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"The role of the Gibberellins in the physiological action of the Yellow-Green 6 gene in tomato, Lycopersicon Escuelentum mill."

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University of Massachusetts Amherst

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"THE ROLE OF THE GIBBERELLINS IN THE PHYSIOLOGICAL ACTION OF THE YELLOW-GREEN 6 GENE IN TOMATO, LYCOPERSICON ESCULENTUM MILL."

A Dissertation Presented

By

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"THE ROLE OF THE GIBBERELLINS IN THE PHYSIOLOGICAL ACTION OF THE YELLOW-GREEN 6 GENE IN TOMATO, LYCOPERSICON ESCULENTUM MILL."

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November, 1970

D E D I C A T I O N

It has been said that behind every 'successful' man is a woman. To the woman - Nancy - this piece of work is dedicated with affection. And for whatever this dissertation is worth, I wish to share it with my family and with Mrs. Eloisa Tiongco Vda. de Perez.

A C K N O W L E D G M E N T S

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The Role of the Gibberellins in the Physiological Action of the Yellow-Green 6 Gene in Tomato, Lycopersicon esculentum Mill.

(November 1970)

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Directed by: Professor William H. Lachman

The tomato mutant yellow-green 6 (yg_6) exhibits pleiotropic characteristics resembling those regulated by the gibberellins (GAs). The yg_6 has yellow-green cotyledons and leaves, greatly elongated and colorless hypocotyl and stems, and no detectable anthocyanin. This study was conducted to determine the relationship between the phenotypic expressions of the yg_6 gene mutation and the GAs.

The yg_6 was found to contain three times as much endogenous GAs as the normal wild type (L. esculentum var. 'cerasiforme' Line 018). The presence of a higher quantity of GAs in the yg_6 than in the wild type was confirmed by gas-liquid chromatography (GLC). Gibberellins A_3, A_8, A_9, A_4 and/ or A_7, and possibly A_5 were identified using GLC in the trimethylsilylated extracts of both tomatoes. However, certain GAs were found to be present in greater quantities in the mutant than in the wild type.

The yg_6 could still respond to applied GA_3 indicating it is not fully saturated with this hormone. The mutant has a lower level of saturation to GA_3 (50 µg/plant) than the wild type (100 µg/plant). GA_3 induced a drastic change in leaf shape from normally serrated (dentate) shape to

smooth edge (entire) shape in both plants. The GA-induced smooth-edged leaves closely resemble the "potato-leaf" character in tomato, a character known to be controlled by a single recessive gene.

The specific activity of L-Phenylalanine ammonia-lyase (PAL) in the $\underline{yg}_6$ was found to be twice that in the wild type. Two concentrations of GA_3 promoted the activity of PAL in both tomatoes; the promotion being slightly greater in the $\underline{yg}_6$ than in the wild type. The $\underline{yg}_6$ mutation did not appear to affect the amount of GA-inhibitors present in the mutant when compared to that in the normal.

GA_3 and Phosfon treatments resulted in nearly mimicking completely the phenotypes of either plants, except for the bright yellow primary shoot as in the mutant. GA_3 induced the pleiotropic characters of the $\underline{yg}_6$ in the wild type, or conversely, Phosfon induced the characteristics of the normal in the $\underline{yg}_6$. Grafting experiments, involving the side-approach and top grafting, did not result in inducing drastic changes in the syndrome of three pleiotropic characters studied.

It is concluded that the syndrome of pleiotropic characters of the $\underline{yg}_6$ mutant, namely faster growth rate, reduced chlorophyll content, absence of anthocyanin, depressed root and top growths, and twice the activity of PAL than in the wild type, could be explained by their being causally related to the GAs.

INTRODUCTION

The genetics of the tomato (Lycopersicon esculentum Mill.) has been extensively studied. The tremendous progress accomplished in elucidating the linkage relationships of tomato mutants was reported recently (41). More than one hundred genetic loci have been established in eleven of the twelve tomato linkage groups (172). There are now a total of 608 known genes compiled, described, and designated with gene symbols, with 328 new genes listed in 1966 (41) to supplement those previously reported (16, 40).

The rapid progress being made today in the fields of plant genetics and plant physiology suggests that efforts to merge these two disciplines in a study of the physiological actions of single mutant genes would prove rewarding. The availability of a great number of tomato mutants with known genetic inheritance provides a ready tool for such an investigation.

One such tomato mutant with promising phenotypic characters is the yellow-green 6 (yg_6). The yg_6 mutant was obtained by irradiation of seeds of the normal wild type, Lycopersicon esculentum var. 'cerasiforme' Line 018 (31). Evidence from F_1 materials (37) indicated that the yg_6 gene and five other "yellow-green" mutants assort independently. Recently, De La Roche and Lachman (61) reported that the yg_6 gene is located at map position 50 between the genes hairless and anthocyaninless on chromosome XI. Whalen (219) and De La Roche (60) concluded that the three plant characteristics comprising the yg_6 syndrome represent a case of pleiotropism rather than a complex locus.

The $\underline{yg}_6$ mutant exhibits the following pleiotropic characteristics (219): yellow-green cotyledons and leaves, greatly elongated and colorless hypocotyl, and no detectable anthocyanins in either the stems or leaves (see Figure 1). The actively growing region is bright yellow with the leaves turning pale green at maturity. These characteristics of the mutant closely resemble those traits regulated by the gibberellins which suggested that the phenotypic expressions of the mutation might be related to the gibberellins (GAs) and to the processes controlled by this group of hormones.

Experiments were designed (1) to determine the relationship of the physiological action and phenotypic expression of the mutant gene to the endogenous GAs, (2) to determine whether the mutation affected the quantity and quality of endogenous GAs, and (3) to determine whether the mutation affected the level of endogenous GA-inhibitors. Attempts were made to induce drastic changes, possibly mimicking the phenotypes of both plants by applications of GA and Phosfon to soil-grown and test tube-cultured seedlings and by grafting. The GA would augment the level of endogenous GAs while Phosfon, an antigibberellin known to block GA biosynthesis (62), would reduce the level of endogenous GAs. Since there is no available information on the identity of the GAs in tomato, the identification of tomato GAs was undertaken by gas-liquid chromatography.

Figure 1. Pleiotropic characteristics of the *yg*$_6$ tomato mutant: yellow-green cotyledons and leaves, greatly elongated and colorless hypocotyl, and no detectable anthocyanin in either the stems or leaves. Two-week old seedlings of the mutant and the wild type (*L. esculentum* var. 'cerasiforme' Line 018) are shown.


FISHER SCIENTIFIC CO
PITTSBURGH • PHILADELPHIA • CHICAGO
NEW YORK • ST. LOUIS • MONTREAL • TORONTO
CENTIMETERS
INCHES
L. esculentum var. cerasiforme Line 018
yellow-green-6
Date sown - 8/12/68
Date photographed - 8/26/68

FIGURE 1

L I T E R A T U R E R E V I E W

Gibberellin-Induced Responses In Higher Plants

The physiological effects of the gibberellins on plant growth have been studied for over thirty two years since Yabuta and Sumiki (189) first reported their success in the crystallization of gibberellic acid. There is a considerable body of literature on the subject and detailed descriptions of the physiological responses of plants to GA are available in a number of reviews (26, 149, 160, 161, 189, 191, 192, 193).

Some of the growth responses now known to be associated with GA were originally described by early Japanese workers in their reports on the "Bakanae" disease of rice (Oryza sativa L.) caused by the fungus Gibberella fujikuroi (SAW.) Wr. (Fusarium moniliforme Sheld.). It was from this fungus that the first GA was derived (190). The symptoms of the "Bakanae" disease were (1) unusual elongation of the seedlings, (2) elongation of the stem until maturity, and (3) appreciable inhibition of root growth.

Rapid stem growth. The most characteristic general response of plants to GA is rapid stem elongation. The pronounced stem elongation resulting from GA treatment could be explained anatomically by enhanced subapical mitotic activity and cell elongation (177). Phinney (156, 161) reported that some, but not all, single gene dwarf mutants of corn responded with normal tall growth when given a continous supply of GA_3. It was reported that the dwarf habit of growth in these dwarf corn mutants was due to shortening of the internodes rather than to a decrease in the number of internodes (161). GA treatment induced normal growth in the

corn mutants by the lengthening of internodes. GA was also found to replace the need of a biennial, gene-controlled rosetted variety of Hyoscyamus niger L. for a cold treatment; GA caused the rosette plant which lacks internodes to bolt and flower (104, 105).

Barton (14, 15) reported that GA could overcome the repressed and dwarf growth habit in the seedling stage of certain "physiologic dwarfs" in Malus and Paeonia without the normal requirement for cold treatment. The term "physiologic dwarf" is distinguished from "genetic dwarf" in that the former is characterized by dwarf growth in the seedling stage which can be overcome by cold or other environmental conditions, while the latter does not respond to environmental factors (160). Attention is directed to the fact that there is as yet no information available on the level of endogenous GA in these plants which would shed light on the physiology of dwarf growth habit in these materials.

Inhibition of rooting, chlorophyll and anthocyanin. Root growth and the syntheses of pigments, chlorophyll and anthocyanin, are the only processes pronouncedly inhibited by GA. There are a number of reports which indicate that GA suppressed root growth in many plants, including pea, bean, and tomato (26, 28, 225) and in red maple (9). Tomato and bean plants when grown in culture solution supplied with GA exhibited significant reduction in dry weight of roots (210).

It is generally known that GA treatment at higher dosage levels results in etiolation or yellowing of the leaves of the plant. When the chlorophyll content of leaves was studied in response to GA treatments, significant reductions in chlorophyll content occurred with increasing GA concentrations in sweet-lime seedlings (128) and in barley

seedlings (197). The paleness of leaves in barley seedlings resulting from GA treatment could be explained by the quantitative changes in pigment components; chlorophylls a and b were decreased, the xanthophylls remain unchanged, while the carotenes were increased (197).

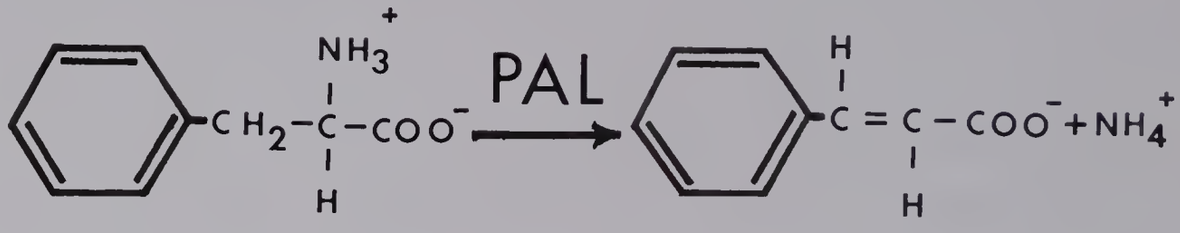
Furuya and Thimann (70) working with two species of Spirodela and Bachelard (7) working with Acer rubrum have shown that GA, at low physiological concentrations, inhibited anthocyanin synthesis. And in red maple, an interrelationship between root formation and anthocyanin synthesis appears to exist (7, 8, 9).

Promotion of L-Phenylalanine ammonia-lyase activity. There are a considerable number of plant enzymes the synthesis and specific activity of which are promoted by GA. In particular, the hydrolytic enzyme from the aleurone cells of barley seed, a-amylase, has been extensively studied. The classic work from Varner's laboratory (212) demonstrated that GA was responsible for the de novo synthesis of a-amylase. The a-amylase induction and release from isolated aleurone cells or endosperm-half of barley seed by GA (133, 148) was adapted into a biological assay which was specific for GA (42, 148, 158).

L-Phenylalanine ammonia-lyase (E.C.4.3.1.5) (PAL) is another plant enzyme whose activity could be promoted by GA. PAL which was initially discovered in barley tissues by Koukol and Conn in 1961 (103) was originally known as phenylalanine deaminase. PAL catalyzes the removal of ammonium ions from L-phenylalanine to yield trans-cinnamate (see chemical reaction, page 7).

Engelsma (68) first reported what appears to be a slight promotion of activity of PAL resulting from GA treatment (10^{-3}M) in gherkin

hypocotyl segments. Cheng and Marsh (36) found a 60 per cent increase in PAL activity in the stem of dwarf pea within 24 hours following GA treatment, and 3-fold higher PAL activity in GA-treated tissues than in the untreated tissues during a 10-day period.



L-Phenylalanine trans-cinnamate

REACTION CATALYZED BY L-PHENYLALANINE AMMONIA-LYASE

Recently, Reid and Marsh (170) demonstrated GA-induced increase in specific activity of PAL in a number of species including dwarf-1 corn mutant and its normal tall sibling, normal tomato and pinto bean seedlings. They also found high PAL activity in the juvenile form of Hedera helix and the apparent absence of the enzyme in the adult form. It has been suggested that the difference in growth form in Hedera could be explained in part by a difference in the level of endogenous GA since Robbins (174) was able to reverse the adult form to the juvenile form by GA application. The adult form is characterized by short shoots and the ability to flower while the juvenile form is characterized by elongated internode and the absence of flowering.

Mutant Genes and the Level of Endogenous Gibberellins

The terms "gibberellin" and "gibberellin-like" substances will be used according to the definitions proposed by Phinney and West (160) with the former term restricted to substances defined by both biological and chemical properties while the latter term defined by biological properties only. The term gibberellin is restricted to compounds which have a gibbane skeleton (see diagram, p. 21) and biological activity as promoting cell division and/or cell elongation in plants. Gibberellin-like substances are those with requisite biological activity in appropriate intact dwarf mutant tests and in other bioassays specific to GA. These bioassays include the 5 dwarf mutants of Zea mays L., the dwarf mutant of Pisum sativum L., the single gene mutant (dwarf 'kidachi' cultivar) of Pharbitis nil Chois., and the gene-controlled rosetted variety of Hyoscyamus niger L. Other bioassays which are relatively specific to GA but not necessarily involving shoot growth are also used by some investigators. Some of these are the a-amylase barley endosperm assay (148, 158), lettuce hypocotyl (69), cucumber hypocotyl (27), lettuce seed germination (29), and others (18, 57).

Attempts to explain the growth differences in certain plants through the participation of gibberellic acid have been made. Phinney (156) working with 10 single gene mutants of Zea mays L. which exhibit the dwarf habit of growth, found that only 5 mutants dwarf 1 (d_1), d_2, d_3, d_5, and anther ear 1 (an_1) responded to the gibberellins tested (GA_3, GA_2, GA_1). These mutants responded with normal growth and became indistinguishable from the normal tall corn (156, 157). It was suggested that these GA-responding mutants might have aberrant reactions

controlling different steps in the synthesis of endogenous gibberellins (159).

Brian and Hemming (25) were the first to report a differential response to GA in which certain dwarf cultivars of pea (Pisum sativum) of unknown genotype, broad bean (Vicia faba) and French bean (Phaseolus multiflorus) of known genotype were found to respond more to GA than the normal cultivars. Several single gene mutations which control the ability of the dwarf genotypes to respond to GA were reported in pea for the le gene (23), in Lolium perenne Aitch. for the d gene (44), and in Zea mays for the d_1, d_2, d_3, d_5, and an_1 genes (156, 157). Similar to the responses of the corn mutants, the other single gene mutants were restored by GA treatment to phenotypically normal plants.

Radley (166) reported that the dwarf 'Meteor' cultivar of peas appears to have the same level of GA-like substances as the tall cultivar, 'Improved Pilot'. On the other hand, Köhler and Lang (99) showed the presence of GA-like materials in the tall pea, 'Alaska', but not in the dwarf pea, 'Progress No. 9'. Phinney (157) working with the 5 GA-responding mutants and normal corn found appreciable amounts of GA-like substances in normal tall corn. However, among the 5 GA-responding mutants, the extracts from the mutants d_3, d_5, and an_1, separated by either paper or column chromatography, did not show any GA-like activity. The total GA in the mutants d_1 and d_2 when compared to that in the normal corn was found to be less than half that of the total GA in the normal. Phinney (157) concluded that the 5 dwarf mutant genes are responsible for the dwarf growth habit through the control of the amount of endogenous GA. The correlation with the level of GA-like substances

in tall plants compared to the lowered amounts or absence in certain dwarf plants was presented as evidence for a causal relationship between GA and the dwarf growth habit. At this point it should be noted that all mutant genes studied so far solely involved those controlling dwarf growth habit.

Growth differences between early- and late-flowering varieties of Trifolium and the presence or absence of bolting in Hyosyamus have been studied in relation to the amounts of gibberellins in these plants. Stoddard (188) reported that the late-flowering red clover (Trifolium pratense L.) which responded more to added GA than the early-flowering type on the bases of stem elongation, tillering, leaf shape and growth habit was found to contain less GA. This result fits the observation that the rate of stem elongation is initially slower in late-flowering varieties. Lang (105) found an appreciably higher level of GA in bolting than in non-bolting plants. He also reported that a certain GA-like-substance was observed in bolting but not in non-bolting ones. From these limited data, Lang (105) concluded that a causal relationship may be inferred between the quantity and quality of GA-like substances and the presence or absence of bolting. Similarly, Harada and Nitsch (79) and Nitsch (136) reported that there was a positive correlation between the amount of GA-like substances and bolting in Chrysanthemum morifolium Ram. cv. 'Shuokan', a cold-requiring plant, and in Rudbeckia speciosa Wenderoth., a long day plant. The accumulated evidence would indicate that gibberellin may be the primary limiting factor in the bolting of at least some long day and cold-requiring rosetted plants (79, 136).

Endogenous Inhibitors Interfering with GA

The concept that growth of plants is the net result of the influence of growth-promoting versus growth-inhibiting materials had been proposed as early as 1947 by Larsen (107). Since then this proposal was invoked in several studies to explain the results obtained. Radley (166) who found no difference in the level of GA-like substances in either the dwarf or tall cultivar of peas suggested that the growth dissimilarities between these cultivars could be explained by a difference in the inhibitor level. Phinney (157) also suggested that an alternate explanation for the dwarf growth habit of certain maize mutants that were found to contain no GA-like activity or less amounts of GA could be the accumulation of higher than normal amounts of inhibitors of GA-induced growth. Bentley (17) and Hemberg (87) have reviewed the evidence for the probable regulatory roles of inhibiting substances. It should be pointed out that there is some skepticism about some growth inhibitors on the grounds that their effects are non-specific.

Köhler and Lang (99) presented evidence for the presence of inhibitors in immature seeds of lima beans which interfere with the response of dwarf peas to GA. These inhibitors have no activity when applied to a particular bioassay alone but they decreased the response of bioassay materials to GA. They also reported that the quantity of these substances seems to be inversely correlated with the growth of tall and dwarf peas. Tall peas, in which growth is rapid in the dark seem to contain relatively low amounts of these materials while dwarf peas in which growth is likewise rapid in the dark contain lower levels. In

contrast, high levels of the inhibitors were found when the dwarf peas were grown in the light and where growth was markedly inhibited. Köhler and Lang (99) concluded that these inhibitors could participate in the growth regulation of plants, particularly by an interplay with GA.

