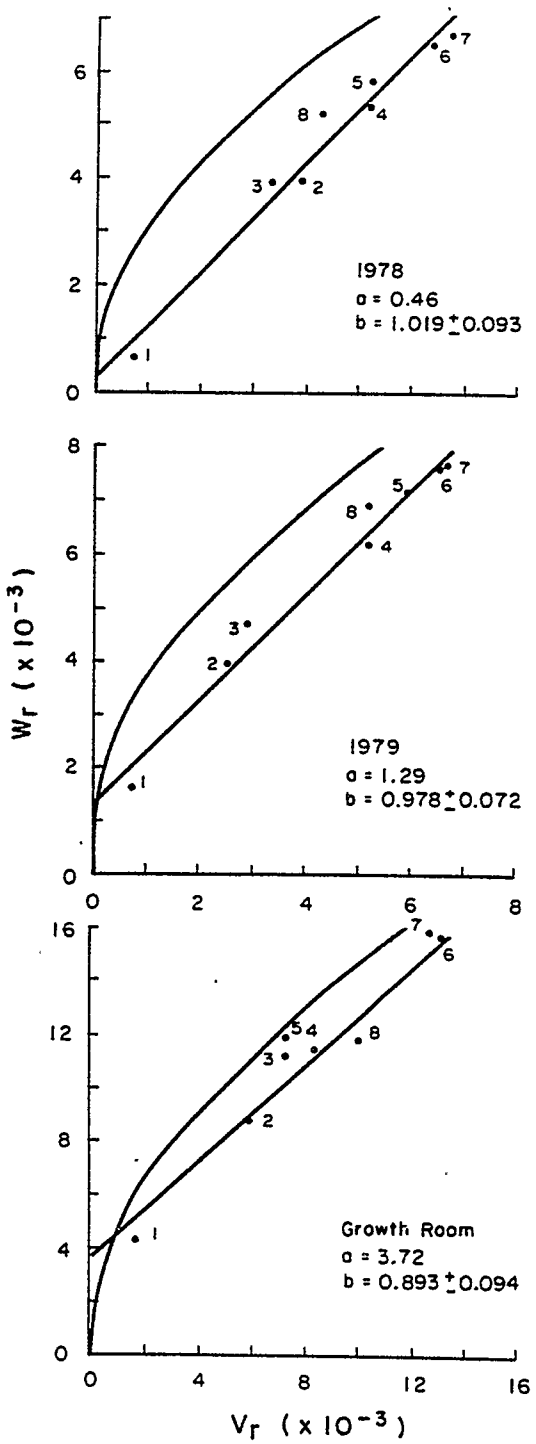


Fig. 2. Wr/Vr graphs for CHU to flowering, 1978, 1979, and Growth Room.

and dominance (H_1 and H_2) components were similar, indicating that both were involved in the control of CHU to flowering, confirming the conclusion from (Wr + Vr) and (Wr - Vr) values (Table 2). H_1 and H_2 values were almost equal, indicating that positive (u) (increased CHU) and negative (v) allele frequencies were equal over all loci. The value of $H_2/4H_1$ was close to the maximum value of 0.25, which arises when $u = v = 0.5$ at all loci. While the F value, representing the ratio of dominant to recessive alleles, varied widely, the negative value from field trials may indicate that more recessive than dominant alleles were present in the inbreds.

The dominance ratio $(H_1/D)^{0.5}$ confirmed the earlier conclusion of incomplete dominance (Table 2). Because the value of F, and therefore the value of the ratio $F/2(D\cdot(H_1-H_2)^{0.5})$, fluctuated widely, conclusions regarding specific dominance level at various loci could not be made. Incomplete dominance for early flowering has been previously reported (12, 19, 151).

The heritability estimates in the broad- and narrow-sense were high (Table 2) but were somewhat lower than those obtained by Bonaparte (12). The high estimates of narrow-sense heritability suggest that selection for early flowering should be effective. The broad-sense heritabilities indicate that environmental effects were lower in the growth room than in the field.

While the diallel analysis of Hayman (63) is useful for evaluating the mode of inheritance, the analysis of Griffing (55) is particularly useful to the breeder as general combining abilities (GCA) are calculated for each inbred parent and specific combining abilities (SCA) are cal-

Table 2

Analysis of variation for corn heat units to flowering

	Field			Growth
	1978	1979	Mean	room
Components (± SE)				
D	8064 ± 649	10987 ± 325	9525	24604 ± 623
H_1	6251 ± 196	6013 ± 1020	6132	15958 ± 495
H_2	6139 ± 235	5614 ± 719	5877	14080 ± 254
F	-2251 ± 137	-325 ± 475	-1288	3965 ± 2498
Derived Values				
$(H_1/D)^{0.5}$	0.883	0.736	0.810	0.805
$H_2/4H_1$	0.247	0.245	0.246	0.232
Heritability (%)				
Narrow	68.7	73.3	71.0	71.4
Broad	89.0	90.8	89.9	93.7

culated for hybrids. In this manner, performance of specific genotypes can be evaluated. Since Griffing's analysis is widely used, the effect of the CHU transformation on this analysis was also studied. In all three trials, both GCA and SCA were significant ($P < 0.01$).

In considering the relative importance of GCA, Griffing's Method 3 or 4 should be used to remove effects of inbreeding depression and heterosis that would inflate specific combining ability variances. The analysis of this value for both flowering-time and CHU to flowering in field trials revealed that relative importance of GCA decreased when the CHU transformation was used (Table 3). Flowering-time and CHU to flowering were largely determined by GCA effects and, thus, GCA estimates were higher than those of SCA, for all studies.

GCA estimates for flowering-time and CHU to flowering were generally consistent across years and environments (Table 4). The GCA of CM7 and CL5, however, varied between field and growth room studies, possibly reflecting a genotype-environment interaction. The GCA range in the growth room exceeded that of field studies as both high and low extremes were amplified. GCA estimates were more consistent after transformation to CHU.

In summary, a transformation of CHU was effective in relieving a disturbance of the simple additive-dominance model of inheritance of flowering-time. This transformation has a physiological basis and may alleviate certain apparent disturbances to the simple model of inheritance of developmental characteristics. Consistency across time or environment is increased by this transformation, leading to a more constant estimation of relative performance and combining abilities.

Table 3

Combining ability components of variance and relative importance of general combining ability

	Flowering-time		CHU	
	1978	1979	1978	1979
GCA component of variance	4.196	6.834	2702	2299
SCA component of variance	0.951	0.305	1713	1317
Relative importance of GCA	0.898	0.978	0.759	0.777

Table 4

Estimates of general combining ability effects for flowering-time and CHU to flowering for eight corn inbreds

Parent	Flowering-time		CHU		
	1978	1979	1978	1979	Growth room
1. 66A4-2	-3.98	-5.11	-117.1	-100.6	-161.2
2. CG8	-1.45	-1.83	-19.9	-33.9	-13.7
3. CM7	1.82	0.86	21.9	17.7	-3.2
4. CL5	-1.02	-0.91	-2.0	-17.2	12.5
5. CM49	1.55	1.25	15.0	24.4	18.9
6. CL3	1.24	0.98	24.1	18.8	54.0
7. W103	0.02	0.98	19.1	18.8	41.7
8. 66D34-1	2.12	3.52	63.4	66.3	96.6
s.e. (any effect)	0.36	0.29	4.1	6.3	9.6
s.e. (difference of any two effects)	0.55	0.43	6.2	9.5	12.4

I.2 Inheritance of tillering and flowering-time in early maturing maize.

Abstract

A diallel cross and F_2 populations derived from eight early maturing maize inbreds were used to investigate the inheritance of tillering and flowering-time (anthesis), and the possible relationship between tillering and flowering-time. Incomplete dominance for increased tillering was observed; potence ratios, representing the overall degree of dominance, ranged from 0.26 to 0.52. Dominance for early flowering ranged from incomplete with a potence ratio of 0.55 to overdominance with a potence ratio of 1.40. Broad-sense heritabilities were low for both characters. The genetic component of variation for tillering was due to general combining ability effects; specific combining ability effects were not significant. A significant negative linear relationship between tillering and flowering-time was found. Lack of independent assortment of tillering and flowering-time in F_2 populations indicated that the two characters are genetically related.

Introduction

The potential for the production of tillers in maize is genetically controlled and probably related to the degree of introgression of teosinte, a profusely tillering ancestor of maize (126). However, actual tiller development of a plant is a function of this genetic potential coupled with environmental factors, principally fertility, moisture availability, and plant population density (28). Although certain profusely tillering races of maize may be better adapted to withstand nutrient and moisture stress, tillering in commerical hybrids is generally considered undesirable (28). Consequently, relatively little work dealing with the inheritance of tillering has been conducted.

Dominance for increased tillering was incomplete in crosses involving a non-tillering Missouri dent and two multi-tillered popcorn varieties and variation across years was large, indicating low heritability of tillering (39). Although Rogers (117) observed incomplete dominance for increased tillering in two maize-teosinte hybrids, he also found incomplete dominance for reduced tillering in four other hybrids. Thus, variation in degree of dominance for tillering exists.

Stuber et al. (134) observed no significant association between tillering and duration to tasseling in progeny from maize crosses involving two populations developed in North Carolina. In inbreds adapted to southern Alberta, we have observed a positive relationship between increased tillering and early flowering (Major and Rood, unpublished).

This study was undertaken to investigate the inheritance of tillering

and the apparent relationship between tillering and flowering-time in eight early inbreds.

Materials and Methods

The previously described (Chapter I.1.) diallel cross was again used. Diallel crosses, including reciprocals and inbred self-crosses, were made in 1977. In 1978, F_1 plants of which 66A4-2 was the male parent were self-crossed to produce F_2 seed. The inbreds and the diallel hybrids were grown in 1978. The inbreds, F_1 hybrids, and the F_2 lines were all grown in 1979. On 15 May 1978 and 15 May 1979, four seeds were hand-planted in each of 14 hills spaced 36 cm apart in rows 75 cm apart. After emergence, each hill was thinned to two plants resulting in a population density of 75,000 plants/ha. A split-plot design with generations as main block was employed; two replications were grown in 1978 and four in 1979, with single-row plots of each genotype per replication. Fertilizer was applied at a rate of 440 kg/ha of 26-13-0 and weed control with 1.1 kg/ha atrazine and 3.4 kg/ha sutan was excellent. Soil moisture was brought to field capacity by sprinkler irrigation in July and August in 1978 and 1979, and immediately after planting in 1979.

The diallel genotypes were also grown in a high-ceiling controlled environment growth room as previously described (Chapter I.1.).

Flowering-times, designated as the first day anthers extruded from the glumes, were recorded for individual plants in the field and growth room, and the number of tillers (excluding the primary tiller) were counted at flowering. For each of the genotypes or F_2 lines, about 100 plants were monitored. The mean tiller number of each plot was used for the parents and single-cross hybrids in the diallel analysis.

Daily maximum and minimum air temperatures were used to calculate

corn heat unit (CHU) values (14, 87). Cumulative CHU from emergence to anthesis were calculated for each plant. Three-point moving averages of frequency distributions of CHU from emergence to anthesis were calculated.

Combining ability analysis was conducted on diallel data using Griffing's (55) fixed model method 1 and 3 analyses with the modification of Thompson (139). Analyses of F_2 data were conducted using the method described by Mather and Jinks (89).

For each cross, midparent (m) and additive (d) values were calculated from inbred data. The dominance component (h) of genetic variation was calculated using a weighted least squares analysis of F_1 and F_2 data (89).

Potence ratios (PR) (89) were calculated from the F_1 and F_2 data sets using a least squares analysis in which:

$$PR = [n_{F_1}(h_{F_1} / 0_{F_1}^2) + n_{F_2}(h_{F_2}/0_{F_2}^2)]/[(n_{F_1}/0_{F}/^2 + n_{F_2}/0_{F_2}^2)\underline{d}]$$

where n_{F_1} and n_{F_2} were the number of F_1 and F_2 individuals considered and $1^{\times 2}_{F_1}$ and $0^2_{F_2}$ were the F_1 and F_2 variances. Potence ratios represented overall degree of dominance, a value less than one but greater than zero indicating incomplete dominance, a value of one indicating complete dominance, and a value greater than one representing overdominance.

Broad-sense heritabilities were calculated from estimates of the genetic and environmental variances as described by Mather & Jinks (89).

Results and Discussion

Highly significant ($P < 0.01$) genotypic effects for tillering in the field were observed and partitioning of the genetic variation revealed highly significant ($P < 0.01$) general combining ability (GCA) effects. When parental inbreds were included in the analysis (Griffing's Method 1), specific combining ability (SCA) effects were significant ($P < 0.05$) in 1979. However, analysis without inbred data (Griffing's Method 3) showed no significant SCA effects. Thus, the significant SCA effects using Griffing's Method 1 analysis principally reflected inbred depression. These results indicated that an accurate estimate of tillering of an F_1 hybrid can be made on the basis of the GCA of its parent inbreds.

Significant reciprocal effects were not detected. Consequently, reciprocals and replications were pooled to produce half-diallel arrays (Table 5). Mean tiller number ranged from 0.0 - 3.3 for the inbreds and from 0.0 - 2.8 for F_1 hybrids. Variable dominance for increased tillering was observed. Incomplete dominance was observed in F_1 hybrids having a multi-tillered parent such as 66A4-2 or CG8 (Table 5). Complete dominance was observed in some hybrids from 66D34-1 and overdominance was often observed when two single-stalked inbreds were crossed, particularly if one of the parents was CM7 (Table 5).

Mean tiller number of the F_1 hybrids was higher than that of the inbreds in 1978 and 1979 but the difference was significant only in 1979. This heterosis represented dominance and overdominance for tillering. Potence ratios, representing overall degree of dominance, ranged from 0.26 (66A4-2/CG8) to 0.57 (66A4-2/CL5) (Table 5). Average

Table 5. Tiller number per plant averaged over replications and reciprocals for 1978 (upper values) and 1979 (lower values).

Parents	66A4-2	CG8	CM7	CL5	CM49	CL3	W103	66D34-1
66A4-2	2.83	1.83	1.08	1.75	1.58	1.79	1.00	1.29
	3.33	2.83	2.21	2.03	1.61	2.11	1.86	2.44
CG8		1.67	1.13	0.92	0.42	0.75	0.83	1.71
		1.61	1.39	1.44	1.67	1.45	1.22	1.69
CM7			0.08	0.83	0.25	0.54	0.25	1.25
			0.00	1.05	1.25	0.75	1.11	1.39
CL5				0.17	0.08	0.13	0.38	0.63
				0.17	0.61	0.28	0.75	0.89
CM49					0.08	0.04	0.08	0.13
					0.17	0.75	0.36	1.06
CL3						0.00	0.00	0.46
						0.17	0.50	0.58
W103							0.00	0.25
							0.00	0.75
66D34-1								0.50
								0.85

potence ratio was 0.37, indicating that dominance was incomplete. This variable level of dominance is consistent with previous reports (39, 117).

Low broad-sense heritabilities (Table 6) indicated that only about half of the variation in the F_2 in the field was a result of genetic effects. Low heritabilities indicating a large environmental influence on tillering is consistent with Emerson's (39) results.

Variances of the segregating F_2 populations generally exceeded variances of the inbreds and F_1 hybrids (Table 6). High variance was encountered for the F_1 hybrid 66A4-2 x W103 and for the inbred W103. Although high variance was also detected for the inbred 66D34-1, the variance of its F_1 hybrid 66A4-2 x 66D34-1 was low.

Tillering in the growth room was low compared to field trials, and was only observed in 66A4-2 hybrids; thus, significant GCA effects for inbreds other than 66A4-2 were all negative (Table 7). The GCA estimates obtained for the growth room, however, were consistent with those obtained from the field study (Table 7).

In the inbreds, GCA effect in field trials was correlated with inbred tillering (Table 7). GCA values of the multi-tillered inbreds, 66A4-2 and CG8, were high. GCA values of non-tillered inbreds, CM7, CL5, CM49, CL3, and W103 were similar and low. The intermediate tillering inbred, 66D34-1, had a GCA between those of the multi-tillered and non-tillered inbreds.

The GCA estimates for tillering were negatively correlated ($P < 0.01$) with GCA estimates previously reported for corn heat units (CHU) to flowering-time (Chapter I.1.). This indicated that the two traits

Table 6. Inheritance characteristics for tillering in early maturing maize.

Female[1] parent	Number of auxillary tillers					Components of variation		Potence ratio (PR)	Broad-sense heritability (%)
	mid-parent value (m)	F_1		F_2		additive (d)	dominance (h)		
		mean	variance	mean	variance				
CG8	1.73	1.94	0.56	1.50	1.45	0.80	0.21	0.26	58.8
CM7	0.95	1.57	0.50	1.14	0.82	1.58	0.62	0.39	35.4
CL5	0.85	1.81	0.47	1.10	1.03	1.68	0.96	0.57	55.8
CM49	0.80	1.49	0.59	0.71	0.88	1.73	0.69	0.40	45.1
CL3	0.79	1.24	0.52	0.56	0.99	1.74	0.44	0.26	54.4
W103	0.74	1.50	0.88	0.83	0.92	1.74	0.71	0.41	30.8
66D34-1	1.27	1.67	0.35	1.01	0.68	1.27	0.40	0.32	26.2
$\bar{X}$	1.02					1.51	0.58	0.37	43.8

[1]Male parent for all crosses was 66A4-2.

Table 7. General combining ability effects for tiller number of eight early maturing maize inbreds.

Inbred	Tiller number		
	field		
	1978	1979	growth room
66A4-2	0.91a	1.00a	1.41a
CG8	0.42b	0.45b	0.00b
CM7	-0.10cd	0.06bc	-0.12bc
CL5	-0.16cd	-0.36cd	0.02b
CM49	-0.43d	-0.33cd	-0.18bc
CL3	-0.28cd	-0.30cd	-0.13bc
W103	-0.42d	-0.43d	-0.50c
66D34-1	0.04bc	-0.04bcd	-0.44c

a-d Within columns, GCA effects followed by the same letter do not differ ($P > 0.05$) using Duncan's multiple range test.

were correlated in the parental inbreds but did not provide information about genetic relationship between profuse tillering and early flowering.

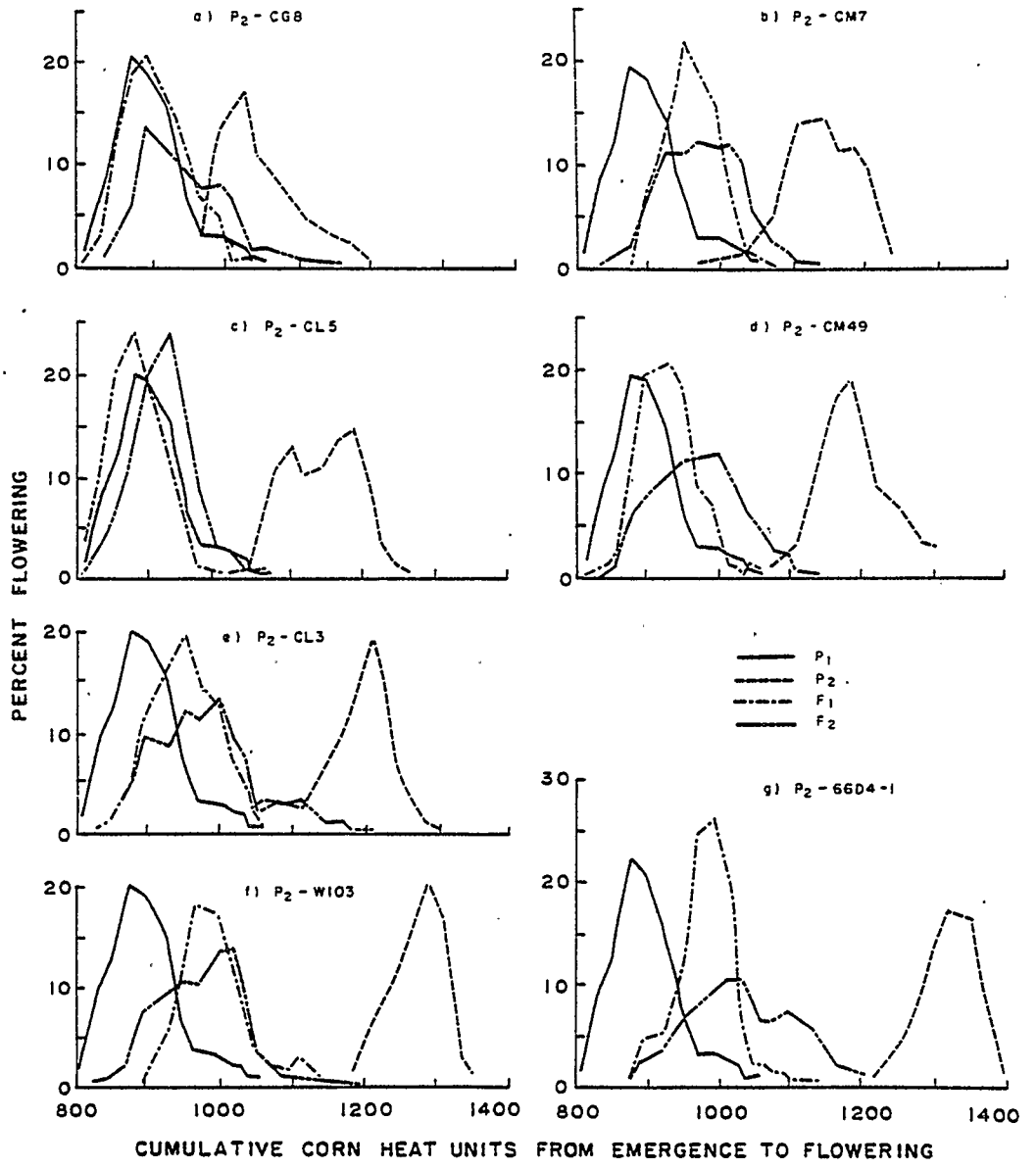
A transformation to corn heat units (CHU) was an effective means of alleviating an apparent disturbance to the simple additive-dominance model of inheritance of flowering-time in the diallel data set of this study (Chapter I.1.). Incomplete dominance for early flowering was observed and narrow- and broad-sense heritabilities were high. The segregating F_2 populations in this study offered further opportunity to study the inheritance of flowering-time.

The bimodal F_2 frequency distribution obtained for 66A4-2 x CG8 (Fig. 3a) indicated that the two parental inbreds probably differed by a single gene for flowering-time. The F_1 mean was the same as the 66A4-2 mean, representing complete dominance of the early allele. A bimodal F_2 frequency distribution for tillering in 66A4-2 x CG8 was also observed but codominance rather than complete dominance was indicated for tillering.

The bimodal F_2 distributions observed for 66A4-2 x CL3 (Fig. 3e) and 66A4-2 x 66D34-1 (Fig. 3g) were probably the result of 2 cool days of rain rather than genetic segregation. The general absence of bimodal F_2 distributions strongly suggested that polygenic differences were present between 66A4-2 and inbreds other than CG8.

Dominance for early flowering was observed for all genotypes as both F_1 and F_2 means were closer to the early than late parent (Fig. 3). The F_1 hybrid, 66A4-2 x CL5, flowered earlier than the early parent (66A4-2). While the slight difference in the F_1 could have been attributed to increased vigor of the hybrid, the early F_2 mean indicated

Fig. 3. Frequency distribution for cumulative corn heat units (CHU) from emergence to flowering in parental inbreds and F_1 and F_2 generations grown in the field in 1979. For all crosses, P_1 was 66A4-2.


a) P2 - CG8
b) P2 - CM7
c) P2 - CL5
d) P2 - CM49
e) P2 - CL3
f) P2 - W103
g) P2 - 66D4-1
P1
P2
F1
F2
PERCENT FLOWERING
0
10
20
30
800
1000
1200
1400
CUMULATIVE CORN HEAT UNITS FROM EMERGENCE TO FLOWERING

that heterosis was truly occurring (Fig. 3c). This heterosis may not be due to overdominance of a specific gene, but rather may result from CL5 containing one (or more) dominant early alleles lacking in 66A4-2. Dominance for increased tillering was also highest in the 66A4-2 x CL5 hybrid and F_2 population (Table 8). Incomplete dominance for early flowering was observed in hybrids other than 66A4-2 x CG8 and 66A4-2 x CL5 and, consequently, potence ratios were less than one (Table 8). This is consistent with previously reported data (12, 19, Chapter I.1.).

Broad-sense heritabilities (Table 8) were low compared to earlier estimates based on diallel performance (Chapter I.1.). Although the diallel and F_2 involved the same genotypes and took place in the same field, the methods of estimating heritability were different. The estimation of heritability used in this study was based on the analysis of data from individual plants and, consequently, more variation was detected than in the diallel analysis in which a single flowering value for each row was considered.

A scaling test (89) of data from this F_2 study indicated that duration to flowering generally conformed to the simple additive-dominance model of inheritance. A transformation to CHU was an effective means of reducing an F_2 scaling test coefficient for the 66A4-2 x CM49 F_2 from a significant ($c = 2.981 \pm 1.288$, $P < 0.05$) to an insignificant ($c = 31.36 \pm 26.59$) level. Thus, consistent with the previous diallel analysis (Chapter I.1.), transformation to CHU alleviated an apparent disturbance to the simple additive-dominance model of inheritance of flowering-time.

Table 8. Inheritance characteristics for corn heat units (CHU) to flowering-time in early maturing maize.

Female[1] parent	Mid-parent value of CHU (m)	Components of variation		Potence ratio (PR)	Broad-sense heritability (%)	Correlation coefficient (r) between F_2 anthesis and tillering
		additive (d)	dominance (h)			
CG8	978	-72	-59	0.82	44.4	-0.52**
CM7	1021	-115	-63	0.55	0.6	-0.45**
CL5	1022	-116	-162	1.40	8.8	-0.28**
CM49	1042	-135	-117	0.86	45.3	-0.43**
C	1042	-136	-95	0.70	53.4	-0.56**
W103	1069	-163	-122	0.75	37.7	-0.45**
66D34-1	1116	-210	-137	0.65	59.5	-0.05
$\bar{X}$	1046	-135	-108	0.82	35.7	-0.39

[1]Male parent for all crosses was 66A4-2.

**$p < 0.01$.

Tillering and flowering-time did not segregate independently in the F_2. Highly significant negative correlations between tiller number and flowering-time were observed for all F_2 populations except 66A4-2 x 66D34-1 (Table 8). The mean linear correlation coefficient (r) of the seven populations was -0.39. When the non-segregating parental and F_1 data were included, the correlation coefficient was -0.71. This value was similar to the correlation coefficient between leaf number and flowering-time in this population (r = 0.78), a relation that serves as the basis of one maturity classification of maize (3, 18).

This relationship between tillering and flowering-time probably indicated that either genes controlling the two traits were linked or a pleiotropic effect existed, i.e., the two traits were controlled by the same gene(s). If linkage was the cause, loci must have been close since no extremely early, non-tillering individuals were observed--plants that would have resulted from crossing-over between the loci.

Direction of dominance was consistent with a genetic association as early flowering plants were multi-tillered and incomplete dominance for both early flowering and increased tillering existed. The behavior of 66D34-1 and its F_2 was inconsistent with a pleiotropic relationship. This inbred, the latest in the study, was multi-tillered (Table 5) and no correlation between tillering and earliness was observed in the 66A4-2 x 66D34-1 F_2 (Table 8). Data from the other seven inbreds were, however, consistent with a genetic relationship between tillering and flowering-time.

A genetic relationship between tillering and earliness would make the production of extremely early non-tillering genotypes difficult if

close linkage is involved, and impossible if a pleiotropic effect is responsible. A genetic relationship would explain the behavior of certain Canadian genotypes including Howe's Early Alberta and Gaspe Flint, two extremely early, profusely tillered cultivars.

I. 3. Diallel analysis of leaf number, plastochron, and plant height of early maturing maize.

Abstract

The diallel cross from eight early maturing maize inbreds was used to investigate inheritance and determine combining abilities for leaf number and plant height, two correlates of flowering-time, and plastochron. Dominance for increasing leaf number was almost complete. Overdominance for increasing plant height and overdominance for decreasing plastochron were also observed. Broad-sense heritabilities were 88, 74, and 78% for leaf number, plastochron, and height in the growth room. Narrow-sense heritabilities were lower, particularly for height. Neither reciprocal effects or specific combining ability effects were significant for leaf number, plastochron, or plant height in field or growth room trials. Thus, hybrid performance was determined by general combining abilities of parental inbreds. Leaf number and plastochron were positively correlated with flowering-time. Leaf number was also correlated with height. Overdominance for decreasing plastochron explains how dominance for increasing leaf number and incomplete dominance for decreasing flowering-time may act simultaneously and indicates that heterosis, in the form of overdominance, occurs for development in maize.

Introduction

Final leaf number and plant height are readily measurable characters that are positively correlated with flowering-time in maize (24). Leaf number is largely determined by genotype and serves as the basis of one index of maize maturity (3, 18). Within a genotype, leaf number increases with increasing temperature and, in some genotypes, with increasing photoperiod (66, 70). Correlative changes between plant height and flowering-time have also been reported after changes of temperature and photoperiod (22, 70). A genetic association between height and flowering-time is indicated by the finding that selection for early flowering leads to a correlated reduction in plant height (141).

Production of early maturing maize cultivars based on combined selection for decreased leaf number and height as well as early flowering will only be effective if these characters are under the same genetic control. Incomplete dominance for early flowering (12) and increased leaf number (12, 84) and overdominance for increased plant height (48) have been reported. Plant height can be partitioned into leaf number and internode length; overdominance for the latter has been reported and explains overdominance for plant height (51).

A phenotypic relationship between leaf number and flowering-time is quite reasonable. Increasing the vegetative phase of the life cycle leads to increases in leaf number and a delay in flowering. Thus, a correlation between the two is expected. However, the inheritance of the two is not explained simply by a pleiotropic relationship involving length of the vegetative phase. While incomplete dominance of both characters is consistent with a genetic association (12), direction of

this dominance is opposite to that which is predicted by a pleiotropic model. Dominance for early flowering and increasing leaf number are observed while early flowering is correlated with decreasing rather than increasing leaf number.

This study was undertaken to investigate the inheritance of leaf number and height as well as plastochron. The association between these characters and flowering-time was considered, with special reference to the possible genetic association between leaf number and flowering-time.

Materials and Methods

A diallel cross from eight inbreds of maize was grown in field trials in 1978 and in a controlled environment growth room as previously described (Chapter I.1.).

Dates of emergence and four- and eight-leaf stages, defined as the emergence of the ligule of the fourth and eigth leaves, respectively (59), were recorded. Growth room plastochron was defined as one-quarter of the time interval between the four- and eight-leaf stages. Because a wet spring hampered accurate recording of the four-leaf stage in the field, field plastochron was calculated as days to tassel emergence divided by final leaf number. After the four-leaf stage, twist-ties were placed above the fifth leaf to allow accurate leaf number counts following sesescence of the lower leaves. Flowering-times were defined as the times to first emergence of the anthers from glumes. Final leaf number and plant height were recorded about 1 week after anthesis.

The genetic analysis was based on the diallel cross technique of Hayman (63) as described by Mather and Jinks (89). Narrow- and broad-sense heritabilities were calculated using the formula of Mather and Jinks (89). Combining ability analysis was also conducted, using Griffing's (55) fixed model method 1 and 3 analyses with the modification of Thompson (139).

Results and Discussion

Leaf Number

On the basis of previously reported results for mean duration to flowering in field trials in 1977, 1978, and 1979 (Chapter I.1.), the inbreds (66A4-2, CG8, CL5, CM49, CL3, W103, and 66D34-1) are referred to here as array members 1 to 8, respectively.

Leaf number for the inbreds ranged from 7.9 (66A4-2) to 12.6 (66D34-1) in the field and from 8.0 (66A4-2) to 14.0 (CL5 and 66D34-1) in the growth room (Table 9). For the hybrids, it ranged from 9.9 (66A4-2 x W103) to 12.9 (CM49 x CL3) in the field and from 10.0 (66A4-2 x CL5) to 15.8 (CM49 x CL3) in the growth room. The mid-parent leaf number was lower than the F_1 mean in both field and growth room trials (Table 10).

Genetic analysis revealed a similar inheritance pattern in both field and growth room trials and highly significant genotype effects ($P < 0.01$). As partitioning of the genotypic variation revealed that reciprocal effects were not significant, reciprocals were pooled to produce half-diallel arrays. Variance of each row (Vr) and the covariance between the parents and their F_1 hybrids in each row (Wr) were calculated for each member of the array (89). Differences in the sum of variances and covariance (Wr + VR) across replicates were significant ($P < 0.01$) indicating that non-additive genetic variation existed for leaf number. Differences between covariances and variances (Wr - Vr) were not significant, indicating that non-additive variation occurred solely in the form of independently distributed dominance effects (89).

Table 9. Leaf number, plastochron, and plant height of eight early maturing maize inbreds.

Inbred	Leaf number		Plastochron (days/leaf)		Height (cm)	
	Field	Growth room	Field	Growth room	Field	Growth room
66A4-2	7.9	8.0	4.5	4.7	102	173
CG8	9.4	12.0	5.4	5.5	122	156
CM7	10.8	12.5	4.6	4.0	165	162
CL5	10.3	14.0	4.5	4.3	130	177
CM49	11.8	12.0	4.3	3.3	133	148
CL3	10.7	12.0	3.9	4.5	111	135
W103	9.8	9.0	5.0	4.0	151	170
66D34-1	12.6	14.0	4.1	5.5	152	211

Table 10. Parental and F_1 means and heterosis for leaf number, plastochron, and plant height of early maturing maize grown in field and grown room trials

	Leaf number		Plastochron (days/leaf)		Height (cm)	
	Field	Growth room	Field	Growth room	Field	Growth room
Parental mean	10.4	13.8	4.52	4.46	133	166
F_1 mean	11.4	14.4	3.15	4.04	186	217
Heterosis†	1.0**	0.6*	-1.37**	-0.42**	53**	51**

† $(\bar{X}_{F_1} - \bar{X}_P)$

*,** Statistically significant $P < 0.05$ and 0.01, respectively.

Joint regression analysis of Wr versus Vr was highly significant ($P < 0.01$) and replicates within the field or growth room trials were in agreement with respect to member position. The linear regression coefficient (b) differed significantly from zero but not significantly from unity (Fig. 4). Thus, analyses of the (Wr - Vr) and regression offered support for the adequacy of the simple model of inheritance for leaf number.

The positive intercept of the Wr/Vr graph (Fig. 4) indicated that dominance for leaf number was incomplete. Position of array members indicated that with the exception of 66D34-1 (8), relative level of dominance of the inbreds was relatively constant across environments (Fig. 4). Inbreds CM7 (3), CL5 (4), and CM49 (5) were closest to the Wr intercept indicating that they contained principally dominant alleles. These inbreds were intermediate in leaf number (Table 9). Inbred W103 (7) was next on the graph, followed by CL3 (6), CG8 (2), and 66A4-2 (1). The latter three inbreds had the fewest leaves and contained principally recessive alleles with respect to leaf number. Thus, from the graph, incomplete dominance for increased leaf number was observed.

Both additive (D) and dominance (H_1 and H_2) components were involved in the control of leaf number (Table 11), confirming the conclusion from (Wr + Vr) and (Wr - Vr) values. In the analysis based on field data, H_1 and H_2 were similar, indicating that positive (u) (increasing leaf number) and negative (v) allele frequencies were about equal. The value of $H_2/4H_1$ table was slightly below the maximum value of 0.25, which arises when $u = v = 0.5$ over all loci. The F value was

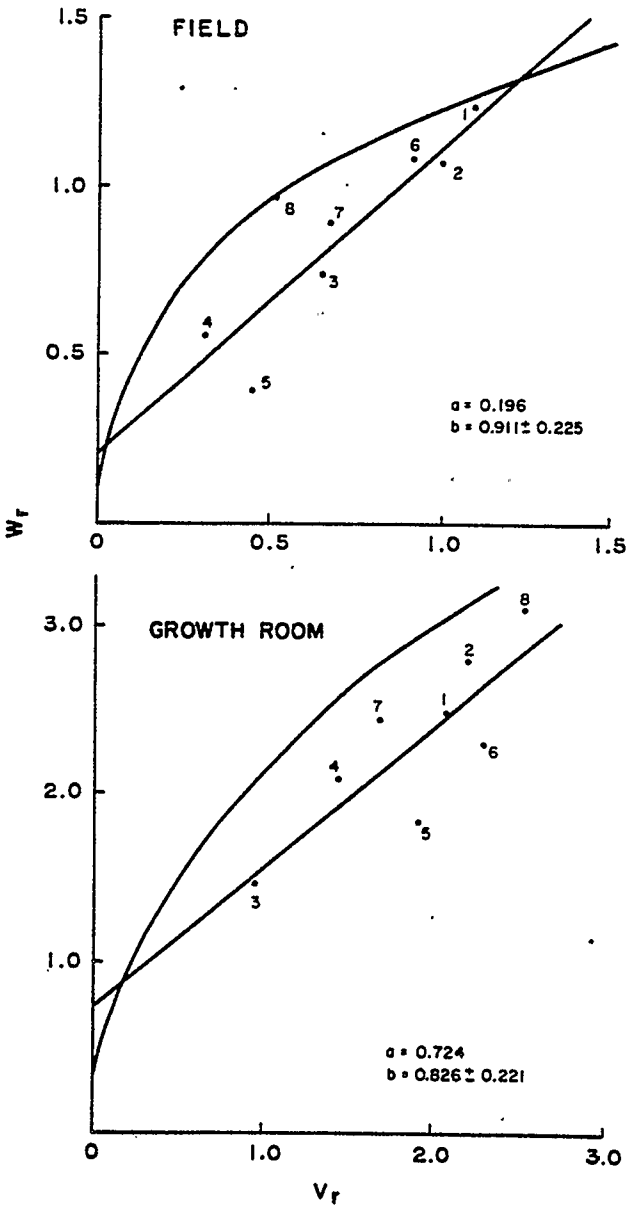


Fig. 4. W_r/V_r graphs for leaf number of maize from field and growth room.

positive, indicating that there were more dominant than recessive alleles in the parent inbreds. The dominance ratio $(H_1/D)^{0.5}$ was almost 1.0 in field and growth room trials indicating that dominance for increased leaf number was almost complete (Table 11). Dominance for increased leaf number has been previously reported although the level has varied (12, 19, 84).

