PLANT GROWTH REGULATORS AS TOOLS FOR RECONSTRUCTING THE EVOLUTION OF PLANT FORM

G. P. Cheplick[1]
Department of Botany, Miami University
Oxford, Ohio 45056

ABSTRACT: Life history characteristics of plant species are subject to regulation by genes and the expression of these genes may be regulated, in turn, by growth regulators responding to environmental cues. Mutations which result in changes in levels of endogenous hormones or other growth regulators at specific points in development could lead to an altered phenotype with an adaptive advantage. These mutations eventually become incorporated into an evolving population and assume importance by resulting in a new phenotypic trait, such as lobed leaf shape, which may be a component of species strategy. When compared to an extant non-mutant population (P), the new mutant population (P´) may be considered as a different taxonomic unit at a subspecific level. If the genetic potential still exists in P´ for the expression of the original phenotype of P, an experimenter should be able to apply phytohormones or other regulatory substances at a specific point in the development of individuals of P´ and obtain the phenotype common in P. This contention requires that growth regulators function as gene de-repressors and that certain point mutations result in changes in growth regulator levels within a plant. In this way, plant growth regulators may be useful to those systematists investigating phylogenetic relationships among closely related plant taxa and population ecologists interested in plant life history evolution.

*   *   *

Introduction

"...growth substances and hormones act as transducers of perceived environmental cues and control a wide range of structural and functional adaptations that define the limits of elastic strain and enable a plant to survive environmental challenges."
   --Salisbury and Marinos (1985)

The life history strategy of a plant species entails a suite of morphological, biochemical, and physiological characteristics which enable individuals to survive and reproduce in a particular habitat (Jain, 1979). Ecologists have recognized the adaptive nature of many of these characteristics, but often speculate on the evolution of certain adaptations without substantial evidence concerning the earlier stages of evolutionary development from which they emerged. For example, two related plant species may exhibit slightly different leaf morphologies, yet it may be difficult to decide which species exhibits the derived condition. In this paper I propose that plant growth regulators could provide a useful tool by which relationships between plant taxa and the evolution of certain aspects of plant life history might be better elucidated. I use the term growth "regulator" in a general way to refer to the entire range of morphoregulatory substances found in plants.

A number of growth regulators influence plant growth and development by regulating RNA-directed protein synthesis (Key, 1969; Leopold and Kriedemann, 1975; Wareing and Phillips, 1978; Sen, 1985). Filner and Varner (1967) have shown that gibberellins can cause de-repression of the gene responsible for alpha amylase synthesis in barley aleurone cells. Hence, gibberellin is allowing the expression of a gene which is normally repressed in a tissue lacking the hormone. Theologis (1986) has reviewed

*   *   *

the evidence for the "gene activation hypothesis" which proposes that auxin regulates the synthesis of mRNAs coding for proteins needed in the growth process. If other growth regulators can similarly allow the expression of previously "quiescent" genes, some of which influence morphological characteristics defining a life history strategy rather than only affecting biochemistry, a model system exists whereby the application of growth regulators may open up previously suppressed pathways of morphogenetic development.

The purpose of this paper is to demonstrate the potential usefulness of plant hormones in understanding phylogenetic relationships among closely related plant taxa, and how the evolution of subtle changes in plant form may be brought about by changes in hormone levels. First, a brief summary of morphoregulatory mechanisms and the role of plant growth substances will be presented; second, adaptive changes in leaf morphology will be considered in detail as an example of a specific hormone-mediated effect; and third, a hypothetical scheme depicting the origin and nature of an evolutionary change which leads to an adaptive leaf morphology will be developed.

Morphoregulatory Mechanisms

In plants, growth regulating substances have the potential to influence evolutionary change by directly or indirectly affecting different stages in the hierarchical chain of events proceeding from gene action to cells to organ morphology. Bachmann (1983) proposed that regulatory genes provide a possible mechanism for the control of organ-specific gene expression by producing gene products that coordinate the transcription of the regulated structural genes. The specificity in gene action is determined by some external signal such as a phytohormone. In some cases, the genes involved in the synthesis and release of hormones and the genes determining the cell-specific synthesis of a hormone receptor can function as the "regulator genes of tissue differentiation" (Bachmann, 1983).