Endogenous inhibitors which selectively suppress the growth induced by GA have also been extracted from seeds of the carob tree (Ceratonia siligua) by Corcoran and Phinney (47) and Corcoran et al. (48), and from a brown algae by Radley (167).

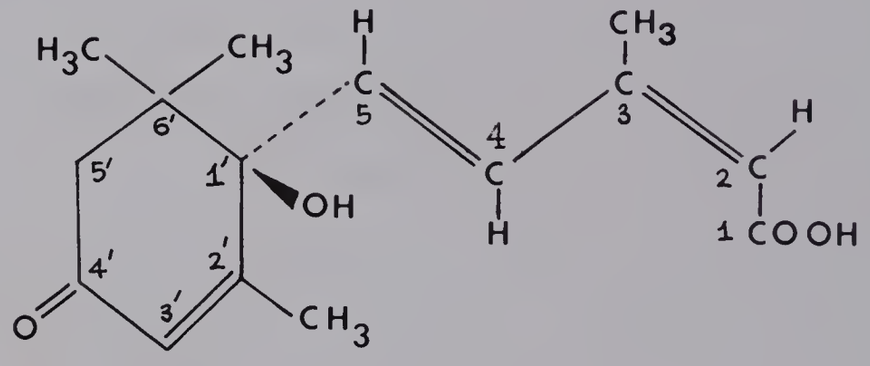
Abscisic Acid and GA

In agreement with the observation of Köhler and Lang (99), abscisic acid (ABA) could indeed be the growth inhibitor which could participate in the regulation of growth of plants by an interplay with GA. ABA has been found to antagonize the effect of GA in various bioassay tests for GA, including the lettuce hypocotyl, the dwarf maize leaf section, the oat leaf section, and the pea epicotyl tests (215).

Although the study of ABA was initiated only 7 years ago (142, 143) this hormone now ranks in importance with the auxins, gibberellins, and cytokinins as major plant growth regulators (see recent review, 2). Inhibitory substances, like "dormin" extracted from Acer (45), the abscission-accelerating substance from yellow lupins (46), and growth inhibitor from plum shoots (13) were later shown to be identical with "Abscisin-II". Recently, the name "Abscisic Acid" was adopted in place of "Abscisin-II" (Addicott et al., 1). However, the term "dormin" could still be used to describe substances which appear to function as "endogenous dormancy-inducers" (64, 65) while the term "Abscisin-I",

whose structure is still not elucidated, should be held in abeyance (2).

Irving and Lanphear (91) and Irving (90) found an inhibitor, extracted from short day-treated Acer negundo, which appears to be similar to ABA. When Acer was given the short day treatment the ABA-like substance levels were highest, while GA-like activity was greatest when the treatment included long days. Since short day photoperiod enhances the induction of cold hardiness while long day photoperiod does not, it appears that hardening process is more closely related to a build-up of ABA levels than to a reduction of GA levels.



STRUCTURE OF (S) - ABSCISIC ACID, the naturally occurring form.

Chemical Name: 3-methyl-5-(1'-hydroxy-4'-oxo-2',6',6'-trimethyl-2'-cyclohexen-1'-yl)-cis, trans -2,4-pentadienoic acid

ABA has been found to counteract or inhibit GA-induced responses, in particular, germination, growth and senescence (5, 38, 98, 209, 226). Paleg (148) reported that ABA prevented the hydrolysis of starch in barley seeds during germination, a process promoted by GA. Chrispeels and Varner (38) found that ABA appears also to inhibit the syntheses of proteases, ribonucleases, and other hydrolytic enzymes whose syntheses

in aleurone layers are promoted by GA. It was concluded that ABA has an apparent specific effect on the syntheses of certain enzymes in barley seed.

Van Overbeek (144, 145) has shown that it is possible to turn the growth of Lemna minor off and on by manipulating the concentrations of ABA and benzyladenine in the medium. ABA inhibits while benzyladenine reverses the ABA effect. Neither auxin nor GA could counteract the inhibitory effect of ABA on the growth of Lemna. The drastic reduction in growth induced by ABA was preceded by a striking reduction in synthesis of nucleic acid while the resumption of growth by benzyladenine treatment was preceded by a resumption in the rate of synthesis of nucleic acid. Van Overbeek (144) suggested that the "Monod's Concept" (127) of allosteric effectors of an enzyme complex could account for the interaction between ABA and benzyladenine. In Lemna, it was concluded that ABA affects primarily the synthesis of DNA and secondarily the synthesis of RNA (144). In contrast in radish, ABA was reported to affect RNA synthesis more directly than DNA synthesis (215) since mature leaf disc was employed in which DNA synthesis is not detectable.

Earlier, Wareing et al. (215) have reported that the inhibitory effect of ABA on the growth of leaf sections of tall normal maize could be overcome by GA, but not on d_1 mutant maize. It was postulated that ABA does not interact directly with GA but that it affects GA biosynthesis somehow. Later, evidence for this suggestion was presented (214). Wareing et al. (214) found that ABA applied to excised shoots of corn leads to a marked reduction in the levels of certain endogenous GAs in both normal tall and d_1 mutant. Likewise, when ABA was applied to intact,

whole plants of Spinacia oleracea the large increase in the level of GAs which normally occur when they are transferred from short day to long day was inhibited. At the biochemical level, it was suggested that the primary effect of ABA was the inhibition of RNA synthesis, which in turn might lead to inhibition of enzyme synthesis and hence to inhibition of GA production (145, 146, 214).

It is interesting to note that in several cases, the effects of ABA are temporary and only by repeated treatments will a response be induced (65, 187). For instance, ABA applied to young or still vigorously growing plants failed to produce leaf abscission, which is one of the major assay for ABA activity (187). Addicott and Lyon (2) concluded that these results show that plant tissues are well adapted to ABA and that the tissue can respond readily to applied ABA either with some observable response or in certain circumstances, by rapid inactivation of exogenous ABA.

Growth Retardants

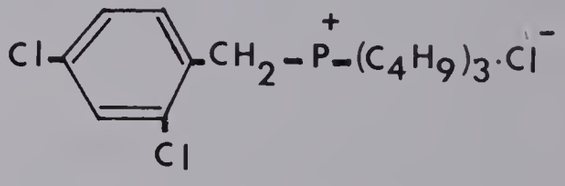
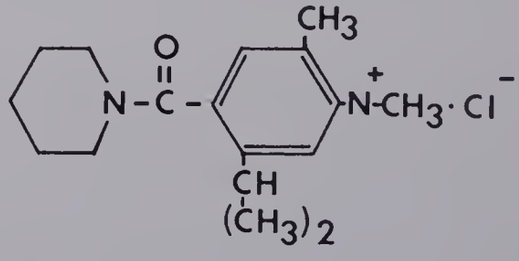
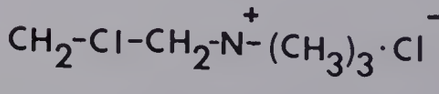
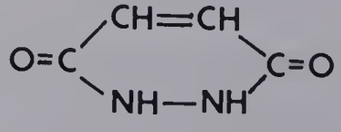
The growth retardants, Phosfon, AMO, and CCC, with their highly specific and selective inhibition of particular step(s) in GA biosynthesis, are without question a powerful tool in studying the physiological effects of the gibberellins.

In a review Cathey (33) used the term "growth retardant" to include all chemicals that slow cell division and cell elongation and regulate plant height without formative effects. In contrast, "growth inhibitor" like maleic hydrazide suppresses apical growth by completely inhibiting cell division (211). A typical growth response to added chemical

retardants is suppression of stem or shoot elongation. There are also a number of reports (123, 211, 221) which indicate that these retardants not only alter plant growth toward shorter height but also result in darker green leaves.

These synthetic compounds which give the same general growth responses with a wide range of plant species, exhibit no similarities in structure to each other or to the gibberellins (211). The chemical structures and chemical names of three growth retardants and one growth inhibitor are shown in the table below, (for comparison to the structure of gibberellin A_3 see page 23).

GROWTH RETARDANTS AND GROWTH INHIBITOR

COMMERCIAL NAME	CHEMICAL NAME	CHEMICAL STRUCTURE
Phosfon or Phosfon D	2,4-dichlorobenzyl tributyl phosphonium chloride	
AMO 1618	2-isopropyl-4-dimethyl amino-5-methyl phenyl-1-piperidine-carboxylate methyl chloride	
CCC or Cycocel	2-chloroethyl trimethyl ammonium chloride	
Maleic Hydrazide	1,2-dihydropyradzine-3,6-dione	

Growth responses to the retardants tend to be opposite to those obtained with GA (224). Also the growth retardants and GA can counteract each other's effects and when added simultaneously they exhibit a mutually antagonistic effect (211, 224, 225). Sachs (178) presented an anatomical basis of the action of growth retardants. He found that the greatly reduced stem elongation resulting from AMO application was due to the inhibition of cell division and cell elongation in the subapical meristematic region. GA, on the other hand, promoted subapical cell division and cell elongation and shoot growth (177).

Lockhart (111) using kinetic analysis concluded that Phosfon and CCC interact competitively with GA on stem elongation in the sense that the effects of the retardants could be completely overcome by a saturating dose of GA. He suggested that these chemicals retard stem elongation by reducing the activity of GA. Paleg et al. (150) working with GA-induced a-amylase production in the barley seed showed that these retardants do not interfere with GA action. In contrast, evidence was presented which show that the retardants inhibit GA biosynthesis (11, 96, 135, 179).

AMO and CCC were found to inhibit the biosynthesis of GA_3 in cultures of Gibberella without suppressing the growth of the fungus (96). In another report (135), as little as 0.1 mg/l of CCC was found to reduce GA_3 production by the fungus by one-half and it was concluded that CCC and, by analogy, AMO were selective inhibitors of GA synthesis in Gibberella. Phosfon, which was reported earlier (80) to have no inhibitory effect on GA production in Gibberella because of the effective destruction of the compound by the fungus was later (179) shown to inhibit

GA production when cell-free preparations of the fungus were used.

Baldev et al. (11) presented evidence showing that growth inhibition in higher plants caused by the retardants was actually mediated through the inhibition of GA biogenesis. AMO, when supplied to the young, isolated pea fruit cultures, inhibited the accumulation of GA-like substances by about 60 per cent and did not affect the growth of the seeds. CCC, similarly, caused a reduction of GA-like substances in the seeds of Pharbitis nil (231).

Direct chemical evidence to show that these compounds inhibit certain enzymatic steps in the biosynthesis of GA awaited the work of Dennis et al. (62). They found that AMO, Phosfon and Phosfon S inhibited the formation of kaurene from mevalonate and caused the accumulation of geranylgeraniol in endosperm preparations using Echinocystis. This result was confirmed later when similar inhibitions of the formation of kaurene from mevalonate by AMO were found in preparations from pea seeds (3) and fruits (73). Cross and Myers (56) confirmed the findings that GA_3 production and kaurene formation were inhibited by AMO and CCC in the Gibberella. These results would allow us to use the term "anti-gibberellins" to refer to growth retardants, namely Phosfon, AMO, and CCC. All 3 retardants, inhibit the reaction step from trans-geranylgeranyl pyrophosphate to copalyl pyrophosphate whereas Phosfon in addition inhibits the following 2 reaction steps: (1) copalyl pyrophosphate to form cyclic diterpenes, and (2) copalyl pyrophosphate to form kaurene [(-)-kaurene-16-ene] (106).

Present Status of Gibberellin Research

The most recent review on the gibberellins was by Lang (106) in which a comprehensive survey of the chemistry and biosynthesis of the hormone was presented. A tremendous advance in the identification of the naturally occurring GAs and at least six bound GAs, and in the elucidation of GA biosynthesis was made in only about 4 or 5 years (106). Earlier reviews on GA include the one on the early history of GA (189, 191, 192); the responses of economic plants to GA (222); the chemistry of the early fungal GAs (26); the GA as native plant growth regulators (160); the applied aspects of the GAs (193); the physiology of GA (161); the physiological effects of GA (149); the GA biosynthesis (55); and the effect of GA on nucleic acid and protein metabolism (97).

At this writing, there are now 29 chemically identified GAs, as compared to the 13 GAs at the time of Paleg's review in 1964 (149). The use of improved techniques and better instrumentation were responsible for this progress in the identification of natural GAs. These include the adaptation of combined gas chromatography and mass spectrometry in the elucidation of the identity of GA (114, 115, 116), nuclear magnetic resonance spectroscopy (76, 208), infrared spectroscopy (131, 132, 201), mass spectrometry (199, 227, 230), and gas chromatography (34, 89).

The feasibility of gas chromatography for the separation and identification of pure GAs was first reported by Ikekawa and Sumiki (89). Methyl esters of nine GAs were successfully separated and identified using two columns, 1.5% SE-30 and 2% QF-1-0065. Both Pryce *et al.* (164) and Sitton *et al.* (186) were able to apply gas-liquid chromatography (GLC)

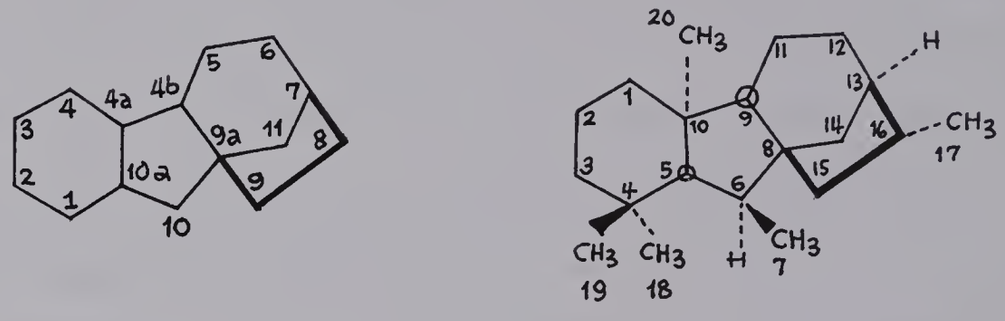
in the identification of GAs in bean extracts and of GA precursors in roots respectively. Sweeley et al. (196) reported a safe method of silylating carbohydrates by the use of trimethylsilyl derivatives (TMS). TMS derivatives of GA, ABA, and 3-Indoleacetic acid, both pure and partly purified plant extracts, were suitably identified and their quantities measured (59). Earlier, Cavell et al. (34) succeeded in identifying several GAs from crude and purified acid fractions, prepared from immature seeds of Phaseolus, using both methyl ester and TMS ether of this methyl ester. GLC offers the advantages of separating the hormones from interfering compounds present in the plant extracts, and adaptability to both qualitative and quantitative work. It should be noted that the greatest problem GLC methods have is the necessity for acquiring authentic samples, which are not always available , for direct comparison. However, retention times of at least 23 GAs separated in about 4 different columns are available in the literature (89, 115).

Compared to gas chromatography, results from combined gas chromatography-mass spectrometry techniques are definitive and exact. Reference mass spectra of at least 24 GAs as methyl esters and TMS ether of methyl esters are available in the literature for comparison to provide conclusive identification of GAs without need of authentic samples (19, 199, 200, 227).

In order to avoid further confusion in naming the new GAs, MacMillan and Takahashi (122) have proposed the use of the A numbers designation for all naturally occurring, fully identified compounds with the gibbane skeleton and the appropriate gibberellin properties. The more recently isolated GAs with such trivial names as Bamboo GA, Pharbitis GA and

Canavalia-I GA were given the new designations respectively as follows: A_{19}, A_{20}, and A_{21} (Appendix, Table I). It should be noted that the gibberellin A numbers do not necessarily follow the order of discovery.

The present system of systematic nomenclature of the GAs is based on the gibbane skeleton (see diagram). However, there is now a proposal to change the parent skeleton from gibbane to gibberellane (176).



THE STRUCTURES OF GIBBANE AND GIBBERELLANE. Heavy lines or wedges indicate bonds lying above the plane of the ring system; broken lines indicate bonds lying below this plane [Adapted from Lang, 1970 (106)] ent=enantiomer

19-C and 20-C gibberellins. The 29 GAs presently known are listed in Appendix, Table I, including their trivial names, molecular formula, molecular weight, natural sources, and references. At the time this dissertation was being written up, a similar table and all 29 structural formula were published by Lang (106).

Of the 29 GAs, 16 belong to the 19-C category and the remaining 13 belong to the 20-C category. The former category are all mono-carboxylic acids with the COOH group in position 7 (refer to the numbers on the gibberellane structure, see diagram), and have a lactone

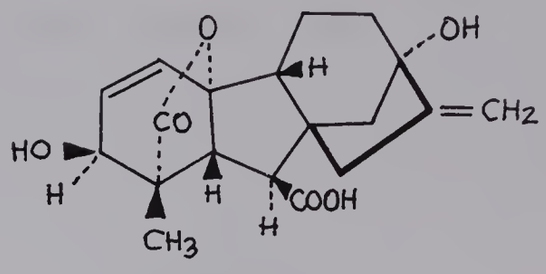
configuration in the A ring due to the loss of the extra C atom. The latter category have COOH groups in position 7 and 18 and some also in 20, while still others have CHO (aldehyde) group in position 20.

It is interesting to note that a structural difference with regard to the OH in positions 3 and 13 seems to occur in GAs obtained only from Gibberella and/or only from higher plants. For example, if a GA which was derived from the fungus has only one OH, it is always in the position 3 (e.g. GA_{13} and GA_{14}), while GA from plants always has it in the position 13 (e.g. GA_6). This difference appears to be a reflection of the existence of two major pathways of GA synthesis (106).

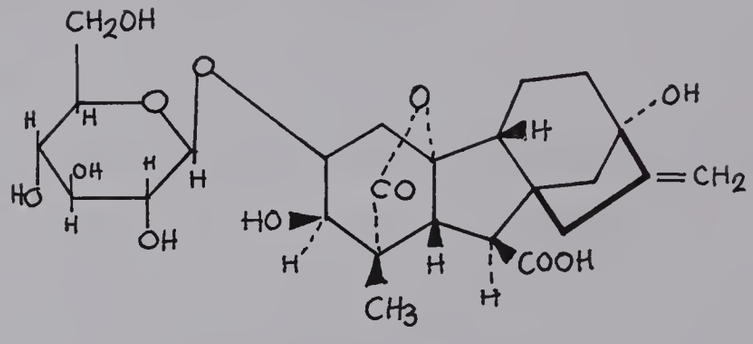
The "Conjugated" gibberellins. The occurrence of "bound" GAs, "water-soluble", or "butanol-soluble" GAs has been reported by several investigators since 1961 when McComb (124) first reported the occurrence of extracts which exhibit GA-like activity but was of a polar nature. In contrast with the acidic or "typical" GAs, these substances cannot be extracted from acidified aqueous solutions with ethyl acetate but can be extracted with n-butanol. Such water-soluble GAs have been reported in immature seeds of the Japanese morning glory (Pharbitis nil Chois.) by Murakami (129), Ogawa (140, 141), and Zeevaart (231). These substances were also reported to occur in seeds and seedlings of tomato (151), tulip bulbs (6), in immature seeds of Pharbitis nil Chois. (208).