Heritabilities for leaf number were moderate (Table 11) and far lower than those previously reported by Bonaparte (12) who used a similar diallel analysis. However, he used a far greater range of leaf number than that reported here. Consequently, genotypic differences for leaf number would be expected to have been higher in his material and relative importance of genotypic effects correspondingly larger. Hence, larger heritabilities in Bonaparte's (12) study are understandable.

Plastochron

Plastochron for inbreds ranged from 3.9 (CL3) to 5.4 (CG8) days/leaf in the field and from 3.3 (CM49) to 5.5 (CG8 and 66D34-1) days/leaf in the growth room (Table 9). For the hybrids, it was shorter than that of either parent in the field and growth room, and, hence, the F_1 mean was lower than the parental mean (Table 10). This heterosis was the result of overdominance for reduced plastochron.

The Wr/Vr regression coefficient for field data was significantly different from unity (Fig. 5a), indicating a failure to conform to the simple additive-dominance model of inheritance. Consequently, Hayman (63) analysis on the field plastochron data was not completed although one piece of information was gained--five array members were located

Table 11. Analysis of genetic variation for leaf number, plastochron, and plant height of early maturing maize

	Leaf number		Plastochron	Height
	Field	Growth room	Growth room	Growth room
Components of variation (± SE)				
D	1.88 ± 0.36	4.64 ± 1.83	0.621 ± 0.132	412 ± 58
H_1	1.69 ± 0.38	3.84 ± 1.13	0.680 ± 0.056	2581 ± 375
H_2	1.46 ± 0.31	2.78 ± 0.59	0.597 ± 0.042	2238 ± 230
F	0.73 ± 0.25	2.23 ± 2.23	0.258 ± 0.093	562 ± 46
Derived values				
$(H_1/D)^{0.5}$	0.967	0.929	1.028	2.603
$(H_2/4H_1)$	0.218	0.170	0.217	0.227
Heritabilities (%)				
Narrow-sense	52.7	63.4	45.3	11.5
Broad-sense	79.9	88.4	73.5	77.6

below the Wr = 0 axis indicating that overdominance for plastochron occurred in the field (Fig. 5).

The different levels of heterosis for plastochron (Table 10) and differences in Wr intercepts of the Wr/Vr graphs (Fig. 5) for field and growth room trials may have arisen from two sources. The parent inbreds respond differently to different temperature regimes and, thus, an interaction of genotype x environment exists for plastochron (unpublished). Also, the plastochron estimates from field trials were based on rates of leaf production up to tassel emergence while plastochron from the growth room trials were based on rate of leaf production between the four- and eight-leaf stages.

Analysis of the growth room plastochron data satisfied requirements of Hayman's (63) model, i.e., (Wr - Vr) did not differ among replicates and the joint regression coefficient was not different from unity (Fig. 5).

Genotype effects were highly significant ($P < 0.01$) but reciprocal effects were not significant. Reciprocals were pooled to produce half-diallel arrays. A significant ($P < 0.01$) Wr/Vr regression was observed and, as previously noted, the regression coefficient (b) was not significantly different from unity (Fig. 5).

The intercept of the Wr/Vr graph was near the origin, indicating that dominance was almost complete (Fig. 5). As indicated by position on the graph, inbreds CL3 (6) and W102 (7) contained principally dominant alleles while 66A4-2 (1) and CM49 (5) contained principally recessive alleles; other inbreds were intermediate. The dominance (H_1 and H_2) and additive (D) components of genetic variation were about equal (Table 11). The ratio $(H_1/D)^{0.5}$ was about 1.0, indicating that

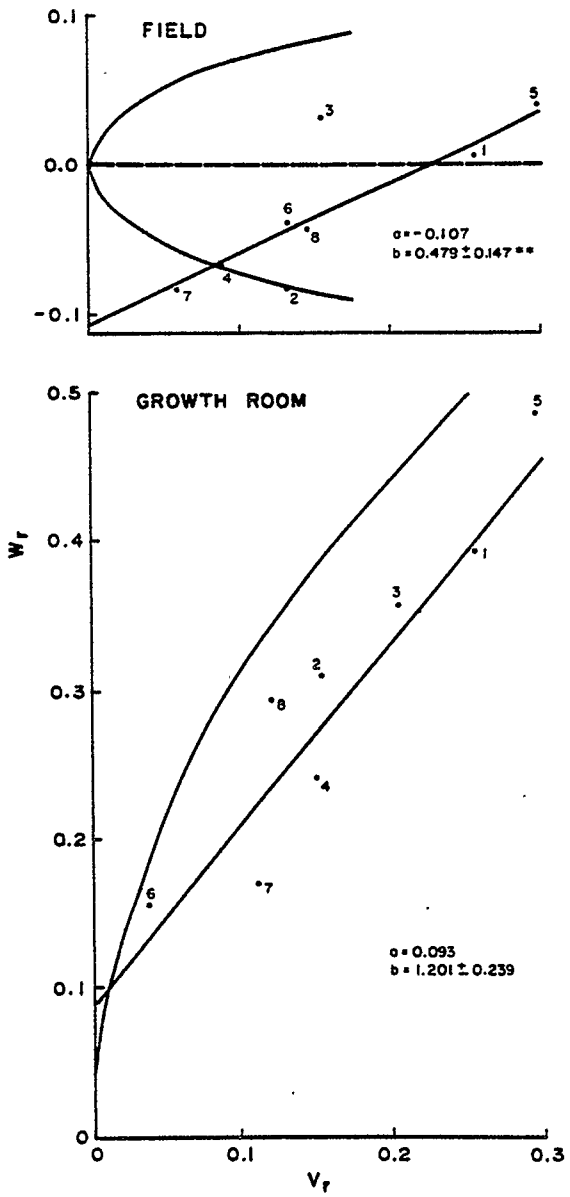


Fig. 5. W_r/V_r graphs for plastochron of maize from field and growth room. **Regression coefficient (b) significantly different from 1.0, $p < 0.01$.

dominance for plastochron was complete.

The ratio $H_2/4H_1$ was less than 0.25, indicating that positive (increasing plastochron) and negative allele frequencies were unequal in the parent inbreds (Table 11). The positive value of F indicated that there were more dominant than recessive alleles regardless of positive or negative direction. Heritabilities were lower than those of leaf number, indicating that there was a greater environmental influence on plastochron than on leaf number.

Height

Plant height for inbreds ranged from 102 (66A4-2) to 165 cm (CM7) (Table 9). Hybrids were consistently taller than either parent; this heterosis was the result of over dominance for increased height. As was found for leaf number and plastochron, genotypic effects for height were highly significant ($P < 0.01$). Reciprocal effects were not significant and reciprocals were pooled to produce half diallel arrays. Analysis of Wr and Vr satisfied requirements of Hayman's (63) model. (Wr - Vr) differences across arrays were not significant and a significant Wr/Vr regression was observed in which the regression coefficient (b) did not differ significantly from unity (Fig. 6). In the graphs for both field and growth room, the Wr intercept was far below the origin, indicating over dominance for increased height. Position of array members on the Wr/Vr graph varied widely among environments (Fig. 6). The error values for the field data were quite large and array member positions varied among replicates thus disturbing the requirements of Hayman's (63) model. Consequently, analysis was continued on only the

growth room data.

Dominance (H_1 and H_2) components of genetic variation exceeded the additive component (D) supporting overdominance for increased plant height. The ratio $(H_1/D)^{0.5}$ (2.6) far exceeded 1.0, which would arise when dominance was complete and offered a quantification of the level of overdominance. Overdominance for plant height in maize has been reported previously (48). Since height can be partitioned into leaf number and internode length and since dominance for leaf number was observed, overdominance for internode length was expected. An analysis of internode length showed a significant Wr/Vr regression but, in both field and growth room trials, the coefficient was significantly below 1.0 (data not presented). In both graphs, the Wr intercept was far below the origin, verifying that overdominance occurred. Overdominance for internode length is consistent with previous reports (51).

H_1 and H_2 dominance components of variation for height were about equal, indicating that positive (increasing height) and negative allele frequencies over all loci were about equal (Table 12). The value of $H_2/4H_1$ confirmed this conclusion. The narrow-sense heritability for height was very low indicating that response in plant height due to selection would be slow (Table 12). Broad-sense heritability was much higher, however, indicating that height was principally a result of genetic variation. The large differences between the narrow and broad heritabilities indicated that much of the genetic variation was non-fixable.

This inheritance of height in the growth room may not necessarily reflect response in the field. Light intensity in the growth room was

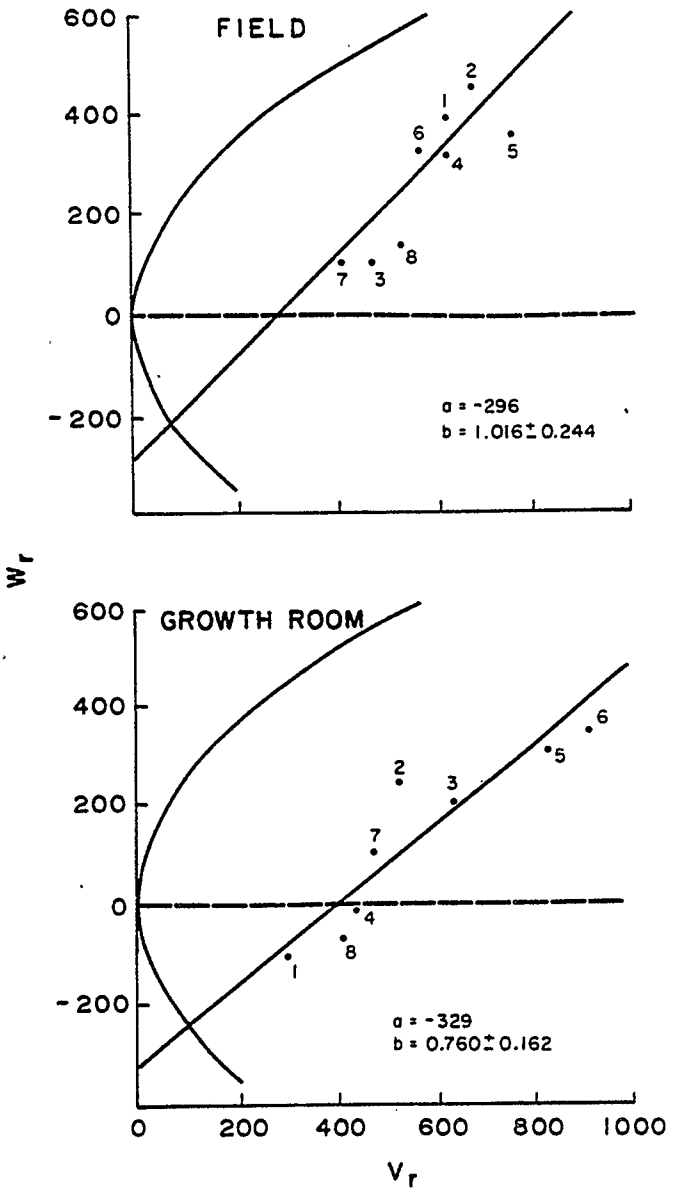


Fig. 6. W_r/V_r graphs for plant height of maize from field and growth room.

far below "full sunlight" and hence photosynthate production, and consequently growth, would be altered. Further, spectral differences of irradiance in the growth room and field may have lead to modifications in shoot elongation (44). These maize genotypes also respond differently to growth room conditions indicating that an interaction of genotype x environment exists for growth and developmental characters (Chapter II.1., Chapter II. 3.).

While the Hayman (63) analysis is the most useful means of considering the adequacy of the simple additive dominance model of inheritance (89), Griffing's (55) combining ability analysis is particularly useful to the breeder. General combining abilities (GCA) are generated for inbreds indicating their average contribution to hybrid performance; and sepcific combining abilities (SCA) are calculated for hybrids indicating significant deviation of specific hybrids from the expected performance based on GCA of the parent inbreds.

With respect to the importance of GCA and SCA, analysis of the three characters - leaf number, plastochron, and plant height - was similar. Analysis using Griffing's (55) Method 1 (parents, F_1, and reciprocals) indicated that both GCA and SCA effects were significant. If inbred data were removed (Griffing, Method 3), SCA effects were not significant. The difference in the conclusions based on Method 1 and 3 analyses was a result of the relatively poor performance of the inbreds and represented inbred depression or, alternately, hybrid vigor rather than true SCA effects. With respect to leaf number, plastochron, and plant height, GCA effects were adequate to predict the performance of hybrids. These GCA effects can only be determined by considering

Table 12. Estimates of general combining ability effects for leaf number, plastochron, and plant height for eight early maturing maize inbreds.

Inbred	Leaf number		Plastochron		Height	
	Field	Growth room	Field	Growth room	Field	Growth room
66A4-2	-1.10 a*	-2.35 a	-0.364 a	-0.146 ab	-23.3 a	-15.9 a
CG8	-0.11 bc	-0.54 b	0.076 cd	0.197 c	- 4.2 bc	- 5.8 ab
CM7	0.46 cd	1.99 d	0.050 cd	0.088 bc	17.1 e	6.0 bc
CL5	-0.34 b	-0.70 b	0.092 cd	-0.080 abc	- 2.3 cd	2.6 bc
CM49	0.76 d	0.74 c	-0.031 bc	-0.291 a	3.8 cd	0.4 bc
CL3	0.28 cd	0.93 c	-0.203 ab	0.061 bc	-13.4 ab	- 6.9 ab
W103	-0.47 b	-0.63 b	0.216 d	0.03 bc	7.6 de	7.1 bc
D34-1	0.58 d	0.56 c	0.178 cd	0.135 bc	13.9 e	12.6 c

*Within columns, GCA effects followed by the same letter do not differ ($P < 0.05$) using Duncan's Multiple Range Test.

performance of a number of hybrids produced from a specific inbred.

Estimates of GCA effects for leaf number, plastochron, and plant height are shown in Table 12. Relative GCA estimates for leaf number and height across environments were very similar. While some agreement in an inbred's GCA across the three characters indicated similar genetic control, this agreement was not complete. Rankings of GCA for the inbreds for leaf number, plastochron, and plant height in the growth room were: 1, 4, 7, 2, 8, 5, 6, 3; 5, 1, 4, 7, 6, 3, 8, 2; and 1, 6, 2, 5, 4, 3, 7, 8. Order of GCA for flowering-time was: 1, 5, 2, 3, 4, 7, 6, 8 (Chapter I. 1.). Although correlations between GCA estimates for these three characters were observed, these correlations were far from perfect.

Highly significant ($P < 0.01$) positive correlations between flowering-time and leaf number, between flowering-time and plastochron, and between leaf number and plant height were observed in both field and growth room trials (Table 13). In the growth room, leaf number and plastochron were also correlated. The correlation between leaf number and flowering-time, and leaf number and plant height is consistent with previous reports (24, 66).

In this study, height was not correlated with flowering-time (Table 13). Since height was correlated with leaf number and leaf number was correlated with flowering-time, height should have been related to flowering-time, through leaf number. The reason it was not detected in this study may have been that the maturity range of material represented only a fraction of the maturity range of maize, as all inbreds were early maturing. In studies involving larger maturity

Table 13. Linear correlation coefficients (r) between flowering-time, leaf number, plastochron, and plant height, of early maturing maize grown in field (upper values) and growth room (lower value) trials

	Leaf number	Plastochron	Plant height
Flowering-time	0.553**	0.288**	0.134
	0.581**	0.567**	0.137
Leaf number		0.037	0.245**
		0.460**	0.291**
Plastochron			0.074
			0.069

** Significant correlation ($P < 0.01$).

ranges, correlations between plant height and flowering-time have been reported (24, 66).

Overdominance for short plastochron explains how incomplete dominance for both short flowering-time and increased leaf number can take place in the same system. When early and late inbreds are crossed, the hybrid tends to flower early and produce more leaves than the mid-parent value. Plastochron is shorter than in either parent and, hence, heterosis, in the form of overdominance, is observed for this developmental character. Genotypic variation of plastochron explains the lack of a perfect correlation between flowering-time and leaf number. Length of the vegetative phase in which leaves are produced is not the only variable affecting final leaf number since the rate of leaf production (plastochron) also varied. The lack of a correlation between plastochron and leaf number in field trials (Table 13) indicates that a maturity index based entirely on leaf number may not be valid due to genotypic variation for plastochron. Dominance for increased leaf number and overdominance for short plastochorn indicated that the production of early maturing maize hybrids with far more leaves than inbreds of corresponding maturity should be possible.

II. 1. Responses of early maize inbreds to photoperiod.

Abstract

Twelve early-maturing inbred maize (Zea mays L.) lines were grown in controlled environments at photoperiods of 14, 16, 18, 19, 20, 21, 22, 23, or 24 hours. Days from emergence to anthesis increased as photoperiod increased for 10 of the 12 inbreds. The photoperiod response of the inbreds was adequately described by a three-line model previously used for other plant species. Genotypic variability for the photoperiod response was identified.

The basic vegetative phase, which is the time from emergence to anthesis in optimal photoperiod conditions, ranged from 37 to 57 days. The photoperiod sensitivity, expressed in days delay per hour increase of photoperiod, ranged from 0 to 2.5. The maximum optimal photoperiod, which is the longest photoperiod at which no photoperiod-induced delay in anthesis is observed, ranged from 14 to 24 hours and the critical photoperiod, above which no further delay in anthesis occurs, ranged from 21 to 24 hours. The photoperiod-induced phase varied from 0 to greater than 17 days. A lack of correlation between components of the photoperiod response suggests independent regulation of these components.

Introduction

Photoperiod is a major factor influencing the rate of development and, therefore, adaptation of crop plants. This influence is particularly important to agriculture in temperate climates such as exist in Western Canada where, even at the southern extreme, the maximum natural photoperiod exceeds 17 hours (40, 121). Producing cultivars whose development is not delayed by long days becomes more important as one moves north, since the natural photoperiod extremes increase while the frost-free season generally decreases.

Delays in tassel initiation or flowering of maize (Zea mays L.) grown under long photoperiods have been reported by many workers (13, 22, 43, 49, 70, 79, 90, 137). Day neutral genotypes have also been identified (42, 133). Hence, a genotypic component to the photoperiod response of corn exists. Francis (41) suggested that, in general, temperate-adapted varieties show lower photoperiod sensitivity than tropical varieties. Hunter et al. (70) examined four maize cultivars, ranging in maturity rating from extremely early to late and found that, under controlled environments, lengthening photoperiod delayed tassel initiation in all but the earliest cultivar. Further, for the three sensitive cultivars, photoperiod sensitivity increased with increasing maturity rating, thus supporting Francis' hypothesis.

By modifying and applying a model developed for rice by Vergara and Chang (142), Major (85) examined the photoperiod response of 18 cultivars representing nine temperate crop species. Figure 7 adapted from Major (85), represents a generalized response of a short-day plant to photoperiod. The basic vegetative phase (BVP) has been defined as the number

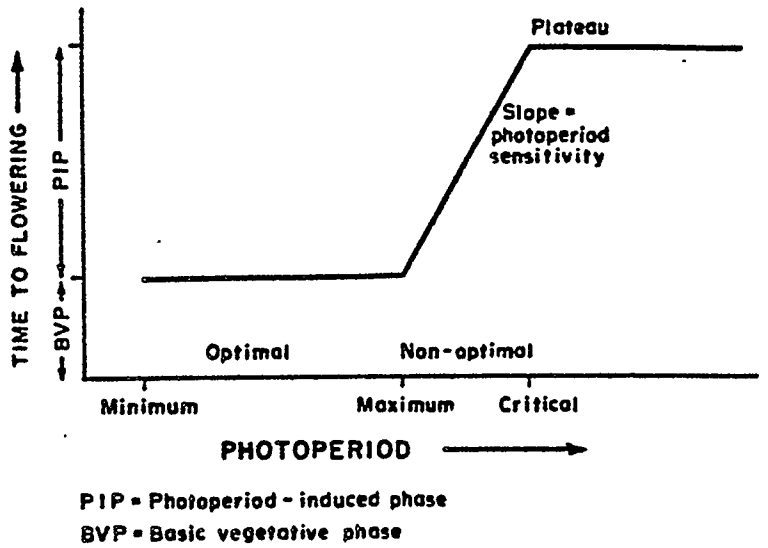


Fig. 7. Generalized response of a quantitative short-day plant to photoperiod (adapted from Major 1979).

of days required to reach floral initiation in optimal photoperiods (142). Since photoperiod affects time to tassel initiation in maize but not the interval between tassel initiation and anthesis (first pollen shed) or silking (13, 79), time to anthesis may be used to evaluate BVP, removing the need for destructive sampling. Thus, in maize BVP may be operationally defined as time to anthesis rather than tassel initiation. The maximum optimal photoperiod (MOP) of a short-day plant is the maximum photoperiod at which the BVP is observed (i.e., no photoperiod delay) (17). Photoperiod sensitivity is the delay in flowering per unit increase of photoperiod at longer than optimal photoperiods. A critical photoperiod may exist above which flowering will not occur (qualitative short-day plants) or, alternatively, a critical photoperiod may exist above which no further delay in flowering occurs (Fig. 7). The photoperiod-induced phase (PIP) results from the delay in flowering at non-optimal photoperiods. The duration of the PIP is the difference between the BVP and the number of days required at the critical photoperiod.

Much of the previous research into the photoperiod response of maize has included tropical cultivars (41, 43). Since day lengths during the growing season are longer at higher latitudes, the photoperiod response of temperate cultivars should be investigated.

The objective of this study was to investigate the photoperiod response of a number of temperate maize inbreds and to measure the BVP, photoperiod sensitivity, maximum optimal and critical photoperiods, and the PIP of these inbreds.

Materials and Methods

Twelve early maturing inbred maize lines representing a wide range of genetic origin were chosen for this study. CL3 and CL5 were developed at Lethbridge, Alta.; CM7 and CM49 at Morden, Man.; CG11 at Guelph, Ont.; and W103 and WD at Madison, WI. 66A4-2 was developed from Howes Early Alberta, a very early open-pollinated variety; F64-11-6-1 from Funk's G2A; and 7275-13-1 from Funk's 7275. 66D34-1 and GELI-2, both of European origin, were developed from INRA 260 and the open-pollinated cultivar "Gelber Badischer', respectively.

Three kernels of each inbred line were planted in 25- x 15-cm plastic pots, containing a mixture of soil, sand, and peat in the ratio of 2:1:1 and thinned to one plant per pot after emergence.

Two plants of each inbred line were grown in each run in controlled environment cabinets (Controlled Environments Ltd., Winnipeg, Man), with either 14-, 16-, 18-, 19-, 20-, 21-, 22-, 23-, or 24-hour photoperiods at a 25/15°C day/night temperature regime. Three separate runs were made at 14-,16-, 18-, and 20-hour photoperiods, two runs at 22- and 24-hour photoperiods, and one run at 19-, 21-, and 23-hour.

A basic 14-hour photosynthetic photoperiod was extended by using one-third of the cabinet's incandescent lights to bring the photoperiod to the appropriate length. For all treatments, the day temperature was maintained for 14 hours. Increases and decreases in temperature were at the rate of 5°C/hour. Irradiance, measured with an ISCO model SRS spectroradiometer (Instrument Specialities Company Inc., Lincoln, NB), was 808 uEinsteins sec^{-1} m^{-2} in the 400-750 nm range from 20 cool white fluorescent bulbs and 45 incandescent bulbs (40 W) during the high

intensity regime and 68 uEinsteins sec^{-1} m^{-2} from 15 incandescent bulbs (40 W) during the low intensity extension of the basic light period.

Plants were observed daily, and the dates of plant emergence and anthesis (anthers first extruded from glumes) were recorded for each plant. Maximum optimal and critical photoperiods were estimated from initial data plots. At longer than optimal photoperiods (i.e., the PIP), linear and quadratic regression analyses were performed with days to anthesis as the dependent variable and photoperiod as the independent variable. Mean days to anthesis at optimal photoperiods was used as the BVP value. Mean days to anthesis at greater than critical photoperiods were used as plateau values. The intercepts of the linear regression line with the BVP and plateau were the corrected maximum optimal and critical photoperiods, respectively. The regression coefficient (b) was considered to be the photoperiod sensitivity. The PIP was calculated by subtracting the BVP from the plateau value.

Analyses of variance were conducted on days between emergence and anthesis at the nine photoperiods, and on the BVP's, photoperiod sensitivities, PIP's, and maximum optimal and critical photoperiods. Significant differences between inbreds or between treatments were detected using Duncan's Multiple Range Test.

Results and Discussion

Time to anthesis was highly significantly ($P < 0.01$) affected by inbred (genotype) and photoperiod. Significant ($P < 0.01$) interaction between these two factors reflected genotypic variability for the photoperiod response (Fig. 8).

Over the photoperiod range examined, the responses of five inbreds, 66A4-2, 66D34-1, CL5, GELI-2, and WD were best described by a single line. Only a maximum optimal photoperiod and a basic vegetative phase (BVP) were identified for CG11, CM49, 7275-13-1, and CL3, while only a critical photoperiod was observed for CM7 and F64-11-6-1. The response of these seven inbreds was best represented by a two-line plot. Both optimal and critical photoperiod were observed for W103, which was best represented by a three-line plot.

Although it is easy to visualize a two- or three-line response, statistical analysis is difficult (46, 67). One method of comparing fit of a three-line and a quadratic photoperiod response model is to compute correlation coefficients and lack of fit F-values for linear and quadratic regression analyses of complete data sets and data sets from the PIP only. This technique has limitations as response abnormalities, including increased variability at long photoperiods, may make a weighted least squares or transformation of data before regression analysis appropriate. Despite these possible drawbacks, this analysis was carried out (Table 14). Correlation coefficients were consistently higher with data from only the PIP (column 1 vs. 6). When linear regression analysis was carried out on the complete data set, significant lack of fit F-values were obtained for inbreds CM49, CL3, F64-11-6-1,

Fig. 8 Days from emergence to anthesis vs. photoperiod for 12 early maize hybrids. Solid lines represent plots obtained by fitting the data into the model shown in Fig. 7.

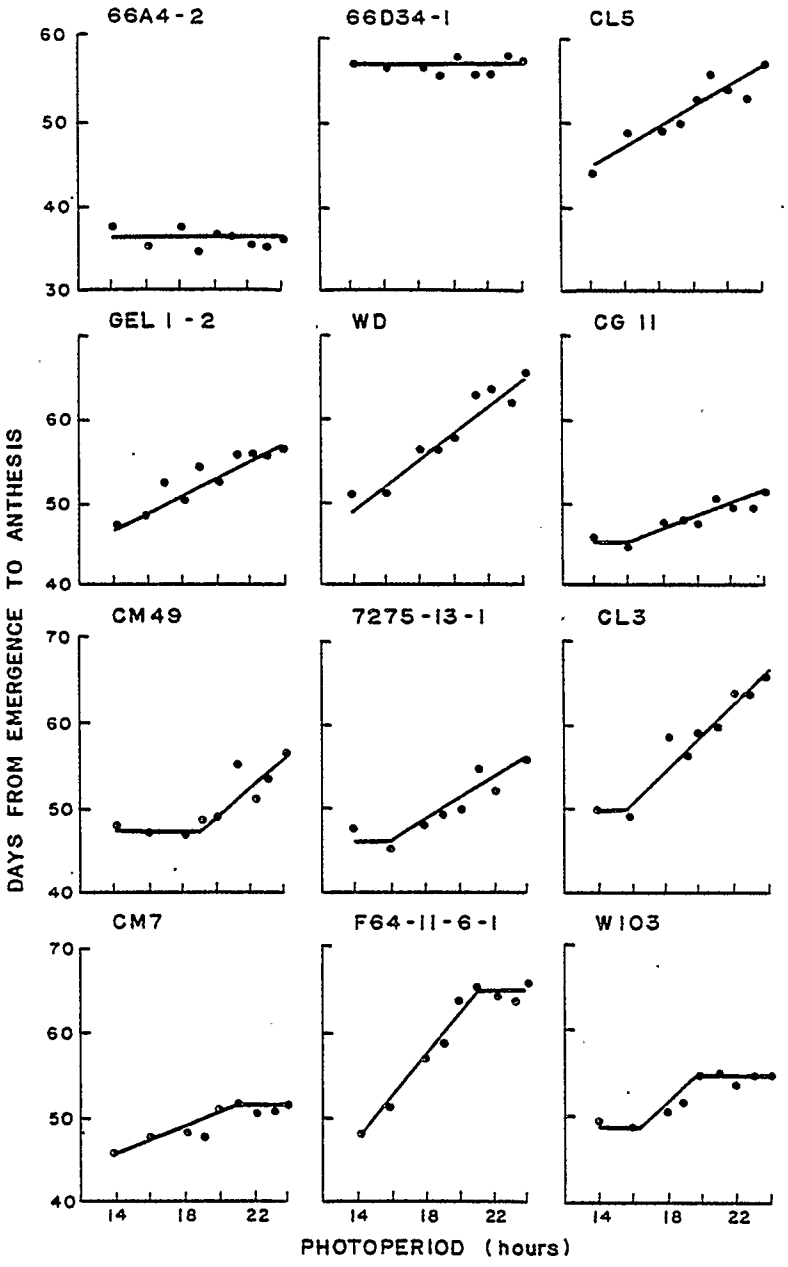

66A4-2
66D34-1
CL5
GEL 1-2
WD
CG 11
CM49
7275-13-1
CL3
CM7
F64-11-6-1
W103
DAYS FROM EMERGENCE TO ANTHESIS
PHOTOPERIOD (hours)
30
40
50
60
70
14
18
22

Table 14. Correlation coefficients (r) and lack of fit F-value for complete data sets and data from the photoperiod-induced phase of 12 early maize inbreds for the linear and quadratic regression analyses of days from emergence to anthesis vs. photoperiod.

	Complete data					Data from PIP only				
	Linear		Quadratic			Linear		Quadratic		
	r	F-value lack of fit	r	F-value lack of fit	Improve-ment	r	F-value lack of fit	r	F-value lack of fit	Improve-ment
	1	2	3	4	5	6	7	8	9	10
66A4-2	0.406	0.98	0.488	0.76	1.92					
66D34-1	0.350	0.81	0.438	0.73	1.28					
CL5	0.935	1.22	0.940	1.14	1.52					
GELI-2	0.869	1.73	0.893	1.15	3.02					
WD	0.938	2.28	0.938	2.77	0.24					
CG11	0.580	2.17	0.641	1.95	2.63	0.896	1.94	0.917	1.36	2.93
CM49	0.707	4.45**	0.821	2.32	10.14**	0.792	2.65	0.859	2.19	2.54
7275-13-1	0.870	2.14	0.879	2.22	1.52	0.935	1.06	0.942	0.77	2.00
CL3	0.959	4.74**	0.923	5.34**	0.86	0.963	3.39	0.966	3.72*	1.29
CM7	0.902	0.75	0.914	0.47	2.14	0.875	0.27	0.88	0.35	0.12
F64-11-6-1	0.936	4.70**	0.969	1.53	20.43**	0.970	0.29	0.969	0.41	-0.08
W103	0.699	3.33*	0.716	3.68*	0.92	0.793	2.10	0.793	3.31	0.03

*, **Statistically significant $P < 0.05$ and $P < 0.01$, respectively.

and W103. Significantly improved fit was obtained for CM49 and F64-11-6-1 with quadratic regression analysis on the complete data set. Lack of fit F-values became nonsignificant for all inbreds when only data from the PIP were analyzed. Lack of fit F-values were reduced for all inbreds when analysis was carried out only on PIP data (column 2 vs. 7). Thus, the three-line response model offered a better fit than quadratic regression analysis.

Analyses of the PIP with a quadratic regression did not significantly improve the data fit over linear regression analyses (Table 1, column 10). This study yielded data suggesting that, in the sensitive range of the response, flowering of maize is a linear function of photoperiod.

Genotypic variability was found for all components of the photoperiod response for the 12 early maize inbreds (Table 15). Three BVP classes were recognized. The first (66A4-2) had a BVP of 35.7 days, flowering about 10 days earlier than all other inbreds. The second (CG11, CM49, 7275-13-1, CL3, and W103) had BVP values ranging from 45.3 to 49.4 days. The third (66D34-1) had a BVP value of 57.0.

For the remaining five inbreds, CL5, GELI-2, WD, CM7, and F64-11-6-1, days required to reach anthesis were still decreasing at the shortest photoperiod examined. If, as suggested by Francis (41), 14.5 to 15.0 hours is the optimal photoperiod (which he referred to as the "critical day length") for many maize genotypes, one would expect the response curves of these inbreds to level off at about 14 hours and BVP values would be close to those in Table 15. These genotypes would be in the second BVP class. However, Francis' concept of a constant maximum optimal photoperiod is based on work with only a few genotypes and

Table 15. Basic vegetative phase (BVP), photoperiod sensitivity (regression coefficient, maximum optimum photoperiod (MOP), critical photoperiods, photoperiod-induced phase (PIP), and maximum photoperiod-induced delay calculated from the regression analysis between days from emergence to anthesis and photoperiod for 12 early maize inbreds.

Inbred	BVP	MOP	PIP	Critical photo-period	Photoperiod sensitivity	Maximum delay PIP/BVP
	days	hours	days	hours	days/hour	%
66A4-2	36.5 c*	24 a	0 b	24 a	0 d	0
66D34-1	57.0 a	24 a	0 b	24 a	0 d	0
CL5	≤45†	≤14	≥11.9	24 a	1.19 bc	≥26.4
GELI-2	≤47	≤14 c	≥ 9.9	24 a	0.99 c	≥21.1
WD	≤50	≤14 c	≥15.7	24 a	1.57 bc	≥31.4
CG11	45.3 b	16.1 bc	6.9 a	24 a	0.88 c	15.3
CM49	47.1 b	19.1 b	9.4 a	24 a	1.94 ab	20.0
7275-13-1	46.6 b	16.0 bc	10.5 a	24 a	1.31 bc	21.3
CL3	49.3 b	15.6 bc	17.7 a	24 a	2.11 ab	35.9
CM7	≤46	≤14 c	≥ 5.5	20.9 b	0.80 c	≥12.0
F64-11-6-1	≤47	≤14 c	≥16.2	20.9 b	2.45 a	≥34.5
W103	49.4b	16.3 bc	5.6 a	19.4 b	1.76 ab	11.3

* a-d Within columns, means followed by the same letter do not differ ($P = 0.05$) using Duncan's Multiple Range Test.

† BVP values ≤ a given value indicates that the number of days required to reach anthesis was still decreasing at the lowest photoperiod tested.

photoperiods. Since the present study shows significant genotypic variability for MOP, a classification of these four inbreds into the second BVP group is inappropriate.

To determine BVP values for the four unclassed inbreds, screening at lower photoperiods must be made. This was not done in the present experiment as a 14-hour, high-light-intensity, photosynthetic period was presented, supplemented by 0 to 10 hours of low-intensity photoperiod extension. Dropping the photoperiod below 14 hours would have led to a reduction in the photosynthetic period, possibly confounding the photoperiod effect (143).

Flowering of inbreds 66A4-2 and 66D34-1 was not delayed by increasing the photoperiod. Photoperiod sensitivity, or slope of the regression line of the PIP, for these day-neutral inbreds was not significantly different from zero. Maximum photoperiod sensitivity observed was 2.45 days delay/hour increase of photoperiod (F64-11-6-1). There was no correlation between BVP and photoperiod sensitivity (Table 16). This does not support the hypothesis that temperate adapted maize genotypes should show low BVP correlated with low photoperiod sensitivity (41), although 66A4-2, the earliest genotype examined, was day neutral. It must be noted that genotypes included represent only a small portion of the maturity range available as all were selected for potential adaptation to northern climates. Hence, extrapolation to the overall corn population is inappropriate.

Evidence for independent regulation of photoperiod characteristics is found in the lack of correlation between photoperiod response components (Table 16). Only maximum optimum photoperiod and photoperiod

Table 16. Linear correlation coefficients of photoperiod response characteristics of 12 early maize inbreds.

	BVP	Photoperiod sensitivity	Maximum optimum photoperiod
Photoperiod sensitivity	0.153		
Maximum optimum photoperiod	-0.030	-0.623**	
Critical photoperiod	-0.070	-0.329	0.282*

*, ** Statistically significant $P < 0.05$ and $P < 0.01$, respectively.

sensitivity were significantly correlated. This correlation resulted from consideration of the day netural inbreds 66A4-2 and 66D34-1 which had MOP of 24 and photoperiod sensitivity of 0. Analyses excluding data from the day netural inbreds showed no significant correlations.