Auxins and other hormones have been useful to physiologists studying mechanisms of cellular expansion (Taiz, 1984). In one example of a dramatic cellular change, phytohormones were used to induce the differentiation of tracheary elements from mesophyll cells (Church and Galston, 1988). Gibberellins affect the extensibility of cell walls and thus cell elongation (Jones, 1982). In addition, Stoddart (1982) noted that gibberellin "may affect synthesis and/or secretion of wall precursors by transcriptional or translational control." Sen (1985) pointed out that almost nothing was known about the molecular basis for the effects of hormones on initiation and plane of cell wall growth, which are so important in tissue differentiation. Perhaps the gene products of activated or de-repressed loci relevant to the present discussion are cell wall-associated proteins or enzymes which alter the mechanical properties of the cell wall. Stebbins (1986) hypothesized that proteins associated with intercellular membranes and the intracellular cytoskeleton were the primary gene products determining patterns of morphogenesis. Because of the integrated nature of plant development (Wardlaw, 1965; Wareing, 1977; Sachs, 1986, 1988; Trewavas, 1986), pronounced changes in cellular elongation rates in plant tissues will ultimately affect morphological phenotypes (Digby and Firn, 1985; Dale, 1986; Green, 1986, 1987).

Hydroxyproline-containing proteins, which appear to be ubiquitous constituents of plant cells, provide an example of molecular regulation of cell enlargement and/or division with significant effects on morphology (Basile, 1980). The morphoregulatory role of these proteins is illustrated by experimental application of chemical antagonists to leafy liverworts. The antagonists, which interfere with the synthesis or function of the regulatory proteins, induce a type of leaf development in different liverwort species that is not normally expressed in the genera or families to which the species belong (Stebbins and Basile, 1986). Because many of these differences can be related to differences in relative amounts of cell division and/or

cell enlargement, Basile (1980) proposed that certain hydroxyproline-containing proteins bound to the cell wall act to suppress development of highly localized populations of cells. Additional cellular interactions within developing organs could also influence plasticity in plant development, with growth hormones playing a major role (Sachs, 1986). Mutations of genes coding for enzymes or other proteins that are important in the synthesis or transport of growth substances are likely to have major effects on morphogenesis and may be largely responsible for changes in adult form noted by plant evolutionists (Stebbins and Basile, 1986; Sachs, 1988).

An Example

Gibberellic acid (GA) is known to affect a number of morphological and physiological characteristics in many plant species (Jones, 1973) and these traits can be considered as components of plant strategy (Table 1). Changes in endogenous GA levels occurring at specific times in ontogenetic development, whether they be mediated via changes in environmental conditions or differential gene expression, may thus influence features important to growth, survival, and reproduction. Evidence that quantitative changes in GA content occur during plant development is summarized by Moore (1979).

Table 1. Plant characteristics known to be affected by gibberellic acid which can be components of plant strategy.

bud growth	fruit growth	seed germination
cambial activity	internode elongation	senescence
flower corolla growth	leaf growth	sex expression
flower induction	leaf shape	

As an example, consider the effect of GA on leaf morphology. Since different leaf shapes are known to be adaptive in different environments (Givnish, 1979, 1987; Gurevitch, 1988), changes in leaf shape are relevant in an evolutionary context. It is well established that in heteroblastic plants, GA can promote the production of "juvenile" leaf shapes, even in individuals previously producing leaves of the "adult" form (Robbins, 1957; Njoku, 1958: Allsopp, 1962; Wallenstein and Albert, 1963; Borchert, 1965; Feldman and Cutter, 1970a). In other cases, GA treatment has been noticed to mimic the tendency for leaves to become entire with decreased lobing as irradiance decreases (Gray, 1957; Jones, 1985). Endogenous gibberellins as well as other regulatory substances such as auxins (Thimann, 1977; Digby and Firn, 1985) probably play an important role in the regulation of leaf shape and development in a variety of plant species. Digby and Firn (1985) review the relationship between growth substances and leaf growth in greater detail.