Sembdner (185) has introduced the more specific term "conjugated" gibberellin to describe these substances; and suggested the continous use of the term "bound" GAs for substances not yet identified. At present, there are 6 identified conjugated GAs: 5 conjugates between gibberellin A_3, A_8, A_{26}, A_{27} and A_{29} and a glucoside each; and 1 conjugate between

gibberellin A_3 and an acetyl group. These substances have been identified from immature seeds of Pharbitis (208, 228, 229), immature and mature seeds and seedlings of Phaseolus coccineus (182, 184, 185). As an example, the structure of a conjugate GA is given below.



GIBBERELLIN A_3



"CONJUGATED" GA

GA_3-glucoside, 2-O-β-glucosyl-GA_3
MW=508; 1 mole of A_3 and glucose each

Treatment of extracts containing "bound" or "conjugated" GA with acid, alkali or enzyme preparations like ficin or emulsin released "free" or "typical" GAs. The existence of protein-bound GAs was assumed by many authors (86, 94, 124) from their observation that ficin or emulsin treatments released free GAs from bound ones. However, Lang (106) explained that evidence obtained with such a crude enzyme preparation is inconclusive. Sembdner et al. (185) presented evidence which showed the formation of conjugated GAs of the glucosyl-types after application of GA to plants. Conjugated GAs appear to function as storage and as a transport GA (12, 84, 151).

MATERIALS AND METHODS

The yellow-green 6 (yg_6) mutant and the normal wild type tomato (Lycopersicon esculentum var. 'cerasiforme' Line 018), which differ from one another by a single recessive gene, were used in this study. Seeds of the mutant tomato were obtained from Professor W. H. Lachman, Department of Plant and Soil Science, University of Massachusetts, Amherst. Seeds of Line 018 were obtained from Professor R. H. Whalen, Department of Botany-Biology, South Dakota State University, Brookings.

The general procedure in handling tomato seeds and seedlings was as follows: tomato seeds were soaked in aerated distilled water for at least 3 days at which time the radicles had emerged. Germinated seeds were sown in rows on seedflats in a mixture of soil, sand, and peat moss (7:3:2), then covered with white sand and placed in the greenhouse. Ten days after sowing, the seedlings were transplanted to 6-inch plastic pots and were allowed to recover in the shade and take root for 4 days. At the end of this period, the seedlings were considered to be 2 weeks old.

Application of GA and Phosfon

Two-week old transplanted tomato seedlings were given a soil drench treatment of GA_3 (purchased from Nutritional Biochemical Corp.) or Phosfon (gift from Mobil Chemicals). Each 6-inch plastic pot, containing 4 seedlings, was given 250 ml of 10^{-4}M GA_3 or 10^{-3}M Phosfon or distilled water. Twelve plants constituted a treatment.

Drop application of GA_3 onto the shoots of three-week old transplanted tomato seedlings was also performed. Five GA concentrations

were prepared as follows: 1, 10, 50, 100 and 500 µg/0.1 ml. Two drops of surfactant Triton X-100 were added per 100 ml of GA solution to increase the penetration of GAs into the leaf tissues (157). Two drops of the surfactant were also added to the distilled water which was applied to the control plants. Each seedling received 0.1 ml of one of the GA solutions or distilled water only once at the start of the experiment. Twelve plants constituted a treatment.

Growth Rate, Chlorophyll and Anthocyanin

The 2-week old plants were treated once at the start of the experiment and growth measurements were begun immediately. The height of the plant from the soil level to the tip of the highest leaf was measured on alternate days for 2 weeks. At the end of 2 weeks, the chlorophyll and anthocyanin contents were determined. Growth measurements were also made on 3-week old plants treated with 5 concentrations of GA_3 to compare the GA-saturation level between that in the mutant and in the normal tomato.

The method of Arnon (4) was followed in the determination of chlorophyll content. One gram of fresh leaves, sampled from leaflets nos. 2 and 3 (leaflet no. 1 being the growing point), was chopped to pieces and homogenized by using a Virtis "45" homogenizer at medium speed for 1 min in 80% acetone. Sodium bicarbonate was always added to the acetone used in chlorophyll determination for the purpose of reducing excessive destruction of chlorophyll molecules. The homogenized tissue was poured on a No. 1 Whatman filter paper and the filtrate collected into a volumetric flask. The filtrate was made up to a total volume of

25 ml by addition of acetone and from which an aliquot was taken for analysis. The aliquot was diluted with acetone (1 ml aliquot: 5 ml acetone) and read spectrophotometrically at 663 and 645 nm.

The method of Bachelard (7) was followed in the determination of anthocyanin content. The plants were harvested by cutting the stems directly above the soil level. One gram of stem tissue was taken starting from the cut end of the stem. The sample was chopped into small pieces and soaked in 5 ml of 0.1 N HCl for 24 hours. The extract was poured on a No. 1 Whatman filter paper and the filtrate read directly in the spectrophotometer at 510 nm. Acetone containing sodium bicarbonate and 0.1 N HCl were used for the blanks in the chlorophyll and anthocyanin determination respectively. In both pigment estimations, each treatment was constituted of 4 replications.

Test Tube Culture

Seeds were germinated and seedlings raised in the manner described previously except that 7 days after sowing of seeds, the 7-day old seedlings were uprooted and their roots carefully washed. One seedling was transferred into each test tube containing 5 ml of different concentrations of GA or Phosfon or distilled water. The concentrations of GA used were 0.01, 0.1, 1.0, and 10.0 µg/ml while the concentrations of Phosfon used were 10^{-7}, 10^{-6} and 10^{-5} M. Preliminary work showed that Phosfon concentrations of 10^{-4} and 10^{-3} M were highly toxic to 7-day old $\underline{yg}_6$ and wild type seedlings. Test tubes were placed in a growth chamber in which a continous white light (purchased from Champion Lights Co.) and a constant temperature of 25°C was maintained. After a week

under these conditions, the rate of stem elongation and the number of lateral roots were determined. Eight seedlings constituted a treatment.

Preparation of PAL Extract

Seedlings were raised and grown as described previously. PAL activity in the 34-day old $\underline{yg}_6$ and normal wild type was compared. Seedlings were transferred and kept for 3 days in a growth chamber which was exposed to continous irradiation by four 100-watt incandescent bulbs and eight cool-white 40-watt fluorescent lamps. Light intensity was 1,000 foot-candles at the level of the seedlings as measured by a Weston model 765 illumination meter. Seedlings were given distilled water daily for 3 days.

Eight to nine weeks old seedlings were used in determining the effects of two concentrations of GA_3 on PAL activity. Seedlings were transferred and kept for 3 days in the same growth chamber mentioned above. After the second day, 250 ml of 10^{-4} or 10^{-5} M GA or distilled water was poured directly at the base of the plants in each 6-inch pot containing 4 seedlings. Twenty-four hours later, the plants were sampled for PAL activity determinations.

Harvesting was accomplished by cutting the stem directly above the soil level. About 7 to 8 cm of stems, starting from the cut end, served as the experimental material and approximately 3 to 6 stem segments, which weighed about 4 grams, constituted a replication. The tissue was placed on ice-water immediately after harvest.

The method used by Reid (169) in the preparation of PAL extracts was followed. Grinding buffer was prepared by adding 0.3 ml of 2-mercaptoethanol (Sigma Chemical Corp.) for every 100 ml of 0.1 M borate

buffer. The chopped stem sample was ground in grinding buffer (3 ml of grinding buffer to every gram of stem sample) using a Virtis "45" homogenizer at high speed for 1 min. The grinding receptacle was kept in an ice bath during the homogenizing period. The homogenate was filtered through 2 layers of cheesecloth into chilled polypropylene centrifuge tubes kept on ice. The filtrates were spun at 20,000 x G for 10 min. in a Sorvall RC-2 refrigerated centrifuge maintained at 0°C.

The supernate was decanted into another chilled centrifuge tube and 1.5 volumes of acetone (-10°C) was added. The tubes were placed in the freezer (-10°C) for 30 min for precipitation of protein. Later, the tubes were centrifuged at 5,000 x G for 10 min, after which the supernate was decanted and the precipitate rinsed with 10 ml of distilled water. Three ml of 0.1 M borate buffer which contained no mercaptoethanol was added to each tube. Glass stirring rods were used to slowly tease the precipitate back into solution. Clumped precipitate was further broken down into small particles through the use of a disposable pipette. After the protein was thoroughly teased back into solution, the extract was centrifuged at 20,000 x G for 10 min. The resulting supernate which we will designate as the "enzyme extract" was used for PAL assay.

PAL Assay

Enzymic activity was estimated spectrophotometrically at 290 nm. The reaction mixture (3 ml) contained 0.5 ml of 0.2 M borate buffer at pH 8.7; 1.9 ml of distilled water; and 0.4 ml of enzyme extract. The reaction was initiated by adding 0.2 ml of 0.1 M L-phenylalanine when the temperature of the mixture was 30°C. The temperature was maintained

at 30°C throughout the spectrophotometric determination. The blank contained essentially the same reaction mixture except for omitting L-phenylalanine, and in its place 0.2 ml of distilled water was added instead. One unit (U) of enzyme is defined as the amount of enzyme catalyzing the formation of 1 μmole of cinnamate per min at 30°C. Enzymic activities are reported in terms of milliunits (mU) per mg protein and mU per gm fresh weight. Protein content was estimated following the method of Lowry et al. (112). A Beckman DU-2 spectrophotometer was used in all spectrophotometric analyses.

Grafting Experiments

Two types of grafting, the side-approach and top grafting (83), were used in these experiments. In both types of grafting, the yg_6 mutant and the wild type were grafted onto their own rootstock and in addition reciprocal grafts were made between them.

In side-approach grafting, 6 weeks old seedlings grown singly in 4-inch plastic pots in the greenhouse were used. A smooth cut about 2 in. long was made on each stem to be united approximately 3 to 4 inches from the growing point and the two cut surfaces bound tightly together with twine. After 2 weeks, the top of the rootstock plant was severed directly above the graft union and the base of the scion plant was cut below the graft union.

In top grafting, 4 to 5 weeks old seedlings grown singly in 4-inch plastic pots in the greenhouse were used. The top of the rootstock plant was removed by a smooth cut about 1 in. above the cotyledonary node. The scion, comprised of 3 to 4 inches of apical shoot, was prepared by

cutting the stem end into a wedge shape. The wedged-end of the scion was inserted into the split top of the rootstock and the graft held firmly by a rubber band. Newly grafted plants were enclosed completely in a plastic bag for 5 days which provided a high humidity condition sufficient to prevent excessive transpiration. One week later, successful graft unions were consummated.

Extraction of GA and GA-Inhibitors

Tomato seeds and seedlings were germinated and raised in the typical manner described except that seedlings to be used for extraction were not transplanted. Shoots and cotyledons of 6-week old plants were harvested, chopped, and extracted in twice the volume of methanol (1:2 w/v). Flow diagram used in extracting GA and GA-Inhibitors from tomato is shown in Figure 2. After 24 hours of soaking, the sample was homogenized in a Waring blender and filtered through 4 layers of cheesecloth. The filtrate was passed twice through 2 layers of No. 1 Whatman filter paper using a vacuum filtration system. The methanol and water in the filtrate were evaporated *in vacuo*. The dried residue was taken up with distilled water to a total volume of 100 ml which was partitioned against 2 organic solvents. The aqueous residue first was extracted 4 times against petroleum ether (B.R. 30 to 60°C) at pH 5.0, followed by 3 times extraction against chloroform at pH 7.0 (99). The petroleum ether and chloroform phases were collected separately, dried, and later bioassayed to detect the presence of GA-inhibitors using the lettuce seed germination test. The final aqueous extract resulting from chloroform extraction was adjusted to pH 3.0 and designated as the "aqueous acidic extract."

Figure 2. Flow diagram used in extracting gibberellins and gibberellin-inhibitors from shoots and cotyledons of 6-weeks old $\underline{yg}_6$ and normal wild type tomato seedlings.

FLOW DIAGRAM OF EXTRACTING GIBBERELLINS AND GA-INHIBITORS FROM TOMATO

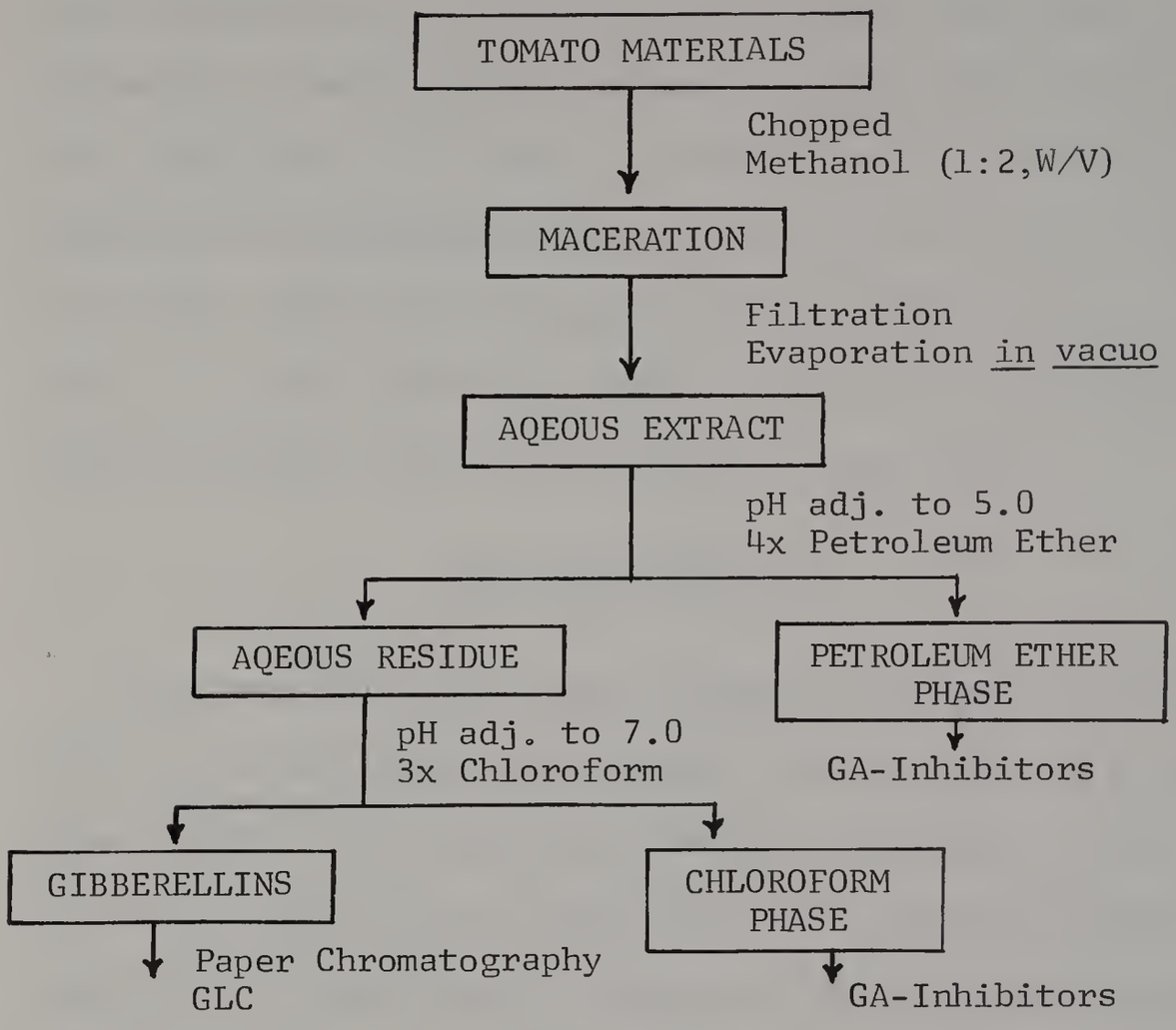


FIGURE 2

Paper Chromatography

A portion of the aqueous acidic extract was applied to a 3MM Whatman chromatography paper as a 1 cm wide streak. The paper was run in descending chromatography in 1-butanol, acetic acid and water [95:5:30 v/v/v (139)] for 30 cm (about 12 hours), then air dried and cut into 10 strips, each corresponding to 0.1 of an R_f unit. Each strip was eluted separately with 50 per cent acetone-water and the resulting eluate was dried. The dried eluate was taken up in distilled water and tested for GA-like activity on the "Halo half-seed" assay (158).

Halo Half-Seed Assay

The "halo half-seed" assay developed by Phinney and Fukuyama (158) was employed in determining the amount of GAs in both the $\underline{yg}_6$ and the wild type tomatoes. This assay has 3 distinct advantages over the other major bioassays, namely (a) very short total time consumed [35 hours for this assay versus 2 weeks for d-1 corn assay (161)], (b) more sensitive to GA than the lettuce hypocotyl (69), dwarf corn and dwarf pea (57) bioassays, and (c) neither kinetin nor IAA has displayed any effect on this barley assay (134).

Barley seeds, variety 'Traill', were obtained from Dr. P. B. Price, Department of Agronomy, South Dakota State University, Brookings. Barley seeds of uniform size were dehusked and sterilized by soaking in 50 per cent H_2SO_4 for 2 to 2 1/2 hours at room temperature. After soaking, seeds were vigorously and repeatedly washed (at least 10 washings) with sterilized water. The seeds were cut in two collecting only the embryoless half-seeds which were kept for 6 hours in sterilized water containing

streptomycin sulfate. It was found that half-seeds soaked in sterilized water for more than 6 hours exhibited a-amylase production even without GA treatment, indicating the presence of natural GAs in the seeds. In contrast, half-seeds soaked for about 6 hours or less require the addition of GA for a-amylase production. After autoclave sterilization, 3.9 per cent solution of potato dextrose agar (purchased from Fisher Scientific Co.) was poured into sterile Petri dishes. The following modifications of Phinney's halo half-seed assay (158) were made. Five half-seeds were placed equidistant from one another on the agar surface in each Petri dish. Each half-seed received 0.1 ml of either distilled water (for the control) or GA_3 of different concentrations or extracts eluted from paper chromatograms. Three operations, cutting of seeds in two, placing half-seeds on the agar surface, and application of GA to the half-seeds, were performed inside a sterile chamber to minimize contamination. Plated and treated seeds were incubated in a growth chamber, with a constant temperature of 25°C, for a period of 25 hours. After incubation, IKI solution (0.5 gm Iodine and 2.0 gm Potassium Iodide in 150 ml of water) was added to the agar plates and the diameter of the halo formed around the seed was measured by using a photographic enlarger. This assay was based on the diffusion of a-amylase into the agar resulting in the formation of a clear halo surrounded by a blue-purple background (see Figure 3).