II. 2. Diallel analysis of the photoperiodic response of maize.

Abstract

A diallel cross from eight early maturing maize inbreds was used to investigate the inheritance of photoperiodic response and the role of this response in adaptation to high latitudes. Days from emergence to flowering increased for six of the inbreds and all of the hybrids as the photoperiod was increased from 14- to 22 hours. The photoperiodic response was divided into a basic vegetative phase, which is the time to flowering under ideal photoperiod conditions, and a photoperiod-induced phase. The maximum optimal photoperiod was the longest photoperiod at which no photoperiod-induced delay of flowering-time was observed; the slope of the photoperiod response was the photoperiod sensitivity.

Additive and dominance components of genetic variation were important for all photoperiod characteristics. Incomplete dominance for decreased basic vegetative phase was observed. Day neutrality, characterized by a maximum optimal photoperiod of 24 hours and a photoperiod sensitivity of 0, was recessive. Heritabilities were high for the basic vegetative phase and low for other photoperiod characteristics. Specific combining ability effects were significant for photoperiod sensitivity and maximum optimal photoperiod; reciprocal effects were not significant. General combining ability (GCA) effects for the basic vegetative phase were correlated with GCA effects for flowering time from field trials in Alberta. For a photoperiod similar to that of 50°N latitude, the average photo-

period-induced delay in flowering-time of the diallel hybrids was 1.5 days. Thus, photoperiod is of only minor importance in the developmental adaptation of the early maturing maize genotypes examined.

Introduction

Delays in tassel initiation or flowering of maize grown under long photoperiods have been shown repeatedly (13, 22, 49, 70, 139). Since maize flowers even under 24-hour photoperiods, most maize genotypes are considered to be quantitative short-day plants (41, 143). Day-neutral genotypes have also been identified, indicating that genotypic variation of the photoperiod response exists (42, Chapter II. 1.).

Although the genetics of photoperiodism have been investigated in tobacco (1), sweet pea (83), and other plant species (23, 54), no generalization regarding dominance of photoperiod sensitivity or day-neutrality can be made (143). In maize, Rogers (117) reported that a weak photoperiod response was dominant to the strong short-day response of teosinte. Furthermore, variable photoperiod response of the teosinte varieties and the corn-teosinte hybrids indicated polygenic inheritance of the photoperiod response. A similar inheritance has been reported for other plant species (23, 54). The wide phenotypic variation in photoperiod response of maize also indicates polygenic inheritance (41, Chapter II. 1.).

In the preceeding Chapter a photoperiod response model, developed by Vergara and Chang for rice (142) and modified by Major (85) was further modified and used to analyze the photoperiod response of 12 short-season maize inbreds. Improvement of data fit using the three-line model rather than a polynomial regression offered support of the model's validity.

In the present study, the previously described model was used to analyze data from a diallel cross to determine the inheritance of

photoperiod response characteristics of maize. Because genetic variation in photoperiod response exists in maize, and photoperiod response may affect developmental adaptation in high latitudes, this study should provide information that will be useful to the breeder attempting to produce genotypes adapted to high latitudes.

Materials and Methods

Eight inbreds of early maturing maize (Zea mays L.) were used. The inbreds CL3 and CL5 were developed at Lethbridge, Alberta; CM7 and CM49 at Morden, Manitoba; CG8 at Guelph, Ontario; and W103 at Madison, Wisconsin; and inbreds 66A4-2 and 66D34-1 at Ottawa, Ontario. Inbred 66A4-2 was developed from Howe's Early Alberta, a very early open-pollinated variety, and 66D34-1 from INRA 260, a European variety. We have shown previously that the latter two inbreds are day-neutral (i.e., no delay in flowering with increasing photoperiod) whereas the other inbreds are photoperiod sensitive. The basic vegetative phases (BVP) for all these inbreds range from 35.7 days (66A4-2) to 56.9 days (66D34-1) (Chapter II.1.).

Crosses to produce diallel genotypes, including reciprocals and inbred self-crosses, were made in the field in Lethbridge in 1977. The photoperiod study was started in September 1977 and continued into 1980 with the use of a large walk-in growth room (Controlled Environments Ltd., Winnipeg, Manitoba). The temperature regime was 25/20° day/night, the day temperature was maintained for 14 hours, and rises and falls in temperature were 5°C/hour. The photoperiod consisted of a 14-hour high intensity (808 υEinsteins sec^{-1} m^{-2} in the 400-750 mm range) photo-syntehtic period and 0 to 10 hours of low intensity (68 υEinsteins sec^{-1} m^{-2}) photo-period extension.

Three kernels of a genotype were planted in 25- x 15-cm plastic pots that contained a mixture of soil, sand, and peat (2:1:1). After emergence, pots were thinned to one plant and, every 14 days, 0.25 g 28-14-14 fertilizer was added to each pot. Two replicates of each

genotype were included in each run. Experimental treatments included 14-, 16-, 18-, 20-, and 22-hour photoperiods with two replications of each. A second set of the 14-, 18-, and 22-hour treatments was grown later.

Since Griffing's (55 diallel analysis showed that reciprocal effects were not significant, reciprocals were pooled to produce half diallel arrays. Consequently, the sample size was effectively doubled for each hybrid and eight data values were available for the 14-, 18-, and 22-hour treatments with four values for the 16- and 20-hour treatments. Thus, 32 data values were considered for each hybrid.

Plants were observed daily and dates of plant emergence and anthesis (anthers first extruding from the glumes) were recorded for each plant. After all eight runs were complete, an analysis of variance was conducted between time to anthesis (flowering-time) and photoperiod.

Photoperiod response characteristics were analyzed as previously described (Chapter II.1.). Flowering-time was plotted against photoperiod for each genotype and maximum optimal photoperiod was estimated. The BVP was calculated as the mean flowering-time at photoperiods equal to or below the MOP. A linear regression of flowering-time versus photoperiod was performed and the regression coefficient (b) was considered to be the photoperiod sensitivity. The intercept of the regression line with the BVP was the corrected MOP. Hayman's (63) diallel analysis, as described by Mather and Jinks (89), was performed on each of the photoperiod response characteristics: BVP, photoperiod sensitivity, and MOP. Narrow- and broad-sense heritabilities were calculated using the formula of Mather and Jink's (89). Griffing's (55) fixed model Method 3 analysis

with the modification suggested by Thompson (139) was used to test significance of general (GCA) and specific combining ability (SCA). Inbred data were excluded in this analysis to eliminate effects of inbred depression that could be mistaken for SCA effects (Chapter I.1.). The relative importance of GCA and SCA was determined as suggested by Baker (6). Estimates of GCA effects were made using Griffing's (55) Method 1 analysis (inbreds included).

Results and Discussion

The inbreds (66A4-2, CG8, CM7, CL5, CM49, CL3, W103, and 66D34-1) are referred to as array members 1 to 8, respectively.

Basic Vegetative Phase (BVP)

The BVP of the inbreds ranged from 37.4 to 56.6 days (Table 17, 66A4-2 and 66D34-1), and values were generally consistent with those previously reported (Chapter II.1.). In all crosses, the BVP of the hybrid was below the midparent value indicating dominance for short BVP and, in some cases, the hybrid BVP was below the shorter parental BVP indicating heterosis.

Variance of each row (Vr) and covariance between parents and their hybrids (Wr) were calculated (89) for each member of the array. Differences in the sums of Vr and Wr across replicates were significant (VR = 17.35, $P < 0.01$) indicating non-additive variation for BVP. Differences between (Wr - Vr) across replicates were also significant (VR = 7.89, $P < 0.01$) indicating non-additive variation other than independently distributed dominance effects. Thus, this data set did not satisfy the simple additive dominance model of inheritance for BVP.

The poor fit with the simple additive-dominance model of inheritance was also reflected in the Wr/Vr graph (now shown). Although a significant regression ($r = 0.86$) was observed, the slope differed from unity ($b = 0.666 \pm 0.165$, $P < 0.05$) and, hence, the Hayman (63) analysis could not be continued on the complete data set.

Jana (71) showed that, in certain situations, selective removal of one or more of the members of a diallel array may improve fit with the

Table 17. Basic vegetative phase (BVP), photoperiod sensitivity, and maximum optimal photoperiod (MOP) of eight early maturing maize inbreds.

Inbred	BVP (days)	Photoperiod sensitivity (days delay/hour)	MOP (hours)
66A4-2	37.4	0	24
CG8	45.0	0.57	15.9
CM7	47.5	0.66	13.2
CL5	45.8	1.11	15.7
CM49	47.5	0.96	14.5
CL3	55.2	1.46	18.0
W103	53.8	1.54	18.0
66D34-1	56.6	0	24

simple model of inheritance. In this study, removal of W103 (7) improved the fit. Analysis of the resulting seven parent diallel showed homogeneity of (Wr - Vr) across arrays (VR = 2.75) and the significant Wr/Vr regression (r = 0.83) had a slope that did not differ significantly from unity (Fig. 9).

The positive Wr intercept of the Wr/Vr plot indicated incomplete dominance and the position of array members indicated that the direction of dominance was for short BVP (Fig. 9). The member with the shortest BVP, 66A4-2 (1), was closest to the intercept and the positions of array members were in numerical order of ascending BVP. Thus, the order of the array members with respect to dominance for BVP was the same as that previously reported for flowering-time in field trials (Chapters I.1 and I.2.).

Both the additive (D) and dominance (H_1 and H_2) components of genetic variation were significant for BVP (Table 18). The additive component exceeded the dominance component verifying incomplete dominance. Overall degree of dominance $(H_1/D)^{0.5}$ was less than one and offered a quantitative estimate of this incomplete dominance. Incomplete dominance for short GVP is consistent with incomplete dominance for short flowering-time, which has been reported previously (12, 50, Chapter I.1. and I.2.).

The ratio $H_2/4H_1$ was less than 0.25, indicating that positive (increasing BVP) and negative allele frequencies over all loci were unequal (Table 18). The positive value of F indicates that there were more dominant than recessive alleles in the parental inbreds. Both broad- and narrow-sense heritabilities were high. The latter, which represents fixable, additive, heritable variation, indicates that

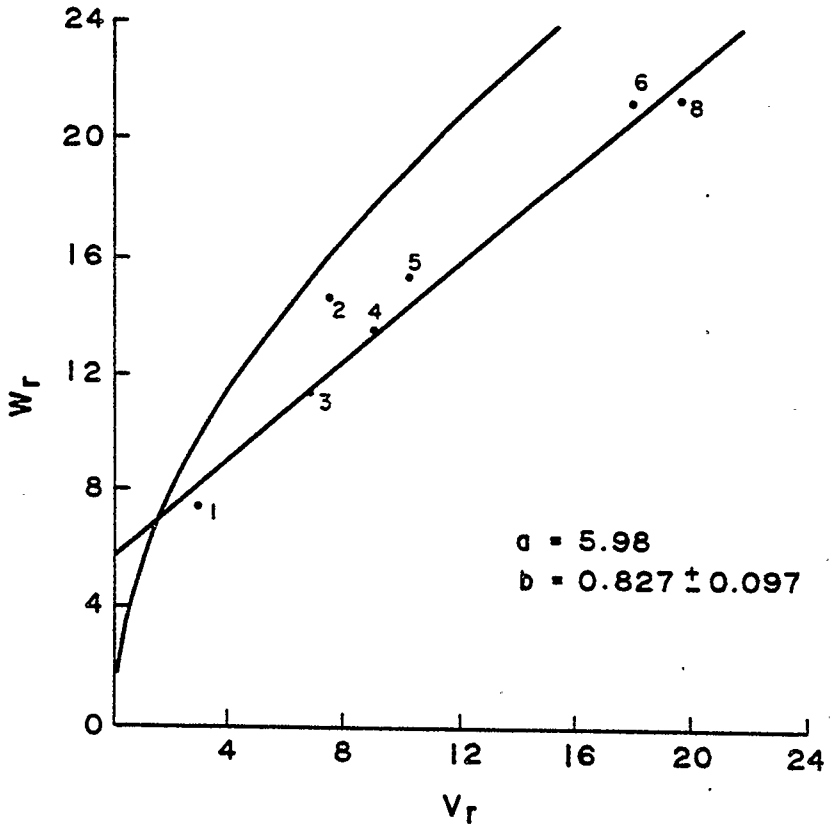


Fig.9 . Wv/Vr graph for basic vegetative phase (BVP) of a diallel cross from eight early maturing maize inbreds.

Table 18. Analysis of genetic variation for basic vegetative phase (BVP), photoperiod sensitivity, and maximum optimal photoperiod (MOP) of early maturing maize.

	BVP (days)	Photoperiod sensitivity (days delay/hour)	MOP (hours)
Components (± SE)			
D	35.5 ± 3.4	0.371 ± 0.165	15.1 ± 2.0
H_1	19.5 ± 1.4	0.577 ± 0.067	36.6 ± 5.5
H_2	15.4 ± 1.4	0.364 ± 0.093	23.2 ± 3.5
F	12.3 ± 4.8	0.495 ± 0.120	29.0 ± 4.1
Derived values			
$(H_1/D)^{0.5}$	0.741	1.372	1.555
$H_2/4H_1$	0.200	0.178	0.158
Heritability (%)			
Broad-sense	96.5	70.6	70.8
Narrow-sense	75.3	23.6	1.8

response to selection for decreased BVP should be rapid. The analysis of derived components of genetic variation produced values for BVP that were quite similar to those obtained in the diallel analysis of flowering-time in field trials of this diallel (Chapter I.1,) and in field trials by Bonaparte (12).

Photoperiod Sensitivity

The photoperiod sensitivities of the inbreds ranged from 0 (day-neutral, 66A4-2 and 66D34-1) to 1.54 days delay per hour increase in photoperiod (W103) (Table 17). No day-neutral hybrids were observed as hybrid photoperiod sensitivity fell between the inbred extremes. Hybrid photoperiod sensitivity varied widely and no general conclusions regarding overall direction of dominance could be made simply on the basis of examination.

Significant differences in (Wr - Vr) across arrays were not detected (VR = 0.41) and the regression (r = 0.79) had a slope that did not differ significantly from unity (Fig. 10). The Wr intercept was below the origin and (Wr - Vr) values for all array members were negative. Hence, overdominance was observed with respect to photoperiod sensitivity. The direction of this overdominance was not consistent, however, as day-neutral array members (1) and (8), as well as the most photoperiod-sensitive array members (5, 6, 7), were clustered far from the origin (Fig. 10). The three array members located closest to the origin (2, 3, 4), and thus containing principally dominant alleles, were intermediate in photoperiod sensitivity. Thus dominance of alleles for intermediate photoperiod sensitivity was observed in these genotypes.

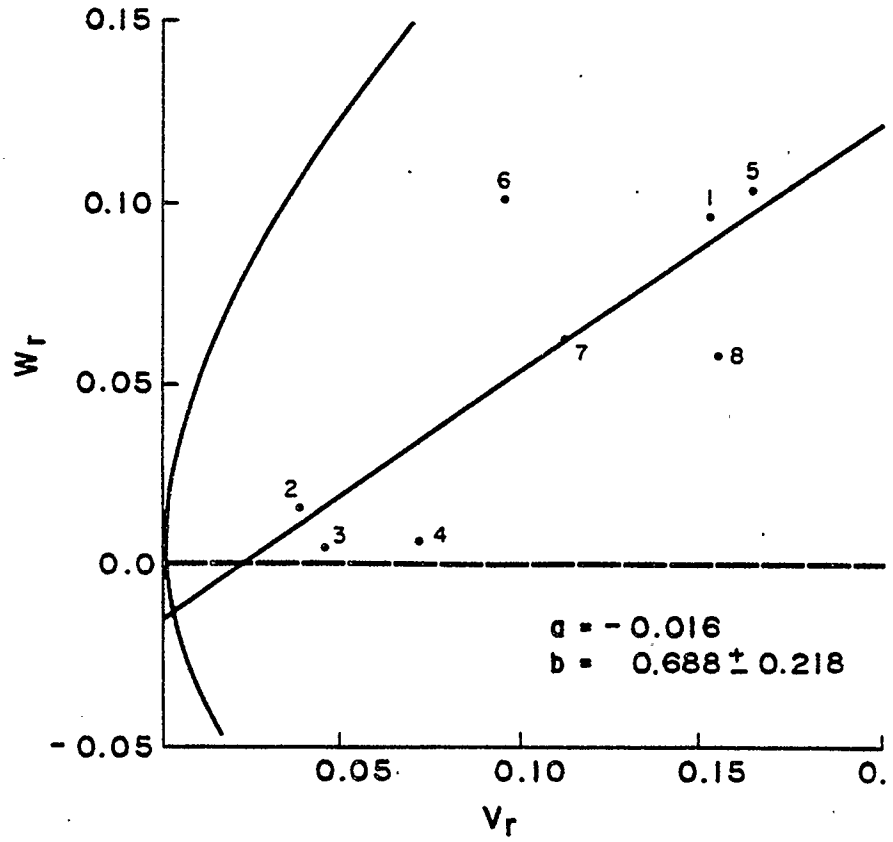


Fig. 10. Wr/Vr graph for photoperiod sensitivity of a diallel cross from eight early maturing maize inbreds.

Additive (D) and dominance (H_1 and H_2) components of genetic variation were significant for photoperiod sensitivity (Table 18). The overall level of dominance $(H_1D)^{0.5}$ was 1.4, a value greater than 1.0, thus indicating overdominance. The value of $H_2/4H_1$ indicated that positive (increasing sensitivity) and negative alleles occurred with unequal frequencies. The positive value of F indicated that the number of dominant alleles exceeded the number of recessive alleles in the parental inbreds.

While broad-sense heritabilties were intermediate (Table 18), narrow-sense heritabilities for photoperiod sensitivity were low. Thus, response to selection for photoperiod sensitivity or insensitivity would be slow.

Maximum Optimal Photoperiod (MOP)

The MOP of the inbreds ranged from 13.2 to 24 hours (Table 17, CM7 vs. 66A4-2 and 66D34-1). As occurred with photoperiod sensitivity, hybrid MOP was variable. Mean hybrid MOP was 15.5 hours and below the mean inbred MOP (17.9 hours). This reflected the recessive behavior of the alleles for a 24-hour MOP, which were in the day-neutral inbreds 66A4-2 and 66D34-1.

Differences in $(W_r - V_r)$ across arrays were not significant (VR = 1.12) and the regression ($\underline{r}$ = 0.75) had a slope that did not differ from unity (Fig. 11). The W_r intercept was below the origin representing overdominance. The day-neutral inbreds (66A4-2 and 66D34-1, $\underline{1}$ and $\underline{8}$) were located farthest from the intercept indicating the presence of recessive alleles. The photoperiod-sensitive array members formed a

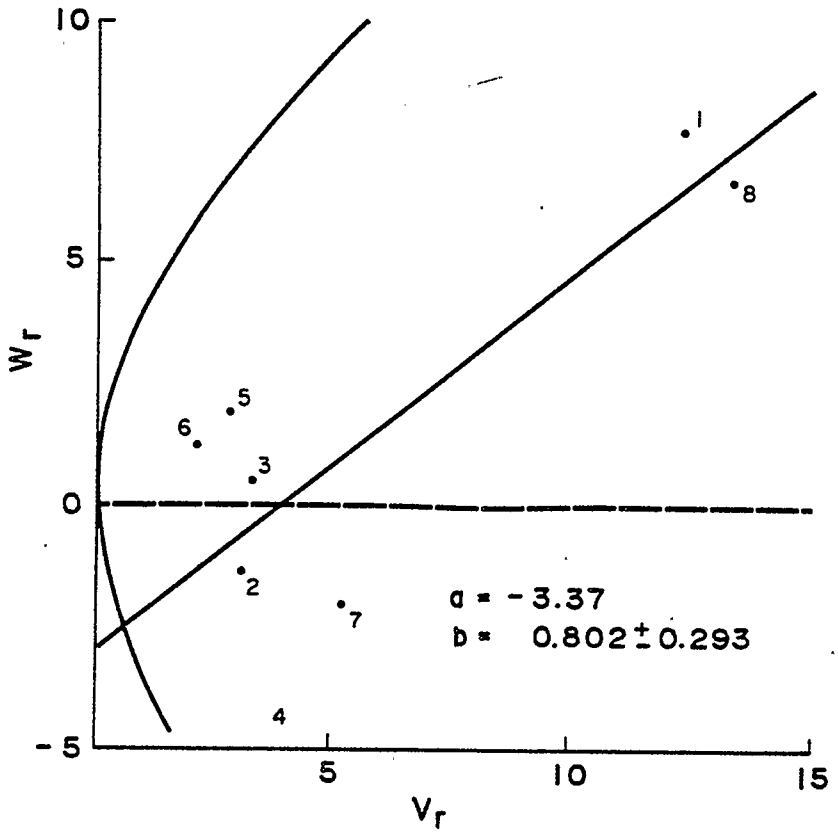


Fig.11 Wr/Vr graph for maximum optional photoperiod of a diallel cross from eight early maturing maize inbreds.

single loose cluster near the W_r intercept (Fig. 11).

Additive (D) and dominance (H_1 and H_2) components of genetic variation were significant for MOP (Table 18). Overdominance was indicated since the value of $(H_1/D)^{0.5}$ exceeded 1.0, thus supporting the conclusion based on the negative W_r intercept. The value of $H_2/4H_1$ indicated unequal frequencies of the positive (increasing MOP) and negative alleles, and the positive value of F indicated a greater frequency of dominant alleles. Thus, alleles for reduced MOP were dominant and more frequent than alleles for increasing MOP. Since the narrow-sense heritability for MOP was very low (Table 18), response to selection for MOP would be slow.

While the Hayman diallel analysis (63) provides the most useful information about the inheritance of a genetic system (89), Griffing's (55) combining ability analysis is very useful to the breeder. Only general combining ability (GCA) effects were involved in the control of BVP. Specific combining ability (SCA) effects were significant ($P < 0.05$) for photoperiod sensitivity and MOP. The relative importance of GCA versus SCA was 0.60 and 0.34 for these two characters. The GCA effects for BVP, photoperiod sensitivity, and MOP ranged from -5.3 (66A4-2) to 2.6 (66D34-1), -0.31 (CG8) to 0.21 (W103), and -0.85 (CL3) to 0.80 (66A4-2), respectively (Table 19).

The order of inbreds for increasing GCA for BVP (1, 4, 2, 3, 5, 6, 7, 8) was very similar to the GCA order (Chapter I.1) for corn heat units to flowering in field trials at Lethbridge, Alberta in 1978 (1, 2, 4, 5, 7, 3, 6, 8) and 1979 (1, 2, 4, 3, 6, 7, 5, 8). This indicates that BVP and field performance are similar and, hence, the photoperiod-

Table 19. Estimates of general combining ability (GCA) effects for basic vegetative phase (BVP), photoperiod sensitivity, and maximum optimum photoperiod (MOP) for eight early maturing maize inbreds.

Inbred	BVP (days)	Photoperiod sensitivity (days delay/hour)	MOP (hours)
66A4-2	-5.3 d*	-0.21 bc	0.80 a
CG8	-0.3 bc	-0.31 c	-0.58 ab
CM7	0.3 b	-0.02 abc	-0.43 ab
CL5	-1.1 c	0.13 a	-0.37 ab
CM49	0.3 b	0.11 a	0.75 a
CL3	1.7 a	0.07 ab	-0.85 b
W103	1.8 a	0.21 a	0.55 ab
66D34-1	2.6 a	0.02 ab	0.13 ab

* Within columns, values followed by the same letter do not differ ($P < 0.05$) using Duncan's multiple range test.

induced delay in flowering is either small or constant. The mean MOP of the diallel hybrids was 15.5 hours, slightly less than 2 hours shorter than the effective natural photoperiod in Lethbridge before tassel initiation (40, 121). The mean hybrid photoperiod sensitivity was 0.795 days delay per hour increase of photoperiod. Thus, the mean photoperiod-induced delay in flowering-time under the natural photoperiod at Lethbridge should be about 1.5 days. This is a very minor delay compared to the BVP range of 18 days, a range that represents only a fraction of the maturity range of maize; thus photoperiodism is of only minor importance in the developmental adaptation of these early maturing maize genotypes. Photoperiodism may be of greater importance if maize adapted to tropical regions is introduced into northern breeding programs to maximize heterosis. Tropical genotypes tend to show higher photoperiod sensitivities than temperate genotypes (41). Hybrids produced by crossing temperate and tropical genotypes may show high photoperiod sensitivity since day neutrality is recessive.

III. Gibberellin physiology of northerly-adapted maize

In GA research (as is the case with research dealing with other phytohormones) there are four principal approaches to the questions regarding a possible role in plant processes. The simplest and most frequently used approach is to add additional GA (usually GA_3) to a plant or plant part and observe resultant changes. However, response to exogenous application may not reflect endogenous role. Following exogenous application it is unlikely that the compound applied is distributed naturally and indeed, it is possible that little or even none of the applied compound reaches certain receptor sites (presently unidentified but assumed to exist) as mobility of the applied compound may be low. The exogenous application rates typically required for an observable response are generally far higher than endogenous levels implying that problems with mobility and compartmentalization are very significant. Almost all of the early work and most of the current work with exogenous GA application relies on the use of gibberellic acid (GA_3). This reliance stems from the availability of GA_3 which is produced in copious quantities by the fungus Gibberella fujikuroi. However, GA_3 is not always native in higher plants and its high efficacy and persistance may impart different biological activity than other GAs. Indeed, the high biological activity of GA_3 may underly its usefulness to the fungus.

The next simplest means of assessing the possible role of GAs in a plant system is to reduce the endogenous pool through the addition of metabolic blocks such as AMO-1618 or CCC. Unfortunately, these growth inhibitors typically block more than ent-kaurene and hence, plant

response to the exogenous application of the inhibitor cannot not be simply attributed to a reduction in GA level.

Probably the most meaningful single approach to questions regarding possible role of GAs in plants is an analysis of endogenous GA levels. This has been the approach described in the following three chapters. In these studies, levels of endogenous GA-like substances have been estimated through bioassay after purification. While it is assumed that levels of GA-like substances offer a meaningful reflection of actual GA levels, this assumption has not been adequately tested. Further, the possible involvement of compartmentalization is entirely disregarded as an entire plant or organ is homogenized.

It is well known that the efficacy of different GAs varies widely and some GAs are biologically inactive (25). Biological involvement is further complicated as different bioassays respond differently to different GAs (25). If the GA responsible for GA-like activity in a given sample is known, the efficacy of that GA standard in the specific bioassay used may be used to calculate the approximate amount of GA present. For example, GA_{19} is about as active as GA_3 in the Tan ginbozu dwarf rice assay while GA_{20} is somewhat less active (25). If we assume that Hedden et al (65) have characterized the most abundant GAs of maize we may conclude that the component from SiO_2 peak IV which co-chromatographs on reverse-phase HPLC with GA_{19} is indeed GA_{19}, the ug GA_3 equivalents should approximate the ug GA_{19}. The peak of GA-like activity which co-chromatographs with GA_{20} on SiO_2 and reverse-phase HPLC is probably in part, GA_{20} and is probably underestimated.

If the biologically active GA(s) of rice and maize are similar

(which would be consistent with the equivalent efficacy of various GAs in the dwarf rice and dwarf maize assays (25)) then the reduced efficacy of the GA_{20}-like substance may actually represent reduced endogenous efficacy as well. Thus, for estimating levels of endogenous, biologically active GAs, bioassay has one advantage over chemical analysis. However, the qualitative ambiguity and quantitative variability of bioassay is a distinct disadvantage relative to definitive chemical methods.

Assessments of endogenous levels of GA-like substances can provide data suitable for correlative analyses but do not provide evidence for causal associations between GAs and plant growth or development. When supplemented with data from manipulative studies, causal associations may be suggested. Thus, endogenous analyses will provide the basis for the following three chapters.

The final approach in research regarding possible roles of phytohormones and plant growth and development consists of investigations into biosynthesis and metabolism. All research carried out during the course of this thesis project dealt with the metabolism of ^{3}H - labelled GAs. Radioactively labelled compounds are simply detected and ^{3}H labelled GAs have been previously made in quantities adequate for manipulative metabolic studies. It is hoped that analyses of the movement and metabolism of exogenously applied ^{3}H labelled GAs is indicative of the fate of endogenous GAs. However, this suggestion has not been adequately researched and hence, all research based on metabolism of exogenously applied GAs must be viewed cautiously. As was the case with respect to experiments based on the exogenous application of cold GAs,

effects of compartmentalization and mobility of different GAs cannot be easily analyzed or controlled. Thus, results from these metabolic studies can only hint at the natural internal processes within the maize plant.

III. 1. Effects of Genotype, Temperature, and Developmental Stage on Gibberellin Content of Maize.

Abstract

Growth rates and levels of endogenous gibberellin-like substances (GA-like) were measured in two northerly adapted maize inbreds and their F_1 hybrid grown under cool (soil 12°C, air 20/10°C day/night) and warm (soil 17°C, air 25/15°C) temperature regimes. Growth rates and the levels of GAs were reduced under the cool temperature conditions for all genotypes. The least vigorous (in terms of vegetative growth) genotype, inbred CM49, had the lowest GA levels under both temperature regimes. The hybrid CM7 x CM49 had the greatest shoot dry weights and, at most harvest times, the highest GA content. GA levels generally fell after tassel initiation and then rose to maximal levels at the onset of internode elongation. At this stage, CM49, CM7, and CM7 x CM49 contained 284, 3865, and 6921 ng GA_3 equivalents per 100 meristems, respectively, under the warm temperature conditions. Consistent with a lower level of endogenous GAs, the less vigorous inbred was more responsive to exogenous application of GA_3 or $GA_{4/7}$ than the vigorous inbred, CM7. After exogenous application of the GAs, an initial promotion of internode elongation was followed by a reduction of vegetative growth in both genotypes. Thus, endogenous GA level is dependent on genotype and is affected by temperature and, particulary, developmental stage.

Poor seedling vigor under cool spring conditions is a major factor limiting maize production at northerly latitudes (86). Although it is known that genotypic variability in temperature sensitivity of maize

exists for growth and development (29, 16) little is known about the regulatory mechanism underlying the different responses of maize genotypes to cool temperatures (i.e., cool temperature vigor).

Exogenous application of GA_3 hastens cool temperature germination and seedling emergence of maize (9, 56) and sorghum (151). Sugarcane seedlings show a greater growth response to GA_3 under cool than warm temperatures (15, 76) and similar interactions between GA_3 and temperature are observed in other grasses (76, 146). Further, Reid et al (113) and Radley (111) reported that the endogenous GA-like content of wheat was reduced at cool temperatures. These observations suggest that GA content of maize may be reduced at cool temperatures.

The response of maize to exogenous GA_3 is dependent on genotype (101, 102) and timing of application (58). Inbreds have been shown to be more responsive to GA_3 than hybrids, and response of intermediate genotypes is associated with the degree of inbreeding (101, 102). Although inbreds vary in vegetative growth and cool temperature vigor, these are generally more vulnerable than hybrids to prolonged cool temperature exposure (unpublished data of S.B.R. and D.J.M.). These findings suggest that endogenous GAs may be involved in the control of cool temperature vigor.

This study was initiated to investigate the possible association between growth under cool temperatures and endogenous GA-like level, in genotypes varying in cool temperature vigor. The endogenous GA-like substances of two inbreds and their F_1 hybrid were also compared.

Materials and Methods

Plant Materials

Seeds of two northerly adapted maize (Zea mays L.) inbreds, CM7 and CM49, were obtained form J. Giesbrecht, Agriculture Canada Research Station, Morden, Manitoba. After two cycles of selection for cold vigor in CM7, self-crosses and CM7 (male) x CM49 (female) outcrosses were made in field trials at Lethbridge, Alberta, in 1978. Following harvest, seed was stored at 5°C.

Ten seeds of a genotype were planted in each 20 x 25 cm plastic pot. The bottom 5 cm of each pot was packed with sand, the upper 20 cm was filled with "Cornell mix", a peat-like mixture with added nutrients (3). Pots were placed in troughs of circulating water in which temperature was maintained at 12°C (cool regime) or 17°C (warm regime). Air temperature in the growth room (Controlled Environments Limited, Winnipeg, Manitoba) was 20/10°C (day/night, cool regime) or 25/15°C (warm regime); the day temperature was maintained for 14 h, and rises and falls in temperature were 5°C/h. A 14-h photoperiod (970 uEinsteins m^{-2} sec^{-1}) was presented and all pots were watered twice daily.

One pot of each genotype was planted five days before the planting of all other pots. Following the emergence of the ligule of the third leaf, plants were dissected daily in order to determine the time of tassel initiation. Tassel initiation was defined as the time of first appearance of tassel ridges on the apical meristem. The first harvest took place 2 days before tassel initiation in CM7 x CM49 and three successive harvests followed at about 4-day intervals. At each harvest, shoots of 5 plants were excised at the root crown, dried, and weighed.

Heights to the uppermost extended leaf tip of 10 plants were also measured. Healthy, uniform plants were harvested, bisected longitudinally, and shoot half-cylinders containing apical meristems were excised. These were frozen with solid CO_2, lyophilized, and leaf tissue teased away from the apical meristematic cone. About 50, 35, 30, and 20 meristems were bulked for GA extraction at harvests one to four, respectively.

Two replications of this experiment were carrried out. Samples from runs 1 and 2 were analyzed for GAs simultaneous and values presented here are the means of these 2 runs.

Extraction

Freeze-dried apical meristematic cones were extracted for GAs as previously described (78, Chapter IV.1.). However, meristematic tissue (5g) was homogenized in 200 ml methanol-H_2O (80:20) and shaken for 12 h at 4°C. After solvent partitioning, purification of the acidic, ethyl acetate-soluble fraction using poly-N-vinyl-poly-pyrollidone (53) and charcoal-celite columns (113) was followed by gradient elution chromatography on Woelm SiO_2 partition columns (33). The resulting 26 fractions were bioassayed at serial dilutions for GA-like activity using a modified (78) Tan-ginbozu dwarf rice assay (97).

Biologically active non-polar (fractions 5-8), and polar (fractions 15-20) SiO_2 partition column fractions were bulked, re-run on a second SiO_2 column and the biologically active non-polar or polar fractions were again bulked and further chromatographed using reverse phase high performance liquid chromatography (HPLC) as previously described (80).

The resultant fractions were bioassayed for GA-like activity as above.

Exogenous application

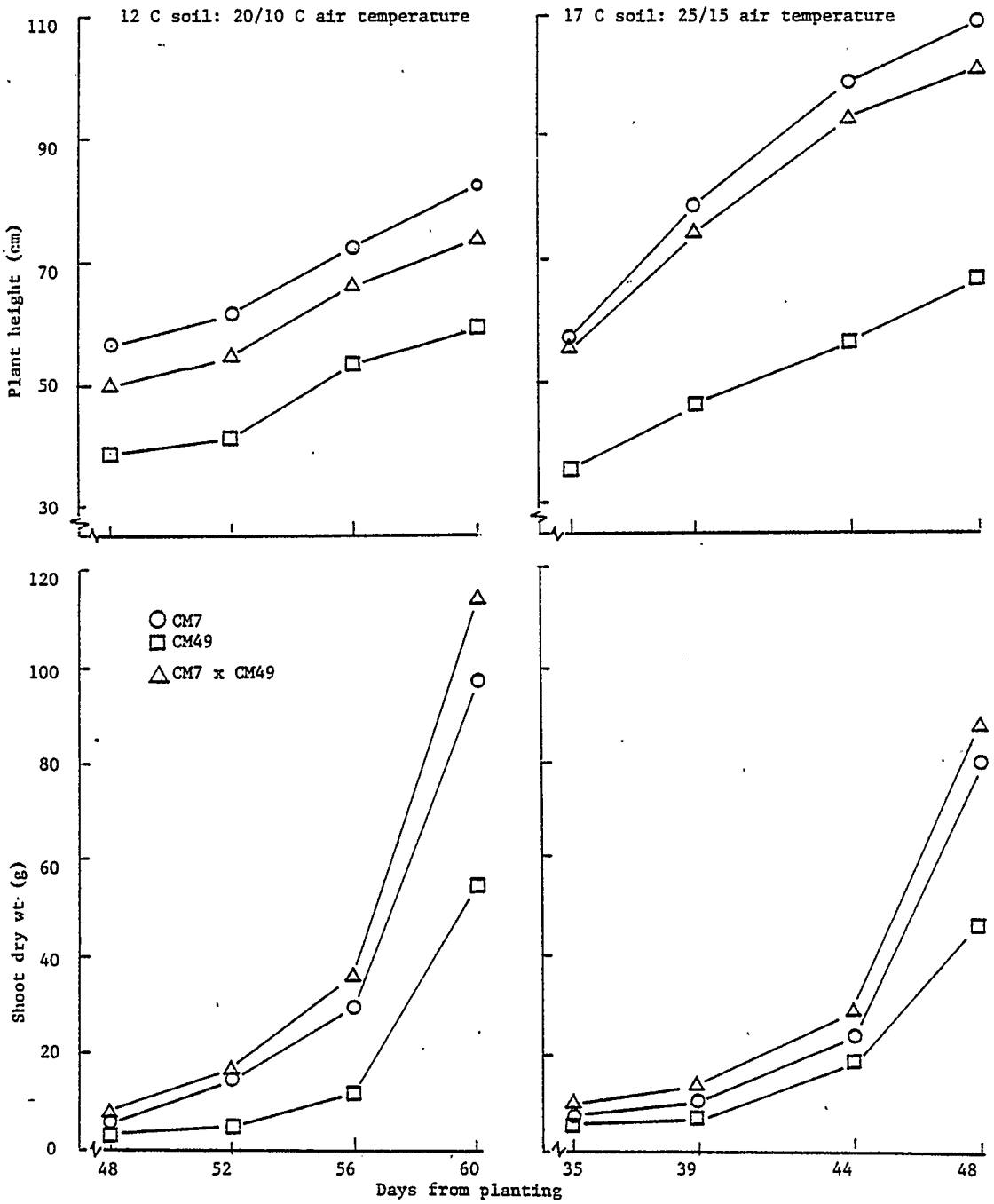
Seeds of CM7 and CM49 were planted in the field at Lethbridge, Alberta, on May 13, 1980. Soil temperature (10 cm depth) was 12.8°C at planting. A single application of 0.1, 1, or 5 mg of GA_3, GA_4 (89:11; GA_4:GA_7) or $GA_{4/7}$ (58:42; GA_4:GA_7) in 1 ml EtOH:H_2O (10:90) was injected into the whorl of each plant on July 2, 1980. At this time plants were at the 4-leaf stage (ligule of leaf 4 visible), tassels were 4 mm in length and tassel ridges were visible. Plant heights were measured 14 d after treatment and final plant growth characteristics (Table 22) were determined on August 14, 1980. Leaf area of the eight leaf was measured with a Wescor LI 3000 portable area meter and the degree of sex reversion was rated on a scale from 1 (no reversal) to 5 (complete sex reversal of the apical inflorescence to the female form) (See: Chapter III.3.).