Feldman and Cutter (1970a) have shown that if whole plants of Centaurea solstitialis were grown in a culture on media containing GA, the leaves produced were simple and entire; however, plants on a control medium formed lobed leaves. Culturing of excised leaf primordia on media with and without GA demonstrated that GA could cause a leaf primordium which would normally produce lobed leaves to form entire leaves (Feldman and Cutter, 1970b). The mechanism of GA action was not known. In cotton (Gossypium arboreum), it is known that leaf shape is controlled by multiple alleles (Hutchinson, 1934), but according to climatic conditions either narrow or broad leaves may be produced (Hutchinson, 1936). The narrow-leaved types are found in areas of low rainfall, whereas the broad-leaved types predominate in more mesic habitats. Hutchinson (1936) concluded that, depending on environmental conditions,

the broad or narrow leaf form has a selective advantage, and broad leaves are a
"primitive character" in cotton. In addition, Hammond (1941) speculated that the
control of leaf shape gene expression could be mediated by ."hormone diffusion" in
this same species.

If GA is in fact influencing leaf development by regulating gene expression, it
might be considered that exogenously applied GA could reveal the derived nature of a
currently adaptive leaf morphology. Such a contention assumes the genetic
potentiality for producing the more primitive morphology still exists in the extant
species under investigation (Rosenblum and Basile, 1984; Stebbins and Basile, 1986).

It is, of course, possible that exogenous growth regulator application might
induce deviant forms unrelated to any ancestral condition and not mediated via
genetic regulation. In addition, the more advanced condition could involve <u>increased</u>
endogenous GA levels. However, careful comparisons among closely related taxa in
terms of morphological and genetic similarities in conjunction with other taxonomic
data indicative of phylogenetic relationships should help in unravelling questionable
situations. Measurements of endogenous hormone levels would also help to clarify the
relationship of regulator levels to morphological condition. In this regard, it is
notable that in a recent review on the evolution of plant form, Sachs (1988) called
for an "analysis of ontogeny based on comparative studies" and stated that the most
promising differences to consider were those involving single factors and single
developmental processes. He also stressed the importance of obtaining, "wherever
possible", concentrations of the relevant growth substances and details of the
molecular mechanisms involved.

Although necessarily somewhat speculative, an evolutionary senario relevant to a
biochemically mediated change in morphology can be envisioned as follows. Individ-
uals of a plant species in a certain habitat normally synthesize significant quan-
tities of GA in their leaf primordia at a specific point in ontogeny such that entire
leaves result. This leaf form is adaptive in this habitat. If a mutant arises so
that significant quantities of GA are not available to the leaf primordia at this
same developmental stage or pulses of GA occur at specific times during development,
lobed leaves result. Provided lobed (or dissected) leaves were selectively
advantageous to individuals possessing them under certain environmental situations
(e.g., Lewis, 1969; Gurevitch, 1988), the mutant leaf form could become widespread in
a population and ultimately replace the now "primitive" entire leaf form. The
genetic potential to produce entire leaves may not be entirely lost however, and an
investigator could induce the production of the primitive leaf type by application of
GA to the young leaf primordia at the appropriate time in development (Rosenblum and
Basile, 1984). Thus, the derived nature of the lobed leaf morphology is revealed.
In this regard, Stebbins (1986) has stated that morphological differences between
plant species "can be mimicked by applying known chemical agents" under controlled
conditions and provided a few examples where this had been done. Sachs (1988) noted
that changes in phytohormones could change the location of intercalary growth in the
leaves of pea plants and concluded that in developing pea leaf primordia there was a
capacity for intercalary growth that was not normally expressed.

What other evidence is there that in some species leaf shape is under genetic
control? In <u>Tropaeolum</u>, Whaley (1939) showed leaf shape to be controlled by an
epistatic relationship between two gene loci. The genetic control of leaf shape in
cotton (<u>Gossypium</u>) has been extensively analyzed by Hammond (1941), and Sachs (1988)
speculated that these shape-controlling genes could act by changing the supply of
phytohormones such as auxin. Also, related work has shown that genes controlling
shape may be expressed in other plant organs as well (Sinnott, 1935). Common garden
studies of <u>Geranium sanguineum</u> collected from different geographical locations
revealed that there were genetic differences between populations in the phenotypic
expression of leaf dissection (Lewis, 1969). Although shape-controlling genes are

not necessarily tied to the synthesis of phytohormones, they must directly or indirectly produce gene products having some morphoregulatory role. For example, gene products which affect cytoskeleton orientation and cellular dimensions ultimately determine integrated developmental patterns in plant leaves and shoots (Digby and Firn, 1985; Green, 1987).