Standard dose response of 'Traill' barley seeds to different concentrations of GA_3 was also studied. The following GA_3 concentrations were tested: $.5 \times 10^{-4}$, 1×10^{-3}, 5×10^{-3}, 1×10^{-2}, 5×10^{-2} and 1×10^{-1} μg GA_3/0.1 ml.

Figure 3. "Halo half-seed Assay". Five embryoless half-seeds of barley were placed equidistant in each Petri dish with the cut surface faced down on the potato dextrose agar medium. Assay is based on GA-induced diffusion of a-amylase from the seed into the agar resulting in the formation of a clear halo surrounded by a blue-purple background. Half-seeds (left), which did not receive GA, showed no halo formation while those which received GA (10^{-3} ug GA/half-seed) (right) showed the halo after IKI solution was added.

FIGURE 3

Lettuce Seed Germination Bioassay

The lettuce seed germination assay for GA-inhibitors was based on the ability of GA to overcome the inhibitory effect on germination of lettuce seeds by extracted inhibitors. Fifty lettuce (Lactuca sativa var. 'Grand Rapids') seeds (gift from Ferry-Morse Seed Co.) were germinated on No. 1 Whatman filter paper in each 6-cm round plastic culture dish at 25°C for 2 days. The dried petroleum ether and chloroform residues were first taken up in distilled water (1 ml of water for every 100 gm of fresh shoot sample), then centrifuged for 2 to 3 min at low speed before dilution with water to the following concentrations: 1/2, 1/10, 1/100, 1/1,000, and 1/10,000. The resulting diluted extracts were designated as the "inhibitor solutions". Two GA concentrations (0.01 and 0.1 µg/Petri dish) were used. A total of 2 ml of inhibitor solution or inhibitor solution plus GA solution was added to each Petri dish at the beginning of the experiment. The control consisted of distilled water. Each treatment was replicated twice.

Preparation of Trimethylsilyl Ethers

The method of Davis et al. (59) was followed in preparing the trimethylsilyl ether derivatives using the reagent bis-(trimethylsilyl) acetamide (BSA). A known and equal quantity of aqueous acidic extract, from the yg_6 or wild type tomatoes, was placed separately in 4 ml test tubes to which 1 ml of the reagent BSA (purchased from Pierce Chemical Co.) was added. The test tubes were capped, shaken vigorously and allowed to stand for at least 30 min to ensure satisfactory silylation. Standards of gibberellins A_1, A_3, A_4, A_5, A_7, A_8, and A_9 (gifts from A. Lang,

MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing to Dr. H. V. Marsh, Department of Plant and Soil Sciences, University of Massachusetts, Amherst) were silylated with BSA reagent in an identical manner.

Gas-Liquid Chromatography

Two types of gas chromatographic instruments, the Perkin-Elmer Model 900 and the Varian Aerograph HY-FI Model 600-D, were used in this study. The Perkin-Elmer Model 900 with dual columns and dual flame ionization detectors was employed using the following parameters:

a) 5% SE-30 column, 5 ft x 1/8 in., acid washed and DMCS treated Chromosorb W, initial temperature of 70° C maintained for 6 min followed by programming at 10° C/min to a final temperature of 290°C,

b) 5% SE-52 column, 9 ft x 1/8 in., acid washed and DMCS treated Chromosorb W, initial temperature of 150° C maintained for 6 min followed by programming at 5° C/min to a final temperature of 290° C,

c) 5% OV-22 column, 4 ft x 1/8 in., acid washed and DMCS treated Chromosorb W, initial temperature of 150°C maintained for 6 min followed by programming at 20° C/min to a final temperature of 290° C.

The carrier gas used with Perkin-Elmer instrument was N_2 at a flow rate of 40 ml/min.

The Varian Aerograph was equipped with a flame ionization detector and isothermal temperature control. GLC was carried out using 5% SE-30 column coated on 60/80 mesh, 5 ft x 1/8 in., acid washed, DMCS treated Chromosorb W. Carrier gas was N_2, at a flow rate of 25 ml/min. A column temperature of 70° C for the first 6 min was employed followed by programming at 10° C/min to a final temperature of 290° C. The injector and detector temperature were maintained at approximately 200° C. In all instances, a total sample of 3 μl was injected into the gas chromatograph.

Identification of Gibberellins by GLC

The following technique was employed to identify the gibberellins from the yg_6 and wild type tomatoes: (1) comparison of the retention times of the various GLC peaks of the tomato extracts with the retention times of 7 standard GAs on 3 different chromatographic columns, and (2) co-chromatography. Co-chromatography was performed by adding either one or two or more trimethylsilylated standard GAs to a sample of silylated tomato extract which was then injected into the GLC. The added standard GAs caused an increase in the sizes of the particular tomato GLC peaks with which they co-chromatographed, confirming that the standard GA and tomato peak in fact have the same retention time.

RESULTS

Growth Rate, Chlorophyll and Anthocyanin

Gibberellic acid pronouncedly induced stem elongation (25, 26, 160, 189, 223), significantly reduced chlorophyll content (128, 197) and inhibited anthocyanin synthesis (7, 8, 9). The $\underline{yg}_6$ tomato mutant exhibits a syndrome of three pleiotropic characteristics which resemble those traits regulated by excess GAs: yellow-green cotyledons and leaves, greatly elongated and colorless hypocotyl, and no anthocyanins. To test this possibility, GA and Phosfon, an antigibberellin known to block GA biosynthesis (62), were applied to both the mutant and the normal wild type tomatoes to induce drastic changes and possibly mimicking the phenotypes of both plants. The effects of GA and Phosfon on stem growth, chlorophyll and anthocyanin contents are shown in Figure 4 and Table I. The stems of the untreated 2-week old $\underline{yg}_6$ elongated faster than the stems of the untreated 2-weeks old wild type seedlings (Figure 4). GA (10^{-4}M) caused the stems of $\underline{yg}_6$ and the wild type to elongate at the same rate, suggesting that the $\underline{yg}_6$ was not fully saturated with endogenous GA. On the other hand, Phosfon (10^{-3}M) decreased the stem growth rate of both plants.

The chlorophyll and anthocyanin contents of untreated and GA-and Phosfon-treated plants 14 days following treatment are shown in Table I. The untreated $\underline{yg}_6$ contained significantly less chlorophyll than the untreated wild type. GA significantly reduced the chlorophyll contents of both $\underline{yg}_6$ and the wild type. In contrast, Phosfon did not affect the amount of chlorophyll in the wild type but increased the amount of

Figure 4. Effects of GA and Phosfon on the stem growth of $\underline{yg}_6$ and wild type tomatoes applied as soil drenches to 2-week old transplanted seedlings. The control received distilled water. Seedlings were treated once and height measurements were begun immediately. The height of the plants from the soil level to the tip of the highest leaf was measured on alternate days for 2 weeks. Twelve plants constituted a treatment.

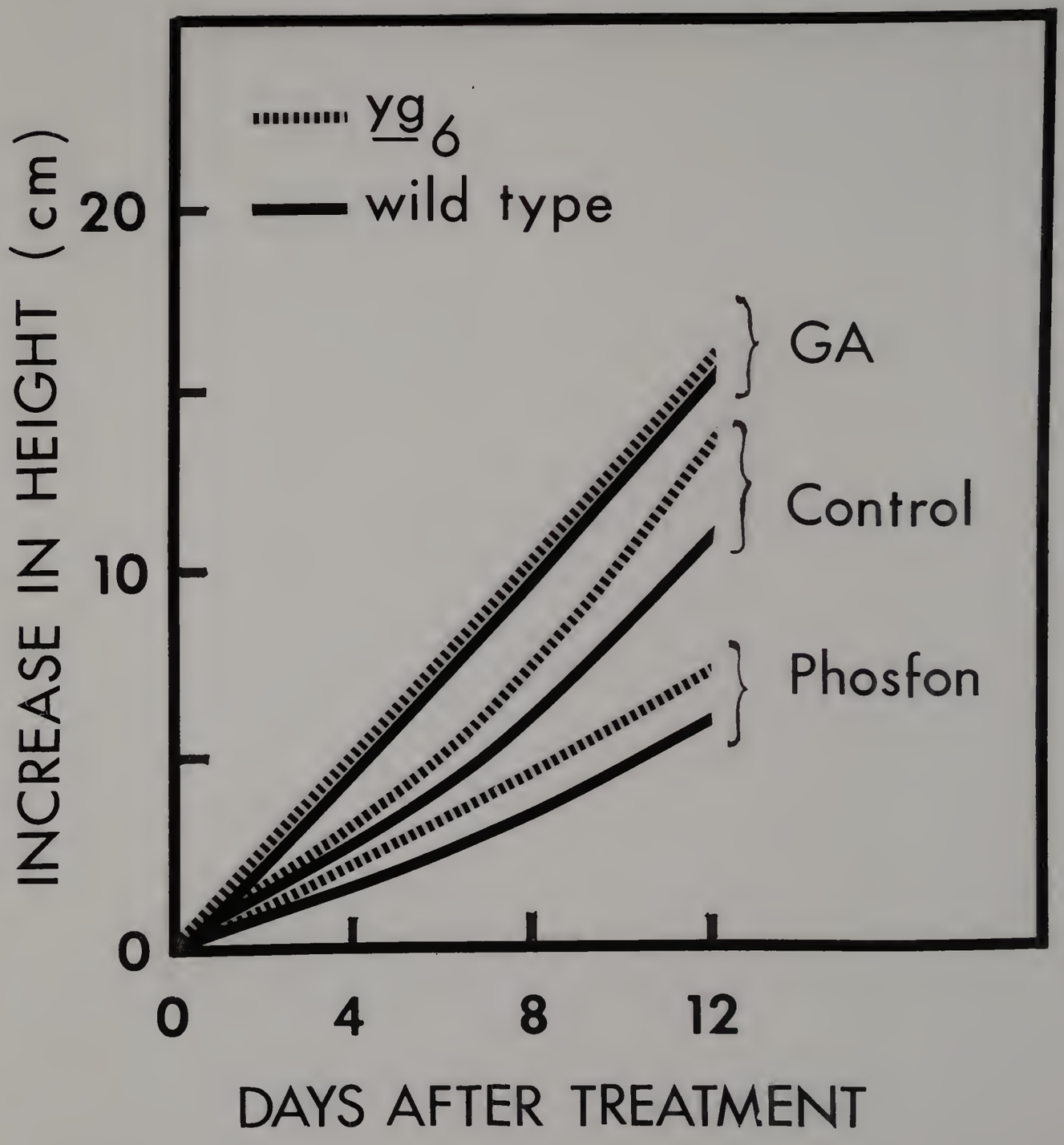

yg6
wild type
GA
Control
Phosfon
INCREASE IN HEIGHT (cm)
20
10
0
0
4
8
12
DAYS AFTER TREATMENT

FIGURE 4

Table I. Effects of GA and Phosfon on the Chlorophyll and Anthocyanin Contents of yg_6 and Normal Wild Type Seedlings.

GA and Phosfon were applied as soil drenches to 2-week old transplanted tomato seedlings. Distilled water was given to the control plants. About 250 ml of 10^{-4}M GA_3 or 10^{-3}M Phosfon or water was applied to each 6-inch plastic pot containing 4 seedlings. Seedlings were treated once and 2 weeks later, the chlorophyll content in the Nos. 2 and 3 leaflets (No. 1 leaflet refers to the main terminal shoot) and anthocyanin content in the stem directly above the soil level were determined.

Treatment	Chlorophyll		Anthocyanin	
	wild type	yg_6	wild type	yg_6
	mg./g. fresh wt.		A_{510nm}	
Control	5.4 ± 0.1[1]	4.0 ± 0.1	0.155 ± 0.002	N.D.[2]
GA (10^{-4}M)	4.2 ± 0.2	3.3 ± 0.2	N.D.	N.D.
Phosfon (10^{-3}M)	5.6 ± 0.2	4.8 ± 0.7	0.400 ± 0.029	0.245 ± 0.033

[1] Results are averages of 4 replications each; standard deviations are given.

[2] N.D. = anthocyanin not detected.

chlorophyll in the mutant to that in the untreated normal wild type. The untreated $\underline{yg}_6$ possessed no detectable anthocyanin while the wild type contained anthocyanin (Table I). Anthocyanin formation in the GA-treated wild type was completely inhibited as indicated by the absence of anthocyanin. On the contrary, Phosfon promoted anthocyanin formation in both plants.

In a separate experiment, GA (10^{-4}M) was also applied as a soil drench to 3-week old tomato seedlings. Similar to that of 2-week old plants, the stems of untreated 3-week old $\underline{yg}_6$ seedlings had a much higher increase in stem growth than that of the untreated 3-week old wild type seedlings (Figure 5). GA increased stem growth in both plants and it should be noted that the GA-treated wild type exhibited a stem growth increase comparable to that of the untreated $\underline{yg}_6$ seedlings.

GA and Phosfon treatments resulted in nearly mimicking completely the phenotypes of either plants, except for the bright yellow primary shoot as in the mutant. GA induced the syndrome of three characters of the mutant in the normal wild type, or conversely, Phosfon induced these three characters as in the normal tomato in the mutant. GA promoted the stem growth of the normal tomato to a rate comparable to that of the untreated $\underline{yg}_6$ (Figure 5), reduced the chlorophyll content of the normal to that of the $\underline{yg}_6$ and inhibited anthocyanin formation in the wild type (Table I). On the other hand, Phosfon reduced the stem growth of the $\underline{yg}_6$ (Figure 4), increased the chlorophyll content of the $\underline{yg}_6$ to that of the normal and induced the formation of anthocyanin in the mutant (Table I). These observations substantiate the suggestion that the syndrome of pleiotropic characters of the mutant is related to the effects of GAs.

Figure 5. Effect of GA (10^{-4}M) on the stem growth of $\underline{yg}_6$ and wild type tomatoes applied as soil drench to 3-week old transplanted seedlings. The control received distilled water. Heights of plants were measured the same manner described in Figure 4. Twelve plants constituted a treatment.

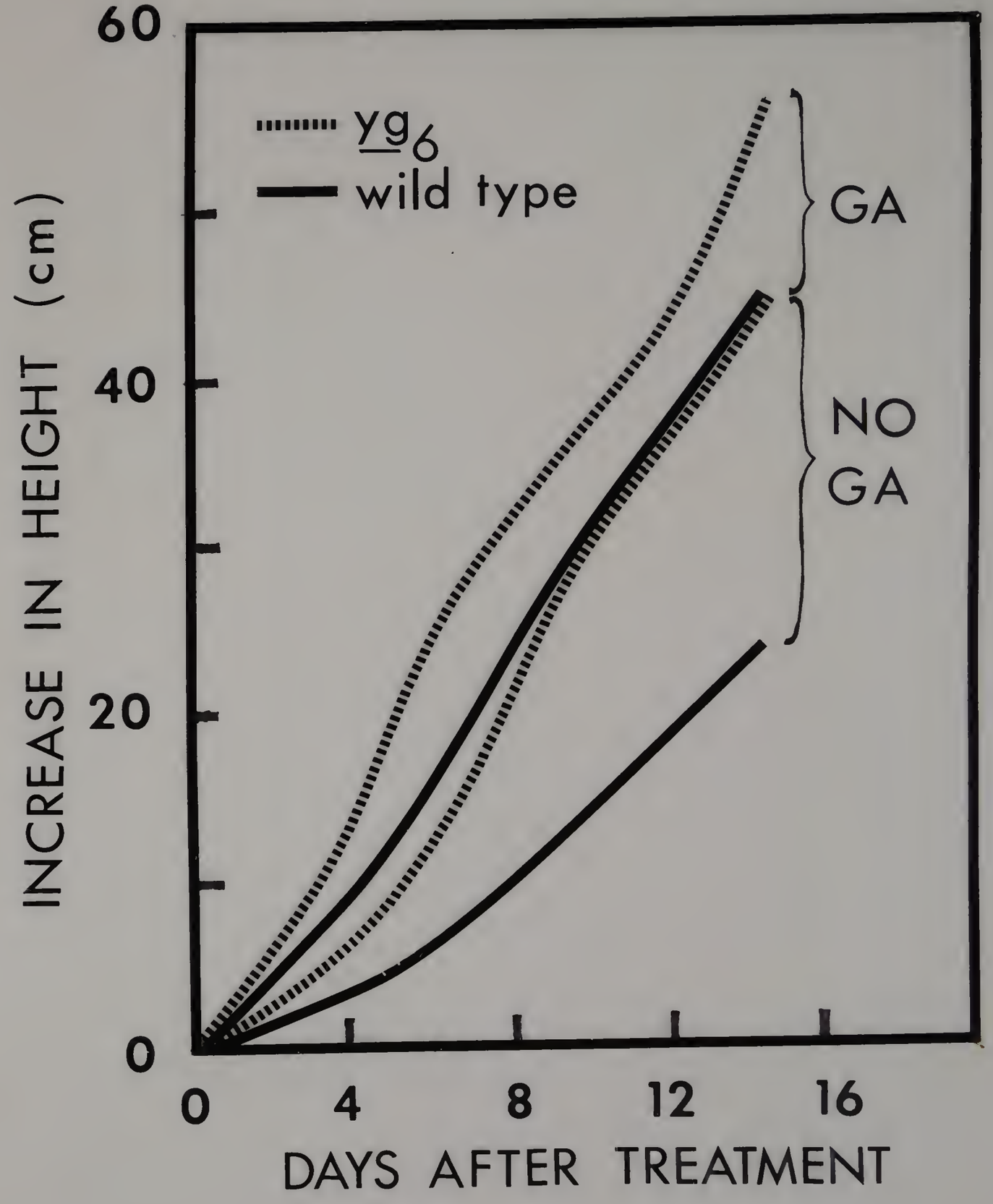

60
40
20
0
INCREASE IN HEIGHT (cm)
yg6
wild type
GA
NO GA
0
4
8
12
16
DAYS AFTER TREATMENT

FIGURE 5

Root Growth

Depressed root growth is another plant characteristic suggestive of the level of gibberellins in the plant. For this reason, the dry weight of roots of untreated $\underline{yg}_6$ and wild type plants were determined. Five-week old mutant tomato seedlings were found to exhibit a lower (significant at P=0.05) dry weight of roots and shoots than the normal tomato seedlings of the same age (Table II). The total dry matter in the mutant tomato was one-half that found in the normal tomato. When the effects of GA on root growth were studied, as little as 1.0 μg GA/plant applied to the shoot was found to be effective in reducing (significant at P=0.05) root growth in both $\underline{yg}_6$ and wild type by about 75 and 73 per cent respectively (Table III). GA at 30 μg/plant appears no more effective in reducing root growth in the $\underline{yg}_6$ and wild type than GA at 1.0 μg/plant.