Results and Discussion

Both temperature and genotype had significant ($P < 0.01$) effects on plant height and shoot dry weight. Inbred CM49 was the slowest growing genotype under both temperature conditions (Fig. 12). The inbred CM7 was slightly taller than the hybrid, CM7 x CM49, but the hybrid had greater shoot dry weights. Differences between the shoot dry weights of CM7 and CM7 x CM49 were significant after day 52 under the cool temperature regime and on day 35, 39, and 48 under the warm temperature regime. Thus, incomplete dominance for increased height and apparent "overdominance" for increased shoot dry weight were observed. "Overdominance" for increased height was previously observed for these and other northerly adapted genotypes (Chapter I.3.). However, final height as measured in the previous study was principally a function of internode length, whereas the plant height reported here represents leaf length. Internode elongation had just begun in CM7 x CM49 at day 48 under the warm temperature regime and had not yet started at day 60 under the cool regime. Data from previous studies showed that internode elongation would start just after day 60 in CM7 under similar cool temperature conditions (unpublished data of S.B.R. and D.J.M.). Since dominance for rapid development is observed in maize (Chapters I.1., I.2., I.3.), elongation in CM7 x CM49 would probably also start soon after day 60.

Under both temperature regimes, tassel initiation occurred about 1 to 2 days earlier in CM7 and CM7 x CM49 than in CM49. The timing of initiation was similar in the hybrid and in CM7, occuring 2 days after the first harvest at each temperature condition. This dominance for

Fig. 12. Changes in plant height and shoot dry weight of two maize inbreds and their F_1 hybrid grown under two temperature regimes.


12 C soil: 20/10 C air temperature
17 C soil: 25/15 air temperature
Plant height (cm)
110
90
70
50
30
Shoot dry wt. (g)
120
100
80
60
40
20
0
CM7
CM49
CM7 x CM49
48
52
56
60
35
39
44
48
Days from planting

early tassel initiation is consistent with the previously reported dominance for early flowering-time (Chapters I.1. and I.2.). A possible confounding effect due to the dissimilar phenology of the genotypes should be overcome by the consideration of a sequence of samples.

Four peaks of GA-like activity were observed in the SiO_2 fractions (Fig. 13). The non-polar first peak (fractions 3-6) was absent in hybrid tissue (Tables 20 and 21) and tended to be the peak with the lowest levels of GA-like activity, expressed in terms of GA_3 equivalents. This peak was not observed previously in meristematic tissue from another northerly-adapted maize inbred, 66A4-2 (Chapter III.3.). The non-polar second peak co-chromatographed with GA_4, which is native in oats (106), and rice (81), and also co-chromatographs with GA_{20}, which is native in maize (65). The polar fourth peak co-chromatographed on SiO_2 with GA_{19}, which is native in maize (65), and with GA_1 and GA_{29} which may be native to maize (65), and GA_3, which is native to oats (106) and other Graminae.

When the fourth peak was further chromatographed on radial Pak-A using HPLC, about 99% of the activity of the polar peak co-chromatographed with GA_{19} while only 1% co-chromatographed with GA_1 (Fig. 14). The absense of a very polar GA_{17}-like peak of activity (e.g., Fr.21+, SiO_2 partition column) may be due to the low efficacy of GA_{17} in the dwarf-rice bioassay (25). GA_{17} is native in maize (65).

In all but two harvests, levels of endogenous GA-like substances were greater under the warm than cool temperature regime (Tables 20 vs 21). Thus, consistent with the previous observations with wheat (111, 113) cool temperature reduced levels of GA-like substances in maize.

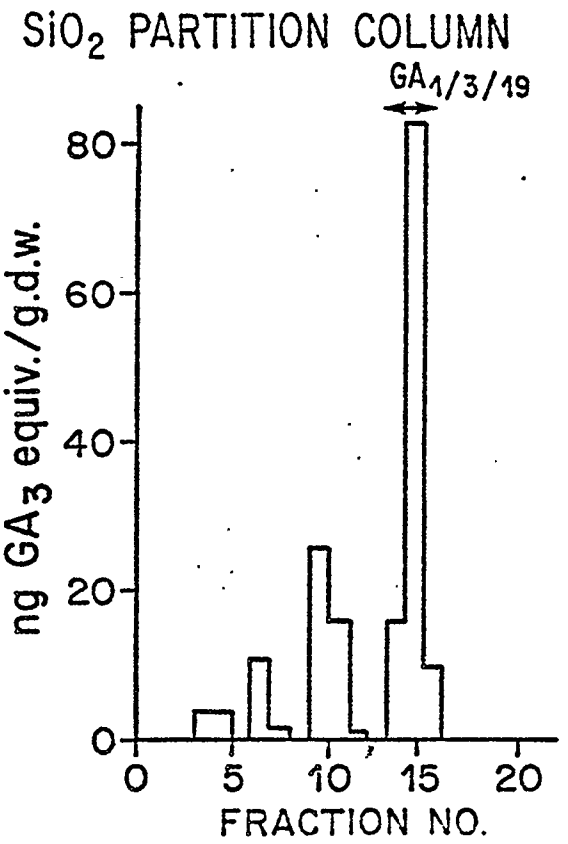


Fig. 13. Representative spectrum of GA-like substances from maize apical meristems as determined by bioassay in serial dilution on dwarf rice cv. Tan-ginbozu.

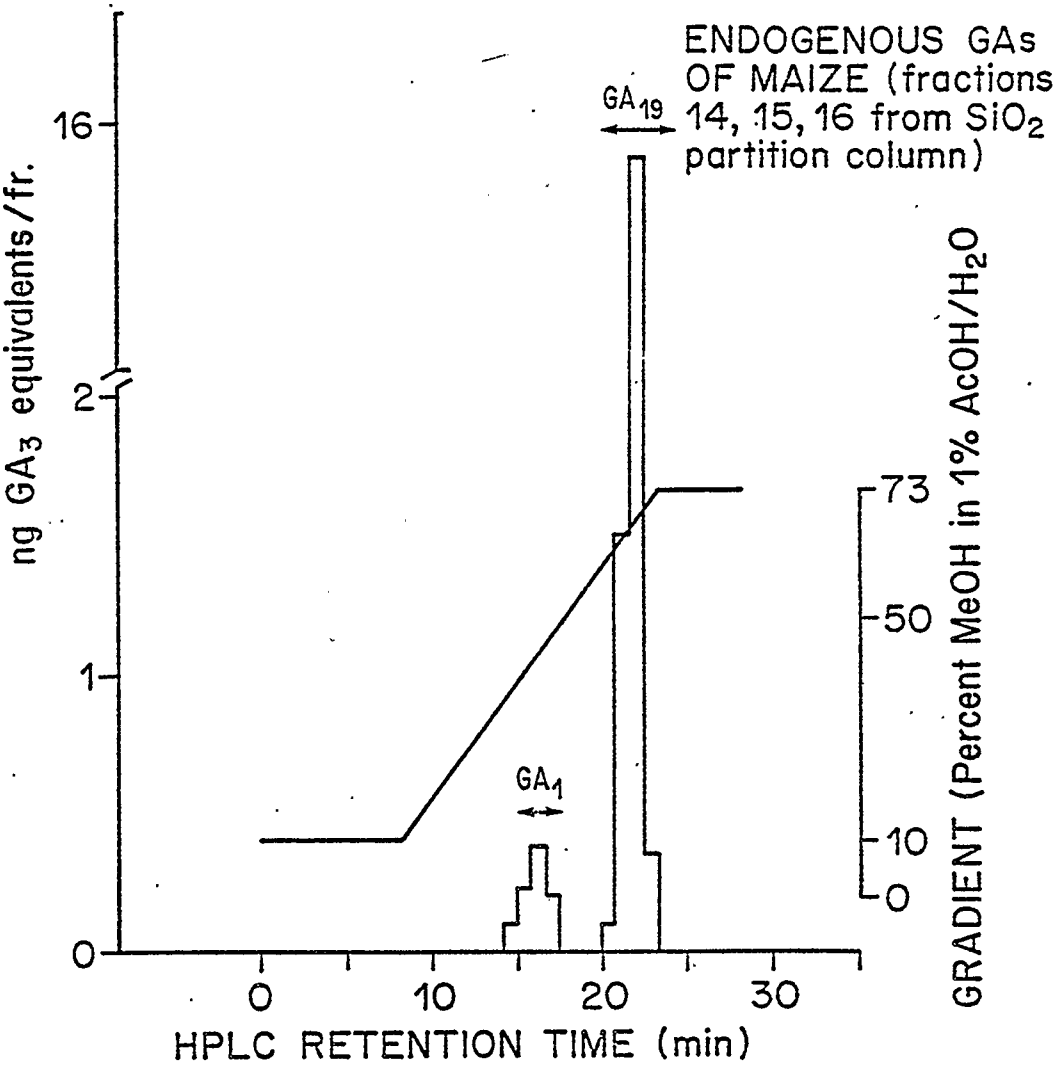


Fig. 14. GA-like activity of reverse phase HPLC fractions from a polar peak of GA-like activity from SiO_2 fractions 15-18, as determined by bioassay on dwarf rice cv. Tan-ginbozu.

The changes in GA-like level during development and the different levels of GAs in the three genotypes, indicate that temperature was not the only factor influencing GA content. In all harvests but one, the least vigorous (in terms of vegetative growth) genotype, CM49, had the lowest GA-like level (Tables 20 and 21). As a result of the relatively complex extraction and purification procedure and the inherent variability of quantitation by bioassay, only 3-fold or greater differences should be considered significant. Such differences between CM49 and the vigorous genotypes (CM7 and CM7 x CM49) were consistently observed (Tables 20 and 21).

The endogenous GA-like substances of the hybrid were not only greater than the least vigorous inbred, CM49, but also tended to be greater than that of CM7, the vigorous inbred (Tables 20 and 21). Under both temperature conditions GA levels in the hybrid were higher at the final harvests which took place just prior to, or at the start of, internode elongation. Heterosis, in the form of apparent "overdominance", for increased internode elongation is observed in maize (Chapter I.3.). Since a principal effect of exogenous GA_3 application is a promotion of internode elongation, it is logical that any "overdominance" for increased GA-like content at the fourth harvests should be associated with "overdominance" for internode elongation.

Under the warm temperature regime, levels of GA-like substances in the hybrid were very high just prior to tassel initiation (Table 20). Dry weights of the hybrid shoots were significantly ($P < 0.01$) greater than weights of shoots of either inbred at this harvest. While the high GA level may have been related to this vegetative vigor, it is also

possible that some of the GA-like activity may have been associated with tassel initiation (Chapter III.3.). GA content of the hybrid fell to its lowest value 7 days after tassel initiation (Table 20). Drops in activity following tassel initiation were also observed in CM7 under the cool regime and in CM49 under both the warm and cool regimes. It is possible that this drop in GA level after tassel initiation may coincide with microsporocyle meiosis and relate to sexual development of the apical inflorescence (Chapter III.3.).

For all genotypes, maximal GA levels were observed at the fourth harvest. As previously noted with regard to the hybrid, the high GA-like levels at the fourth harvest are correlated with the onset of internode elongation.

The vegetatively less vigorous inbred, CM49, showed a greater elongation response to exogenous application of GAs than the more vigorous CM7 (Fig. 15). This is consistent with predictions based on endogenous levels. Since CM49 had lower levels of endogenous GAs than CM7 (Tables 20 and 21), CM49 was expected to show a greater response to exogenous application of GAs.

Although GA_4 and $GA_{4/7}$ significantly ($P < 0.01$) promoted elongation, GA_3 had the greatest effect (Fig. 15). The application on CM49 of 0.1 mg $GA_{4/7}$ was significantly more effective than 0.1 mg GA_4. $GA_{4/7}$ also tended to be more effective than GA_4 at other application rates on both inbreds (Fig. 15). The greater efficacy of $GA_{4/7}$ than GA_4 in this study is consistent with previous reports regarding efficacy in dwarf-maize bioassays. The C-1,2 double bond found in GA_7 (and in GA_3) which is

Table 20. Gibberellin-like contents of apical meristems of maize plants grown at 17°C soil: 25/15°C (day/night) air temperature conditions, determined by the dwarf rice (cv. Tan-ginbozu) assay. Data given as ng of GA_3 equivalents per 100 meristems.

Genotype	Fraction numbers	Days from planting			
		35	39	44	48
CM7 x CM49	3- 6	-	-	-	-
	7-10	153	80	25	-
	11-14	61	90	76	598
	15-20	928	472	26	6323
	Total	1142	642	127	6921
CM7	3- 6	21	7	118	103
	7-10	58	92	128	1156
	11-14	2	18	22	1448
	15-20	151	232	237	1157
	Total	233	349	505	3865
CM49	3- 6	10	41	-	6
	7-10	43	54	9	44
	11-14	85	26	26	132
	15-20	25	103	77	101
	Total	163	225	111	284

Table 21. Gibberellin-like contents of apical meristems of maize plants grown at 12°C soil: 20/10°C (day/night) air temperature conditions, determined by the dwarf rice (cv. Tan-ginbozu) assay. Data given as ng of GA_3 equivalents per 100 meristems.

Genotype	Fraction numbers	Days from planting[1]			
		48	51	56	60
CM7 x CM49	3- 6	-	-	-	-
	7-10	6	21	80	201
	11-14	19	27	232	431
	15-20	91	100	191	1887
	Total	116	148	503	2519
CM7	3- 6	25	78	-	-
	7-10	38	132	16	221
	11-14	40	31	62	151
	15-20	86	-	26	297
	Total	191	242	104	669
CM49	3- 6	-	10	4	66
	7-10	98	29	19	37
	11-14	33	53	13	298
	15-20	24	-	61	267
	Total	156	92	98	668

[1]Plants were at the same developmental stage at day 39 under the warm temperature regime (Table 1) and at day 51 under the cool temperature regime (Table 2).

Fig. 15. Increases in heights of 2 maize inbreds 14 days after exogenous application of GAs.

Height (% of control)
100
200
300
0.1
1.0
5.0
mg GA per plant
GA4/7
GA3
GA4
CM 7
CM 49

absent in GA_4 (and in GA_1) leads to a promotion of activity in the $\underline{d}_1$, $\underline{d}_2$, $\underline{d}_3$, and $\underline{d}_5$ GA bioassays (25).

After the initial rapid elongation in the GA-treated plants, vegetative growth was reduced relative to control plants (Table 22) a response also noted for Parker's Flint maize (101). Consequently, final height was reduced following GA_3 treatment (Table 22). The promotion of final height previously reported in other studies probably resulted from sustained GA application as well as genotypic variation (101, 102). The initial rapid internode elongation may have altered the photosynthetic source-sink relationships such that leaf growth (Table 23) and possibly root growth (104) were reduced. Consequently, subsequent growth could be reduced as a result of the smaller leaf areas (Table 23). The balance of endogenous GAs and other hormones which relate to photosynthate source-sink relationships in maize (114) may have been disturbed by the exogenous application of GAs. As a result, final heights, dry weights (Table 22), and leaf areas (Table 23), were reduced. The reduction of leaf area, which is consistent with a previous report (20 was principally due to a reduction in leaf width; leaf length of CM49 was not affected (Table 23). The assimilatory and photosynthetically active leaf surfaces were reduced even further than the leaf areas since leaf rolling and folding, and incomplete leaf separation resulting in "harp-like" structures, were observed. These effects from exogenous GA application, as well as chlorosis, decreased tassel branching, and the tendency towards sex reversion (feminization) of the apical inflorescence (Table 22) are consistent with previous reports (58, 99, 101, 102). Shoot diameters

Table 22. Growth characteristics of maize inbreds CM7 and CM49 with or without exogenous application of 1 mg GA_3.

Inbred	Final height (cm)	Shoot dry wt. (g)	Shoot diameter, distance above root crown: 5 cm	Shoot diameter, distance above root crown: 20 cm	Number of tassel branches	Degree of sex reversion[1]
CM7						
control	145.3 ± 3.3	68.3 ± 5.2	2.53 ± 0.26	1.91 ± 0.17	12.3 ± 0.7	1.0 ± 0.0
GA_3	61.7 ± 1.7	9.3 ± 2.2	0.77 ± 0.13	1.30 ± 0.08	6.0 ± 0.9	2.3 ± 0.3
% of control	42.5	13.6	30.4	68.1	48.8	230
CM49						
control	101.6 ± 2.2	50.7 ± 5.4	2.00 ± 0.22	1.40 ± 0.14	4.6 ± 0.3	1.0 ± 0.0
GA_3	64.0 ± 2.5	20.0 ± 3.0	0.87 ± 0.11	1.30 ± 0.08	1.0 ± 0.0	3.5 ± 0.7
% of control	63.0	39.4	43.5	92.9	21.7	350

[1]Scale from 1 to 5; 1 represents normal male tassels with no indications of male sterility or feminization, 5 represents complete sex reversion of the apical inflorescence to the female form.

Table 23. Leaf characteristics of maize inbreds CM7 and CM49 with or without exogenous application of GA_3

Inbred	Leaf		
	Width (cm)	Length (cm)	Area (cm^2)
CM7			
Control	6.40 ± 0.05	48.8 ± 0.4	1739 ± 77
GA_3	2.41 ± 0.32	32.2 ± 1.1	402 ± 75
% of control	37.6	66.0	23.1
CM49			
Control	4.54 ± 0.09	39.7 ± 0.4	1035 ± 74
GA_3	2.15 ± 0.16	38.8 ± 1.1	579 ± 49
% of control	47.4	97.7	55.8

were reduced, particularly the diameters of the basal two or three internodes (Table 22). Another result of GA treatment, which has not previously been reported, was a change in color of the basal two internodes from green to purple-red, a color change associated with increased accumulation of a water soluble, diethyl ether- or ethyl acetate-insoluble red anthocyanin (see also Sando et al. (122) for a further description of this maize pigment). These changes may have resulted from a GA-induced alteration in carbohydrate metabolism (60) or alternately, from increased exposure to sunlight following the early internode elongation (10).

In conclusion, levels of endogenous GA-like substances in maize were correlated with vegetative vigor at low temperatures. The less vigorous inbred had the lowest GA content and was more responsive to exogenous GA application than the vigorous inbred. An F_1 hybrid tended to have higher levels of endogenous GAs than either the two parental inbreds. These observations, coupled with the reports that inbreds are more responsive than hybrids to exogenous GA_3 (101, 102) suggest that the reduced vegetative vigor associated with inbreeding depression may result in part from a reduction in endogenous GA level.

III.2. Gibberellin level as a possible phytohormonal basis for heterosis in maize.

Abstract

Under 25/20°C temperature conditions increased in plant height, leaf area, and shoot dry weight were more rapid in an F_1 hybrid than in either of its parental inbreds. The level of endogenous GA-like substances of apical meristematic cylinders was also higher in the hybrid than in either inbred although qualitative differences in GAs of the three genotypes were not apparent. No consistent differences in ABA content of the three genotypes was observed. In all genotypes, GA-like and ABA levels per cylinder rose prior to rapid shoot elongation. Specific GA levels per gram of tissue were apparently highest prior to tassel initiation while ABA levels per gram were highest during tassel initiation. The observation that heterosis for GA-like level was correlated with heterosis for maize growth is consistent with previous reports that inbreds are more responsive to exogenous GA_3 than maize hybrids. Thus, it is possible that the low endogenous GA level of inbreds is a major factor limiting growth and endogenous GA content may provide a hormonal basis for certain aspects of 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 (130, Chapter I.3.). Indeed, the impressive increases in yield of maize which farmers have repeatedly produced in the 20th century have largely resulted from the increased use of hybrid seed (37,46). 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 (130).

The involvement of plant hormones in heterosis is an attractive possibility since these endogenous regulators affect numerous growth processes. An alteration 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 (130), Sinha and Khanna (130) note that no correlative relationships between endogenous hormone levels and hybrid vigor have been described prior to 1975. We are unaware of any subsequent reports of such correlative relationships.

In the previous chapter we observed that growth rate and endogenous gibberellin(s) (GA) content were correlated across three maize genotypes grown under two low temperature regimes. In the previous study, dominance for increased growth rate was observed in a hybrid grown under the low temperature conditions. Apparent "overdominance" had been repeatedly observed when the same maize genotypes were grown under warmer growth cabinet or field conditions (Chapter I.3.). Thus, the next logical question to be addressed was: under favorable temperature conditions

are growth rates and endogenous GA contents still correlated; is heterosis for increased GA content observed to accompany heterosis for growth rate? The present study was initiated to investigate this question. Additionally, since ABA is often assigned as inhibitory role (91) in the regulation of plant growth, we hypothesized that ABA might also be involved in the regulation of growth rate in maize inbreds and their hybrid.

Material and Methods

Plant Materials

Two previously described (Chapters I.1. and II.1.) maize inbreds, CM7 and CM49, and their F_1 hybrid, CM7 x CM49, were included in the study. Ten seeds of a genotype were planted in each 22 x 22 cm plastic pot filled with "Cornell mix" (11). Pots were placed in a walk-in growth room (Controlled Environments Ltd., Winnipeg, Man.) in which a 25/20°C (day/night) temperature regime was presented with rises and falls in temperature of $5^{o}C\ h^{-1}$. A 14 h thermoperiod and photoperiod were presented in which PAR was 808 uEinsteins $sec^{-1}\ m^{-2}$. Pots were watered twice daily.

Six, 15, 21, 28 and 38 d after seedling emergence (emergence - 3 d after planting), the shoots of 5 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 L1 3000 area meter), and shoot dry weights were measured. From these data, mean relative growth rate (RGR) and mean net assimilation rate (NAR) were calculated as:

$$RGR = \frac{(\log_e W_2 - \log_e W_1)}{(t_{2-t1})}$$

$$NAR = \frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{(A2-A1)\ (t2^{-t}1)}$$

where W, A and t represent dry weight, leaf area and day of harvest 1 or 2 (110).

At 15, 21, and 28 d after emergence, shoot cylinders were excised

by cutting the shoots at the root crown and 5 (d 15 and 21) or 10 cm (d 28) above this point. These shoot cylinders contained the apical meristems and were immediately frozen in dry ice, then lyophilized.

GA analysis

Four replicate samples of 25, 10 or 5 (d 15, 21, and 28) cylinders for each inbred and its hybrid (36 samples in total) were extracted for GA as previously described (78). After separation by solvent partitioning (33) and purification of the acidic, ethyl acetate soluble fraction by PVPP (53) and charcoal:celite (32) columns, gradient elution SiO_2 partition chromatography (30, 109) was accomplished. Detection and quantification of GA-like substances was achieved using a modified (78) Tan-ginbozu dwarf-rice u-drop assay (97).

ABA analysis

After removal of aliquots for GA bioassay, SiO_2 partition column fractions 2 and 3 were bulked, dissolved in MeOH, filtered (0.5 um Millipore FH) and a spike of 1.1×10^4 dpm $[^3H]$-ABA (24 Ci/mmol from Amersham) (purified by isocratic reverse-phase HPLC-RC) was added to the bulked fractions from each extract. Spiked extracts were injected through a Waters 3 U6K injector into a Waters HPLC equipped with 2 model 6000A pumps, a model 660 solvent programmer, with fixed (254 nm) and variable wavelength (set at 264 nm) UV absorbance monitors, and flourometer (mm excitation, 265 nm emission). The HPLC column was a semi-preparative 50 cm x 9.4 mm (i.d.) Whatman Partisil-10 ODS-2, M9, and eluant was 100% MeOH 1.6 ml min^{-1}. In this isocratic separation

mode, authentic cis-trans ABA eluted at 10.6 min. After extract injection, fractions were collected from 8 to 14 min and the presence of the [^{3}H]-ABA marker in aliquots from 10 to 11.5 min was verified using liquid scintillation spectrometry. This [3H] ABA-containing fraction was taken to dryness in vacuo and derivatized with ethereal diazomethane. The extent of derivatization was determined by liquid scintillation spectrometry after separating the free acid from ABA-Me on reverse-phase HPLC using a 25 cm x 4.6 mm Whatman Partisil 10 ODS-3 and isocratic elution with MeOH:H_2O:AcOH (64:33.6:0.4 (v/v)). Under these conditions, cis-trans ABA-Me eluted 3 min after trans-trans ABA (136).

The fractions from the ODS-3 HPLC column containing [3H] ABA were solubilized in EtOAc and injected into a 1.4 m x 0.4 cm column packed with 2% SE30 in a Packard model 430 GLC fitted with a model 902 Ni^{63} ECD (125). Injector, oven and detector temperatures were 210, 180 and 225°C and isothermal chromatography was carried out using 30 ml min^{-1} N_2. Retention times of cis-trans and trans-trans ABA-Me were 7.92 and 11.72 min, respectively. In eight randomly assigned extracts, UV irradiation was carried out after initial GLC-ECD, and the sample was rerun on GLC-ECD.

Exogenous application of ABA

In a greenhouse at Lethbridge, Alberta, hybrid maize plants (genotype Mo17 x B73) were grown as previously described. After emergence of the ligule of the third leaf (during the vegetative phase), daily applications of 0.0264, 0.264, 2.64, or 26.4 ug ABA (Sigma Chem. Co., St. Louis, synthetic mixed isomers ca 90% pure) in 0.1 ml 2% EtOH, 2% EtOH, or

pH 4.5 (with HCL) 2% EtOH were injected into the leaf whorl.

Commencing on application day 10, weekly measurements of height to the uppermost extended leaf tip and number of ligules emerged (leaf number) were recorded. Plants were observed daily to determine timing of tassel emergence from the whorl, anthesis (anther exertion) and silking, as well as to consider other morphological changes.

Results and Discussion

Growth

As previously observed (Chapter I.3.) the hybrid grew far more rapidly than either parental inbred under these environmental conditons (Figs. 16 and 18). The hybrid was taller and produced greater leaf areas at all harvests (Figs. 16, 17). 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 the heaviest (Fig. 18).

It had previously been suggested that heterosis for maize growth is the result of an initial advantage of larger hybrid embryos (5). However, in our study RGR and NAR of the hybrid were greater than those of parental inbreds, and we think it unlikely that initially larger embryos could account for increased RGR and NAR. Others have also concluded that superior hybrid growth cannot be explained simply in terms of an initial advantage of the embryo (130).

With the exception of a jog in the hybrid growth curve (Fig. 18) which led to a very low RGR value for the interval d 21 to 28, RGR of the hybrid exceeded that of either inbred (Table 24). Thus, dry weight increases per g dry weight were greater in the hybrid and growth rate was more rapid. A smiliar trend was observed with respect to dry weight per unit leaf area (NAR, Table 25). In all three genotypes, NAR values were highest during rapid shoot elongation (Table 25).

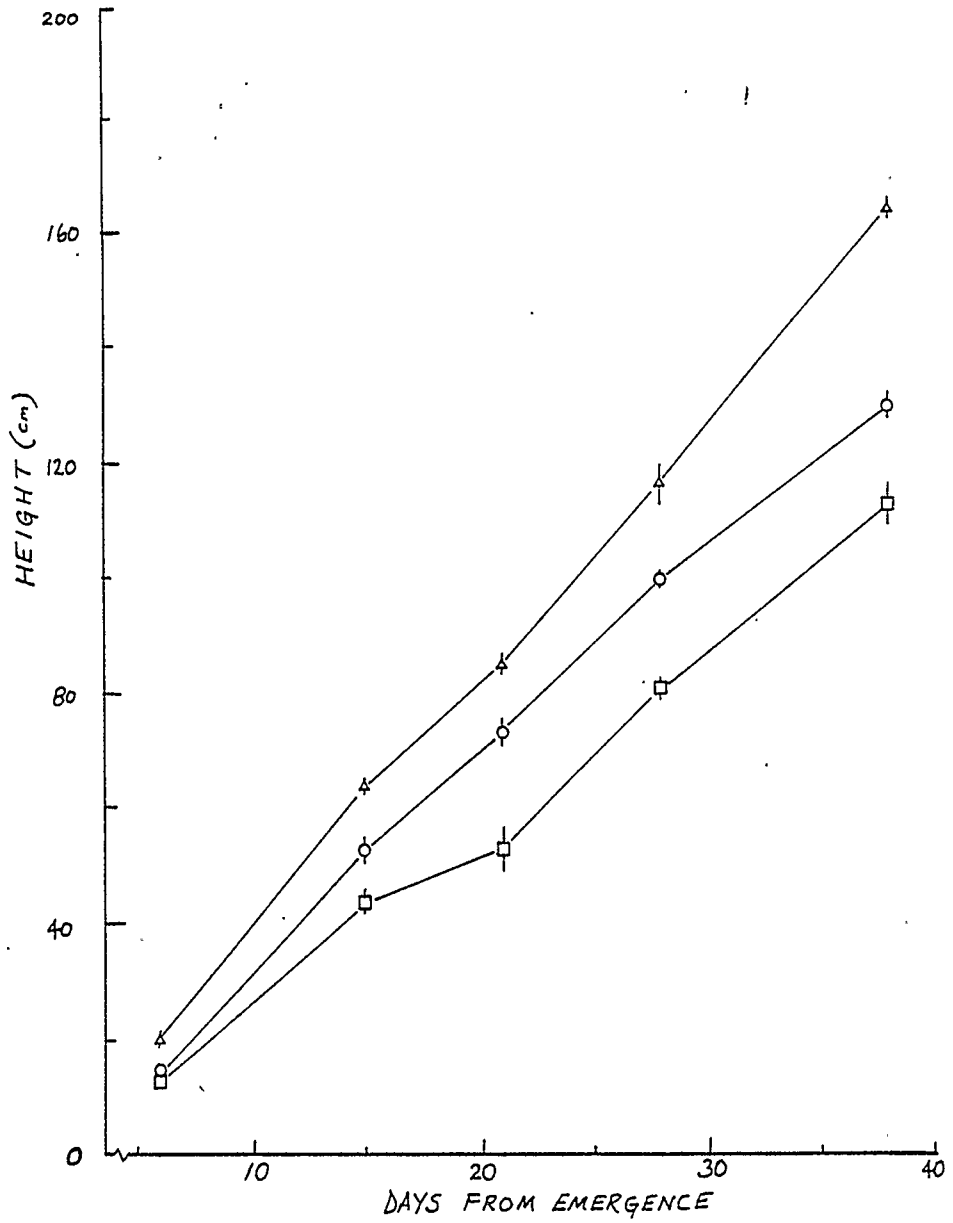


Figure 16. Plant height of two maize inbreds, CM7 (O) and CM49 (□), their F_1 hybrid, CM7 x CM49 (△) at five dates after seedling emergence in a controlled environment room with day/night temperature of 25/20°C. Vertical bars represent standard errors.

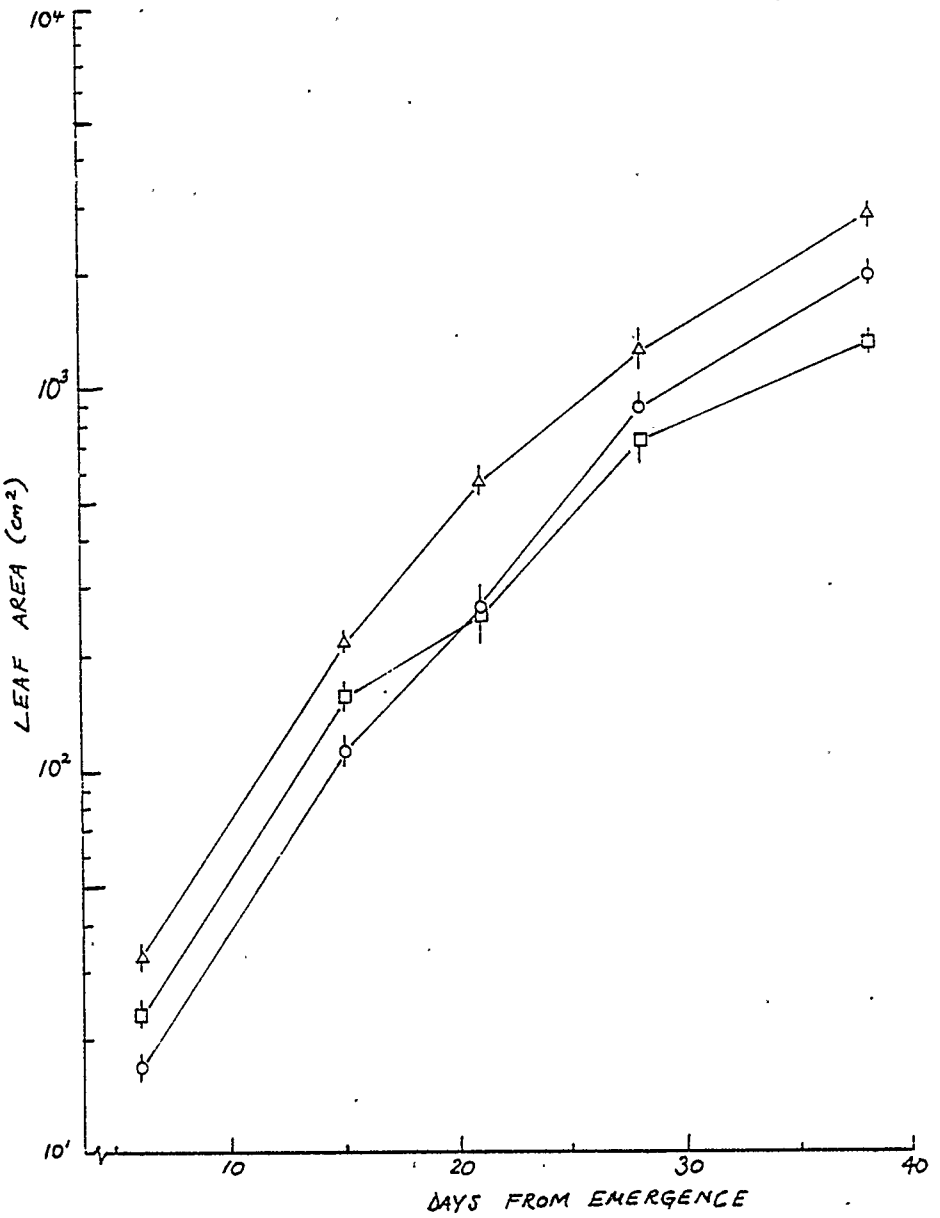


Figure 17. Leaf area (cm^2/plant) of two maize inbreds, CM7 (O) and CM49 (□), and their F_1 hybrid, CM7 x CM49 (Δ) at five dates after seedling emergence in a controlled environment growth room with day/night temperature of 25/20°C. Vertical bars represent standard errors.