What evidence is there that genetic changes in plants may affect endogenous levels of GA? The best evidence comes from studies of genetic dwarfism in crop plants which can be corrected by GA application (Nelson and Burr, 1973). These dwarfing genes interfere with a specific step in gibberellin synthesis (Phinney and West, 1960), resulting in reduced amounts of endogenous GA in the mutant when compared to the non-mutant genotype. It is conceivable that analogous mutants can arise which alter endogenous levels of other morphoregulatory substances such that changes in cytoskeleton and mitotic spindle orientation or cellular dimensions translate into an altered leaf/shoot morphology (see examples in Stebbins (1986) and Green (1987)).

An Hypothetical Model

If it is assumed that GA can act via stimulation of RNA synthesis at normally repressed gene loci, and that genetic changes (i.e., mutations) may affect endogenous GA levels, a simple scheme can be developed to explain how changes in morphology may arise (Fig. 1). An operon system of genetic regulation will be used, in which a structural gene is transcribed only after GA removes repressors from a certain operator gene (Glasziou, 1969). Evidence that such systems of regulation occur in higher plants is reviewed by Scandalios and Baum (1982). Bachmann (1983) utilized a comparable operon model to describe a possible genetic mechanism for the control of organ-specific gene expression in plants. Similarly, a recent scheme for auxin-regulated genes depicts protein repressors and activators as being involved in the regulatory process (Theologis, 1986).

Using leaf morphology as an example again, the present scheme proposes that in a non-mutant individual of some hypothetical species, gene A codes for an inducer molecule which activates structural gene X during some stage of development (Fig. 1A). This latter gene is important for the production of an enzyme (E_1) needed for GA synthesis. This enzyme could be kaurene synthetase, which is important in the synthesis of kaurene, a key intermediate in the GA biosynthetic pathway (Moore, 1979). Hence, activation of structural gene X results in increased GA synthesis and increased levels of endogenous GA. The GA can de-repress structural gene Y, which codes for another factor (E_2) important to a developmental pathway leading to the production of entire leaves (Fig. 1A). In some way, GA alters patterns of cell division in the developing leaf primordia so this leaf type results (Feldman and Cutter, 1970b; Jones, 1985). Enzyme E_2 might be a hydroxyproline-protein or important in the synthesis of any substance that influences cell wall extensibility or elongation.

If gene A were to mutate and produce an "inducer" which was no longer functional, the entire developmental pathway could be altered such that a new phenotype emerges, lobed leaves (Fig. 1B). This may occur because 1) structural gene X is no longer transcribed, 2) E_1 is no longer synthesized, 3) GA is no longer synthesized, 4) structural gene Y is no longer transcribed, and 5) E_2 is no longer synthesized. Gene A′ is, in effect, analogous to the "dwarfing genes" mentioned earlier.