Effect of GA and Phosfon Supplied in Culture Solution

Further studies were conducted to determine the effects of GA and Phosfon on stem elongation and root formation when supplied directly to the roots through the culture solution. Seven day old seedlings were grown in test tubes containing distilled water (control) or solutions of GA or Phosfon. After a week the stems of the $\underline{yg}_6$ in distilled water had grown twice as much as the wild type (3.4 vs. 1.6 cm). In addition, the $\underline{yg}_6$ had fewer lateral roots per seedling (8.8 average) than the wild type (13.1 average).

In Table IV are shown the effects of GA (expressed in per cent of control) on the rate of stem elongation and total number of lateral roots in the $\underline{yg}_6$ and the wild type seedlings. The net increase in stem growth

Table II. Dry Weight of Roots and Shoots of yg_6 and Normal Wild Type Seedlings.

Seedlings were 2-weeks old when transplanted and were 5-weeks old when sampled. Dry weight was based on 10 plants.

Genetic Material	Dry Weight (mg./plant)		
	Roots	Shoots	Total
yg_6	42.7 ± 8.4[1]	117.5 ± 12.1	160.2
wild type	73.0 ± 7.5	305.1 ± 42.5	378.1

[1] Average ± variance which represents 95% confidence limits.

Table III. Effects of GA on the Dry Weight of Roots of yg_6 and Wild Type Seedlings.

Seedlings, which had been transplanted, were given drop application of GA solutions. Each plant received a total volume of 0.1 ml GA by using a micropipette. Control plants received distilled water. After 2 weeks, the dry weight of roots was determined.

Genetic Material	Control	GA (µg/plant)	
		1.0	30.0
yg_6 [1,2]	7.7 ± 1.0[a] (100)	5.8 ± 0.8[b] (75)	5.0 ± 0.8[bc] (65)
wild type	29.6 ± 5.9[d] (100)	21.7 ± 1.6[e] (73)	20.0 ± 4.1[de] (68)

[1] Means of 10 plants expressed in mg. dry weight ± variance at 95% confidence limit. Data in parentheses represent per cent reduction based on the control.

[2] Means in the same row followed by different letters are significantly different, p=0.05.

Table IV. Effect of GA on Stem Elongation and Total Number of Lateral Roots of yg_6 and Wild Type Seedlings.[1]

Seven day old seedlings were uprooted and their roots carefully washed. One seedling was placed into each test tube containing 5 ml of GA solutions at various concentrations or distilled water alone. Plants were kept in a growth chamber with continous white light at 25°C. After a week under these conditions, the rate of stem elongation and the total number of roots were determined. Eight seedlings constituted a treatment.

GA	Stem Elongation		Number of Roots	
	wild type	yg_6	wild type	yg_6
µg/ml	% Promotion[2]		% Inhibition	
0	100.0	100.0	0.0	0.0
0.01	137.3	95.0	2.3	0.0
0.1	171.5	106.1	29.2	20.5
1.0	193.7	112.2	31.3	26.1
10.0	233.5	121.3	40.5	27.0

[1] The rate of stem elongation was based on the lengths of the seedlings at the start and at the end of the experiment a week later.

[2] Per cent based on the control.

in both plants was promoted by GA over the concentration range of 0.01 to 10.0 µg/ml. The wild type exhibited a linear promotion of stem growth over the entire range; whereas the mutant did not show such a linear relationship. At 10 µg GA/ml, the increase in stem elongation in the wild type was twice that of the mutant (234% and 121% respectively). This result suggests that the wild type probably has a higher level of saturation to applied GA than the mutant. On the other hand, GA inhibited lateral root formation in both plants. The degree of root inhibition in both plants increased in proportion to the GA concentration.

The effects of Phosfon on stem elongation and root formation are shown in Table V. Phosfon inhibited the net increase of stem elongation in both the mutant and the wild type. The inhibition of stem growth increased with the increasing Phosfon concentration (10^{-7} to 10^{-5} M). The mutant appears to be slightly more sensitive than the normal wild type is to Phosfon as indicated by a slightly higher inhibition of stem elongation especially in the higher concentrations. The total number of lateral roots of both plants was reduced to almost the same extent by increasing Phosfon concentration.

Saturation Levels and Leaf Shape

To determine the saturation levels of GA in the yg_6 and wild type seedlings, 5 concentrations of GA were each applied once to the shoots of 3-week old plants. As shown in Figure 6, the 3-week old yg_6 had a greater growth rate (40 per cent more) than the wild type. Increasing concentrations of GA progressively stimulated the rate of stem elongation in both plants until a saturation point was reached, with 50 µg GA

Table V. Effect of Phosfon on Stem Elongation and Total Number of Lateral Roots of yg_6 and Wild Type Seedlings.[1]

The same cultural conditions as in Table IV were provided except that Phosfon solutions, at concentration range from 10^{-7} to 10^{-5}M, were used.

Phosfon	Stem Elongation		Number of Roots	
	Wild Type	yg_6	Wild Type	yg_6
Molar		% Inhibition[2,3]		
0	0.0	0.0	0.0	0.0
10^{-7}	25.0	28.0	25.0	18.1
10^{-6}	42.3	56.8	88.8	86.3
10^{-5}	88.4	100.0	100.0	97.7

[1,2] See Table IV.

[3] Preliminary work showed that Phosfon concentrations of 10^{-4} and 10^{-3}M were highly toxic to the 7-day old yg_6 and wild type seedlings resulting to death of seedlings.

Figure 6. Saturation levels of 3-week old $\underline{yg_6}$ and wild type tomato seedlings to applied gibberellin. Stem growth between 2nd and 8th day following treatment. Standard deviations of the means are given. Twelve plants comprised each treatment.

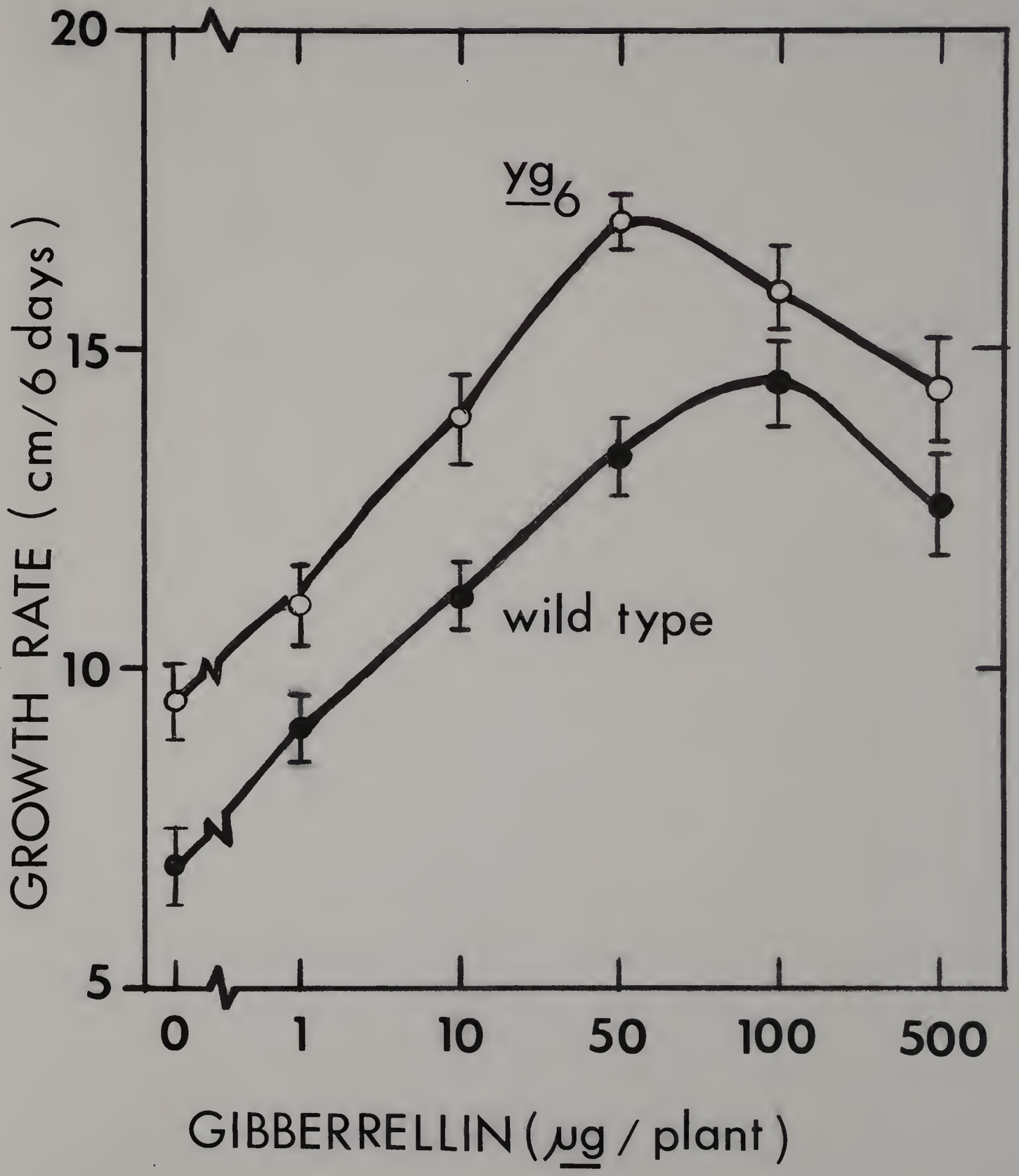

20
15
10
5
GROWTH RATE (cm/6 days)
yg6
wild type
0
1
10
50
100
500
GIBBERRELLIN (µg / plant)

FIGURE 6

apparently saturating the $\underline{yg_6}$ while 100 μg GA appears saturating to the wild type.

One interesting observation made was the pronounced effect of GA on the shape of new leaves. All 5 concentrations of GA induced drastic changes in leaf shape and caused the new leaves that formed on both $\underline{yg_6}$ and wild type plants to develop from normally serrated (dentate) shape to smooth edged (entire) leaves (see Figure 7). Plants treated with 1.0 μg GA reverted back to the production of normal serrate-shaped leaves 5 weeks after the initial treatment while those treated with 10, 50, 100, and 500 μg GA continued to produce new leaves with smooth edges for 7 to 8 weeks. In addition, it was observed that in both plants the stems became increasingly thinner and leaves more chlorotic as the concentrations of GA increased.

L-Phenylalanine Ammonia-Lyase

The specific activity of L-phenylalanine ammonia-lyase (PAL) in the stem of the mutant (2.10 $\pm$ 0.16) was about double that of the wild type (1.11 $\pm$ 0.07) (Table VI). The observed higher specific activity of PAL in the $\underline{yg_6}$ was reflected in the greater amount of enzyme in the extracts of the $\underline{yg_6}$ than in the extracts of the wild type. Similar results were found in a duplicate assay performed during the same day.

The effects of two concentrations of GA on the activity of PAL in these plants were also determined. As shown in Table VII, the two concentrations of GA promoted PAL activity in both plants. However, there was a greater promotion of PAL activity by the two concentrations of GA in the mutant than in the wild type. The increased PAL activity in the

Figure 7. Drastic change in leaf shape induced by GA in both tomatoes from the normally serrated (dentate) shaped-leaf to smooth edged (entire) leaf. All 5 concentrations of GA used, 1, 10, 50, 100 and 500 µg GA/plant, induced a drastic change in shape in the newly formed leaves.

FIGURE 7

Table VI. L-Phenylalanine Ammonia-Lyase Activity of yg_6 and Wild Type Plants.

Seedlings, which have been transplanted when they were 2-weeks old, when 34 days old were kept for 3 days in a growth chamber before stem samples for PAL assay were taken. Preparation of PAL extract and description of PAL assay are presented in the Materials and Method Section.

Genetic Material	mU/gm. Fresh Weight	Specific Activity (mU/mg. Protein)
yg_6	1.33 ± 0.19[1]	2.10 ± 0.16
wild type	0.90 ± 0.05	1.11 ± 0.07

[1] Average of 3 replications; standard deviation values are given.

Table VII. Effect of Two Concentrations of GA on L-phenylalanine Ammonia-Lyase Activity in the yg_6 and Wild Type Plants.

Transplanted plants (8 to 9 weeks old) which were grown in the greenhouse were placed in a growth chamber and kept for 2 days, at which time 2 GA concentrations were applied as soil drench. To each 6-inch plastic pot containing 4 plants, 250 ml of each GA concentration was poured directly at the base of the plants. Distilled water was given to plants designated as control. Twenty-four hours after GA treatment, L-phenylalanine ammonia-lyase activity in the stems were assayed. Each number in the table was based on two replications each.

Genetic Material	Expt. No.	GA Conc. (M)	mU Per Gm. Fresh Weight	Specific Activity (Per Cent of Control)
yg_6	1	Control	0.47	100
		10^{-5}	1.25	270
		10^{-4}	1.35	286
	2	Control	1.25	100
		10^{-5}	1.76	189
		10^{-4}	0.98	313
Wild Type	1	Control	1.01	100
		10^{-5}	1.48	119
		10^{-4}	1.67	165
	2	Control	1.01	100
		10^{-5}	1.51	118
		10^{-4}	3.33	268

GA-treated plants seems to be reflected in most cases by a corresponding increase in the amount of enzyme in the fresh stem tissue.

Grafting Experiments

Rick (171) has demonstrated modifications of certain phenotypic characters of a monogenic but highly pleiotropic tomato mutant toward the normal when grafted as scions on normal stocks. Böhme and Scholz (21) have reported the normalizing effect of grafting a chlorophyll mutant of tomato onto normal plants. In view of these results, grafting experiments were performed in attempts to induce drastic changes and possibly mimic the phenotypes of both plants.

A total of 217 successful grafts, employing two types of grafting, were obtained (Table VIII). There were 53 successful grafts between wild/wild (refers to scion/stock combination); 53 wild/$\underline{yg}_6$; 64 $\underline{yg}_6$/wild; and 47 $\underline{yg}_6/\underline{yg}_6$. No drastic change in phenotypes of either the scion or stock resulted from the grafting experiments. It was observed that the $\underline{yg}_6$ scion grew better when grafted on a wild type stock than on $\underline{yg}_6$ stock. Conversely, the wild type scion exhibited an apparent reduction in growth when grafted on a $\underline{yg}_6$ as compared to its growth on a wild type stock (Figure 8).

Some changes in the greenness and in the anthocyanin coloration of the $\underline{yg}_6$ and wild type scions could be discerned. When the amounts of these two pigments were determined 3 weeks after top grafting, slight increases or decreases of both pigments in the scions were noted depending upon the kinds of stocks involved (Table IX). For example, there was a slight increase in the chlorophyll content of the $\underline{yg}_6$ scion

Table VIII. Total Number of Successfully Grafted Plants Employing 2 Types of Grafting.[1]

TYPE OF GRAFTING	GRAFT COMBINATION				TOTAL
	wild/wild	wild/yg_6	yg_6/wild	yg_6/yg_6	
Spliced Approach	10	26	26	10	72
Top Grafting	43	27	38	37	145
TOTAL	53	53	64	47	217

[1] Procedures for grafting are given in the Materials and Method Section.

Figure 8. Four top-graft combinations involving $\underline{yg}_6$ and wild type (Parent). Note the better growth of $\underline{yg}_6$ scion when grafted on a wild type rootstock.

FIGURE 8

Table IX. Effect of Top Grafting on the Chlorophyll and Anthocyanin Contents in the Scions.

The procedure for top grafting was given in the Materials and Methods Section. Three weeks after the plants were grafted, the chlorophyll and anthocyanin contents were determined. Leaflets numbers 2 and 3 (starting from the shoot of the scion) was used to determine the chlorophyll content while the stem part, sampled 1 in. above the graft union, of the scion was used to determine the anthocyanin content. Procedures in Table I were followed in the determination of the amounts of the 2 pigments.

Pigments	Graft Combination (scion/stock)			
	wild/wild	wild/*yg*$_6$	*yg*$_6$/wild	*yg*$_6$/*yg*$_6$
Chlorophyll[1,2] (mg/g fr. wt.)	10.6 ± 1.4[a]	9.8 ± 0.8[a]	5.0 ± 0.1[b]	4.1 ± 0.8[b]
Anthocyanin (A_{510}^{nm})	0.45 ± .07[a]	0.36 ± .04[a]	0.27 ± 0.7[b]	0.22 ± .03[b]

[1] Results are the averages of 4 replications each; standard deviations are given.

[2] Means in the same rows followed by the same letters are not statistically different.

(5.0 ± 0.1 vs. 4.1 ± 0.8) when grafted on a wild type stock, or there was a slight decrease (0.36 ± 0.04 vs. 0.45 ± 0.07) in anthocyanin in the stems of the wild type scion when grafted on a $\underline{yg}_6$ stock. However these changes were not significantly different.

Halo Half-Seed Bioassay

The Halo half-seed assay proved to be the most sensitive of the bioassays tested. When the dose response curve for the 'Traill' variety of barley was determined, it was found that the halo half-seed bioassay has a limit of sensitivity from 5×10^{-4} to 5×10^{-2} μg GA/half-seed (Figure 9). In comparison, my preliminary work (unpublished) on the range of sensitivity of some of the major assays showed that the lettuce (var. 'Grand Rapids') hypocotyl test is sensitive from 10^{-1} to 10^{2} μg GA/2 ml per Petri dish; the dwarf-1 corn test is sensitive from 10^{-2} to 10^{1} μg GA/plant; while the dwarf pea (var. 'Wando') assay is sensitive from 10^{-3} to 10^{1} μg/plant. These results agree in general with those reported by Brian et al. (30), Crozier et al. (57) and Frankland and Wareing (69).

Amount of Endogenous Tomato GAs

In the determination of the amounts of GAs in the $\underline{yg}_6$ and the wild type tomatoes, both paper chromatography and halo half-seed bioassay were used. The GA-like activities of the eluates from the paper chromatograms of the $\underline{yg}_6$ and the wild type, and the response of the 'Traill' seeds to two concentrations of standard GA_3 are shown in Figure 10. Based on paper chromatography and halo half-seed assay, it was found that the $\underline{yg}_6$ tomato contains three times as much endogenous GAs as the wild type tomato (3.9 vs. 1.2×10^{-2} μg GA_3 equivalent/gm fresh weight, Table X).

Figure 9. Standard dose response curve of the "Halo half-seed assay". The embryoless half-seeds of 'Traill' barley variety were used. The diameter of the halo formed around the half-seed is linear with increasing GA concentrations from 5×10^{-4} to 5×10^{-2} µg GA/half seed.