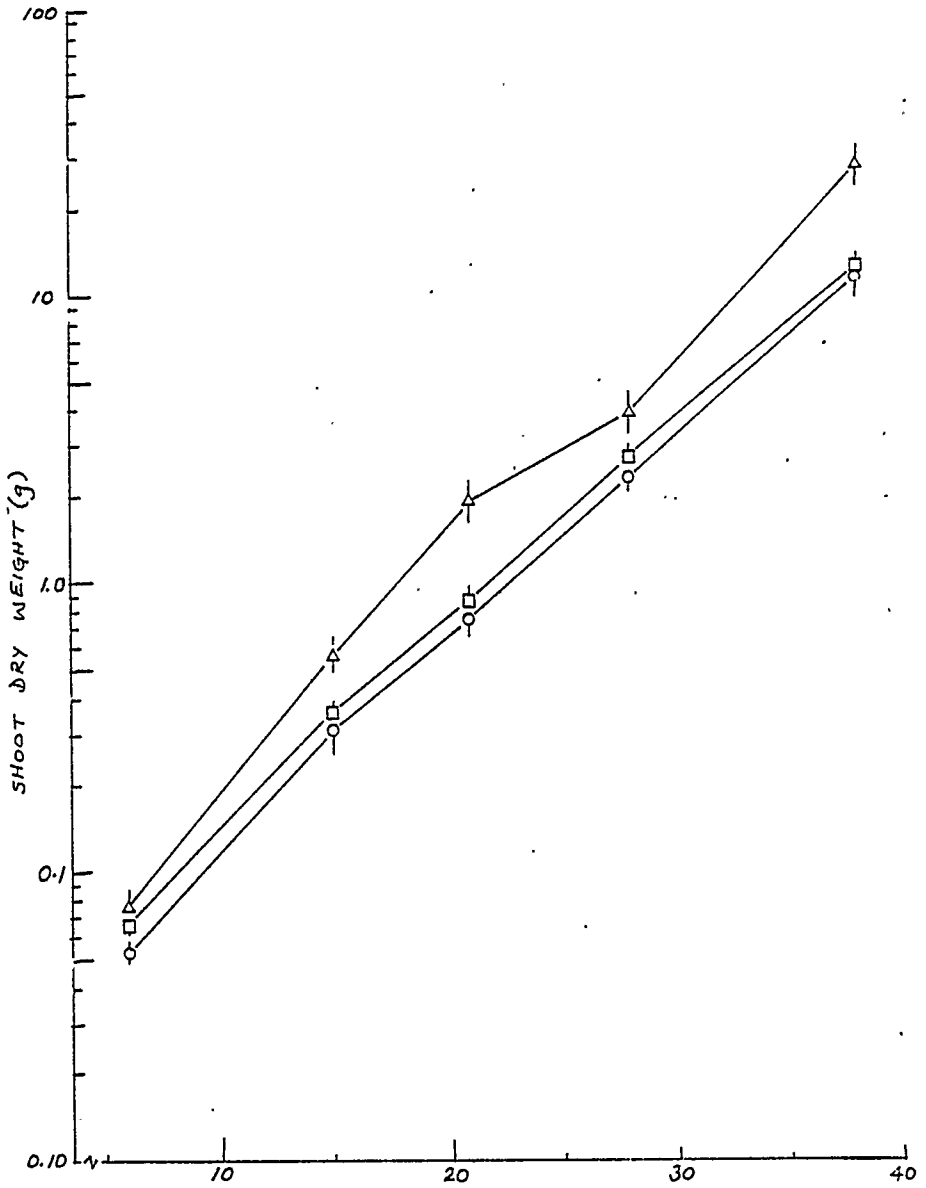


Figure 18. Shoot dry weight (g/plant) of two maize inbreds, CM7 (O) and CM49 (□), and their F_1 hybrid, CM7 x CM49 (Δ) at five dates after seedling emergence in a controlled environment growth room with day/night temperature of 25/20°C. Vertical bars represent standard errors.

Table 24. Relative growth rates (RGR) of two maize inbreds and their F_1 hybrid

	RGR (mg/g/day)				
	Time interval (days from emergence)				
	6-15	15-21	21-28	28-38	6-38
Inbred					
CM7	193	145	165	161	168
CM49	192	138	152	160	163
Hybrid					
CM7 x CM49	226	203	95	201	184

Table 25. Net assimilation rates (NAR) of two maize inbreds and their F_1 hybrid

	NAR ($mg/cm^2/day$)			
	Time interval (days from emergence)			
	6-15	15-21	21-28	28-38
Inbred				
CM7	0.532	0.402	0.507	0.677
CM49	0.378	0.451	0.459	1.051
Hybrid				
CM7 x CM49	0.585	0.641	0.510	1.315

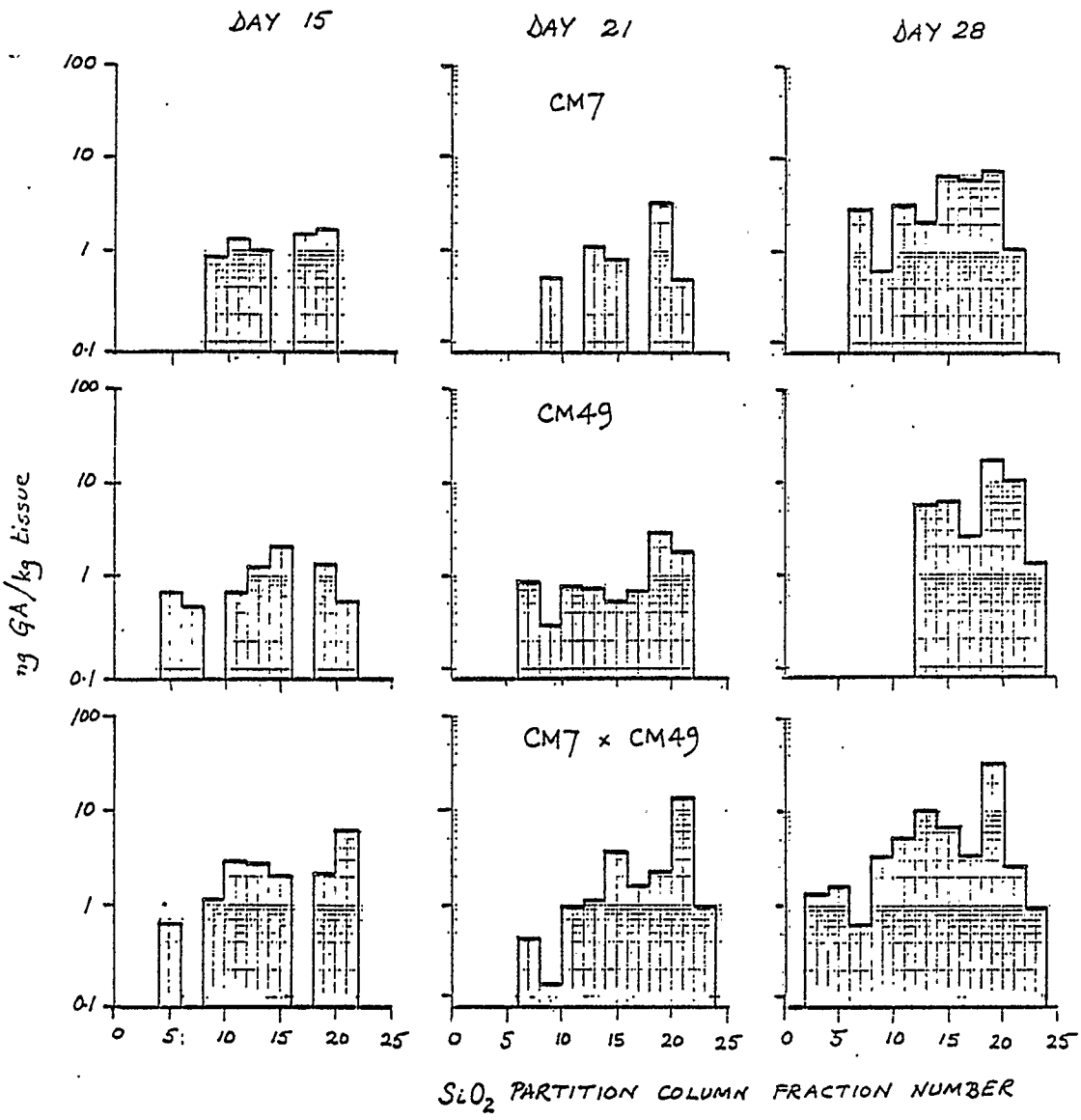


Figure 19. Elution profile of GA-like substances from apical meristem cylinders of two maize inbreds, CM7 and CM49, and their F_1 hybrid, CM7 x CM49, at three dates after seedling emergence.

Table 26. Levels of gibberellin-like substances in shoot apical meristem cylinders of two maize inbreds and their F_1 hybrid

	activity/cylinder (ng GA_3 equivalents)											
	Days from emergence											
	15 SiO_2 fraction				21 SiO_2 fraction				28 SiO_2 fraction			
	3-5	6-8	9-11	Total	3-5	6-8	9-11	Total	3-5	6-8	9-11	Total (fr 1-13)
Inbred												
CM7	0.9	1.8	1.7	4.3±1.4	1.0	2.7	5.0	8.7±1.7	7.0	7.7	5.5	25.3±6.0
CM49	2.0	3.5	4.8	10.2±1.7	1.0	3.2	4.1	8.3±1.4	4.6	20.2	12.2	39.9±2.8
Hybrid												
CM x CM49	3.8	6.1	6.0	16.2±2.4	0.5	8.6	13.6	22.7±1.8	8.0	27.2	53.1	92.0±19.4

Table 27. Specific activity of gibberellin-like substances in shoot apical meristem cylinders of two maize inbreds and their F_1 hybrid

	activity/kg tissue (µg GA_3 equivalent)		
	Days from emergence		
	15	21	28
Inbred			
CM7	78 a	6.2 a	19 a
CM49	100 a	8.2 a	20 a
Hybrid			
CM7 x CM49	109 a	22.9 b	29 a

Within a column, values followed by the same letter do not differ ($P > 0.05$)

Endogenous Gibberellin-like Substances

Three principal regions with GA-like activity eluted from the SiO_2 partition columns (Fig. 19). At least two of these consist of more than a single GA-like substance (Chapters III.1. and III.4.). Thus, at least five distinct, biologically active GA-like substances are present in the extract. However, since all three peaks existed in all three genotypes, and repetitive differences of quantities of GA-like substances in each region were not apparent (Fig. 19, Tables 26 and 27), we have presented total GA-like levels. At harvest 1, 2, and 3, which took place during the vegatative phase, just after tassel initiation and at the onset of rapid shoot elongation, respectively, the hybrid contained higher GA levels per cylinder than either parent (Table 26). When data were analyzed on a per g dry weight basis, the same trend existed, although differences were only significant at day 21 (Table 27). Although total GA activity per cylinder was highest at the onset of shoot elongation, GA activity per g was lower at this time than during the vegetative phase of growth (Table 27). However, a 10 cm cylinder containing the apical meristem as well as basal shoot tissue was harvested on d 28 while a shorter segment, probably containing a relatively larger proportion of apical meristem tissue, was harvested a d 15. Consequently, direct comparison between the two samples is confounded.

Endogenous Abscisic Acid

Since we did not add an internal standard of ABA at the initial extraction step, absolute levels of ABA cannot be estimated with any degree of precision. However, relative differences between extracts

can be noted if one assumes that degradation (losses during work up) will not differ appreciably from sample to sample.

No consistent differences in ABA content of the three genotypes were observed (Table 28 and 29). In all genotypes, ABA level per g dry weight was highest on d 21, right after tassel initiation (Table 29). It should be noted that had we quantified ABA simply by UV absorbance at the HPLC stage, we would have grossly overestimated ABA. Despite the purification on PVPP and charcoal:celite, and separation on SiO_2 partition columns, the extracts at the reverse-phase HPLC stage still contained numerous UV absorbing compounds (in addition to ABA) which co-chromatographed with ABA. The use of two UV detectors set at different wavelengths, and the on-line fluorimeter (ABA characteristically leads to a depression peak in the fluorescence trace) assisted in the recognition of these substances as contaminants. Hence, accurate ABA quantification of ABA in the maize seedling tissue analyzed, was not possible using a single reverse-phase HPLC separation system. Rather, additional (and ideally sequential) chromatographic separation on several diverse columns, as well as the use of different solvent systems (21,30), would be essential for HPLC quantification of ABA from this tissue. Further, since UV absorbance is relatively non-specific, while GLC-ECD is appreciably more specific, the latter must be considered to be the preferred means of ABA quantification.

ABA concentrations were low at the onset of rapid shoot elongation (Table 29). This observation may relate to the finding that ABA generally inhibited shoot elongation when applied exogenously to maize seedlings (Fig. 20). However, the possible role of ABA is unclear, since low levels of ABA caused a slight promotion of elongation in the seedlings (Fig. 20).

Table 28. Abscisic acid levels in shoot apical meristem cylinders of two maize inbreds and their F_1 hybrid

	ng ABA per cylinder		
	Days from emergence		
	15	21	28
Inbred			
CM7	0.86 a[1]	2.22 a	18.75 a
CM49	2.39 b	4.51 ab	18.33 a
Hybrid			
CM7 x CM49	1.26 a	6.70 b	22.86 a

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

Table 29. Specific activity of abscisic acid (ng per gram dry wt.) of two maize inbreds and their F_1 hybrid

	ng ABA per g tissue		
	Days from emergence		
	15	21	28
Inbred			
CM7	17.0 ab[1]	23.4 bc	14.3 ab
CM49	24.8 bc	39.1 c	9.5 a
Hybrid			
CM7 x CM49	8.4 a	37.6 c	7.8 a

[1]Values followed by the same letter do not differ ($P > 0.05$).

There are previous reports of both inhibition and promotion of elongation by ABA in dwarf maize (132), as well as the promotion by ABA of elongation (albeit small) of mesocotyl segments from normal maize (91).

Following ABA application, a previously unreported morphological change was also observed: barren tassels and the complete lack of anthesis (anther exertion) were noted in all plants receiving 2.64 or 26.4 ug ABA daily. Ear production and silking appeared to be normal in these plants. However, this does not mean that ABA specifically inhibits maleness, since application was directly onto the apical meristem, from which the male tassel develops. Indeed, an effect on the tassel might be expected since it was applied just prior to, during, and after tassel initiation. The high (26.4 ug) ABA concentration may have acted in a non-specific herbicidal manner, inhibiting growth and differentiation of the tissue contacted, a possibility supported by the additional observation that final leaf number of these plants never exceeded 15, while the mean leaf number of plants in other treatments was 19.2. Thus leaf initiation and/or development was also inhibited by the high ABA application.

The analysis of endogenous hormonal levels indicated that increased GA content was well correlated with hybrid vigor while ABA levels did not appear to be related to hybrid vigor or its absence. Apparent "overdominance" (performance of the hybrid outside of the performance range of parental inbreds) for growth rate as well as apparent GA levels were observed. The preceeding analysis of endogenous GA-like level are only correlative however, and taken alone, does not imply a causal association. However, other information exists and an integrative analysis suggests that a causal association is possible.

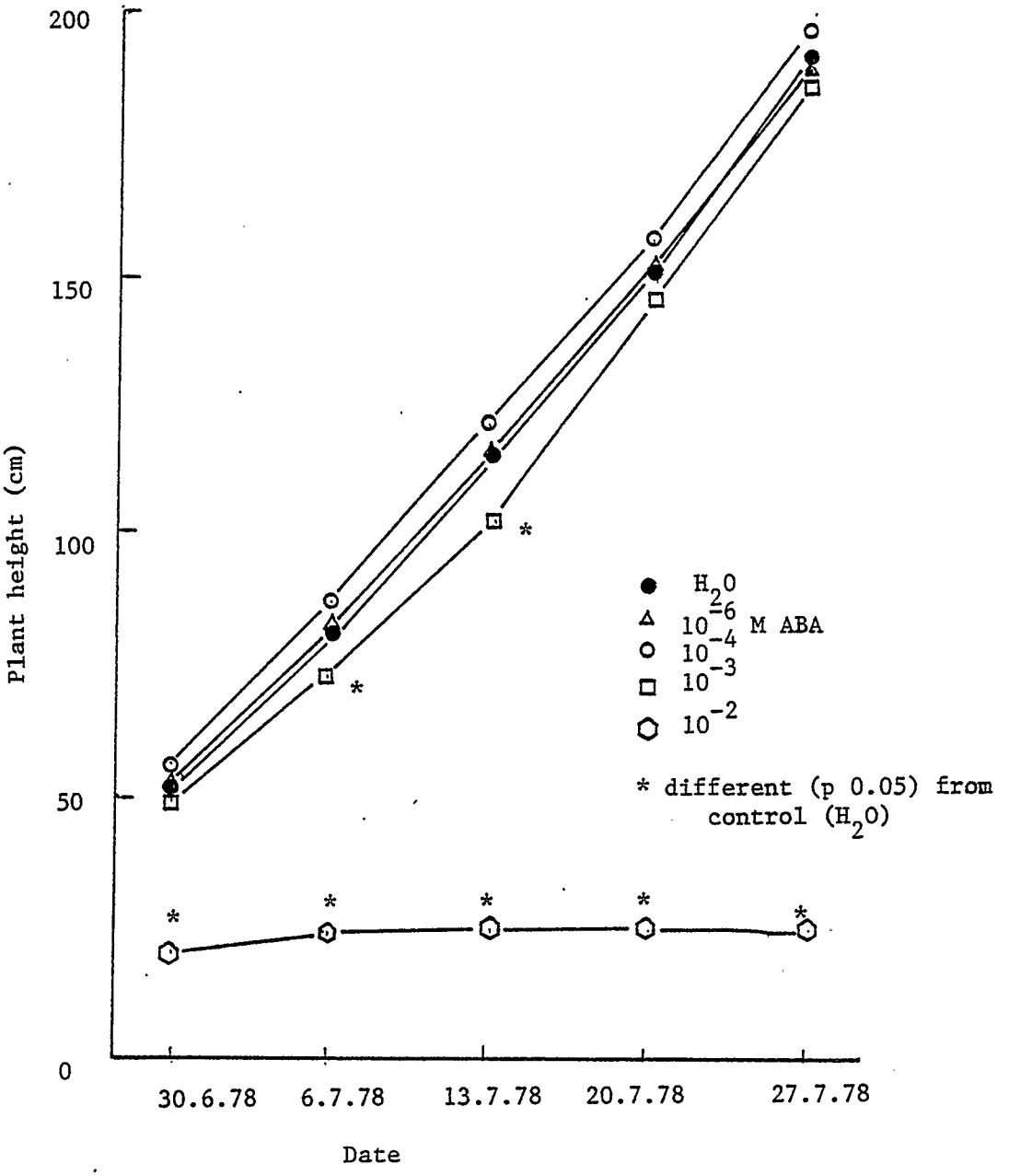


Figure 20. Shoot height of the single cross maize hybrid, Mo17 x B73, between 30 June 1978 and 27 July 1978 as influenced by exogenous applications of four ABA concentrations or water.

Five single gene maize mutants have been identified which are partially or almost totally deficient in GA-like substances (107). These genotypes are phenotypically dwarfed, and the dwarf habit can be overcome through exogenous GA application (107). Thus, a GA deficiency limits growth..

In normal genotypes, growth may also be promoted following exogenous GA application but only a high levels of treatment (20.56).

In normal genotypes, endogenous GA content is correlated with growth rate (Chapter III.1.), and low temperature reduces growth rate as well as endogenous GA content (Chapter III.1.). The same genotypes grown previously (Chapter III.1.) at low temperatures were grown in the present study under more favorable warmer temperature conditions. Growth rates were more rapid under these conditions and endogenous GA levels were higher. Thus, in a number of genotypes levels of GA-like substances are higher under conditions in which growth is rapid.

Taken collectively, these results suggest that endogenous GAs are an important factor regulating the growth rate of maize.

Perhaps most importantly, Nickerson (101,102) previously reported that maize inbreds are more responsive to exogenous GA_3 application than hybrids. Indeed, Nickerson further suggested that sensitivity to GAs was correlated with the degree of inbreeding in maize. This finding is consistent with our observation that inbreds may contain lower levels of endogenous GAs. Hence, the inbreds would be expected to be more responsive to exogenous application. Further, the sensitivity of the inbreds suggests that the low endogenous GA content is a major factor limiting growth. Thus, it is possible that the observed apparent "over-

dominance" for increased GA production may underly at least certain aspects of hybrid vigor in maize.

III.3. Gibberellins and sexuality in maize: changes of endogenous GA-like substances with feminization

Abstract

In developing apical meristems of corn (Zea mays L.) the level of acidic, ethyl acetate-soluble GA-like substances increased to a maximum of 108 ug GA_3 equivalents kg^{-1} dry weight of tissue at inflorescence initiation, and then fell rapidly. At anthesis only a trace (0.2 ug kg^{-1}) of GA-like acitivity remained in the apical (male) inflorescences, while moderate activity (32 ug kg^{-1}), mostly of a non-polar nature, was present in lateral, female, inflorescences.

A sex reversal of the apical inflorescence, from male to female, was elicited by reducing the ambient light intensity. Higher levels of GA-like substances, particularly those eluting from a SiO_2 partition column in the non-polar region, were observed at all harvests in the reverting meristems; levels increased to 180 ug kg^{-1} at inflorescence initiation, then dropped to 122 ug kg^{-1} in the apical (female), reverted meristems. This increase in endogenous GA-like activity with reversion to the female inflorescence is consistent with obersvations that (a) reversion can be obtained with exogenous application of GA_3, and (b) maleness in the ears is enhanced in dwarf mutants of maize apparently deficient in endogenous GAs. Endogenous GAs may thus play a key role in the control of sexuality of corn.

Introduction

Along with an increase in plant height, one of the major morphological changes in maize following exogenous application of GA_3 is an alteration of sexuality. Male florets become sterile and functional female florets develop in the apical inflorescence, or tassel, where normally only male flowers would have developed (58,99,101). The quantity of exogenous GA_3 required to produce a sex reversal is dependent on genotype (101,102) and is only effective in altering sexuality if applied prior to microspore meiosis (58). Results from a number of studies are consistent, however, and suggest that exogenous GA_3 reduces maleness and promotes femaleness in the apical inflorescence of maize (58,99,101,102).

A relationship between endogenous GAs and sexuality is also suggested by the behavior of certain single gene maize mutants. In certain dwarf mutants ($\underline{du}_1$, $\underline{d}_1$, $\underline{d}_2$, $\underline{d}_3$, $\underline{d}_5$), male florets are observed in the lateral inflorescence, or ear, which is normally entirely female (100). The mutants $\underline{d}_3$, $\underline{d}_5$ and $\underline{an}_1$ appear to be GA deficient, while the levels of endogenous GA-like substances in $\underline{d}_1$ and $\underline{d}_2$ are less than half the normal level (107).

A sex reversal of the apical inflorescence can be observed in normal maize grown in northern greenhouses in winter (123), or under cool temperatures (115), the former reversal being prevented if low intensity illumination is provided (115). This reversal was thought to be a true photoperiodic reaction, rather than the result of an alteration in photosynthate production (96).

As the environmental and GA_3-elicited sex reversals are morphologically similar, it seemed possible that the environmentally induced

sex reversion would be accompanied by an alteration in levels of endogenous GAs. In this study, changes in endogenous GA-like substances of the apical meristem during normal development and during an environmentally-induced sex reversal are examined. The environmental control of sex reversal, with particular reference to the importance of light intensity, rather than photoperiod, is also considered.

Material and Methods

Siz maize inbreds, 66A4-2, 66D34-1, 7275-13-1, CG11, W103, and WD were grown in a greenhouse under natural light at Lethbridge, Alberta (Lat. 50°N) during December, 1978 and January, 1979. Effective natural photoperiod, determined by adding 1 h to the daylength (40), was 9.1 h during the interval prior to floral initiation (123). At flowering, degree of sex reversal of the apical inflorescence was recorded on the following scale:

1. No reversal. Tassel branching profuse, only male florets found in the apical inflorescence.
2. Tassel branching reduced, some male sterility.
3. Barren tassels. Little or no tassel branching, no male or female florets appearing.
4. Inflorescences emerging from the leaf whorl, female flowers appearing.
5. Complete reversal. Inflorescenes remaining in the leaf whorl, silks (styles) visible.

The inbred of choice, the early-maturing, profusely tillering 66A4-2 was subsequently grown in a mixture of soil, sand and peat under 20/15°C (day/night, 5°C rise/fall h^{-1}) and an 8 h photoperiod at either high (968 uE m^{-2} s^{-1}) or low intensity (323 uE m^{-2} s^{-1}) using 30 cool white flourescent tubes and 45 incandescent bulbs, or 10 tubes and 15 bulbs, respectively. At harvest (Table 30), cylinders of the meristem were excised from the stem base and immediately frozen with dry ice, lyophilized, and the outer tissue then teased away from the apical meristematic cone.

Meristematic tissue was then extracted at 0°C in MeOH:H_2O (80:20)

Table 30. Age, phenological stage, weight, and number of meristems used in GA extractions of maize tissue.

Harvest No.	Plant age (Days after emergence)	Phenological Stage	Normal Development (high light intensity) Number of meristems	Normal Development (high light intensity) Dry weight (g)	Sex Reversal (low light intensity) Number of meristems	Sex Reversal (low light intensity) Dry weight (g)
1	10	Vegetative	200	3.3	210	2.2
2	17	Tassel initiation	103	6.0	101	2.5
3	24	Microspore meiosis	96	9.9	48	1.3
4 (apical male)	39	Anthesis[1]	25	19.8	36	0.9
4 (lateral	39		7	5.0		

[1]anthers extruding from glumes.

using 20 ml MeOH g^{-1} tissue (75,78). Two ml of phosphate buffer (pH 8.0, 0.5 M) was added for each 10 ml of aqueous MeOH, and the solution was taken to the aqueous phase in vacuo at 35°C. The pH was adjusted to 9.0 with KOH, and the aqueous phase was partitioned 3 x against diethyl ether, the ether phase being discarded. The aqueous phase after adjusting to pH 3.0 with HCl was extracted 5 x with EtOAc. This acidic, EtOAc-soluble fraction was purified on columns of Polyclar AT (PVPP) (53), on charcoal:celite 545 (1g:2g) columns eluted with acetone: H_2O (80:20) (32,152). Chromatography of GAs was accomplished on gradient-eluted Woelm SiO_2 partition columns (30,109). Varigrad chambers 1 to 4 contained 50:50, 65:35, 85:15, and 100:0 formic acid saturated EtOAc: hexane (w/w), respectively. The 26 collected fractions were assayed at serial dilutions of 1/100, 1/200, and 1/400 for GA-like activity on a modified (78) Tanginbozu dwarf rice assay (97). Levels of GA-like substances were estimated by comparison with a GA_3 standard curve (e.g., GA_3 equivalents).

Biologically active non-polar (fractions 4 to 7) and polar (fractions 14 to 17) SiO_2 partition column fractions were subsequently bulked, re-run on a second SiO_2 partition column, bioassayed (78), and the active fractions then chromatographed using reverse-phase HPLC with polyethylene radial-PAK A cartridges packed with uBondapack C_{18}, in a model 100 Radial Compression Module (Waters Assoc.). Samples for HPLC were dissolved in MeOH and filtered through 0.5 um FH Millipore filters before injection onto the column. A linear gradient from 10 to 70% MeOH (aqueous MeOH with 1% acetic acid) was programmed for 15 min at a flow rate of 4 ml min^{-1}, beginning 500 s after sample injection; 50 s (3.33 ml) fractions were collected. Three ml of MeOH was added to each of these fractions which

were dried *in vacuo* prior to bioassay (78).

Results and Discussion

Under the high light intensity 8 hour photoperiod, inbred 66A4-2 showed no signs of sex reversal; profuse tassel branching and functional male florets were observed in the apical inflorescence. Under winter greenhouse conditons an incomplete sex reversal was observed in 66A4-2 (Table 31); apical inflorescences were totally female, but emerged from the leaf whorl. Sex reversal under greenhouse conditons was incomplete in two other inbreds as well: 66D34-1 and 7275-13-1 (Table 31). Reversal in 66D34-1 was least complete: tassel branching was absent, but stamens were functional and ovules absent in the apical inflorescence. The greenhouse sex reversal was complete in the 5 other inbreds examined (Table 31).

Under low light intensity and 8 h photoperiod in growth chambers 66A4-2 underwent a complete sex reversal; only ovules were observed in the apical inflorescence which remained in the leaf whorl. Thus, sex reversal in this day neutral inbred appears to be primarily a function of light intensity.

Distinct changes occur with time in the acidic, EtOAc-soluble GA-like substances in apical meristems of normally developing maize plants (Figure 21, Table 32). Three regions of GA-like activity were observed at most harvest times, eluting in fractions 4 to 7, 8 to 13 and 14 to 18. Total GA-like activity in the meristems rose to a maximum at tassel initiation (day 17) and then fell rapidly (Figure 21). At anthesis, only a trace of GA-like activity was observed in the male apical inflorescences while GA-like activity, particularly in the nonpolar region where many GAs with only one hydroxyl elute (30) was high in the lateral

Table 31. Developmental characteristics of seven short-season maize inbreds.

Inbred	Greenhouse sex reversal[1]	Basic vegetative phase (days)[2]	Photoperiod sensitivity (days delay hr^{-1} increase)
66A4-2	4	35.7	0.10
66D34-1	2	56.9	0.03
7275-13-1	4	49.6	1.27
CG11	5	46.0	0.75
W103	5	49.5	1.05
WD	5	58.1	1.40
CG8[3]	5	--	--

[1] Reversal is based on a 1 to 5 scale, 5 indicating complete sex reversal of the apical inflorescence to the female form.

[2] See chapter "Responses of early maize inbreds to photoperiod".

[3] Photoperiod sensitivity and BVP unknown.

inflorescences in which only ovules were present.

In apical meristems of plants of inbred 66A402 undergoing a sex reversal, there were also three regions of GA-like activity (Figure 21). Since the intermediate peak, occurring in fractions 8 to 13, was absent in 4 of the 9 harvests, it is possible that this peak represents GAs which were rapidly metabolized, possibly to the more polar GAs (e.g., fractions 15+) under certain conditions.

Levels of GA-like activity were higher in the reverting meristems (e.g., days 24 and 39, Table 32, days 10, 17, 24 and 39, Figure 21) than in normally developing meristems. At day 10, at which time the meristems still appear to be vegetative, overall GA-like activity of the reverting meristems was only slightly higher than that of normal meristems, although activity of the non-polar region of reverting meristems was almost three-fold greater than for normal meristems (Figure 21). At tassel initiation (day 17), overall activity of the reverting meristems was higher than normal meristems, principally due to a five-fold increase in non-polar GA-like substances. Since biological activity in the dwarf rice bioassay of many non-polar GAs which elute in this region, such as GA_4, GA_7 and GA_{20}, is far below an equivalent amount of GA_3 (25), these GA-like substances in the meristem extracts probably exceeded the estimates shown in Figure 21 and Table 32.

At day 24, GA-like activity of reverting meristems was almost 100 times that of normal meristems (Figure 21). Phenologically, this harvest took place as floral differentiation was underway, about when microspore meiosis was beginning in the normal tissue (58). Since both male and female primordia are initiated in apical and lateral

Figure 21.

Changes in GA-like substances (as determined by bioassay in serial dilution on dwarf rice cv. Tan-ginbozu) from maize apical meristems, during normal development and during a sex reversal (feminization) of the apical inflorescence. Values above peaks represent total activity of all fractions within a region.

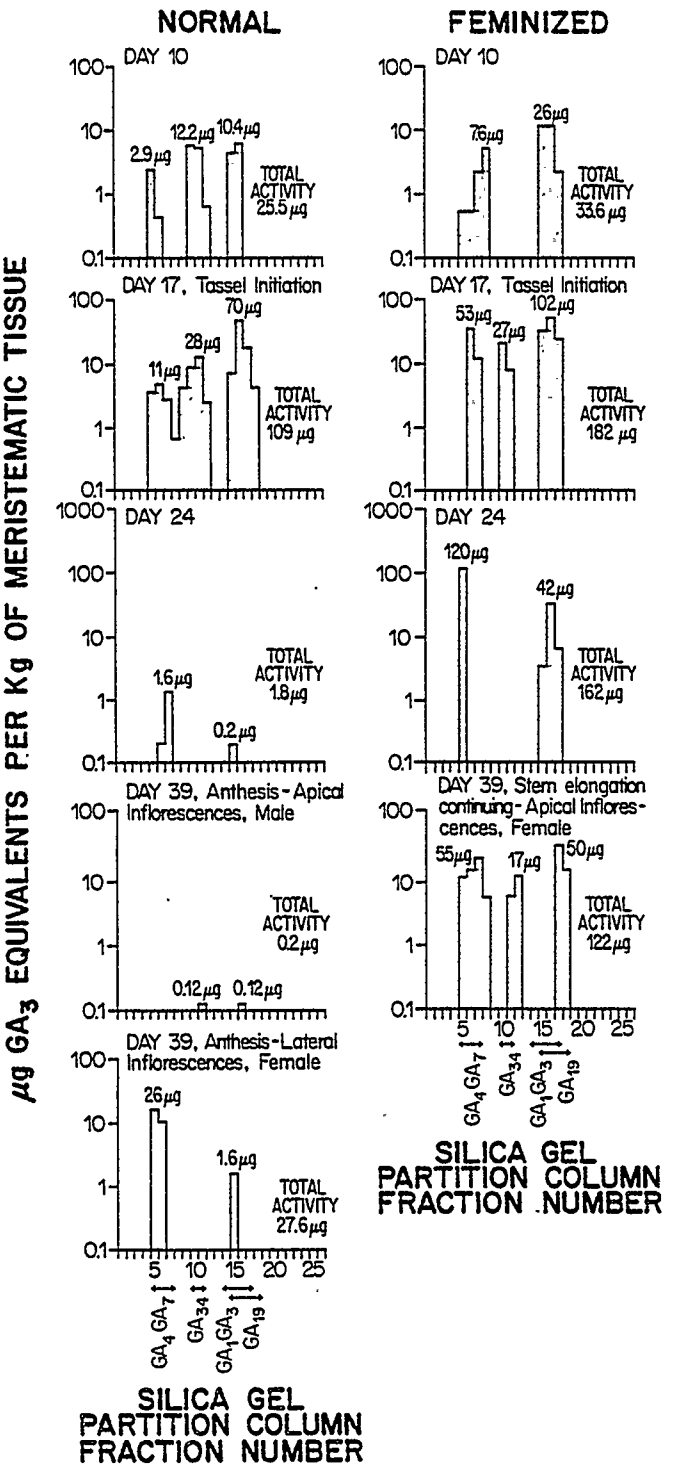

NORMAL
FEMINIZED
DAY 10
2.9μg
12.2μg
10.4μg
TOTAL ACTIVITY 25.5μg
7.6μg
26μg
TOTAL ACTIVITY 33.6μg
DAY 17, Tassel Initiation
11μg
28μg
70μg
TOTAL ACTIVITY 109μg
53μg
27μg
102μg
TOTAL ACTIVITY 182μg
DAY 24
1.6μg
0.2μg
TOTAL ACTIVITY 1.8μg
120μg
42μg
TOTAL ACTIVITY 162μg
DAY 39, Anthesis-Apical Inflorescences, Male
0.12μg 0.12μg
TOTAL ACTIVITY 0.2μg
DAY 39, Stem elongation continuing-Apical Inflorescences, Female
55μg
17μg
50μg
TOTAL ACTIVITY 122μg
DAY 39, Anthesis-Lateral Inflorescences, Female
26μg
1.6μg
TOTAL ACTIVITY 27.6μg
μg GA3 EQUIVALENTS PER Kg OF MERISTEMATIC TISSUE
GA4 GA7
GA34
GA1 GA3
GA19
SILICA GEL PARTITION COLUMN FRACTION NUMBER
SILICA GEL PARTITION COLUMN FRACTION NUMBER

Table 32. Changes in GA-like activity (ng GA_3 equivalents per 100 meristems) with development[1] in normal and sex reverted maize meristems.

SiO_2 Partion Column Fraction	Normal					Reverted (feminized)			
	10d	17d	24d	39d (male)	39d (female)	10d	17d	24d	39d
3 to 7	5	64	16	0	1846	8	12	315	136
8 to 13	20	164	0	8	0	0	64	0	42
14 to 18	17	410	2	8	114	28	247	110	124
Total	42	639	19	16	1969	36	440	425	302

[1]Days after emergence, 17d = tassel initiation.

inflorescences, it is the control of development and abortion, rather than initiation, which determines sexuality (96). The 100-fold increase in GA-like activity at day 24 may thus have occurred at a time which is critical to the sex reversal.

Since lateral inflorescences are initiated long after apical inflorescences (129), a comparison between differentiating reverting apical meristems at day 24 and normal female lateral meristems at day 39 may be valid. On a dry-weight basis, the reverting (female) meristems were higher in GA-like substances (Figure 21) while on a per meristem basis, the normal lateral (female) meristems contained about 5 times the level of GA-like substances of the reverting meristems (Table 32). This greater activity on a per meristem basis reflected the increased size of the lateral meristems (relative to apical, reverted meristems), both in terms of dry weight (Table 30) and number of florets produced. Only 4 to 17 ovules were produced in the reverted meristems, probably as a consequence of the low light intensity. Although quantitative differences existed between these two meristems in terms of overall activity, the ratio of non-polar : polar GAs was very similar (Figure 21).

A 500-fold difference in the level of GA-like activity between the normal and reverted apical inflorescences was observed at the final harvest (day 39). However, since the reverted plants were still elongating on day 39, some of the increased activity in their meristems may be due to GA-like substances related to elongation rather than ovule development.