Should lobed leaves confer an adaptive advantage upon those individuals possessing them, mutant gene A′ could rapidly spread throughout a population. However, since structural gene Y is still present in the genome, exogenous application of GA by an investigator at the appropriate stage of leaf primordia development would result in the production of entire leaves, revealing the derived nature of the now "normal" lobed phenotype. Indeed, such methodology has been used by Rosenblum and Basile
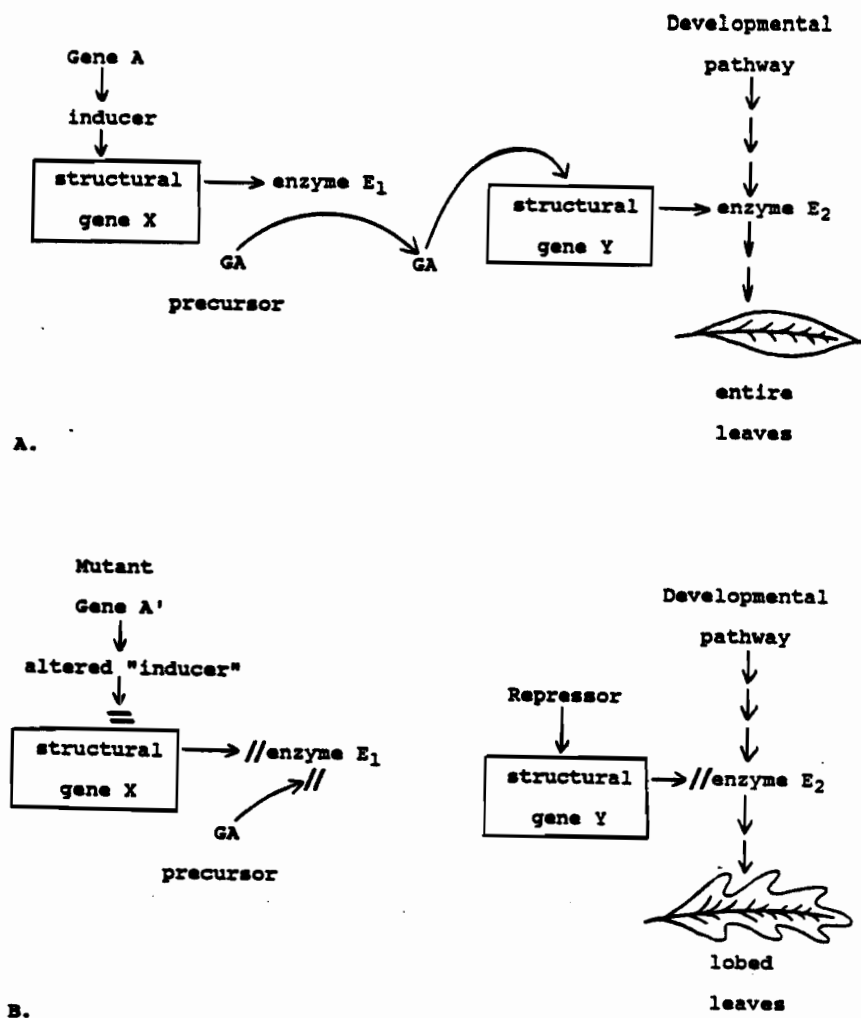
Fig. 1. Hypothetical scheme depicting a change in phenotype as a result of a single
point mutation which affects organ-specific levels of a particular morphoregulatory
substance. GA = gibberellic acid; // = blockage of pathway. A) Initial condition
prior to mutation event. B) Final condition after mutation of gene A. See text
for further explanation.

*   *   *

(1984) to decipher the evolutionary history of Streptocarpus. They showed how
species in the subgenus Streptocarpus which do not normally produce stems could
exhibit morphologies reminiscent of stem-producing species of the subgenus Strepto-
carpella when GA was applied during specific stages of development. Stebbins and
Basile (1986) have proposed the term "phyletic phenocopies" for such changes in form
of a phenotype that mimic the normal form of a related phenotype of a different
taxon.

Close investigation of similar phenomena in other plant groups should prove useful
to systematists investigating phylogenetic relationships among closely related taxa
and plant evolutionary ecologists interested in the adaptive nature of plant form.
The use of growth regulating substances in evolutionary investigations of plant
morphology should contribute substantially to modern efforts to unite two diverse
(and previously disparate) disciplines: plant development and evolutionary biology.

Literature Cited

Allsopp, A. 1962. The effects of gibberellic acid on morphogenesis in *Marsilea drummondii* A. Br. Phytomorphology 12:1-10.

Bachmann, K. 1983. Evolutionary genetics and the genetic control of morphogenesis in flowering plants. Evol. Biol. 16:157-208.

Basile, D. V. 1980. A possible mode of action for morphoregulatory hydroxyproline-proteins. Bull. Torrey Bot. Club 107:325-338.

Borchert, R. 1965. Gibberellic acid and rejuvenation of apical meristems in *Acacia melanoxylon*. Naturwissenschaften 52:65-66.

Church, D. L. and Galston, A. W. 1988. Hormonal induction and antihormonal inhibition of tracheary element differentiation in *Zinnia* cell cultures. Phytochemistry 27:2435-2439.

Dale, J. E. 1986. Plastic responses of leaves. Symp. Soc. Exp. Biol. 40:287-305.

Digby, J. and Firn, R. D. 1985. Growth substances and leaf growth. In: Baker, N. R., Davies, W. J., and Ong, C. K. (eds.) Control of Leaf Growth. Cambridge Univ. Press, Cambridge.