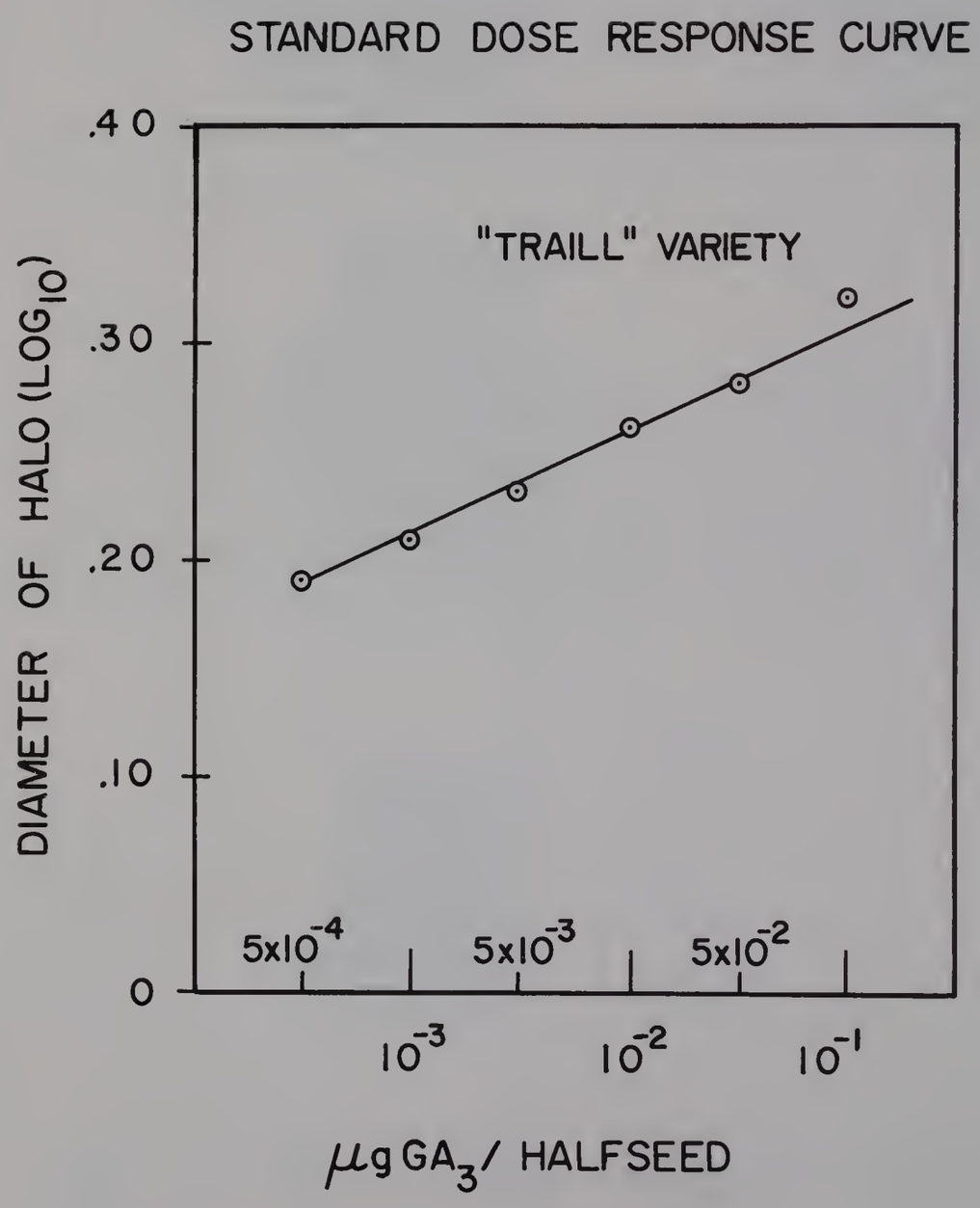

STANDARD DOSE RESPONSE CURVE
"TRAILL" VARIETY
DIAMETER OF HALO (LOG10)
.40
.30
.20
.10
0
5x10-4
5x10-3
5x10-2
10-3
10-2
10-1
µg GA3/ HALFSEED

FIGURE 9

Figure 10. Response of Halo Half-seed Assay to gibberellin-like substances extracted from shoots and cotyledons of $\underline{yg}_6$ and wild type tomatoes. The paper chromatogram was cut into 10 strips, each strip corresponding to 0.1 of an R_f unit. Responses of the assay to 2 GA concentrations are shown (right). Half-seeds which received distilled water did not form any halo. The chromatograms were based on 2 replications. The R_f of gibberellins A_3 and A_7 are indicated.

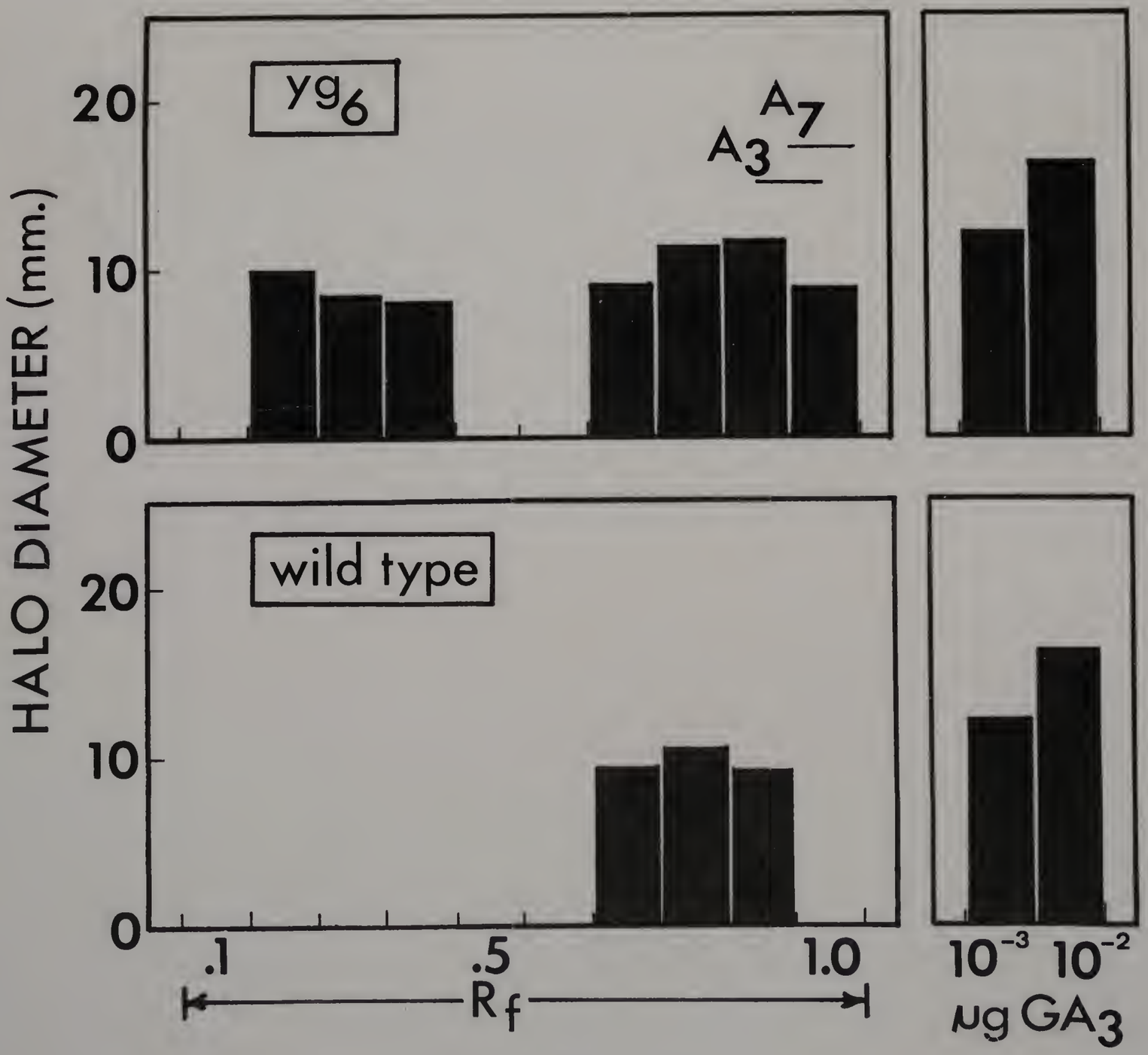

HALO DIAMETER (mm.)
20
10
0
yg6
A7
A3
wild type
.1
.5
1.0
Rf
10-3 10-2
µg GA3

FIGURE 10

Table X. Amount of Gibberellins in yg_6 and Wild Type Seedlings Determined by Halo Half-Seed Assay and Paper Chromatography.

Genetic Material	GA_3-equivalent per gm. fresh weight
	/ug x 10^{-2}
yg_6	3.9 ± 0.4[1]
wild type	1.2 ± 0.0

[1] Average of 2 replications; standard deviation is given.

The presence of a higher quantity of GA-like substances in the $\underline{yg}_6$ mutant than in the wild type was confirmed using gas-liquid chromatography. Equal amounts of trimethylsilylated extracts from the two tomatoes gave similar chromatograms but they differed considerably in the sizes of the GLC peaks. In Figure 11, GLC traces of TMS aqueous acidic extracts from the $\underline{yg}_6$ and the wild type tomatoes are shown. The majority of the GLC peaks of the $\underline{yg}_6$ extract are larger than their corresponding GLC peaks of the wild type extracts, indicating the presence of larger quantities of GAs in the former. As shown in Figure 12, upon increasing GLC sensitivity by 8 times, the resulting GLC chromatogram of the wild type extract revealed a trace similar to that of the $\underline{yg}_6$ trace. This suggests that there were no qualitative differences, only quantitative differences in the endogenous gibberellins of the $\underline{yg}_6$ and the normal wild type tomatoes.

Gibberellin-Inhibitors

Lettuce seeds imbibed with distilled water resulted in 100 per cent germination. In contrast, when materials extracted from both tomatoes by petroleum ether or chloroform extractions were applied with water to the lettuce seeds there was a complete inhibition of germination. The inhibitory effects of these materials, however, could be partially or completely overcome by the addition of commercial gibberellic acid. This phase of the study was made in an attempt to determine whether the $\underline{yg}_6$ mutation affected the production of endogenous GA-inhibitors. The amount or effectiveness of GA-inhibitors was estimated from the same materials from which tomato GAs were determined. No attempt was

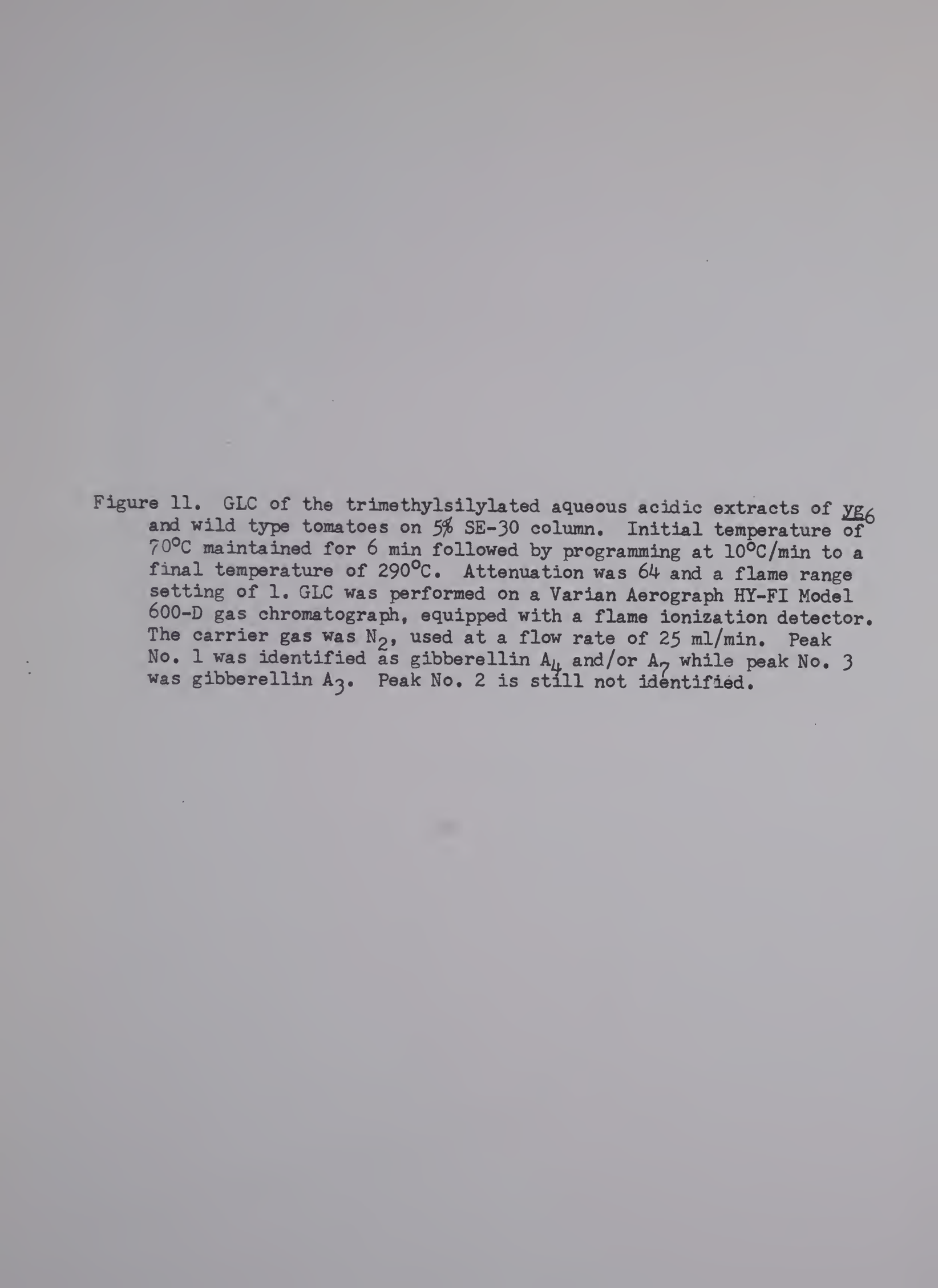


Figure 11. GLC of the trimethylsilylated aqueous acidic extracts of *yg*$_6$ and wild type tomatoes on 5% SE-30 column. Initial temperature of 70°C maintained for 6 min followed by programming at 10°C/min to a final temperature of 290°C. Attenuation was 64 and a flame range setting of 1. GLC was performed on a Varian Aerograph HY-FI Model 600-D gas chromatograph, equipped with a flame ionization detector. The carrier gas was N_2, used at a flow rate of 25 ml/min. Peak No. 1 was identified as gibberellin A_4 and/or A_7 while peak No. 3 was gibberellin A_3. Peak No. 2 is still not identified.

FIGURE 11

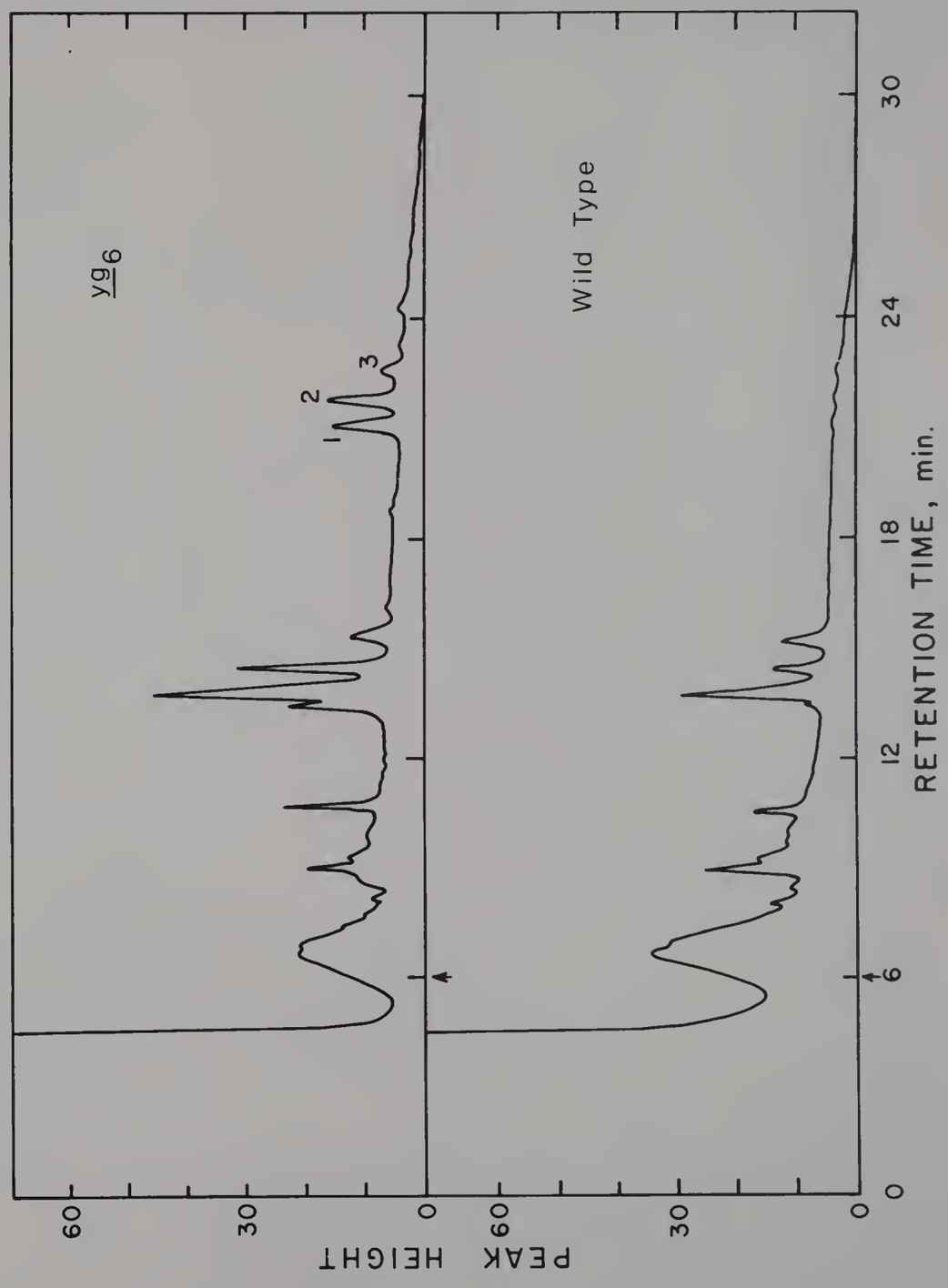

yg6
Wild Type
1
2
3
PEAK HEIGHT
60
30
0
60
30
0
6
12
18
24
30
RETENTION TIME, min.

Figure 12. GLC of the trimethylsilylated aqueous acidic extracts of wild type tomato at a higher sensitivity. Identical chromatographic conditions as in Figure 11 (below) except the sensitivity was increased by 8 times. GLC at the higher sensitivity revealed a similar pattern of peaks between that of the wild type and yg6 extracts (shown in Figure 11, above), indicating no qualitative difference in gibberellins present in the two tomatoes.