Definitive characterization of a number of GAs from maize has been reported (108). Biological activity eluting in the non-polar region

(fractions 3 to 7) and polar region (fractions 14 to 17) of the SiO_2 partition column (Figure 21) co-chromatographed with [^{3}H]-GA_4 and [^{3}H]-GA_1, respectively. In turn, GA_4, GA_7 and GA_{20} are eluted together, as are GA_1, GA_3, GA_{29}, and GA_{19} from the SiO_2 partition column (30). When fractions 3 to 7 from SiO_2 partition columns were run on reverse phase HPLC two biologically active fractions eluted, one in fractions 18-19 co-chromatographed with [^{3}H]-GA_4, the other eluted several fractions later. Fractions 14 to 17 from the SiO_2 partition column were also run on reverse phase HPLC and yielded two peaks of biological activity, both highly active over a range of serial dilutions. One eluted with [^{3}H]-GA_1, the other eluted in fractions 16-17, between [^{3}H]-GA and [^{3}H]-GA_4, behavior which is consistent with GA_{19} (e.g., GA_{19} is active on dwarf rice (25), elutes in the polar region of SiO_2 (30) and between GA_1 and GA_4 on reverse phase HPLC (73)).

Although quantities of GA-like substances were too small for definitive characterization, some tentative conclusions can be drawn from chromatographic behavior. Firstly, the major non-polar grouping could include GA_4 or GA_7, but probably did not include GA_{20}. Secondly, the major polar grouping may have contained GA_1 or GA_3 and GA_{19}. Gibberellins A_1, A_3, A_4, A_7 and A_{19} have been characterized from various members of the Graminae, and GA_1 and GA_{19} are probably native in maize (38, 78, 81, 98, 108).

Thus, large increases in GA-like activity, particularly of a non-polar nature, were observed in apical meristems undergoing a sex reversal. In normally developing plants GA-like activity of the apical (male) inflorescence decreased after inflorescence initiation, while

GA-like activity of the reverting (female) meristems remained high. It has long been known that exogenous GA_3 promoted femaleness in maize (102) and that maleness predominates in dwarf genotypes (100), most of which appear to be deficient in endogenous GAs (107). Our results, together with this information, strongly suggest that GAs are involved in the control of sexuality in maize. High GA levels, particularly GAs of a non-polar nature, favor femaleness, while low GA level favors maleness.

IV. 1. Metabolism of tritiated gibberellins A_{20} and A_4 in maize.

Abstract

After the application of high specific activity [^{3}H]-GA_{20} to 21 day old maize plants, etiolated maize seedlings or maturing maize cobs, a number of [^{3}H]-metabolites were observed. The principle EtOAc-soluble metabolite was tentatively identified as [^{3}H]-GA_1 on the basis of SiO_2 partition chromatography, high resolution isocractic elution reverse-phase HPLC-RC and GLC-RC. Other acidic EtOAc-soluble metabolites were tentatively identified as [^{3}H]-GA_8 and C/D R[^{3}H]-GA_{20}. Numerous BuOH soluble, acidic EtOAc-insoluble conjugate-like metabolites were observed and "identified" by retention times on reverse-phase C_{18} HPLC-RC as "conjugates" of [^{3}H]-GA_{20}, [^{3}H]-GA_1, and [^{3}H]-GA_8. Following a [^{3}H]-GA_4 feed, the principle metabolite was also [^{3}H]-GA_1-like and numerous conjugate-like metabolites were also observed. The direction and rate of metabolism of [^{3}H]-GA_{20} was influenced by developmental stage, time of incubation and tissue studied. Conversion to [^{3}H]-GA_1 was greatest (23% of the MeOH extractable dpm) in 21 day old maize plants. In etiolated maize seedlings C/D R [^{3}H]-GA_{20} was the major acidic, EtOAc-soluble metabolite (7%) while conversion to [^{3}H]-GA_1 was low (4%). In mature maize cobs the level of [^{3}H]-GA_1 conjugate-like compounds was high (27%) and very little acidic, EtOAc-soluble [^{3}H] remained (5%). Mature kernels contained lower levels of conjugate-like compounds (65%) which were principally conjugates of the precursor [^{3}H]-GA_{20}.

Introduction

Hedden et al. (65) have recently reported the characterization by GC-MS of gibberellins A_{53}, A_{44}, A_{17}, A_{19} and A_{20} from immature maize tassels. Further, data from single ion current monitoring (SICM) GC-MS indicated that gibberellins A_1, A_{29} and A_8 are probably also native in maize (65). These GAs are members of the probably early 13-hydroxylation pathway in maize, a pathway which begins with gibberellin A_{12}-aldehyde. Since the $\underline{d}_1$ mutant of maize responds only to the exogenous application of GA_1 and not to the precursor GA_{53} or GA_{20}, Phinney (personal communication) has concluded that the metabolic block in $\underline{d}_1$ prevents the conversion of GA_{20} to GA_1. He further suggests that GA is biologically active only by its conversion to GA_1. Thus, GA_1 is the only biologically active GA in the presumptive pathway in maize. Phinney suggests that biological activity of the other exogenously applied maize GAs in the dwarf maize bioassays is achieved via conversion to GA. (However, Crozier et al. (25) reported that GA_{20} has low activity in the $\underline{d}_1$ bioassay while GA_1 shows no activity in the $\underline{d}_2$ bioassay).

In other plant systems two principal GA metabolites are produced following the application of $[^3H]$-GA_{20}: $[^3H]$-GA_1 and/or $[^3H]$-GA_{29} (36,45,82,112,147). Since these GAs appear to be native in maize, a branch point in the metabolic pathway may exist after GA_{20} (65). The biologically active GA_1 may be produced through 3 hydroxylation or alternately, 2 hydroxylation may yield the biologically inactive GA_{29}. In maize, GA_1 is converted to the biologically less active GA_8 through 2 hydroxylation (26) and a number of potential conjugates exist for most of the GAs in the pathway. Thus, the branch point following GA_{20}

may be an important site of regulation of levels of the biologically active GA(s) and hence should be considered a potential control point. Thus, metabolism of [^{3}H]-GA_{20} was examined during this research project.

Additionally the metabolism of [^{3}H]-GA_4 was investigated. Gibberellin A_4 is native in the cereals Avena sativa (106) and Oryza sativa (81) and in rice the presence of both GA_4 and GA_{34} and the principal GA, GA_{19}, indicates that both the early C-3 and early C-13 hydroxylation pathways exist (81). Even though GA_4 may not be native to maize, it is often an effective precursor of GA_1 (112, 147). Hence, the control of GA_4 metabolism in maize may provide additional hints at control points in the regulation of effective GA level.

Materials and Methods

The metabolism of [^{3}H]-GA_{20} was investigated in maturing maize cobs, husks and kernels, in etiolated maize seedlings, and in 21 day old maize plants. The metabolism of [^{3}H]-GA_4 was investigated in maize cobs and maturing maize kernels only.

Plant Material

1. 21 day old maize plants

Three kernels of the early maturing maize (Zea mays L.) hybrid CM7 x CM49 were planted in each 13 x 20 cm plastic pots filled with a mixture of peat moss and sand. Pots were placed in a growth room in which a 25/15°C (day/night) temperature regime was maintained. After emergence pots were thinned to one plant. Twenty-one days after planting (at which time the plants were still in the vegetative growth phase) 0.22 uCi (about 5 x 10^5 dpm) [^{3}H]-GA_{20} (2.36 Ci/mmol) in 0.4 ml 60% aqueous ethanol was pipetted into the leaf whorl. Twenty-four, 48, 96 and 144 h after the addition of the [^{3}H]-GA_{20} three plant shoots were excised at the soil surface and 4 cm above the surface yielding shoot cylinders which contained the apical meristems. These were rinsed with H_2O and then homogenized in 80% aqueous MeOH (cooled to -40°C).

2. Maturing maize cobs and husks

Plants were raised in field conditions and [^{3}H]-GA_{20} or [^{3}H]-GA_4 was injected into the immature cobs (see Chapter IV.2.). After black layer maturity was reached (27) cobs were harvested and air dried. Husks and kernels were removed and the cobs and husks were frozen, lyophilized, and then homogenized as above (50 ml per g tissue). Three cobs were bulked for analysis of metabolites.

3. Maturing maize kernels

The preparation, harvesting and analysis of [^{3}H]-GA_{20} metabolites in mature maize kernels is described in Chapter IV.2. and only relevant points of comparison will be brought into this section.

4. Etiolated maize seedlings

Mature seeds of the hybrid DK 23 were placed pericarp side down in Petri dishes containing 2 filter paper discs and 5 ml H_2O. Dishes were wrapped in aluminum foil and stored at 22°C for 72 h. Under a green safelight one uCi [^{3}H]-GA_{20} in 30 ul 50% aqueous ethanol was pipetted onto the germinated embryo after the seedling had been turned over (pericarp up). After 16 h incubation in the dark, the seedling was excised from the seed remnant and homogenized in 5 ml -40°C 80% aqueous MeOH.

Extraction, purification and chromatography of [^{3}H]-GA metabolites was performed as outlined in Figure 22. Purification using PVPP was omitted for samples from maize kernels and etiolated maize seedlings. Partitioning against diethyl ether was only performed on the 21 day old seedling tissue and husks, samples which contained moderate amounts of chlorophyll.

Gradient elution SiO_2 partition chromatography has been described previously (30, 109). Forty 10 ml fractions were collected rather than larger fractions previously used, enabling higher resolution. Gradient elution reverse-phase HPLC and isothermal GCL-RC have also been previously described (80). The isocratic elution reverse-phase HPLC-RC was carried out with the HPLC previously described (80) using a Whatman

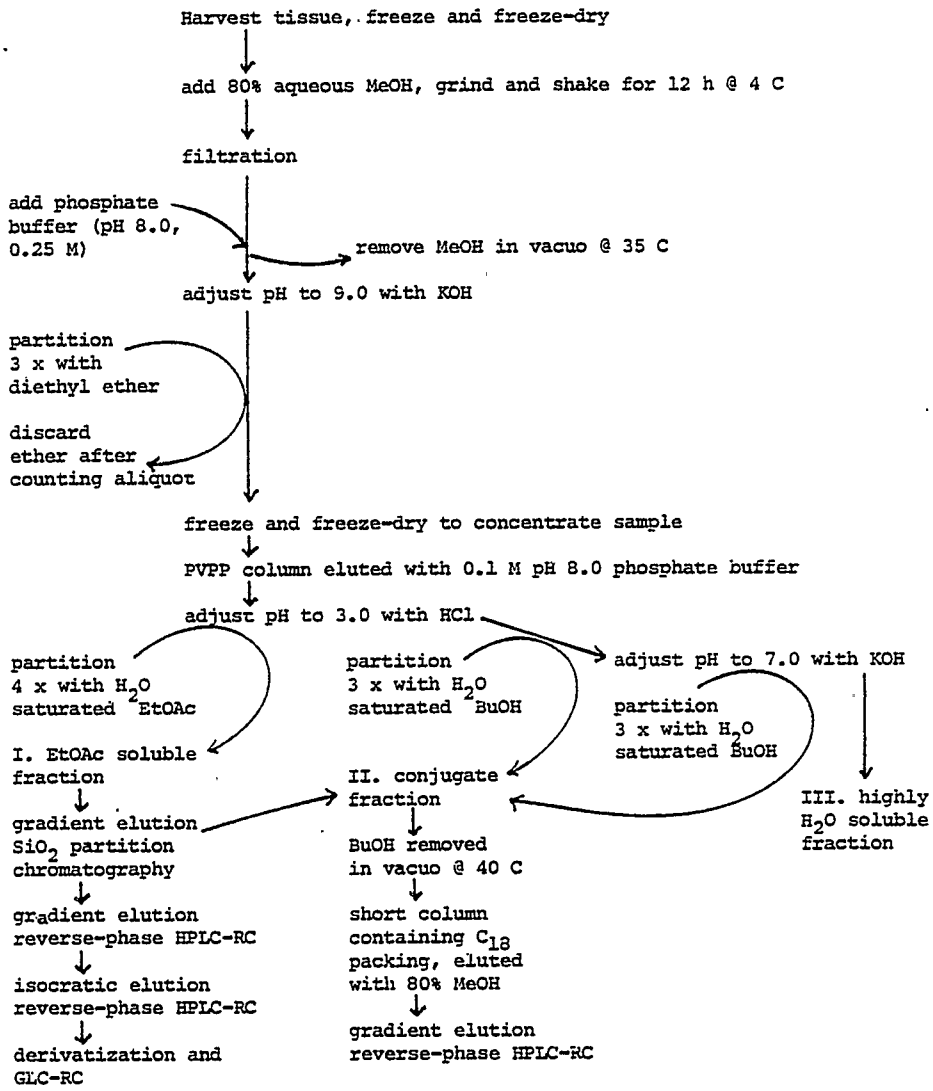


Figure 22. Flow diagram of extraction, purification and chromatography used for analysis of ^{3}H -GAs and ^{3}H -GA metabolites in maize tissue.

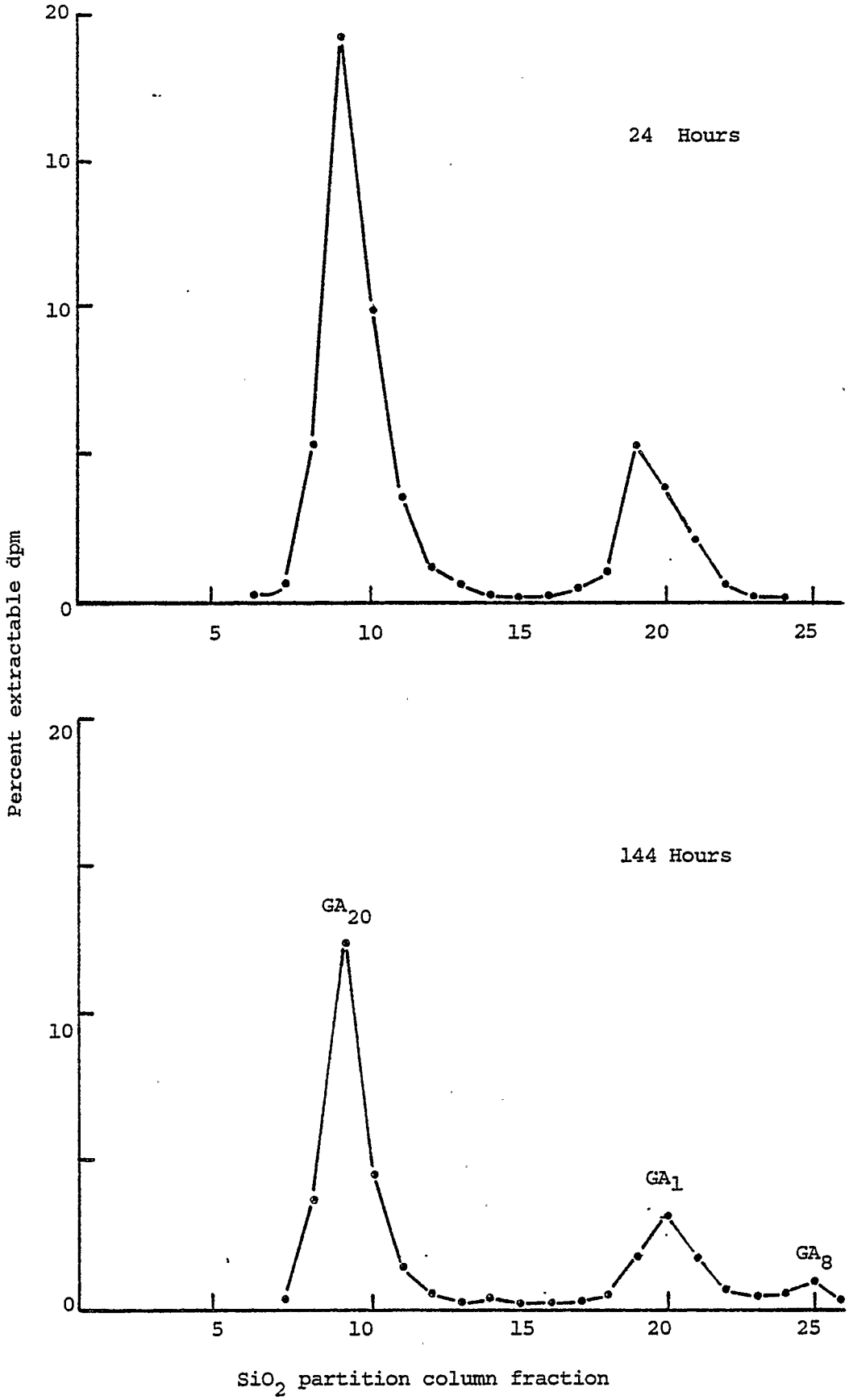
M9 Partisil 10 ODS 2 column (9.4 mm x 50 cm). Eluant was 62.3: 37: 0.7 H_2O: MeOH: Acetic Acid (v: v: v) at 1.8 ml min^{-1}. After HPLC-RC, samples were converted to the MeTMSi derivatives prior to sample injection on GLC-RC.

Results and Discussion

Following the [^{3}H]-GA_{20} feed to the 21 day old maize plants, three regions of radioactivity eluted from SiO_2 partition columns loaded with the EtOAc-soluble fraction (Figure 23). Twenty-four h after addition of the [^{3}H]-GA_{20} most of the [^{3}H] co-chromatographed with authentic [^{3}H]-GA_{20} while a second peak eluted in the more polar region coincidental with [^{3}H]-GA_1. At later harvests a third, even more polar peak eluted which was coincident with authentic [^{3}H]-GA_8 (Figure 23). A less polar shoulder on the [^{3}H]-GA_{20} peak which could have represented C/D R GA_{20} was not observed from these samples.

While there was a rapid initial metabolism of [^{3}H]-GA_{20} (Figure 24) it leveled off after about 48 h. Due to the anatomical organization of the leaf whorl surrounding the apical meristem it probably was not possible to rinse off all of the applied [^{3}H]-GA_{20} that had not been absorbed by the plant. Thus, the [^{3}H]-GA_{20} remaining after 144 h may have been unmetabolized as this was not absorbed. However, the plateau in the level of observed [^{3}H]-GA_1 cannot be similarly explained. Conversion of [^{3}H]-GA_{20} to [^{3}H]-GA_1 presumably occurred within the plant and hence, incomplete uptake does not affect the level of [^{3}H]-GA_1.

Figure 23. Profile of ^{3}H elution from gradient eluted SiO_2 partition columns loaded with extracts from maize plants 24- or 144-h after administering ^{3}H -GA_{20} to the 21 day-old plants.


20
10
10
0
24 Hours
5
10
15
20
25
Percent extractable dpm
20
10
0
144 Hours
GA20
GA1
GA8
5
10
15
20
25
SiO2 partition column fraction

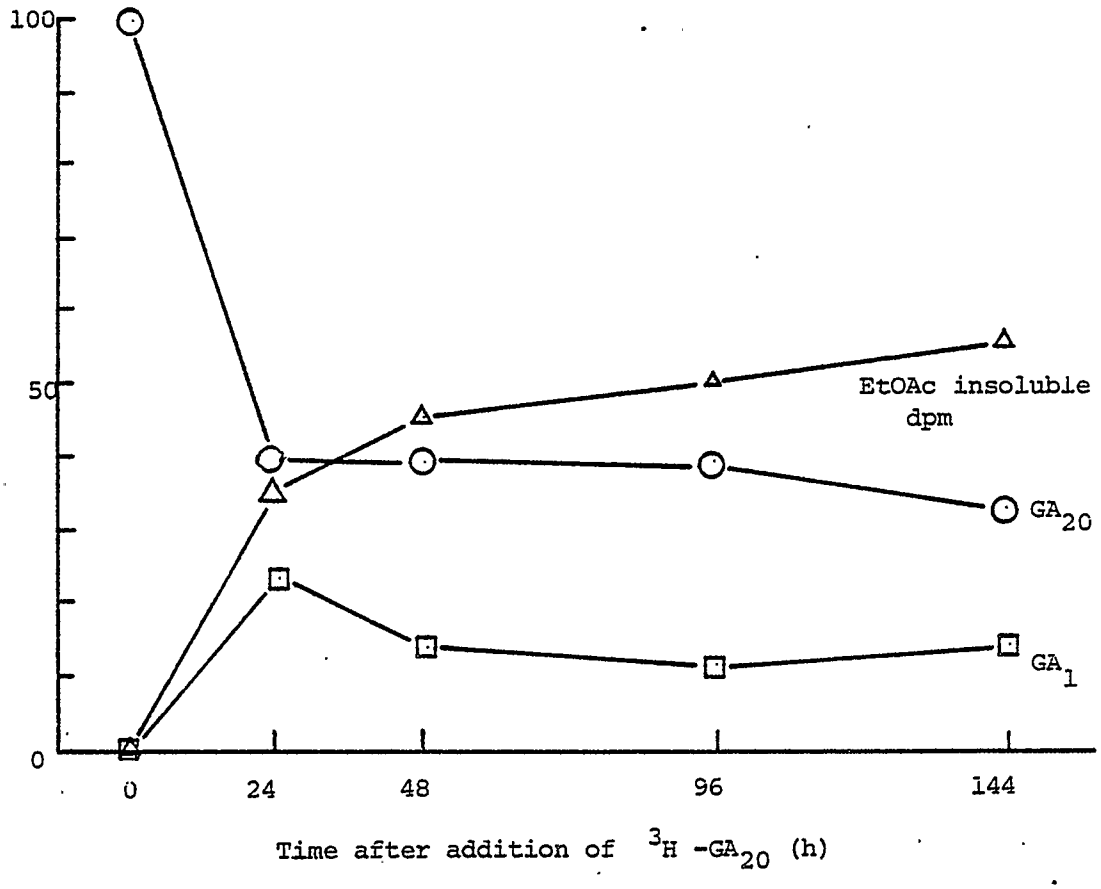


Figure 24. Time-course of changes in ^{3}H labelled compounds following a ^{3}H $-GA_{20}$ feed to 21 day-old maize plants.

SiO_2 partition chromatography does not readily separate GA_1 from GA_{29}, the two principal products from GA_{20} feeds. Since both of these are probably native in maize and GA_{20} is certainly native in maize, it is of particular interest to further characterize the polar peak which co-chromatographed on SiO_2 with $[^3H]$-GA_1.

Jones et al. (73) reported and P. Davies (Cornell University) confirmed (personal communication) that GA_1 and GA_{29} are readily separated on reverse-phase HPLC, even using a low resolution preparative system. Thus the $[^3H]$ peaks from SiO_2 partition chromatography were further analyzed on gradient elution reverse-phase HPLC. The Rt of $[^3H]$-GA_{20} and standards of logical metabolites and some degradation products were determined by reverse-phase HPLC-RC from comparison with metabolites (Figure 25).

The principal EtOAc-soluble metabolite from the feed to 21 day old maize plants which eluted coincidentally with $[^3H]$-GA_1 on SiO_2 partition columns subsequently eluted from C_{18} HPLC columns as a single peak coincident with the Rt of $[^3H]$-GA_1. This peak was collected and subsequently run "isocratically" on reverse-phase HPLC-RC using a 50 cm Whatman Magnum 9 column capable of resolving $[^3H]$-GA_1 from $[^{14}C]$-GA_3 by almost 10 min. When the $[^3H]$-GA_1-like peak was co-injected with a small spike of $[^{14}C]$-GA_3 the $[^3H]$ peak eluted at the same Rt as authentic $[^3H]$-GA_1 (Figure 26). Thus, the $[^3H]$-GA_1-like peak from SiO_2 probably consisted of only a single metabolite which was not $[^3H]$-GA_{29} and very probably was $[^3H]$-GA_1.

While attempts at subsequent GLC-RC of the $[^3H]$-GA_1-like peak from the $[^3H]$-GA_{20} feed to 21 day old maize plants failed, a feel of $[^3H]$-GA_{20} to maturing maize cobs resulted principapply in a chromatographically identical metabolite. After initial purification and SiO_2 chromatography, about 1.5×10^5 dpm of a $[^3H]$-GA_1-like metabolite was run on gradient

Figure 25. C_{18} reverse-phase HPLC radiochromatogram with elution profile of [^{3}H]-GA_{20}, logical metabolites (GA, GA_8), reference standard (ABA) and some degradation products. The chromatogram is a composite trace made by superimposing traces from pairs of [^{3}H]-GA_{20} and one other [^{3}H] compound.

Key:

I. Unidentified degradation product which elutes with injection solvent

II. [^{3}H]-GA_8 (12.53 min)

III. [^{3}H]-GA (25.04 min)

IV. Unkown degradation product of acid-treated [^{3}h]-GA_{20} (27.49 min)

V. cis-trans ABA (28.51 min)

VI. [^{3}H]-GA_{20} (32.05 min)

VII. [^{3}H]-GA_{20}-Me (35.05 min)

VIII. C/D R [^{3}H]-GA_{20} (36.31 min)

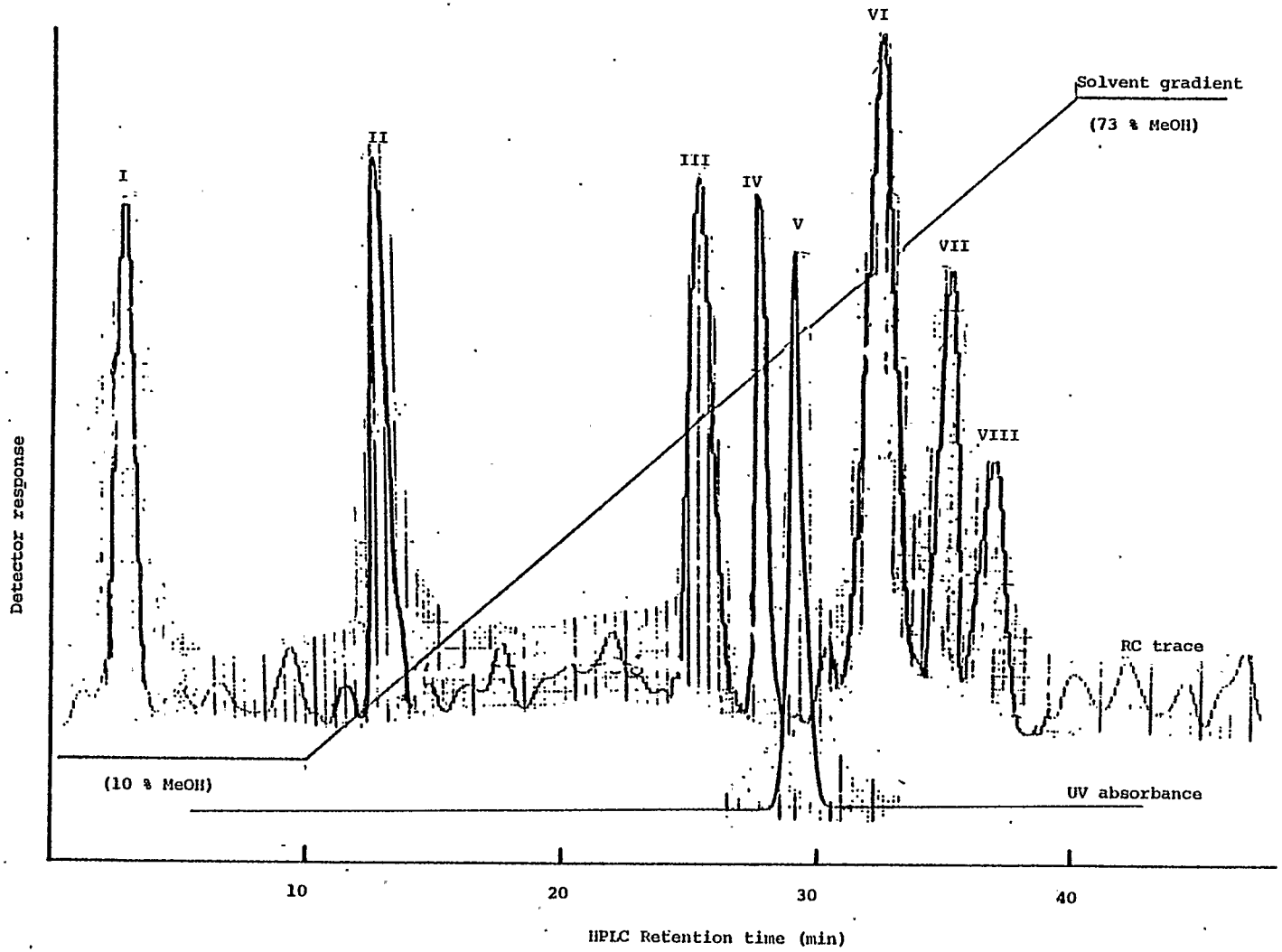
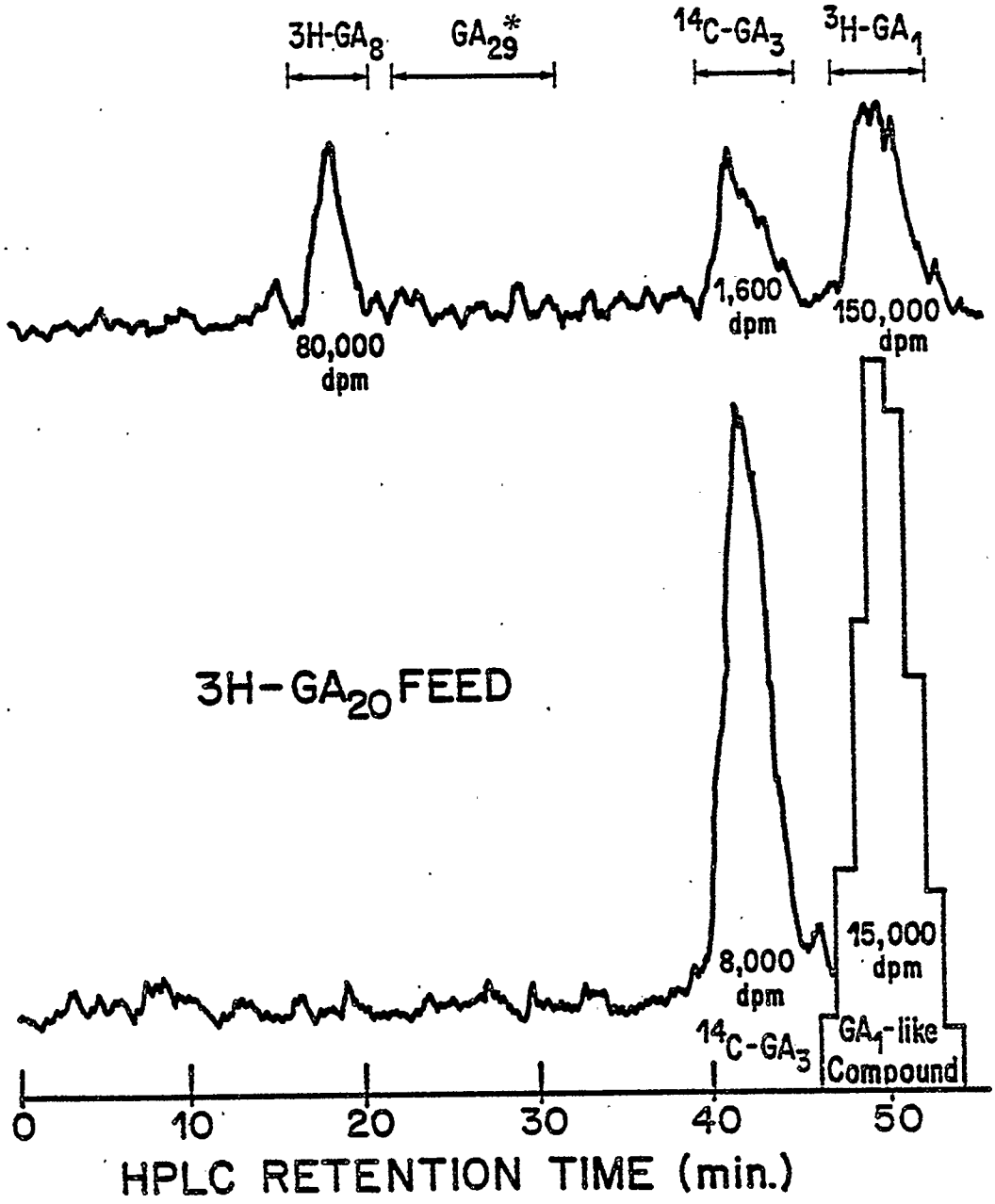

I
II
III
IV
V
VI
VII
VIII
Solvent gradient
(73 % MeOH)
(10 % MeOH)
RC trace
UV absorbance
Detector response
10
20
30
40
HPLC Retention time (min)

Figure 26.

Isocratic elution C_{18} reverse-phase HPLC radiochromatogram with elution of ^{14}C $-GA_3$ and ^{3}H $-GA_1$, and of ^{14}C $-GA_3$ and the acidic, polar ^{3}H -metabolite of ^{3}H $-GA_{20}$ from maize which had co-chromatographed with ^{3}H $-GA_1$ on a gradient eluted SiO_2 partition column.


3H-GA8
GA29*
14C-GA3
3H-GA1
80,000 dpm
1,600 dpm
150,000 dpm
3H-GA20 FEED
8,000 dpm
15,000 dpm
14C-GA3
GA1-like Compound
0
10
20
30
40
50
HPLC RETENTION TIME (min.)

elution reverse-phase HPLC-RC. Radioactivity was adequate for direct RC detection and indicated that the single peak exactly coincided with the Rt of authentic [^{3}H]-GA_1 (24.7 min). After derivation, this metabolite was analyzed by GLC-RC. The MeTMSi derivative of the [^{3}H]-GA_1-like metabolite eluted coincident with authentic GA_1 (Rt 22.5 min) from a 2% SE 30 column. This GLC column easily separates GA_1 from epi-GA_1, another product from [^{3}H]-GA_{20} feeds (35,74). Thus the principal EtOAc-soluble metabolite was tentatively identified as [^{3}H]-GA_1. Consistent with this conclusion is the knowledge that both GA_{20} and GA_1 are native in maize (65) and that [^{3}H]-GA_{20} is converted to [^{3}H]-GA_1 by other plant systems (82,147).

No detectable [^{3}H]-GA_{29} was produced following the [^{3}H]-GA_{20} feeds to maize. As GA_{20} is precursor of GA_{29} (45,112) and as both GA_{20} and GA_{29} are native in maize, the absence of [^{3}H]-GA_{29} was surprising. It is possible that the selection of developmental stages where involvement of GAs in the growth processes are probably high may have led to increased conversion to the biologically active GA_1, with little or no conversion to the virtually inactive GA_{29}. It will be of interest to observe the fate of [^{3}H]-GA_{20} during developmental stages when low levels of biologically active GAs are probably required (e.g., during microspore meiosis in the apical meristem when a low GA level may be required for normal male development (Chapter III.3.)). It would also be interesting to observe the fate of [^{3}H]-GA_{20} in the dwarf mutant $\underline{d}_1$ which is apparently unable to convert GA_{20} to GA_1 (B.O. Phinney, personal communication).

It must be noted that since the [^{3}H]-GA_{20} was labelled principally at the C-2 and C-3 positions, [^{3}H]-GA_{20} loss would occur during C-2 and

C-3 hydroxylation (36). Thus, the conversion of [^{3}H]-GA_{20} to [^{3}H]-GA_1 was probably greater than that which was detected (Table 34) and detection of [^{3}H]-GA_{29} would have been hindered. However, tritium loss by such conversions should have been equal in all experiments and hence, comparison of metabolic rates under different conditions or at different stages should still be valid.

While [^{3}H]-GA_1 was the principal acidic EtOAc-soluble metabolite other EtOAC soluble metabolites were also observed. In the feed to etiolated maize seedlings the principal EtOAc soluble compound (other than [^{3}H]-GA_{20} co-chromatographed on SiO_2 partition chromatography and reverse-phase HPLC-RC with C/D 4 [^{3}H]-GA_{20} (Figure 27). The origin of C/D R [^{3}H]-GA_{20} is uncertain however, as it is know than C/D R [^{3}H]-GA_{20} can be produced simply by treating [^{3}H]-GA_{20} with acid (35). Extracts were repeatedly exposed to hydrochloric, formic and acetic acids during expermental workup. Thus, it is possible that the presence of C/D R [^{3}H]-GA_{20} may be an artifact.

In most samples analyzed, a small [^{3}H]-labelled peak eluted at the Rt of [^{3}H]-GA_8 on SiO_2 and HPLC-RC. Davies and Rappaport (26) have previously shown that [^{3}H]-GA_1 is converted to [^{3}H]-GA_8 in maize and it is known GA_8 is probably native in maize (65). Thus, this very polar EtOAc-soluble metabolite is very probably [^{3}H]-GA_8. As previously noted, the loss of at least some [^{3}H] at the C-2 and C-3 positions during hydroxylation would cause an underestimation of the quantity of GA_8 produced from GA_{20}.

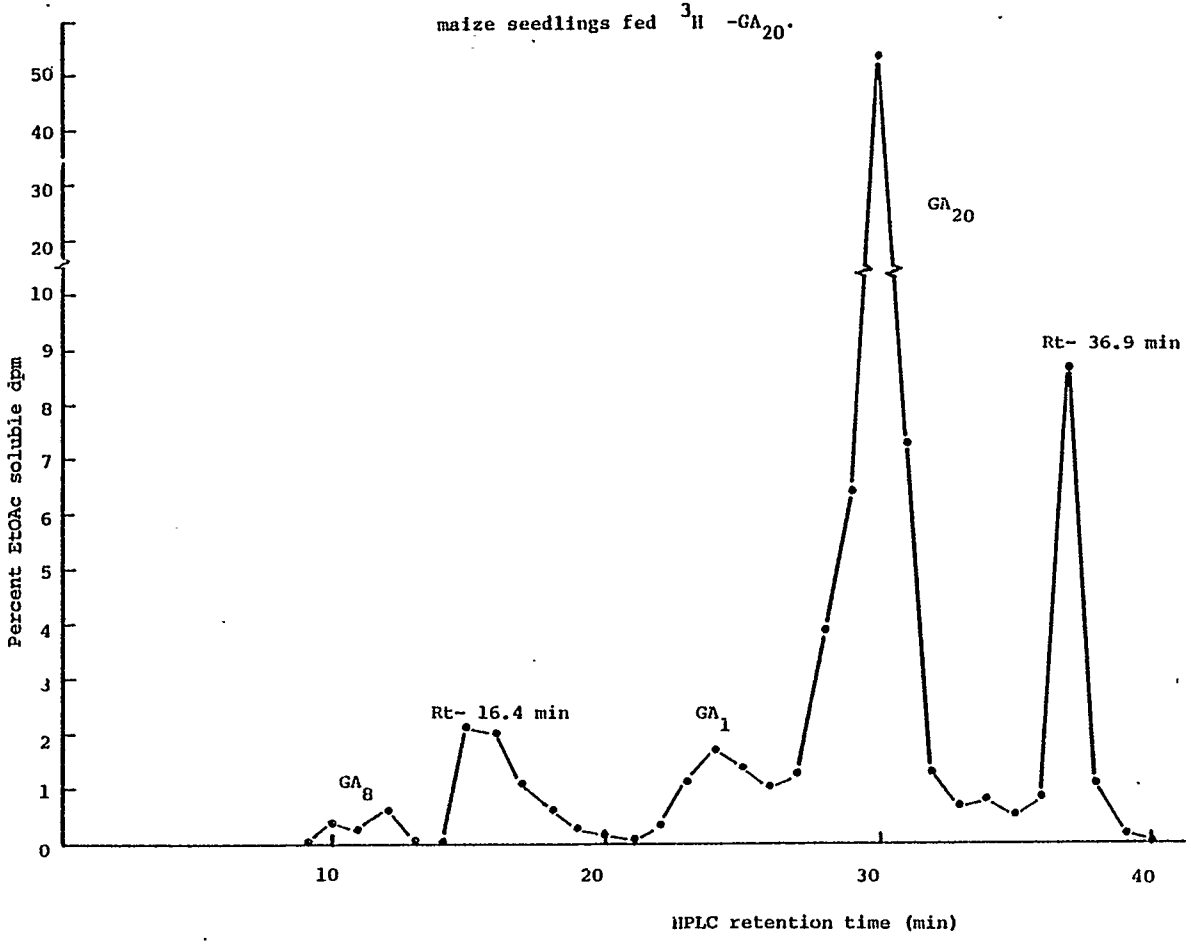
Another minor metabolite was also observed in the [^{3}H]-GA_{20} feed to the etiolated seedlings (Figure 27). With a Rt on HPLC-RC of 16.4 min,

Table 33. Distribution of ^3H following ^3H -GA_{20} or ^3H -GA_4 feeds to maize. Qualitative analysis is based on SiO_2 partition chromatography followed by HPLC-RC. All values represent % of total extractable dpm.

Sample	EtOAc-soluble fraction (GAs)				Conjugate fraction				BuOH insoluble	Total EtOAc insoluble (+ MeOH wash)
^3H -GA_{20} feeds	C/D R	GA_{20}	GA_1	GA_8	C/D R	GA_{20}	GA_1	GA_8		
1. 21 day old plants harvest 24 h		41	23							36
harvest 144 h		32	14	2						53
2. etiolated seedlings	7	54	4	1						18
3. mature cobs		3	2			24	27	2	42	95
4. husks		4	13	5		22	15	3	12	78
5. kernels	4	18	3		52	12	1			74

^3H -GA_4 feeds	GA_4	GA_{34}	GA_1	GA_8	GA_4	GA_{34}	GA_1	GA_8		
1. mature cobs	6		1		29		19	1	43	94
2. kernels	7	5	10	2	66		3	3		73

Figure 27. Profile of ^{3}H elution from C_{18} reverse-phase HPLC of the acidic, EtOAc coluble fraction from an extract of etiolated maize seedlings fed ^{3}H $-GA_{20}$.



this [^{3}H] peak did not correspond to any known metabolite or degradation product (Figure 25).

Following the [^{3}H]-GA_{20} feed to maturing maize cobs in field trials the senesced husks contained more [^{3}H]-GA_1 than [^{3}H]-GA_{20} (Figure 28). The relative abundance of BuOH soluble metabolites which were tentatively grouped as [^{3}H]-GA_1 or [^{3}H]-GA_{20} conjugates tended to be just the opposite, with a far greater abundance of [^{3}H]-GA_{20} conjugates (Figure 29). The analysis of this tissue may be particularly valuable as all [^{3}H] must have been transported to the husks as these were spacially separate from the point of application of [^{3}H]-GA_{20}. The mature maize kernels analyzed (Chapter IV.2.) must also have received [^{3}H] metabolites which had been transported from the maize cobs.

The apparent peak spreading in the HPLC chromatogram from the BuOH soluble fraction of maize husks probably resulted from the large number of conjugate-like compounds which were not adequately resolved when large fractions were collected. Although dpm associated with specific peaks from the BuOH soluble fraction of maize cobs was generally below the detection threshold of the on-line HPLC-RC, higher resolution reverse-phase HPLC-RC analysis was obtained by collecting 20 s fractions (Figures 34, 35). When mature maize cobs (which had been shelled and hence contained no kernels) were analyzed following a [^{3}H]-GA_{20} feed the conjugate fraction contained at least 10 [^{3}H] peaks (Figure 30). Four of the first five peaks co-chromatographed with authentic conjugates of GA_8 and GA_1 (Figures 30, 31). In the absence of authentic conjugates of GA_{20} no tentative identification of BuOH soluble peaks eluting in the less polar regions (Rt about 30 min) could be made. As glucosyl conjugates elute

Figure28. Profile of ^{3}H elution from a gradient eluted SiO_2 partition column loaded with the acidic, EtOAc soluble fraction from maize husks following a ^{3}H -GA_{20} feed to the cob.

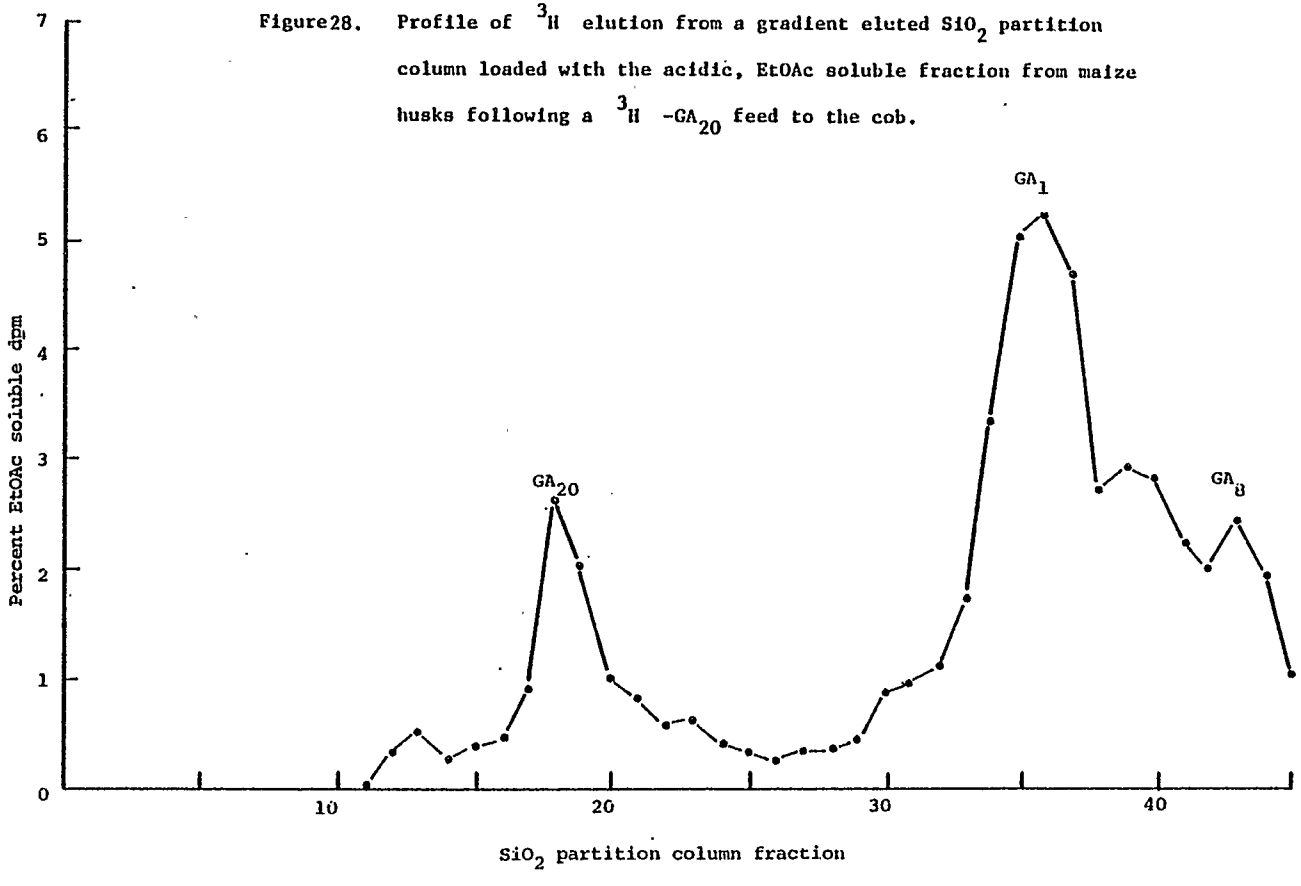
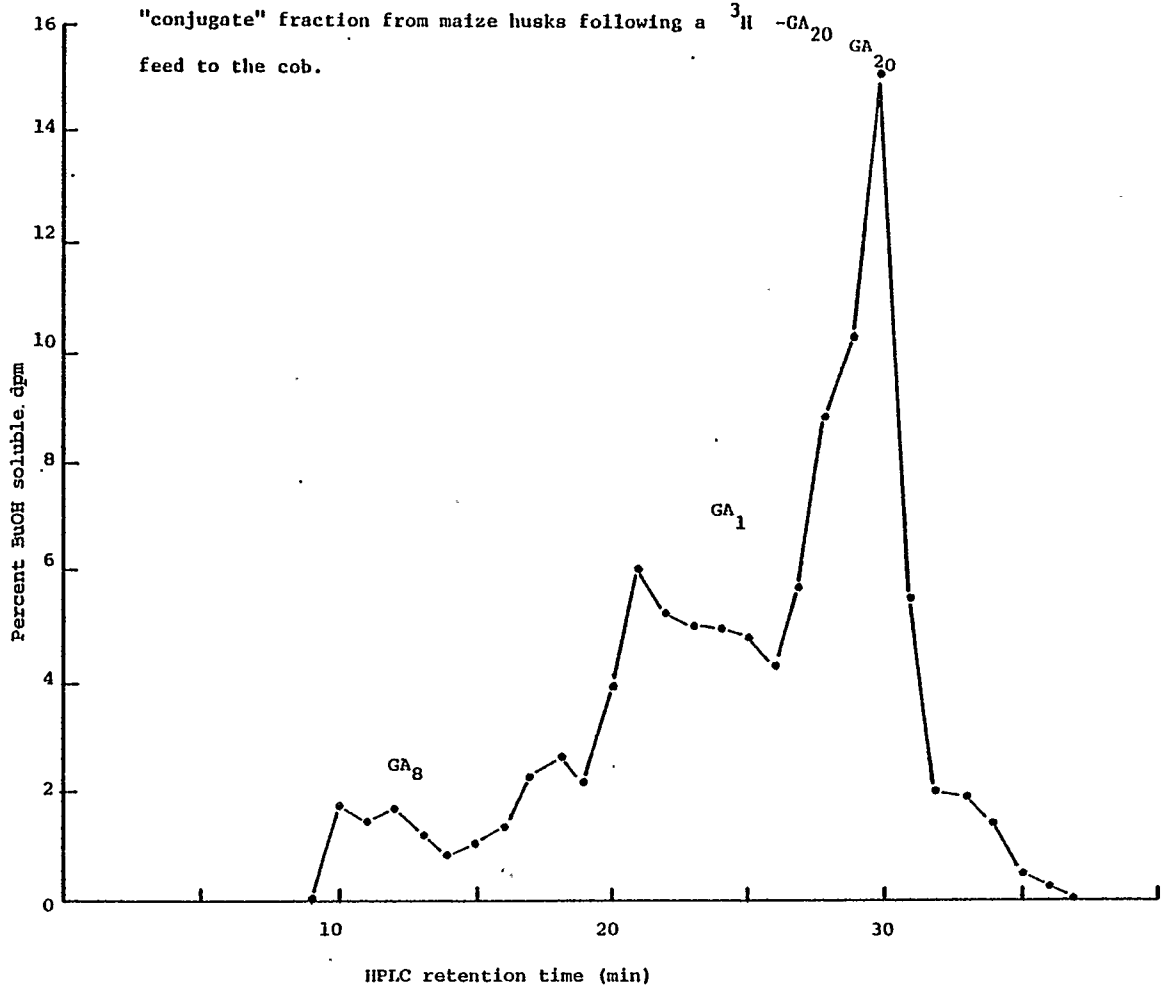


Figure 29. Profile of ^{3}H elution from C_{18} reverse-phase HPLC of the "conjugate" fraction from maize husks following a ^{3}H -GA_{20} feed to the cob.



coincident with or prior to the free acid GA, peak X was probably not a conjugate of [^{3}H]-GA_{20} (Figure 30). Since C/D R [^{3}H]-GA_{20} eluted after [^{3}H]-GA_{20} and also after peak X it is possibl- that peak X represented a conjugate of C/D R [^{3}H]-GA_{20} (Figure 30).

Following the [^{3}H]-GA_4 feed to maturing maize cobs EtOAc soluble [^{3}H] compounds which co-chromatographed on SiO_2 partition columns and reverse-phase HPLC-RC with [^{3}H]-GA_1 and [^{3}H]-GA_8 were observed. Particularly in mature kernels, a peak which eluted from SiO_2 partition columns with the Rt of GA_{34} was also observed (Chapter IV.2.). Analysis of the BuOH soluble conjugate fraction from maize cobs show that at least 10 [^{3}H] peaks were present (Figure 31). Peaks I, III, IV, AND V were tentatively identified as conjugates of [^{3}H]-GA_8 and [^{3}H]-GA_1. The most abundant peak eluted coincidentally with GA_4-GE, which was adequately separated from GA_4-G by this HPLC gradient (Figure 31).

[^{3}H]-GA_4 was less readily metabolized than [^{3}H]-GA_{20} (Table 33) and the conjugate fraction form the [^{3}H]-GA_4 feed contained far higher levels of [^{3}H]-GA_4 conjugates than [^{3}H]-GA_1-like conjugates (Figures 30, 31). As GA_4 has not yet been shown to be native in maize, this decreased metabolism may indicate a reduced ability to metabolize a non-native GA.

It is noteworthy that the fate of [^{3}H]-GA_{20} was dependent not only on the stage at application (Table 33) and incubation time (Figures 22, 23) but also appears to depend on the target tissue. For example, following either [^{3}H]-GA_4 or [^{3}H]-GA_{20} feeds to maturing maize cobs, very little of the [^{3}H] in the cobs partitioned into EtOAc at pH 3.0, while in the husks and kernels increasingly more [^{3}H] compounds were acidic, EtOAc soluble (Table 33 and Chapter IV.2.). The relative abundance of different BuOH soluble metabolites also differed in the different tissues (Figures 30 and 31).

Figure 30. Profile of [^{3}H] elution from C_{18} reverse-phase HPLC of the "conjugate" fraction from maize cobs following a [^{3}H]-GA_{20} feed to the cob.

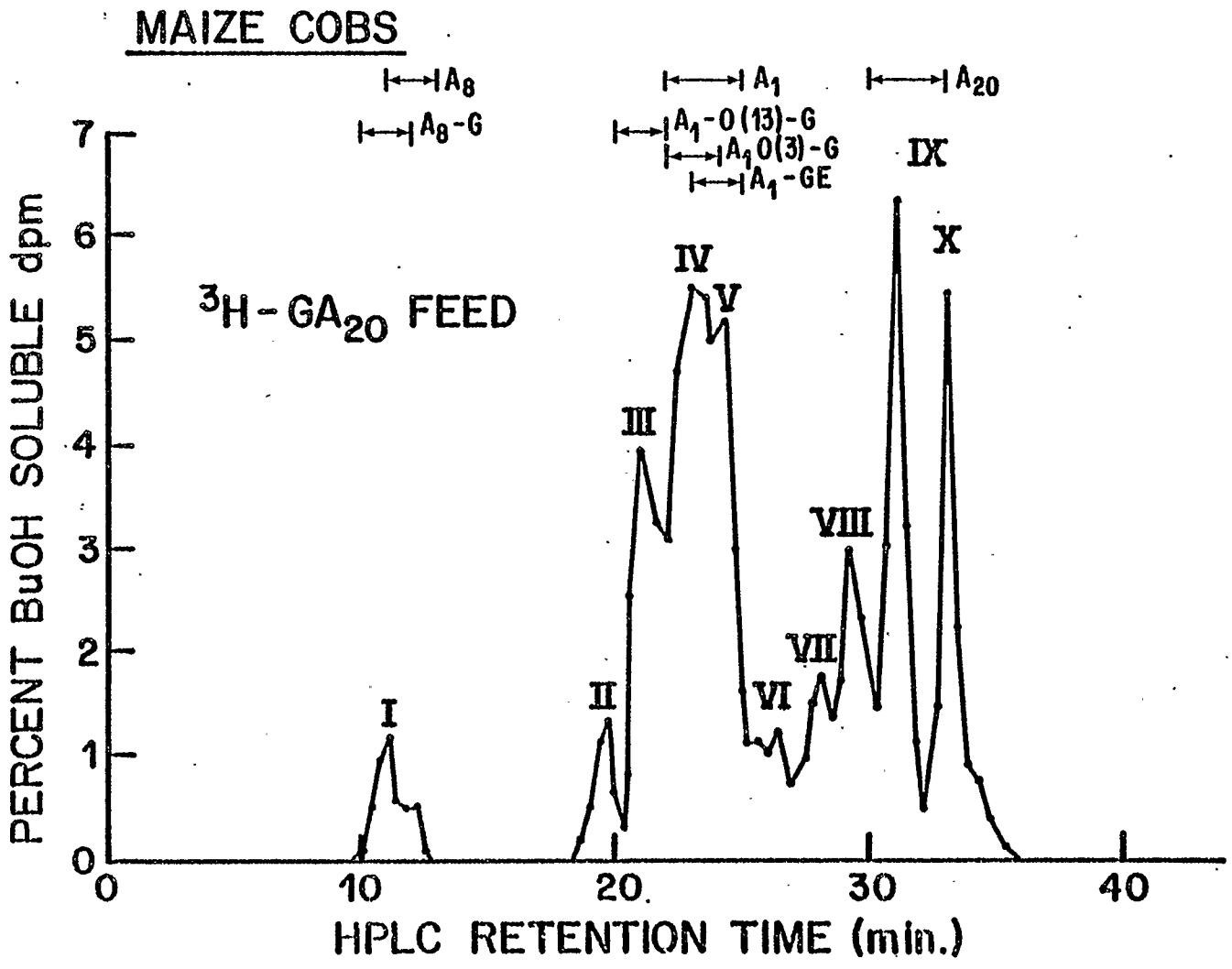
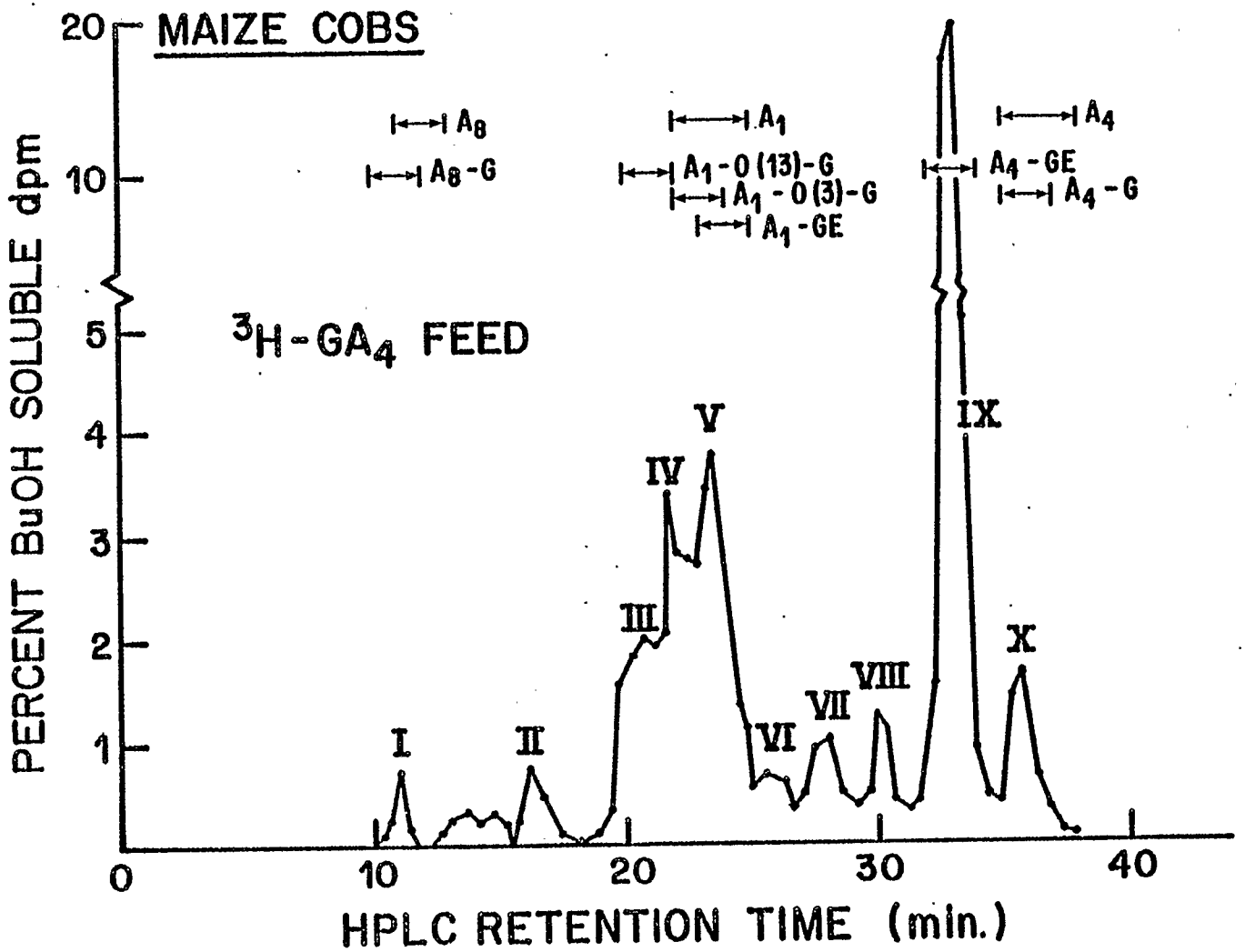

MAIZE COBS
A8
A8-G
A1
A1-O(13)-G
A1O(3)-G
A1-GE
A20
3H-GA20 FEED
I
II
III
IV
V
VI
VII
VIII
IX
X
PERCENT BuOH SOLUBLE dpm
0
1
2
3
4
5
6
7
0
10
20
30
40
HPLC RETENTION TIME (min.)

Figure 31. Profile of [^{3}H] elution from C_{18} reverse-phase HPLC of the "conjugate" fraction from maize cobs following a [^{3}H]-GA_4 feed to the cob.


MAIZE COBS
^{3}H-GA$_4$ FEED
A$_8$
A$_8$-G
A$_1$
A$_1$-O(13)-G
A$_1$-O(3)-G
A$_1$-GE
A$_4$
A$_4$-GE
A$_4$-G
I
II
III
IV
V
VI
VII
VIII
IX
X
PERCENT BuOH SOLUBLE dpm
HPLC RETENTION TIME (min.)
0
10
20
30
40
1
2
3
4
5
10
20

In summary, following a [^{3}H]-GA_{20} feed to maize, the principal acidic EtOAc soluble metabolite was generally a compound which co-chromatographed on a number of different systems with [^{3}H]-GA_1. Additionally, [^{3}H]-GA_8 and C/D R [^{3}H]-GA_{20} were probably produced, although C/D R [^{3}H]-GA_{20} may be an artifact. Numerous conjugate-like compounds were observed and conjugates of [^{3}H]-GA_1 and [^{3}H]-GA_{20} were particularly abundant. Following a [^{3}H]-GA_4 feed, the principal metabolite was also [^{3}H]-GA_1 and again, numerous conjugate-like compounds were observed. The direction and rate of metabolism of [^{3}H]-GA_{20} was influenced by developmental stage, time of incubation and tissue studied.

IV.2. Reversible conjugation of gibberellins *in situ* in maize

Abstract

During seed maturation in maize (*Zea mays* L.) conjugation of exogenously applied [^{3}H] gibberellins (GAs) occurred and these conjugates were subsequently hydrolyzed during imbibition and germination to release the acidic GA moiety. High specific activity [^{3}H]-GA_4 or [^{3}H]-GA_{20} were fed to maize cobs during rapid grain filling and mature kernels were subsequently harvested. In the dry, mature seeds most (80%) of the [^{3}H] was localized in the pericarp and aleurone layer. With imbibition [^{3}H] moved into the starchy endosperm (excluding aleurone) and with germination, the seedling shoot became the principal sink for [^{3}H]. Only 20 to 30% of the [^{3}H] in the dry kernels was soluble in ethyl acetate and most of this was associated with compounds which behaved chromatographically like the precursor [^{3}H]-GA_4 or [^{3}H]-GA_{20}. The principal acidic metabolite from either precursor was a [^{3}H] compound which co-chromatographed with [^{3}H]-GA_1 on SiO_2 partition and C_{18} analytical reverse-phase HPLC columns. This purported [^{3}H]-GA_1 was only a minor component of the ethyl acetate soluble fraction in the dry kernels while most of the [^{3}H] in the dry kernels behaved chromatographically like glucosyl conjugates of precursor [^{3}H] GAs. With imbibition, the [^{3}H] associated with the conjugate fraction decreased and concomitant increases in levels of [^{3}H] GAs were observed.

The natural occurrence of GA conjugates has long been recognized (69,108,135). Often referred to as "bound" or "water-soluble" forms of GAs, these conjugates typically exhibit low biological activity (128) and yeild a more biologically active acidic GA following chemical or

enzymic hydrolysis (124,128).

The physiological role of GA conjugates is not well understood. Although it has been repeatedly suggested that reversible conjugation could provide a means of storing GAs and regulating levels of the biologically active form (e.g., acidic form, except apparently in Lygodium where GA_9-Me is a highly active native antheridiogen (149)) (61,68,69), evidence supporting this hypothesis is mostly indirect. Gibberellin conjugates are preferentially formed during seed maturation and often are present in relatively high levels in maturing seeds (68,69). In bean and wheat seeds levels of acidic, EtOAc-soluble GAs decrease with maturation, and concomitant increases in "bound" or "neutral" GAs have been observed, reversals of this trend being noted upon imbibition (62, 138). In immature bean seeds glucosylation of [^{3}H] GAs does not take place early, rather it occurs as the seeds approach maturation (147,148). And, upon germination, a slight decrease in radioactivity associated with GA_1-glucosyl ester was observed, indicating that reversible conjugation may have been occurring (148). Additional evidence that reversible conjugation of GAs are associated with pea seed maturation/germination is provided by Barendse et al., (8) where [^{3}H]-GA_1 was converted in pea seeds to a highly water soluble compound which apparently yielded [^{3}H]-GA_1 and other radioactive compounds following mild acid hydrolysis. During germination part of the highly water soluble (conjugate?) pool was apparently released, as increases in apparent [^{3}H]-GA_1 and other acidic, EtOAc-soluble metabolites were observed. Unfortunately, GA_1 has not been characterized from pea, and given the methodology at the time, tentative identification of metabolites, including conjugates, was not

possible. Nevertheless, it supports the position that the potential for reversible conjugation in developing and germinating pea seeds is present. However, more recent examinations of the metabolism of native GAs (GA_{20}, GA_{29}) in immature and germinating pea seeds showed no reversible conjugation (45,135). Although one of the native GAs fed to pea (GA_{20}) is a known precursor of GA_1 in some other systems (82,147,148 Chapter IV.1.), in immature pea seeds, GA_{20} does not form GA_1, but is converted to GA_{29} through 28-hydroxylation (45), a step which generally leads to biological inactivation. Thus, it is possible that the apparent reversible conjugation observed by Barendse et al. (8) may have been an artifact brought about through the exogenous application of a foreign GA (e.g., GA_1).

The reversible conjugation of IAA in maturing/germinating maize seeds has now been unequivocally demonstrated (7). The availability of high specific activity [^{3}H] GAs, as well as the application of HPLC-RC and GC-RC for direct analysis of metabolites including [^{3}H] GA conjugates now makes it easier to re-examine the possibility that reversible conjugation of GAs occurs in maturing/germinating seeds.

In the present paper we have examined the metabolism of two [^{3}H] GAs of high specific activity in maturing/germinating maize seeds, a system which responds to exogenous GA application by increased seed weight (e.g., grain yield) (20), enhanced germination, and early seedling growth (9,56). One of the GAs used, GA_{20}, has been characterized from maize tassels (65, 108).

Materials and Methods

Plant Material

The early maturing maize (Zea mays L.) hybrid DK 23 was raised under field conditions at Lethbridge, Alberta, Canada as previously described (Chapter I.1.). After field thinning to a population of 75,000 plants/ha, six uniform plants were selected from within bordered rows for [^{3}H]-GA application.

Radioactive GAs

A number of factors contributed to the selection of [^{3}H]-GA_4 and [^{3}H]-GA_{20} in this study. They are available in high specific activity (1.33 Ci/mmol) and (2.36 Ci/mmol), respectively, and thus effects due to the exogenous application of carrier GA could be minimized. Both are relatively non-polar G_{19} GAs and are readily taken up and metabolized by maize (Chapter IV.1.). And both are relatively early in the several interconversion sequences known for higher plants (64,108), thus providing a reasonable number of acidic interconversion products, as well as GA_4 and GA_{20} per se, which could be conjugated during seed maturation. Both GAs display reasonably high biological activity in many bioassay systems, including the dwarf maize assays (25) and they are both converted by maize to [^{3}H]-GA_1 (Chapter IV.1.). (GA_1 is also native to maize (65)). It has been suggested (B.O. Phinney, ASPP/CSPP Symposium: Hormone Dynamics: Quantitative and Qualitative Regulations; Annual meeting of the ASPP and CSPP, Laval University, Quebec, June 15, 1981) that GA_1 may be the biologically active endogenous GA of maize. Chromatographically similar (e.g., non-polar) GA-like substances are high in immature maize

ears (Chapters III.1. and III.3.) and probably most importantly, GA_{20} is a native maize GA (65,108). While GA_4 is native in other cereals (22) and a GA-like compound which co-chromatographs with $GA_{4/7}$ on SiO_2 partition and reverse-phase HPLC exists in maize (Chapter III.3.) its presence in maize is not proven, and we recognize that its metabolism by maize may not necessarily reflect a natural sequence of events.

Prior to injection, [^{3}H] GAs were purified on gradient-eluted SiO_2 partition columns (30). On 28.08.80 about 1 uCi of [^{3}H]-GA_{20} (140 ng) or 10 uCi [^{3}H]-GA_4 (2.48 ug) were injected in 0.3 ml 50:50 EtOH;H_2O (v/v), 0.5 cm into the shank of each maize ear directly below the cob. Cobs were harvested on 27.09.80, at which time kernels of neighboring cobs had formed black layers, indicating physiological maturity (27). After 21 days of drying indoors with husks removed, kernels were removed from the basal and apical halves of cobs and stored at room temperature prior to germination and extraction.

Germination experiments

For all germination experiments 5 kernels were placed pericarp side down on two 9 cm Whatman #1 filter paper discs in plastic 100 x 15 mm petri dishes. Five ml of glass distilled H_2O were added and the dishes sealed with tape prior to placing inside boxes (which were not totally light tight). The boxes were then placed inside an incandescent-lighted growth chamber at 25°C.

For experiments dealing with the movement of [^{3}H] compounds, kernels or seedlings were dissected and burned (without extraction) in a Packard Model B306 Tri-Carb sample oxidizer; radioactivity was quantified using liquid scintillation spectrometry.

Analysis of [^{3}H] GA metabolites

Kernels, seedlings, or parts thereof, were ground in 15 ml 80% aqueous MeOH (which had been cooled to -40°C) and then shaken at 4°C for 12 h. After filtration, 5 ml 0.1 M pH 8.0 phosphate buffer was added and the MeOH was removed in vacuo at 40°C. The aqueous phase was acidified with 3 N HCl to pH 3.0 and extracted 4x with 10 ml H_2O saturated EtOAc. The aqueous phase was subsequently extracted 3x with H_2O saturated n-BuOH. The pH was then raised with 1 N KOH to 7.0. Two additional extractions with BuOH followed and the acidic and neutral BuOH fractions were bulked. The acidic, EtOAc-soluble fraction was chromatographed on gradient-eluted SiO_2 partition columns as previously described (30, Chapter III.3.). A MeOH wash of the SiO_2 partition column (following the hexane:EtOAc gradient) was added to the bulked BuOH fractions, and this "conjugate" fraction was taken to dryness and chromatographed on C_{18} u-BondaPak reverse-phase HPLC as previously described (80). An on-line HPLC-RC (Berthold) was used to detect [^{3}H] peaks 50,000 dpm; aliquots of HPLC fractions were also taken for liquid scintillation spectrometry. A more complete description of HPLC-RC and subsequent GLC-RC characterization on some of these samples, and on other maize samples after [^{3}H]-GA_4 and [^{3}H]-GA_{20} feeds is given in Chapter IV.1.

In order to process suitable numbers of samples for adequate statistical analyses, a rapid step-elution SiO_2 partition column chromatographic separation of the "precursor" [^{3}H]-GA_4 or [^{3}H]-GA_{20} from their (a) acidic "products", [^{3}H]-GA_1, [^{3}H]-GA_8 and other minor components and (b) from the "[^{3}H] conjugates" was developed. Individual kernels, seedlings, or parts thereof were ground and extracted with 80% MeOH as described above. The

extracts were filtered and taken to dryness with excess MeOH in vacuo at 40°C. The dried samples were solubilized in 50:50 MeOH:EtOAc, followed by two drops of H_2O, prior to loading onto the rapid step-elution SiO_2 partition column.

Ten g saturated (20% H_2O by weight) Woelm SiO_2 were slurried with about 50 ml 95:05 hexane:EtOAc (v/v) (both saturated with formic acid) and poured into a 13 mm i.d. glass column. After low pressure compaction by air, the column height was 14 cm and the column volume, including the SiO_2 bed, was 20.3 ml. Fifty ml 45:55 hexane:EtOAc were flushed through the column. This fraction contained essentially no radioactivity, but did contain much of the pigmentation. The precursor GAs (i.e., [^{3}H]-GA_4 or [^{3}H]-GA_{20}) were eluted in the next seven 10 ml fractions of 55:45 EtOAc:Hexane. The solvent head was allowed to fall to 0.5 cm above the column before changing to the next solvent. The polar [^{3}H]-GA metabolites which were readily EtOAc soluble (i.e., [^{3}H]-GA_1, [^{3}H]-GA_8 and other acidic, unknown [^{3}H] metabolites) were eluted in the next 6 fractions of 05:95 hexane:EtOAc. Finally, 110 ml MeOH were flushed through the column to remove the EtOAc-insoluble [^{3}H] metabolites (e.g., "conjugates"). The three bulked "[^{3}H] precursor", acidic [^{3}H] metabolites, and "[^{3}H] conjugates" fractions were dried in vacuo, solubilized in MeOH and radioactivity in each was determined by liquid scintillation spectrometry. However, since slight variation in the degree of H_2O saturation of the SiO_2 leads to shifts in retention volumes, reverse-phase HPLC analysis of each bulked fraction was carried out on every 11th sample.

The step-elution SiO_2 partition chromatography system was a rapid, efficient means of separating the precursors from their metabolites in

this system. Separation of [^{3}H]-GA_4 or [^{3}H]-GA_{20} from [^{3}H]-GA_1 was complete (Figure 32) and the system provided quantitative recovery of dpm that were known to be associated with the MeOH wash from our usual gradient-eluted SiO_2 partition column (30). Since the dry weight of a single seed (seedling) is low (about 0.15 g) and extracts were generally free of chlorophyll and contained only low levels of other pigments, no additional purification was required prior to step-elution SiO_2 partition column chromatography. SiO_2 fractions were pure enough for analytical reverse-phase HPLC-RC.