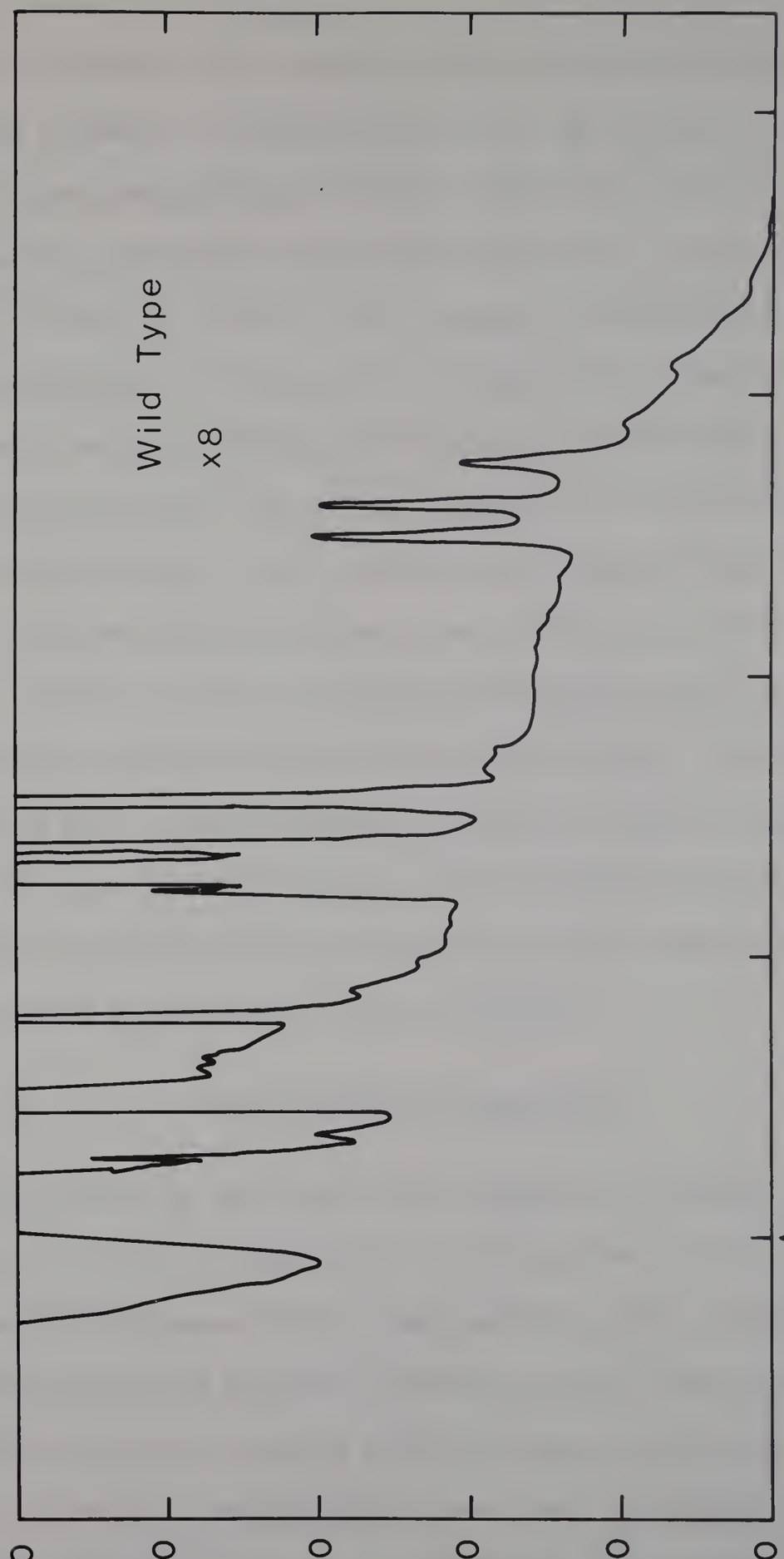

Wild Type
x8
PEAK HEIGHT
100
80
60
40
20
0
0
6
12
18
24
30
RETENTION TIME, min.

FIGURE 12

made in elucidating the chemical nature of the GA-inhibitors.

The presence and relative amounts of GA-inhibitors in the two solvents phases are indicated by their inhibitory effects on lettuce seed germination, expressed in per cent inhibition of germination, and are shown in Tables XI and XII. For example, a relatively greater amount of GA-inhibitors is indicated by a lower percent germination. There appears to be no difference in the amount or effectiveness of GA-inhibitors from wild type and $\underline{yg}_6$ present in the petroleum ether and chloroform residues. The inhibitors from the petroleum ether extraction were still completely inhibitory even at the 2-and 10-fold dilution (Table XI) while those from the chloroform extraction was completely inhibitory at only the 2-fold dilution (Table XII). This suggests that the petroleum ether residues appears to contain slightly higher amounts or more effective GA-inhibitors than the chloroform residues. GA was found to either partially or completely overcome the inhibition of seed germination caused by the diluted GA-inhibitors.

Identification of GAs by GLC

The following techniques were employed to identify the GAs from the $\underline{yg}_6$ and the wild type tomatoes: (a) comparison of the retention times of the various GLC peaks of the tomato extracts with the retention times of 7 standard GAs in 3 different chromatographic columns, and (b) co-chromatography. Since the aqueous acidic extracts of both tomatoes are available, the extracts of both plants were used in the identification studies.

The retention times of 7 standard GAs and of tomato GAs in 3 columns, using the Perkin-Elmer Model 900 chromatograph, are shown in Table XIII.

Table XI. Amount of GA-Inhibitors Obtained After Petroleum Ether Extraction.

Amount of inhibitors was expressed in degree of inhibition of germination of lettuce seeds (var. 'Grand Rapids'). Petroleum ether residue was dried and then taken up in distilled water (1 ml water/100 gm fresh shoot sample). Extract was first centrifuged for 2 to 3 min. before dilution with water. Lettuce seeds which received distilled water gave 100% germination. Each treatment was replicated twice. Each replication was comprised of 50 lettuce seeds placed on No. 1 Whatman filter paper in a 6 cm plastic dish kept at 25°C for 2 days.

	Gibberellic Acid (μg/Petri dish)					
Extract	0		0.01		0.1	
Dilution	wild	*vg*$_6$	wild	*vg*$_6$	wild	*vg*$_6$
	% Germination					
1/2	0	0	0	0	0	0
1/10	0	0	0	0	0	0
1/100	89	81	99	98	100	100
1/1,000	100	97	100	100	100	100
1/10,000	100	98	100	100	100	100

Table XII. Amount of GA-Inhibitors Obtained After Chloroform Extraction.

Inhibitor extract was prepared in the same manner described in Table XI.

	Gibberellic Acid (µg/Petri dish)					
Extract	0		0.01		0.1	
Dilution	wild	yg_6	wild	yg_6	wild	yg_6
	% Germination					
1/2	0	0	0	0	0	0
1/10	61	71	79	78	85	84
1/100	85	89	92	96	100	99
1/1,000	94	97	100	100	100	100
1/10,000	100	98	100	100	100	100

Table XIII. Retention Times of TMS Ether Derivatives of Standard and Tomato GAs.[1]

Gibberellins	Retention Time (min) SE-30[2]	SE-52[3]	OV-22[4]
Standard GAs			
A_1	27.8	31.0	16.3
A_3	27.9	30.2	16.5
A_4	26.8	28.8	16.2
A_5	26.5	28.2	16.1
A_7	26.8	28.6	16.2
A_8	28.7	32.2	16.4
A_9	25.5	26.1	15.8
Tomato GAs			
(A_8)	28.8	(A_8) 32.4	----
(A_1/A_3)[5]	27.8	(A_3) 30.1	(A_3) 16.6
(A_4/A_7)[5]	26.8	----	(A_4/A_7)[5] 16.2
(A_9)	25.6	----	(A_9) 15.8
	----	(A_5) 28.1	----

[1] Perkin-Elmer Model 900 gas chromatograph with dual columns and dual flame ionization detectors was employed. Carrier gas was N_2, used at a flow rate of 40 ml/min.

[2] Initial temperature of 70° maintained for 6 min followed by programming at 10°/min to a final temperature of 290°.

[3] Initial temperature of 150° maintained for 6 min followed by programming at 5°/min to a final temperature of 290°.

[4] Initial temperature of 150° maintained for 6 min followed by programming at 20°/min to a final temperature of 290°.

[5] Not resolved.

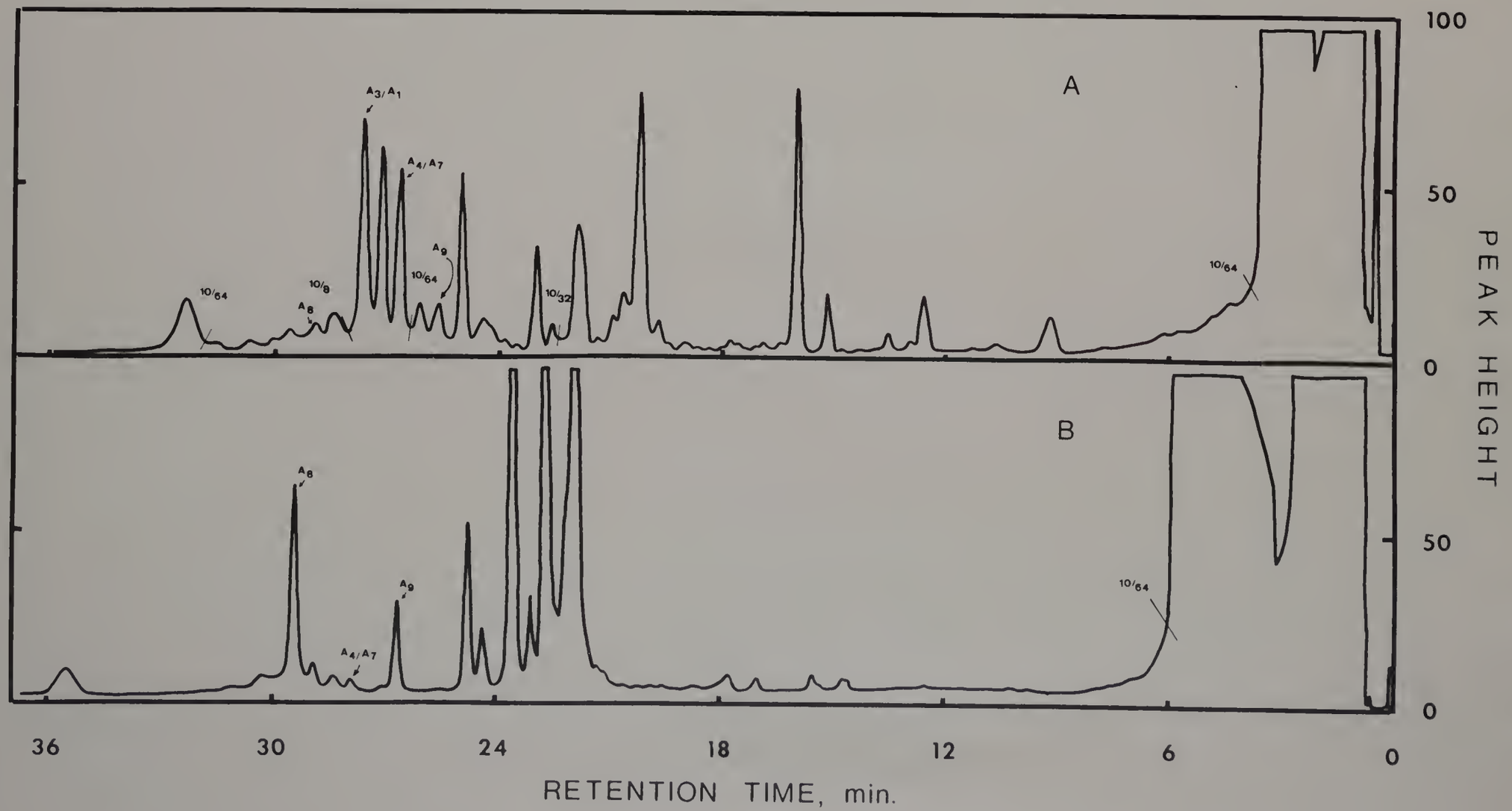
Based on the data in Table XIII, gibberellins A_8, A_3, A_9, A_4, and/or A_7, and possibly A_5 apparently are present in the TMS aqueous acidic extracts of the tomato. No attempt was made to identify the other tomato peaks because of lack of standard GAs. Typical GLC traces of $\underline{yg}_6$ TMS extracts chromatographed in 3 columns, namely 5% SE-30 (Figure 13A), 5% SE-33 (Figure 13C), and 5% OV-22 (Figure 13D) are shown with various peaks, which were identified and labeled correspondingly. The retention times of these labeled peaks are shown in Table XIII.

Previously, 2 chromatographic columns were found to separate standard GAs satisfactorily. Ikekawa and Sumiki (89) reported complete separation and identification of the methyl esters of 9 GAs (A_1 to A_9) using 1.5% SE-30 and 2% QF-1-0065. Similarly, MacMillan and Pryce (115) reported satisfactory separation of methyl esters and in addition, TMS ethers of methyl esters of 23 gibberellins (A_1 to A_{23}) using 2% SE-33 and 2% QF-1. The feasibility of separating several TMS ether derivatives of gibberellins using OV-22 column, in addition to SE-30 and SE-52 columns, were determined. As shown in Table XIII, among the 3 columns studied, under the chromatographic condition used, OV-22 appears to be inadequate in separating the 7 gibberellins. In efforts to improve the separation using OV-22, the temperature program was changed from 20° C/min to 10° C/min. However, no significant improvement in separation resulted.

Another technique employed to identify the tomato gibberellins was co-chromatography. The Varian Aerograph HY-FI Model 600-D chromatograph was used. It was equipped with 5% SE-30 column and supplied with N_2 as the carrier gas at a flow rate of 25 ml/min. It was found that 4 of the compounds from the tomato co-chromatographed with 4 of the standard gibberellins: one with gibberellin A_3 (retention time of 22.5 min), one

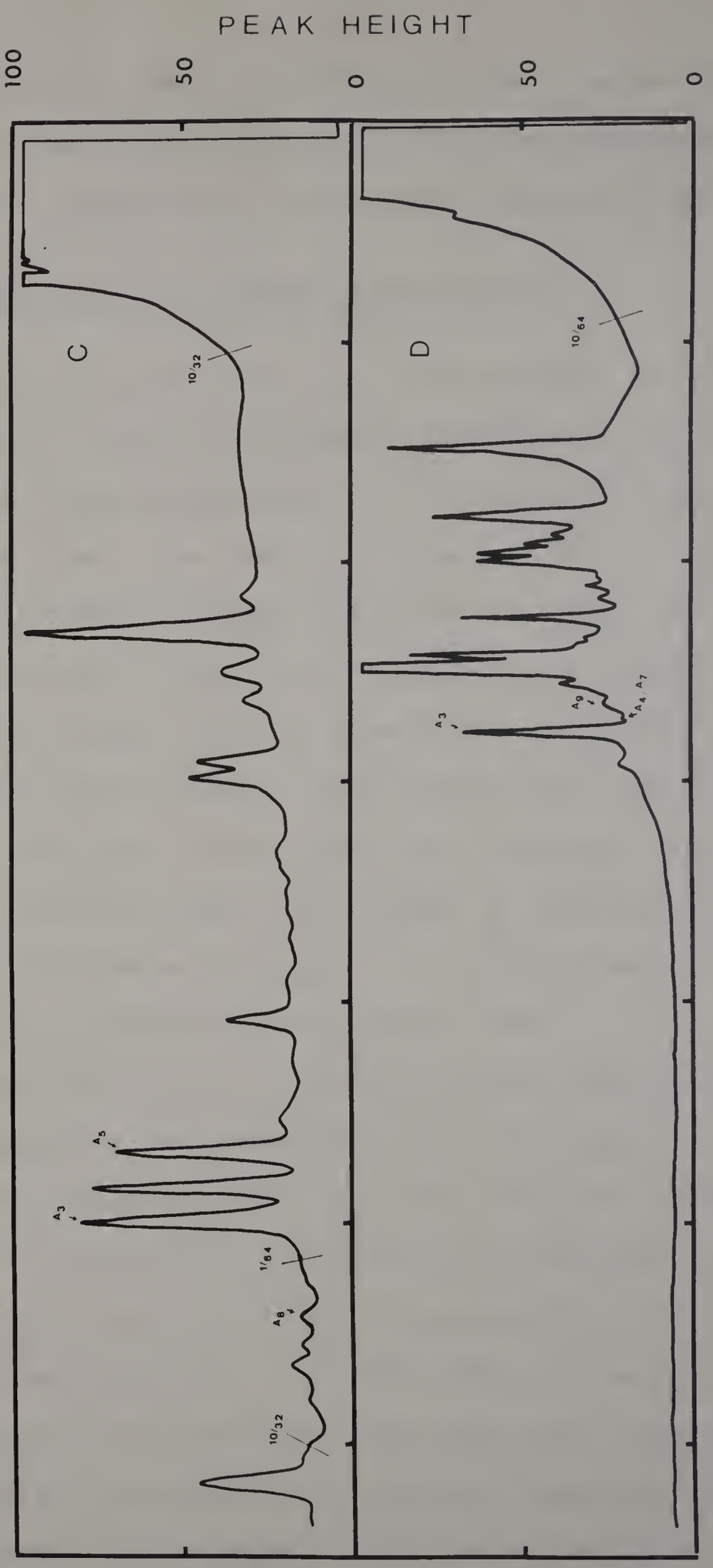
Figure 13A and 13B. GLC of TMS aqueous acidic extracts of yg$_6$ chromatographed on a Perkin-Elmer Model 900, 5% SE-30 column, N_2 carrier gas at 40 ml/min. The column temperature was kept at 70°C for 6 min followed by programming at 10°C/min to a final temperature of 290°C. (A) Traces of trimethylsilylated extracts injected directly into the GLC without paper chromatography, (B) Traces of trimethylsilylated extract, which was eluted from paper chromatogram at R_f 0.0-0.1 and treated with bis-(trimethylsilyl) acetamide, then injected into GLC. The longer retention times of peaks in (B) by at least 1 minute than those in (A) was attributed to manual programing performed in the (B). Identified GLC peaks are labeled appropriately.

FIGURES 13 A and B



Figures 13C and 13D. Typical GLC traces of TMS aqueous acidic extracts of *yg*$_6$ tomato. (C) Chromatographed on 5% SE-52 column, initial temperature of 150°C maintained for 6 min followed by programing at 5°C/min to a final temperature of 290°C, (D) Chromatographed on 5% OV-22 column, initial temperature of 150°C maintained for 6 min followed by programing at 20°C/min to a final temperature of 290°C. GLC performed on a Perkin-Elmer Model 900 equipped with dual columns and dual flame ionization detectors, supplied with carrier gas N_2 at a flow rate of 40 ml/min. Identified GLC peaks are labeled appropriately.

FIGURES 13 C and D



with A_4 and/or A_7 (21.0 min), one with A_8 (23.6 min) and one with A_9 (18.4 min). Gibberellins A_1 and A_5 did not co-chromatograph with any of the compounds present in the aqueous acidic extracts of the tomatoes.

Non-Mobility of Gibberellins A_8 and A_9

A very interesting observation was made concerning the apparent lack of mobility of extracted endogenous gibberellins A_8 and A_9 applied to 3 MM Whatman chromatography paper and developed with 1-butanol, acetic acid and water. When the eluates from R_f 0.0 - 0.1 were treated with BSA and 3 μl sample injected into the GLC, the peaks corresponding to gibberellins A_8 and A_9 were found to be present in considerably larger quantities (Figure 13B) than those obtained with TMS aqueous acidic extracts injected directly into the GLC without paper chromatography (see Figure 13A). In both cases, equal amounts of dried plant extracts were treated with BSA. The presence of larger quantities of GA_8 and GA_9 in the eluates from R_f 0.0 - 0.1 suggests the lack of mobility of these gibberellins in the solvent used.

The eluates from R_f 0.0 - 0.1, which contained more GA_8 and GA_9, contained considerably less gibberellins A_3/A_1, and A_4/A_7 (Figure 13 B) suggesting the apparent mobility of these GAs. This was supported by data from paper chromatography (6 replications, unpublished work) which showed that GA_3 has an R_f of 0.93 while GA_7 has an R_f of 1.0 in the solvent used (See Figure 10). It was also found that GA_3 could be distinguished from GA_7 by its yellowish green color under ultraviolet light while GA_7 could be distinguished by its yellow fluorescence (no UV used) following treatment with 70% H_2SO_4. Both gibberellins are colorless otherwise.

DISCUSSION

The tomato mutant, $\underline{yg}_6$, in addition to exhibiting the syndrome of 3 pleiotropic characters, namely faster growth rate, reduced chlorophyll content, and lack of anthocyanin, has been found to possess depressed root and top growths on a dry weight basis, and to exhibit twice the specific activity of L-phenylalanine ammonia-lyase (PAL) than the normal wild type tomato. These expressions of the $\underline{yg}_6$ mutation suggest an interrelationship between the physiological action of the mutant gene and the gibberellins. It was proposed (154) that the syndrome and other phenotypic characteristics of the $\underline{yg}_6$, including the higher PAL activity, can be explained by one of the following: (1) higher levels of endogenous GAs; (2) less effective endogenous inhibitors; or (3) enhanced sensitivity to GA.

This study showed that the $\underline{yg}_6$ mutant contains three times as much endogenous GAs as the wild type (Table X). The presence of a higher quantity of GAs in the $\underline{yg}_6$ than in the wild type was confirmed by gas-liquid chromatography (Figure 11). There were no qualitative differences, only quantitative differences, in the GAs present in the $\underline{yg}_6$ and the wild type (Figures 11 and 12). Using gas-liquid chromatography, gibberellins A_3, A_8, A_9, A_4 and/or A_7, and possibly A_5 were identified in the aqueous acidic extracts of these two tomatoes. There appears to be no difference in the amounts or effectiveness of GA-inhibitors from the $\underline{yg}_6$ and the wild type.

These results indicate that the syndrome of three pleiotropic characters, and the depressed root and top growths and the higher PAL

activity, could be explained by the mutant possessing higher than normal amounts of endogenous GAs.

The following possibilities may explain the finding that the $\underline{yg}_6$ mutation leads to overproduction of endogenous GAs: the locus is involved either in (1) the production of a "promoter" of the degradation process of GAs, or (2) the production of an "inhibitor" of GA biosynthesis. The normal wild type locus functions to produce more promoter as in (1) or it produces more inhibitor as in (2); either process results in the production of less GAs. In contrast, the mutant locus ($\underline{yg}_6$) functions abnormally and produces less promoter as in (1) or less inhibitor as in (2); either results in production of greater than normal amounts of GAs.

It is noteworthy that certain endogenous GAs are present in greater quantities in the mutant than in the normal tomato. The various steps in the biosynthesis from kaurene to the GAs and also between GAs have been proposed (106). Upon establishing the identity of the other still unidentified GAs in these tomatoes and determining the relative amounts of GAs in both mutant and normal tomatoes, the particular steps in the GA biosynthetic pathway which produce greater than normal amounts of GA could be identified. This information would not only be highly interesting, but could also help elucidate the steps of GA biosynthesis in higher plants.

These investigations with the $\underline{yg}_6$ gene dealt with a mutation controlling higher amounts of endogenous gibberellins. In contrast, previous studies (156, 157, 159) involved attempts to relate single gene dwarf mutations with the lowered level of endogenous GAs. Phinney (157) reported that among 5 GA-responding dwarf corn mutants, 3 mutants ($\underline{d}_3$, $\underline{d}_5$,

and $\underline{an}_1$) did not show any GA-like activity while the other 2 mutants ($\underline{d}_1$ and $\underline{d}_2$) contained less than half as much total GA as the normal tall corn. The correlation with the level of GA-like substances in tall corn compared to the lowered amounts or absence in the dwarf plants are cited as evidence for a causal relationship between GAs and the dwarf growth habit. Köhler and Lang (99) reported the absence of GA-like substances in the dwarf 'Progress No. 9' cultivar of peas and the presence in the tall 'Alaska' cultivar. Lockard et al. (109) found that the gibberellin-like activity was higher in extracts from tall tomato, variety 'Winsall', than in extracts from dwarf tomato, variety 'Tiny Tim'.

Although different gibberellins have been isolated and identified in many plants (106), this is the first report of a more definitive identity of certain tomato GAs. Earlier work in tomato GAs involved paper chromatographic studies. Hill and Selman (88) reported the occurrence of two gibberellin-like substances in the acidic fraction of shoot extracts of tomato (cv. 'Potentate') but the identity was not resolved. Pegg (151) found gibberellin-like substances not only in the acidic fractions but also in the basic and neutral fractions of extracts from seed and etiolated seedlings of the same variety of tomato. Several workers (10, 22, 32) found a gibberellin-like substance with an R_f which coincided with that for either gibberellin A_1 or A_3 in both shoot and root extracts of tomato of different varieties. Lockard et al. (109) recently reported the occurrence of two gibberellin-like substances, the R_f of which corresponded to that of GA_{1+3} and GA_{4+7}, in plant tip extracts of a tall variety of tomato, 'Winsall', and a dwarf variety, 'Tiny Tim'.

The commercial availability of the trimethylsilylating agent, "bis-(trimethylsilyl) acetamide", provides researchers with a simple, rapid, and safe method of silylating gibberellins. This dissertation provides a list of the retention times of 7 gibberellins in the form of trimethylsilyl ether derivatives in 3 chromatographic columns. The above information may prove useful to researchers involved in separation and identification of GAs from plant extracts.

One interesting observation made following the shoot application of 5 concentrations of GA_3 was the complete change in leaf shape of new leaves from normally serrated (indented) shape to smooth edge (entire) shape (Figure 5). Leaf shape alteration similar to the above observation were previously reported in tomatoes by Gray (74) in cv. 'Rutgers' and cv. 'Bonne Best' and by Rappaport (168) in cv. 'Earlypak'. The leaves of tomato typically have cut margins while some varieties have potato-leaf type, so-called because their leaves are broad and entire, like the leaves of potato plants. The GA-induced smooth edged (entire) leaf closely resemble the potato leaf characteristic which is known to be genetically controlled (113). It should be noted that the experiments in which the altered leaf shape were reported used either gibberellin A_3 alone (168, and this dissertation) or both gibberellins A_1 and A_3 (74). Is this alteration in leaf shape a specific effect by particular gibberellins only? GA_3 was also found to induce a transition from juvenile to mature leaf form, as in Ipomoea and Eucalyptus (137, 183) or from mature to juvenile leaf form, as in Hedera sp. var. 'arborescens' (174). A change from opposite to alternate insertion of leaves on stems of Sesamum indicum, the alternate condition normally seen

only on flowering shoots, was reported by Chakravarti (35).

Slight modification in the syndrome of three pleiotropic characteristics studied were observed in the $\underline{yg}_6$ scions of $\underline{yg}_6$/wild grafts and in the wild type scions of wild/$\underline{yg}_6$ grafts. However, these changes are not statistically significant (Table IX). Several reasons may explain the lack of significance: (1) inability of translocating endogenous GAs from the plant part which contains greater amounts to the other which contains lesser amounts; (2) assuming there is translocation occurring, the GAs and/or the GA-precursors from one type of tomato could not function or be interconverted in the other tomato; (3) an insufficient period of time (three weeks) from the onset of successful graft union to the time the data were collected. Possibility (1) is the least attractive explanation in view of the results which show translocation of root-applied GA_3 from both tall and dwarf pea rootstocks to the dwarf pea scions (108). Although it has been shown that GA-like substances extracted from a normal cultivar of tomato ('Potentate') could induce stem elongation when applied to the seedlings of the same tomato (151), it is not unlikely that the GA-like substances from either the $\underline{yg}_6$ or the normal wild type could function when applied to the other. With regard to possibility (3) it is noted that a considerably longer time (more than five weeks) was involved in several grafting experiments in which positive modifications were induced (21, 171).

Reports of positive, immediate modifications have been published. Rick (171) found that phenotype of scions of $\underline{wd}$/ + and +/$\underline{wd}$ grafts ($\underline{wd}$ refers to wilty-dwarf mutant and + refers to the normal tomato, Line 2-72 of 'San Marzano') were greatly modified; size of leaf and thickness

of stem of wd scions of wd/+ were greatly increased, while those characters of + scions of +/wd were greatly reduced. He also found that the influence of stock is dependent not in the stems or leaves but in its root system and suggested that production of a growth-regulating substance by the roots might be responsible for the influence of stock upon scions. Böhme and Scholz (21) reported that the phenotype of the mutant, chloronerva, with respect to morphology, differentiation, and chlorophyll content was changed to that of the normal wild type, 'Bonner Beste', by grafting as well as by application of water extracts from normal plants to the leaves of the mutant. Scholz (181) found that the "normalizing" factor from the normal tomato is a peptide with moderate molecular weight.

On the other hand, several workers have indicated negative findings from their grafting experiments. Wilson and Withner (220) found no modifications in phenotype and in certain major B vitamins in both scions or stocks of grafted tomatoes. Walker (213), grafting tomatoes and Cyphomandra betacea Sendt. reciprocally, found no changes in the phenotypes of tomatoes. Recently, Lockard and Grunwald (108) have reported that grafting tall pea, 'Alaska', and dwarf pea, 'Progress No. 9', onto their own roots or their reciprocal grafts did not change the stem elongation of the stems of the scions regardless of the rootstock they were grafted on. Earlier, Lockhart (110) had observed no increased elongation occurred in the stem of the dwarf rootstock when a tall pea scion was grafted onto a dwarf pea stock. It is noteworthy that in the last two experiments cited (108, 110), the tall and dwarf growth habits were correlated with the amounts of endogenous GAs.

The growth rate studies indicated that the $\underline{yg}_6$ tomato, which still responded to added GA_3, was not fully saturated with endogenous GAs (Figures 2 and 3). The saturation level for the normal tomato (100 μg/plant) was found to be higher than that for the $\underline{yg}_6$ mutant (50 μg/plant) (Figure 4) which likewise suggests the presence of larger amounts of GAs in the $\underline{yg}_6$ than in the normal. In fact, the $\underline{yg}_6$ tomato was demonstrated to contain 3 times as much GAs than the normal tomato (Table X), yet do not grow at a rate 3 times faster than the normal. These observations raise the question of whether any of the tomato GAs is especially functional in promoting tomato plant growth. Not all of a plant's endogenous GAs need necessarily be very active, as they probably include precursors, active and deactivated GAs (57).

GA_3 induced the syndrome of three characters as in the mutant in the normal tomato and conversely, Phosfon induced these three characters as in the normal in the mutant. However, both treatments did not mimic in either plant the bright-yellow primary shoot as in the mutant. The yellow components in leaves are the $\underline{a}$-and $\underline{b}$-carotenes. The bright yellow primary shoot may be explained by (i) presence of higher than normal amounts of total carotenes, (ii) presence of extremely reduced amounts of total chlorophylls, or (iii) combination of possibilities (i) and (ii). Failure to mimic the bright-yellow shoot as in the mutant probably was due to inability in satisfying any of these reasons. Among these possibilities, possibility (iii) is the most attractive. Szalai (197) presented evidence which could be held to support (iii), namely that GA treatment resulted in about 50 per cent reduction in total chlorophylls and about 32 per cent increase in total carotenes. The

other component, the xanthophylls, did not show any significant change in the levels following GA treatment. In this study, it is possible that a more extreme reduction of the chlorophyll content in the normal tomato and the increased amount of carotenes might permit the expression of the bright yellow shoot characteristic in this plant.

Another explanation for the failure to completely mimic the phenotype of the mutant by addition of GA_3 to the normal probably is the dependence of the bright yellow shoot on the presence of a combination of gibberellins in differing amounts. A reason for suggesting this possibility is our finding that certain GAs in the mutant were present in higher amounts than those in the wild type (Figure 11).

SUMMARY

The relationship between the phenotypic expressions of the yellow-green 6 gene mutation in tomato and the gibberellins was investigated.

1. The yg_6 mutant was found to contain 3 times as much endogenous gibberellins as the normal wild type. The presence of a higher quantity of GAs in the yg_6 than in the wild type was confirmed by gas-liquid chromatography.

2. Using gas-liquid chromatography, gibberellins A_3, A_8, A_9, A_4 and/or A_7, and possibly A_5 were identified in the trimethylsilylated extracts of both tomatoes. However, certain endogenous GAs were found to be present in greater quantities in the mutant than in the normal tomato.

3. The yg_6 mutant could still respond to applied GA indicating it is not fully saturated with this hormone. It was found that the mutant has a lower level of saturation to applied GA (50 µg/plant) than the wild type (100 µg/plant). GA promoted the rate of stem growth of the wild type to a rate comparable to that of the untreated yg_6.

4. GA, at two concentrations, promoted the activity of L-phenylalanine ammonia-lyase in both yg_6 and wild type tomatoes; the promotion being slightly greater in the former than in the latter.

5. A drastic change in leaf shape from normally serrated (dentate) shape to smooth edge (entire) shape was induced by GA in both plants. The GA-induced smooth-edged leaves closely resemble the "potato-leaf"

character in tomato, a character known to be controlled by a single recessive gene.

6. The $\underline{yg}_6$ gene mutation did not appear to affect the amount of GA-inhibitors present in the mutant when compared to that in the normal tomato.

7. GA and Phosfon treatments resulted in nearly mimicking completely the phenotypes of either plants, except for the bright yellow primary shoot as in the mutant. GA induced the syndrome of three characters as in the mutant in the normal wild type, or conversely, Phosfon induced these three characters as in the normal tomato in the mutant.

8. Grafting experiments, involving the side-approach and top grafting, did not result in inducing drastic changes in the syndrome of three characters studied.

9. It is concluded that the syndrome of pleiotropic characters of the $\underline{yg}_6$ mutant, namely faster growth rate, reduced chlorophyll content, absence of anthocyanin, depressed root and top growths, and twice the activity of L-phenylalanine ammonia-lyase than the normal wild type, could be explained by their being causally related to the gibberellins.

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A P P E N D I X

Table I. The 29 Gibberellins.

Gibberellin	Provisional Names	Molecular Formula	Molecular Weight	Sources	References
	Gibberellin "A"	$C_{19}H_{24}O_6$	348	Gibberella	75, 190, 195, 198
A_1	Dihydro-gibberellic Acid			Immature Seeds	116, 117, 118, 119, 121, 217, 218
	Bean Factor I			Shoots/Sprouts	81, 95
A_2	- - -	$C_{19}H_{26}O_6$	350	Gibberella	189, 198
	Gibberellic Acid			Gibberella	49, 58, 190, 198
A_3*	Gibberellin "A"	$C_{19}H_{22}O_6$	346	Immature Seeds	67, 201
				Parthenocarpic Seeds	85
				Shoots	81, 129*, 206*
A_4	- - -	$C_{19}H_{24}O_5$	332	Gibberella	203
				Immature Seeds	63, 67, 116
A_5	Bean Factor II	$C_{19}H_{22}O_5$	330	Immature Seeds	116, 117, 118, 119, 132
A_6	- - -	$C_{19}H_{22}O_6$	346	Immature Seeds	116, 121, 122

Gibberellin	Provisional Names	Molecular Formula	Molecular Weight	Sources	References
A_7	- - -	$C_{19}H_{22}O_5$	330	Gibberella	51, 75
				Immature Seeds	63, 67
A_8*	- - -	$C_{19}H_{24}O_7$	364	Immature Seeds	116, 119, 120, 182*, 229*
A_9	- - -	$C_{19}H_{24}O_4$	316	Gibberella	50, 51, 75
				Shoots	81
A_{10}	- - -	$C_{19}H_{26}O_5$	334	Gibberella	77
A_{11}	- - -	$C_{19}H_{22}O_5$	330	Gibberella	Cross and Hanson**
A_{12}	- - -	$C_{20}H_{28}O_4$	332	Gibberella	52
A_{13}	- - -	$C_{20}H_{26}O_7$	378	Gibberella	71
				Immature Seeds	34
A_{14}	- - -	$C_{20}H_{28}O_5$	348	Gibberella	53
A_{15}	- - -	$C_{20}H_{26}O_4$	330	Gibberella	78
A_{16}	- - -	$C_{19}H_{24}O_9$	348	Gibberella	72
A_{17}	- - -	$C_{20}H_{26}O_7$	378	Immature Seeds	115, 163

Gibberellin	Provisional Names	Molecular Formula	Molecular Weight	Sources	References
A_{18}	Lupinus-I	$C_{20}H_{28}O_6$	364	Immature Seeds	100, 102
A_{19}	Bamboo Gibberellin	$C_{20}H_{26}O_6$	362	Bamboo Shoots	131, 204, 205, 206
				Immature Seeds	115, 164
A_{20}*	Pharbitis Gibberellin	$C_{19}H_{24}O_5$	332	Immature Seeds	114, 115, 132, 201, 206*
A_{21}	Canavalia-I	$C_{19}H_{22}O_7$	362	Immature Seeds	202, 206, 207
A_{22}	Canavalia-II	$C_{19}H_{22}O_6$	346	Immature Seeds	202, 206, 207
A_{23}	Lupinus-II	$C_{20}H_{26}O_7$	378	Immature Seeds	101
A_{24}	- - -	$C_{20}H_{26}O_5$	346	Gibberella	82
A_{25}	- - -	$C_{20}H_{26}O_6$	362	Gibberella	Harrison, D.M., and J. MacMillan**
A_{26}*	- - -	$C_{19}H_{22}O_7$	362	Immature Seeds	229*
A_{27}*	- - -	$C_{20}H_{26}O_6$	362	Immature Seeds	229*
A_{28}	- - -	$C_{20}H_{23}O_8$	391	- - -	Koshimizu, 106
A_{29}	- - -	$C_{19}H_{24}O_6$	348	- - -	Takahashi, 106

* Glucosyl-GAs identified
** Personal Communication