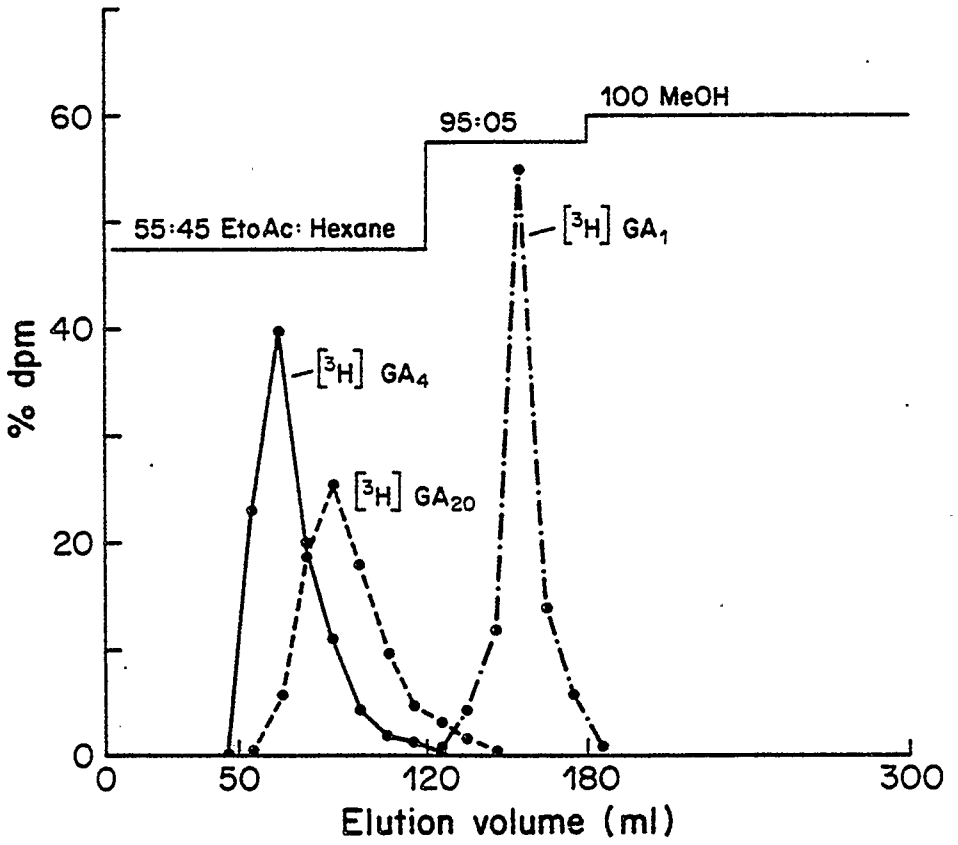


Figure 32. Stepwise elution of [^{3}H] GA_4, [^{3}H] GA_{20} and [^{3}H] GA_1 from SiO_2 partition columns.

Results and Discussion

When [^{3}H]-GA_4 or [^{3}H]-GA_{20} was injected into the shank during the milky stage of kernel maturation, about 10% of the radioactivity was transported into the maturing kernels. The specific radioactivity of the kernels varied widely within a cob, as well as between cobs. For example, kernels from the basal region of a cob fed [^{3}H]-GA_4 contained from 1,000 to 10,000 dpm, with the average for a full, well-formed kernel being about 4,000 dpm. Kernels from the apical region of this cob had consistently few dpm and some had no detectable [^{3}H]. In all cases, basal regions contained higher levels of [^{3}H] than did apical regions of the same cob. This is consistent with the knowledge that kernel growth and development proceeds in an acropetal direction along a cob (140). Analyses were thus restricted to kernels from the basal regions of cobs. It is likely that much of the variability in total dpm from kernel to kernel on a given cob could be attributed to the original location of that kernel during grain filling. Unfortunately, cobs were shelled and the kernels mixed prior to extraction and/or germination, hence the number of extracted seeds required to obtain statistical significance generally exceeded five.

In the mature kernels following a [^{3}H]-GA_4 feed, most (80%) of the [^{3}H] was in the pericarp and aleurone (Figure 33). It was not possible to remove the pericarp from the subtending layers in the dry seed and softening procedures were not used since we feared movement of, or changes in, the [^{3}H] GAs and conjugates.

The starchy endosperm contained virtually no dpm in the dry seeds, while the embryo contained about 20% of the [^{3}H] (Figure 33). The abun-

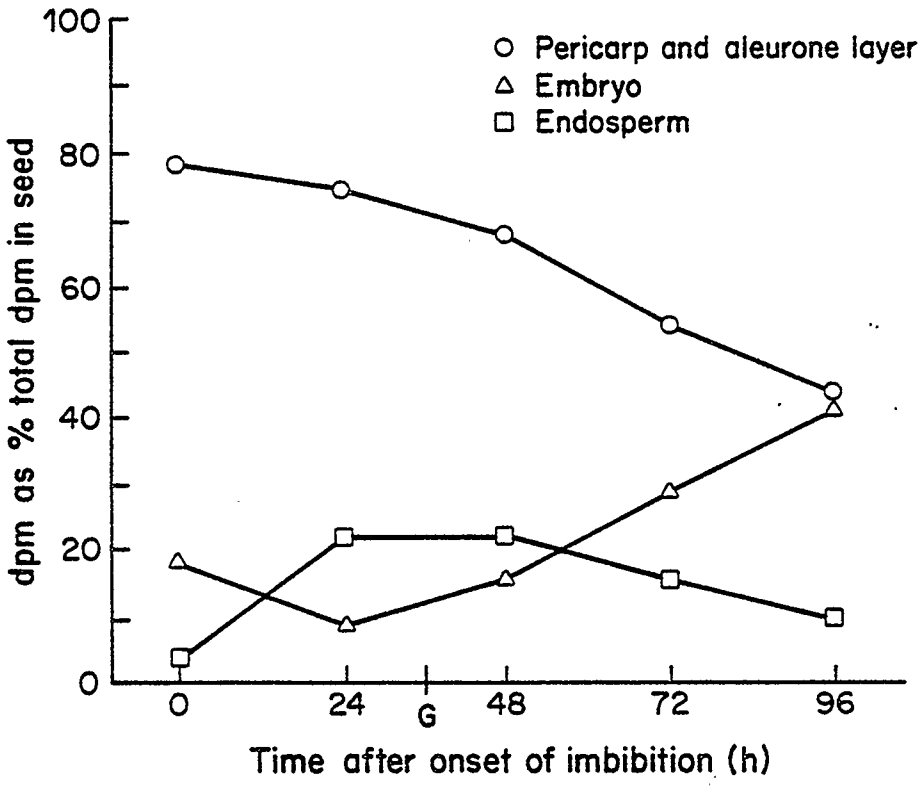


Figure 33. Localization of [^{3}H] in dry and germinating maize seeds following a [^{3}H] GA_4 feed to maize cobs during grain filling.

dance of the [^{3}H] GAs and other [^{3}H] metabolites in the pericarp may explain the lack of promotion by exogenous GA_3 of enzymic hydrolysis of endosperm reserves in embryo-less maize (61). It is probably that sufficient GA is already available near the endosperm. The pericarp/aleurone layer may thus be the major site of GA storage in the mature maize seed.

Upon imbibition, and prior to germination, some of the [^{3}H] moves out of the embryo and pericarp, accumulating in the endosperm (Figure 33). At about the time of germination (e.g., radicle protrusion) the embryo becomes a net importer of the [^{3}H]. By 96 h it is a powerful [^{3}H] sink (Figure 33).

Within the young seedling there is evidence for a predominantly acropetal transport of [^{3}H]. Although the radicle is rapidly growing at 72 h, and is much larger than the young shoot, the shoot contains about 3x the dpm of the radicle. By 96 h the difference between radioactivity in the radicle and the shoot is even greater (Figure 34).

Following a [^{3}H]-GA_4 feed about 30% of the [^{3}H]in dry seeds partitions into EtOAc at pH 3.0, and behaves chromatographically on partition SiO_2 and reverse-phase HPLC systems in a manner similar to acidic GAs (30,33,80). Chromatographic separation on gradient elution SiO_2 partition columns revealed at least 4 acidic [^{3}H] compounds (Figure 35). Chromatographic analyses of I, III, and IV have been previously described (Chapter IV.1.) and for the purposes of this discussion these peaks will be referred to as GA_4-like, GA_1-like and GA_8-like. Peak II eluted from SiO_2 coincidentally with GA_{34}, a logical 2B-hydroxylated metabolite which has been reported from [^{3}H]-GA_4 feeds to other plants (34,144). However, this peak has not been subjected to HPLC-RC and GLC-RC.

The principal acidic [^{3}H] GA in the dry seeds following the [^{3}H]-GA_4

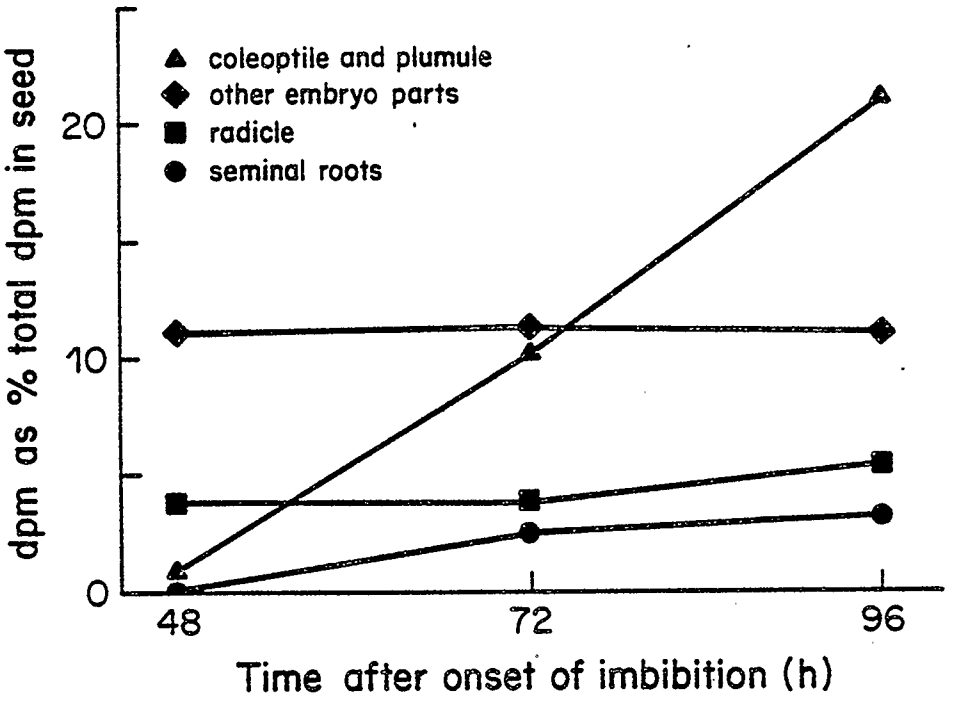


Figure 34. Locatization of [^{3}H] in young seedlings following a [^{3}H] GA_4 feed to maize cobs during grain filling. Germination (radicle protrusion) occurred at about 36 h.

feed was [^{3}H]-GA_1-like; a [^{3}H]-GA_8-like compound was a minor metabolite (Figure 35). Thus, maize is apparently capable of C-2, and C-13 hydroxylations and efficiently metabolized [^{3}H]-GA_4 into GA_1, a compound which is native in maize.

Following a [^{3}H]-GA_{20} feed, about 20% of the [^{3}H] was associated with acidic, EtOAc soluble compounds. These have been tentatively characterized as acidic [^{3}H] GAs. On the basis of chromatographic properties described elsewhere (80, Chapter IV.1.) peaks I, II, III and IV from the SiO_2 partition column (Figure 36) will be referred to as C/D R [^{3}H]-GA_{20}-like, [^{3}H]-GA_{20}, [^{3}H]-GA_1-like and [^{3}H]-GA_8-like. While peak I from the sample shown (Figure 36) is only a very minor component analysis of other samples (particularly samples not processed immediately after extraction) contained larger quantities of the C/D R [^{3}H]-GA_{20}-like compound.

In the [^{3}H]-GA_{20} feed much more of the [^{3}H]found in the dry seed was associated with the "precursor" than occurred in the [^{3}H]-GA_4 feed (Figures 35,36). And, more dpm was associated with [^{3}H]-GA_8 than with [^{3}H]-GA_1 in dry seed which had earlier been fed [^{3}H]-GA_{20}, just the reverse of the trend in [^{3}H]-GA_4 feeds. It was surprising to find different amounts of the [^{3}H]-GA_1-like compound associated with the two feeds. However, the amount of "carrier" was far greater for GA_4 than for GA_{20}. Hence the amount of GA_1 produced from GA_4 may well have been high enough to perturb the system (26) thus reducing apparent metabolism of [^{3}H]-GA_1.

In dry, mature maize seeds about 70% of the dpm following a [^{3}H]-GA_4 feed was associated with the conjugate fraction (Figure 37). Following imbibition, but prior to germination, there was a reduction to about 45% in dpm associated with the conjugate fraction, and a concomitant increase

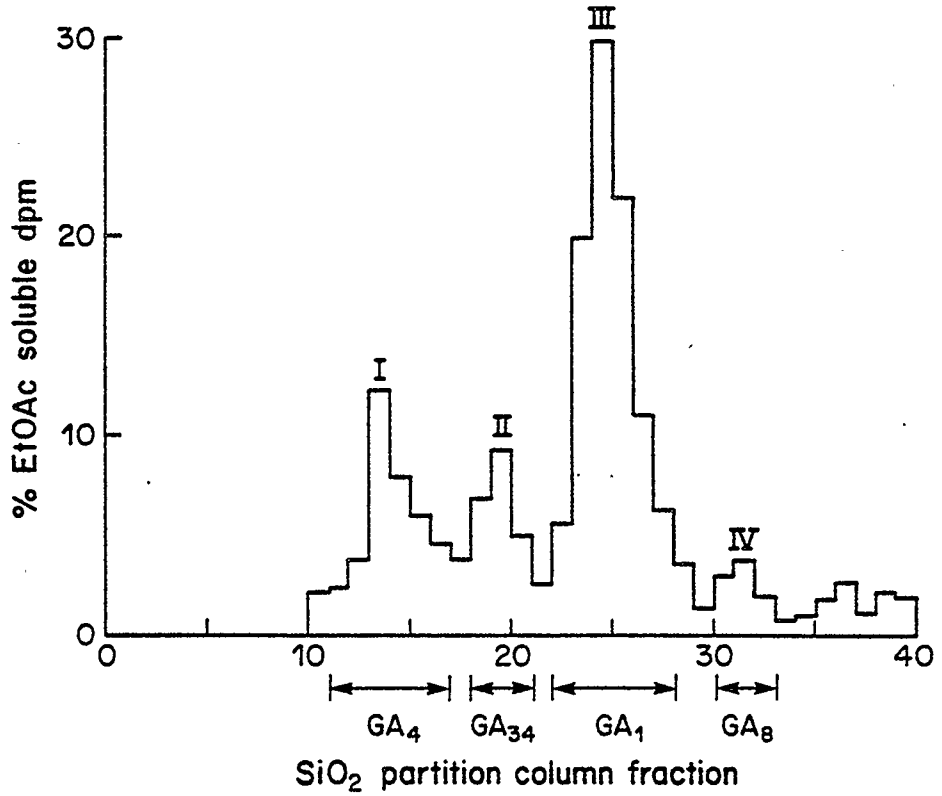


Figure 35. Distribution of [^{3}H] on a gradient eluted SiO_2 partition column loaded with the EtOAc soluble fraction of extracts from seeds following a [^{3}H] GA_4 feed to maize cobs. The [^{3}H] associated with the MeOH wash is not included in the Figure although the MeOH wash dpm were included in the calculation of total EtOAc soluble dpm.

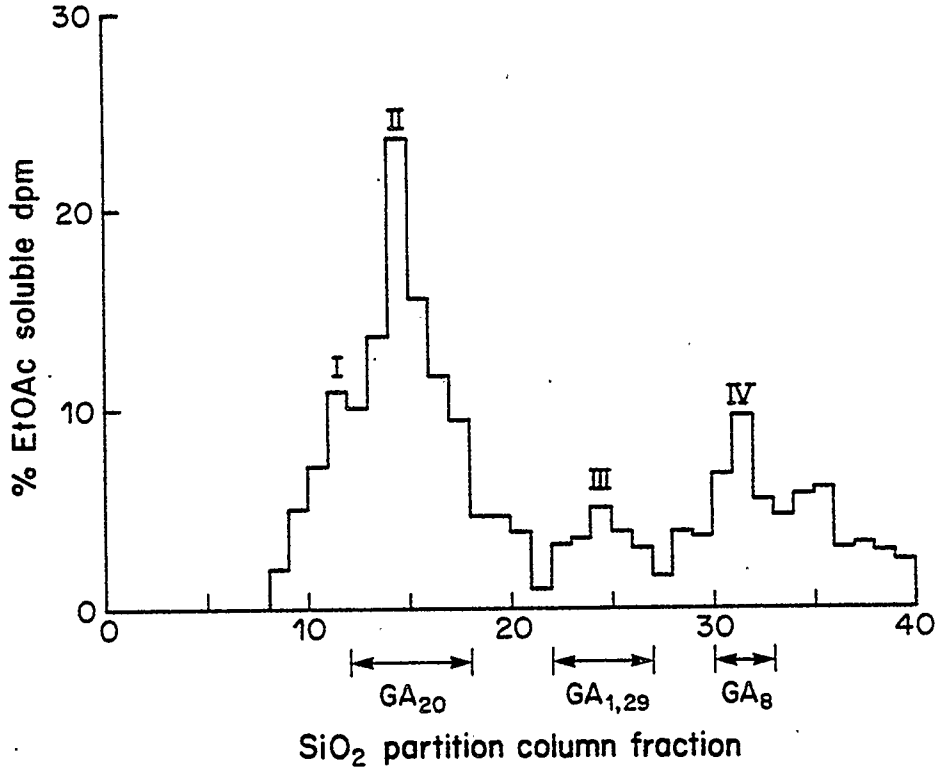


Figure 36. Distribution of [^{3}H] on a gradient eluted SiO_2 partition column loaded with the EtOAc soluble fraction of extracts from maize seeds following a [^{3}H] GA_{20} feed to maize cobs. The [^{3}H] associated with the MeOH wash is not included in the Figure although the MeOH wash dpm were included in the calculation of total EtOAc soluble dpm.

in the [^{3}H]-GA_4 fraction (Figure 37). This trend was statistically reproducible over three separate tests involving [^{3}H] rich kernels from two cobs. Reverse-phase C_{18} u-BondaPak HPLC-RC of the MeOH wash (e.g., conjugate) from SiO_2 partition columns indicated that most of the dpm was associated with a peak which co-chromatographed with GA_4-GE (Figure 38). This HPLC system readily separates GA_4-GE from GA_4-G (80). Statistically significant drops in GA_4-GE dpm associated with imbibition and germination were thus observed. Two other conjugate peaks occurred repeatably following the [^{3}H]-GA_4 feed; these co-chromatographed on HPLC with GA_8-G and with GA_1-O(13)-G (Figure 38). However, there was no change in the percentage of dpm associated with GA_1-G (3%) and GA_8-G (4%) during imbibition. Two other rather sporadic radioactive peaks from the "conjugate fraction" were observed on HPLC. One eluted at Rt 6 min. It may be a degradation product. Another peak, observed particularly from the "conjugate" fraction in dry seed extracts, eluted at Rt 36-37 min from HPLC. This peak co-chromatographed with [^{3}H]-GA_4, and partitioning of the "conjugate" fraction between H_2O and EtOAc (pH 3.0) after storage for 1 week at -20°C (dry) indicated that a slight amount of the [^{3}H]-GA_4 conjugate had been cleaved, thus releasing the [^{3}H]-GA_4. Hence, drying the acidified (e.g., HCOOH present) MeOH was probably responsible for hydrolysis in some of the samples. This ready hydrolysis which releases [^{3}H]-GA_4 is also consistent with the presence of GA_4-GE in the conjugate fraction.

Trends similar to the [^{3}H]-GA_4 feeds in the biological cleavage of conjugates with imbibition were noted in seed fed [^{3}H]-GA_{20} (Figure 39). Upon imbibition, and prior to germination, the percentage dpm associated with the GA_{20} and C/D R GA_{20} conjugates fell, while the percentage dpm

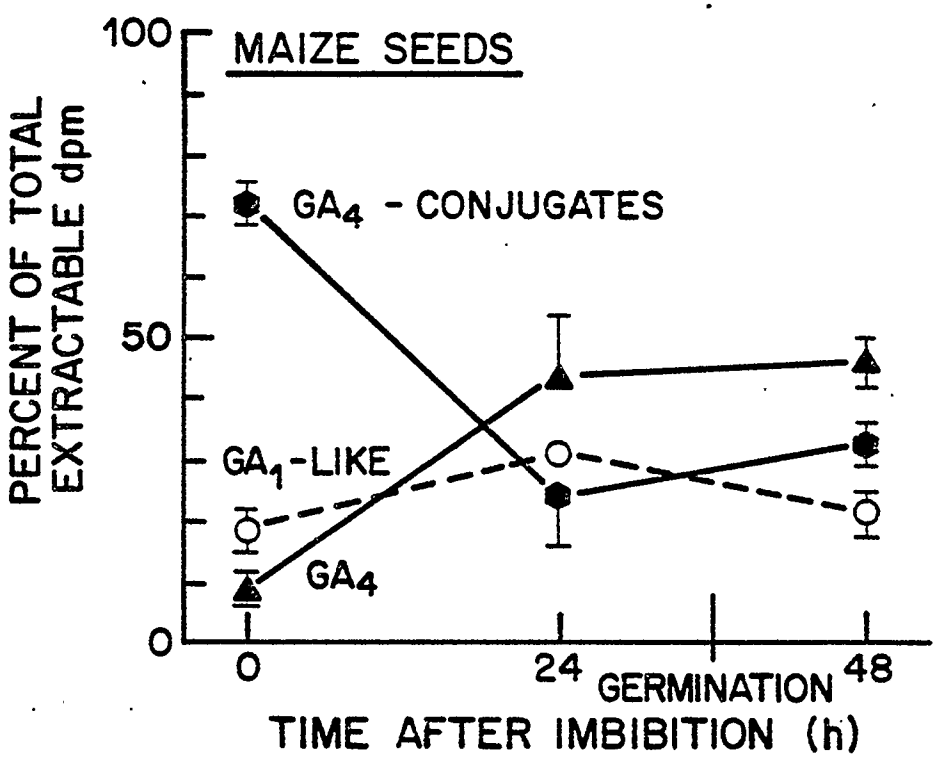


Figure 37. Percentage of [^{3}H] associated with the GA_4, GA_1 and conjugate fractions from step elution SiO_2 partition columns loaded with extracts from dry and germinating seeds following a [^{3}H] GA_4 feed to maize cobs. Subsequent reverse-phase HPLC-RC indicated that almost all of the [^{3}H] in the GA_1 fraction co-chromatographed with [^{3}H] GA_1 while most of the [^{3}H] in the conjugate fraction co-chromatographed with GA_4-GE.

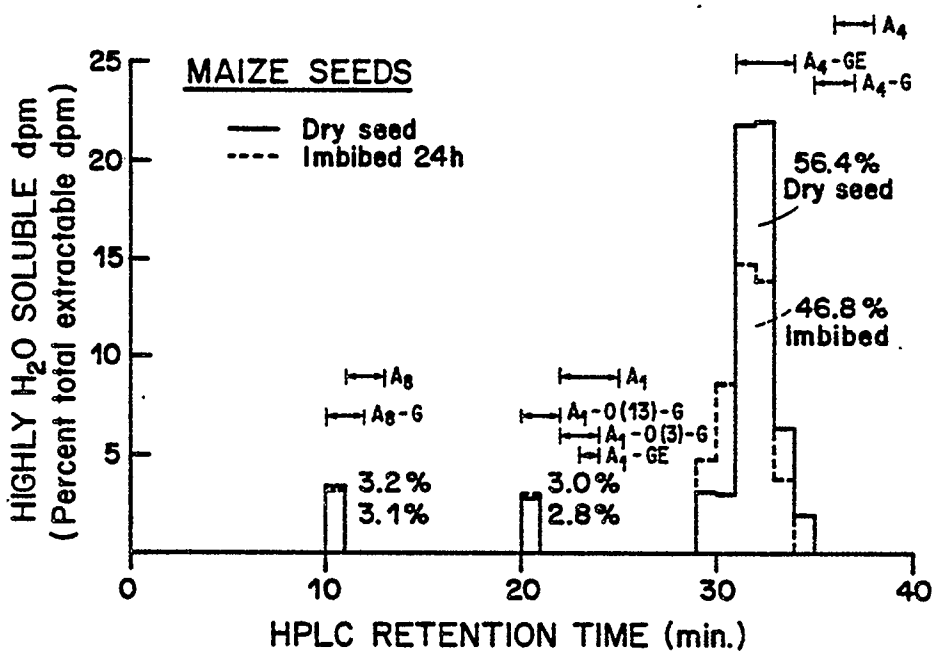


Figure 38. Distribution of [^{3}H] from gradient eluted reverse-phase high performance liquid chromatography (HPLC) columns loaded with the MeOH wash after SiO_2 partition chromatography.

associated with [^{3}H]-GA_{20} and C/D R [^{3}H]-GA_{20} rose. DPM associated with [^{3}H]-GA_1 (and the minor component [^{3}H]-GA_8) also rose, but this was probably the result of hydroxylation of [^{3}H]-GA_{20} after cleavage of the GA_{20} conjugate, since the increase in other [^{3}H]-GA metabolites lagged behind the increase in [^{3}H]-GA_{20} during imbibition and germination (Figure 39).

Analysis of the MeOH wash (e.g., "conjugate" fraction) from the SiO_2 partition column indicated that much of the dpm was associated with a HPLC peak eluting at Rt 36.5 min, about 4 min after [^{3}H]-GA_{20}. Although no standard GA_{20}-conjugates were available, this unknown peak is probably not a glucoside or glucosyl ester of GA_{20}, since glucosyl conjugates typically elute before or coincidental with the free GA on our reverse phase HPLC system (80). Glucosyl ester and glucoside conjugates of [^{3}H]-GA_1, the principal acidic metabolite from [^{3}H]-GA_{20} feeds to maize (26) elute much earlier than [^{3}H]-GA_{20} (80, and Fig. 38). Hence, this peak is not a glucosyl conjugate of [^{3}H]-GA_1. Nor was the peak [^{3}H]-GA_{20} methyl ester, a compound which elutes 3.0 min after [^{3}H]-GA_{20}. It is possible that the peak consists of one or more conjugates of C/D R [^{3}H]-GA_{20} since the free acid C/D R [^{3}H]-GA_{20} elutes 4.6 min after [^{3}H]-GA_{20}. However, it is also possible that the presence of the free acid and conjugate of C/D R [^{3}H]-GA_{20} is an experimental artifact. Durley et al. (35) have previously reported the appearance of C/D R [^{3}H]-GA_{20} and a conjugate (presumed to be a glucosyl ester) of this compound following [^{3}H]-GA_{20} feeds. These authors noted that C/D R [^{3}H]-GA_{20} is readily formed from [^{3}H]-GA_{20} under acidic conditions and suggested that some of the observed C/D rearranged metabolite may have been produced during extraction and chromatography (35). During our work-up,

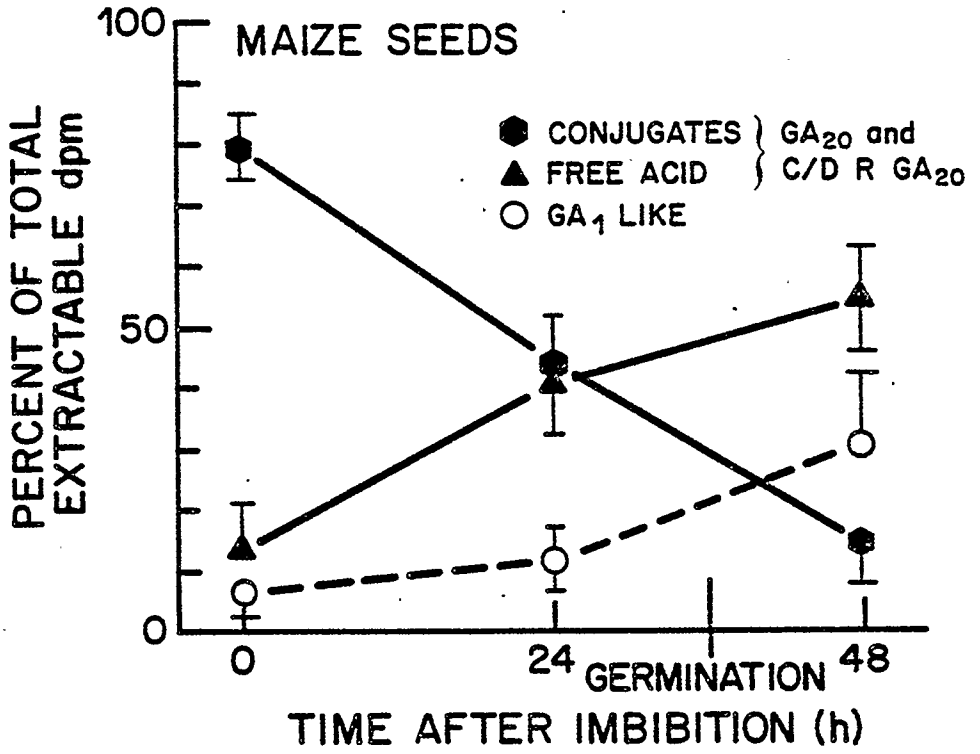


Figure 39. Percentage of [^{3}H] associated with the GA_{20} (and C/D R GA_{20}), GA_1 and conjugate fractions from step-elution SiO_2 partition columns loaded with extracts from dry and germinating seeds following a [^{3}H]-GA_{20} feed to maize cobs.

extracts were exposed to pH 3.0 solutions for varying lengths of time and both the fractions containing [^{3}H]-GA_{20} in the MeOH wash of the SiO_2 partition column were dried in the presence of formic acid. The HCOOH content of the [^{3}H]-GA_{20} fraction was appreciably less than that in the MeOH wash (conjugate fraction). This may explain why most of the conjugate from the dry seed extracts was the purported C/D R [^{3}H]-GA_{20} conjugate while at least half of the acidic [^{3}H] GA was [^{3}H]-GA_{20} with lesser amounts of C/D R [^{3}H]-GA_{20}. Thus, it is possible that the purported C/D R [^{3}H]-GA_{20} conjugate represents a [^{3}H]-GA_{20} conjugate which was "rearranged" during work-up.

In summary, significant reversible conjugation of two mono-hydroxylated C_{19} GAs takes place during seed maturation and subsequent germination in maize. This is consistent with trends noted previously by Barendse et al. (7) and Yamane et al. (148). Further, it strengthens the postulation that reversible conjugation of GAs may play a role in biological regulation of GA level since GAs may be involved in endosperm hydrolysis in maize (61) and early maize seedling growth (9,56,108). Such a means of regulation of "active" GA levels is relevant not only to seed germination, but may also be important in other physiological processes such as the regulation of dormancy (4,57).

Overview and Conclusions

Within the course of these investigations, only the portion of the developmental sequence of maize beginning at imbibition and generally ending at anthesis has been considered. While this limitation was originally imposed out of considerations for time involvement, the selection of the early part of the maize life cycle is particularly valid for the theme of this investigation: developmental adaptation for northerly latitudes. In Southern Alberta, as in most regions with higher latitudes, the onset of the growing season is the least favorable period for maize growth and development. Soil temperatures at planting tend to be about 10°C, far below maize's optimum for growth and development of about 30°C (86). Thus, when the physiologist and breeder attempt to produce northerly-adapted genotypes, seedlings must show satisfactory low temperature vigor. Sub-optimal temperatures typically persist in Southern Alberta until June or early July. During the warmest part of the season (around August) maize development is not severely limited in Southern Alberta. Anthesis of early-maturing genotypes occurs in early June and hence, it is during the portion of the developmental sequence up to anthesis in which low temperatures are regularly presented.

With respect to low temperature vigor an interesting concept has arisen during these experiments: temperature stability. It has been repeatedly observed (Chapters I.1., I.2., III.1., III.2.) that different genotypes respond differently to low temperatures. As a result of this genotype x environment interaction a single equation describing the relationship between maize development and heat accumulation is inappropriate. Generally the corn heat unit equation provides a reasonable

approximation (Chapters I.1., I.2.) but even this is only applicable over an intermediate temperature range (Rood and Major, unpublished). Some genotypes tend to have a relatively constant chronological requirement to anthesis while in others, the number of days to anthesis varies widely under different temperature regimes. In applying a term and concept commonly used when considering variation in yield across environments (yield stability) the phrase temperature stability is appropriate. Genotypes such as CM49 which show little low temperature vigor have low temperature stability while genotypes such as CM7 which grow reasonably well over a wide range of temperatures have high temperature stability (Chapters III.1., III.2.). Although hybrids have only been briefly studied, CM7 x CM49 shows far lower temperature stability than either parental inbred. Conversely, the hybrid is more opportunistic and able to thrive under favorable conditions (Chapters III.1., III.2.). At low temperatures however, the hybrid grows no better than the temperature stable inbred CM7 (Chapter III.1.). With respect to developmental adaptation and temperature stability it is probably that genotypes with high temperature stability are best suited to higher latitutdes and altitudes, regions characterized by large temperature variation over the growing season. However, since these genotypes are less opportunistic they would show less vigorous growth and development under favorable climates and hence, for favorable regions, low temperature stability would be most desireable.

An examination of the portion of the life cycle up to anthesis is also relevant in the study of photoperiodic response. Long days may delay the interval up to tassel initiation while other developmental processes are thought to be day neutral (13,79). For experimental simplicity, anthesis

rather than tassel initiation was observed since an analysis of tassel initiation would require destructive sampling and hence, much larger sample populations. Such a study would not be feasible within the context of limited phytotron space.

A key concept throughout this study has been heritability (Chapters I.1., I.2., I.3., II.2.). This index offers a quantitative description of the relative influence of environment and genotype on a particular character. A given heritability value relates only to the relative influence within a specific environment. Although genotypic variation should be relatively constant across environments, a "fudge-factor" representing the genotype x environment interaction will alter apparent genotypic influence.

How do gibberellins fit into the developmental adaptation of northerly adapted maize? During the course of these investigations it has become apparent that GAs are probably involved in any developmental process examined (Chapters III.1., III.2., III.3., IV.1., IV.2.). Further, genetic variation in endogenous GA content has now been demonstrated (Chapters III.1., III.2.). It is very likely that GAs play regulatory roles in the control of maize sexuality (Chapter III.3.), internode elongation (Chapters III.1., III.2.) and growth rate (Chapters III.1., III.2.). However, in the absence of comparative data dealing with additional genotypes differing in developmental adaptation to northerly latitudes, the role of endogenous GAs in this adaptation is unclear.

Endogenous GA content was well correlated with growth characteristics of two inbreds and their hybrid grown under differing temperature regimes (Chapters III.1., III.2.). Thus, temperature stability for GA content

paralleled temperature stability for growth. However, it must again be cautioned that such correlations must not be given causal status without additonal data from manipulative studies.

The most conspicuous aspect of GA metabolism was the observed variation with developmental change (Chapters IV.1., IV.2.). Changes in metabolism and endogenous GA content are expected if GAs do indeed play an integral role in the regulation of development. This study provides evidence that endogenous GAs act as a mediator between genotype, environment and developmental phenotype of maize. These relationships should be further studied before the role of GAs in developmental adaptation can be fully understood.

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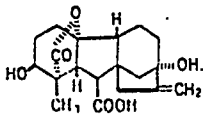

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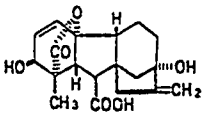

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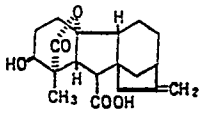
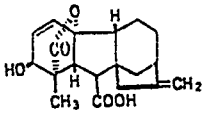
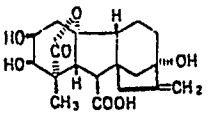


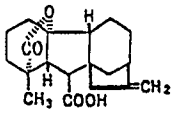
A_4



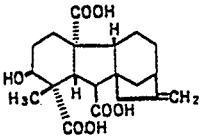
A_7



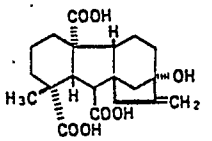
A_8 **



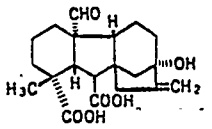
A_9



A_{13}



A_{17} *



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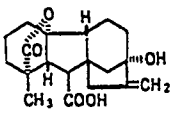
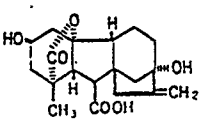
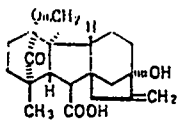


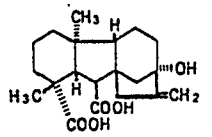
A_{20} *



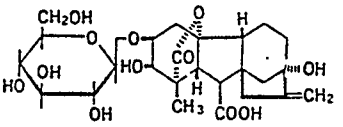
A_{29} **



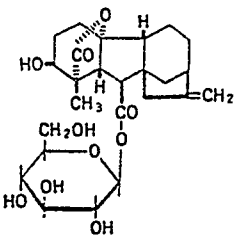
A_{44} *



A_{53} *



A_8 - glucoside



A_4 - glucosyl ester

Appendix I. Structures of gibberellins native to maize and other gibberellins and conjugates involved in these studies. (* native to maize; ** probably native to maize (65))