Feldman, L. J. and Cutter, E. G. 1970a. Regulation of leaf form in *Centaurea solstitialis* L. I. Leaf development on whole plants in sterile culture. Bot. Gaz. 131:31-39.

Feldman, L. J. and Cutter, E. G. 1970b. Regulation of leaf form in *Centaurea solstitialis* L. II. The developmental potentialities of excised leaf primordia in sterile culture. Bot. Gaz. 131:39-49.

Filner, P. and Varner, J. E. 1967. A simple and unequivocal test for de novo synthesis of enzyme: density labelling of barley α-amylase with H_2O. Proc. Natl. Acad. Sci. 58:1520-1526.

Givnish, T. 1979. On the adaptive significance of leaf form. In: Solbrig, O. T., Jain, S., Johnson, G. B., and Raven, P. H. (eds.) Topics in Plant Population Biology. Columbia Univ. Press, New York.

Givnish, T. 1987. Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol. 106:131-160.

Glasziou, K. T. 1969. Control of enzyme formation and inactivation in plants. Ann. Rev. Plant Physiol. 20:63-88.

Gray, R. A. 1957. Alteration of leaf size and shape and other changes by gibberellins in plants. Amer. J. Bot. 44:674-682.

Green, P. B. 1986. Plasticity in shoot development: a biophysical view. Symp. Soc. Exp. Biol. 40:211-232.

Green, P. B. 1987. Inheritance of pattern: analysis from phenotype to gene. Amer. Zool. 27:657-673.

Gurevitch, J. 1988. Variation in leaf dissection and leaf energy budgets among populations of *Achillea* from an altitudinal gradient. Amer. J. Bot. 75:1298-1306.

Hammond, D. 1941. The expression of genes for leaf shape in *Gossypium hirsutum* L. and *Gossypium arboreum* L. II. The expression of genes for leaf shape in *Gossypium arboreum* L. Amer. J. Bot. 28:138-150.

Hutchinson, J. B. 1934. The genetics of cotton. X. The inheritance of leaf shape in Asiatic Gossypiums. J. Genet. 28:437-513.

Hutchinson, J. B. 1936. The genetics of cotton. XVI. Some observations on the inheritance of form and size in Asiatic cottons. J. Genet. 32:399-410.

Jain, S. K. 1979. Adaptive strategies, polymorphism, plasticity, and homeostasis. In: Solbrig, O. T., Jain, S., Johnson, G. B., and Raven, P. H. (eds.) Topics in Plant Population Biology. Columbia Univ. Press, New York.

Jones, H. G. 1985. Adaptive significance of leaf development and structural responses to environment. In: Baker, N. R., Davies, W. J., and Ong, C. K. (eds.) Control of Leaf Growth. Cambridge Univ. Press, Cambridge.

Jones, R. L. 1973. Gibberellins: their physiological role. Ann. Rev. Plant Physiol. 24:571-598.

Jones, R. L. 1982. Gibberellin control of cell elongation. In: Wareing, P. F. (ed.) Plant Growth Substances 1982. Academic Press, New York.

Key, J. L. 1969. Hormones and nucleic acid metabolism. Ann. Rev. Plant Physiol. 20:449-474.

Leopold, A. C. and Kriedemann, P. E. 1975. Plant Growth and Development. McGraw-Hill Book Company, New York.

Lewis, M. C. 1969. Genecological differentiation of leaf morphology in Geranium sanguineum. New Phytol. 68:481-503.

Moore, T. C. 1979. Biochemistry and Physiology of Plant Hormones. Springer-Verlag, New York.

Nelson, Jr., O. E. and Burr, B. 1973. Biochemical genetics of higher plants. Ann. Rev. Plant Physiol. 24:493-518.

Njoku, E. 1958. Effect of gibberellic acid on leaf form. Nature 182:1097-1098.

Phinney, B. O. and West, C. A. 1960. Gibberellins as native plant growth regulators. Ann. Rev. Plant Physiol. 11:411-436.

Robbins, W. J. 1957. Gibberellic acid and the reversal of adult Hedera to a juvenile state. Amer. J. Bot. 44:743-746.

Rosenblum, I. M. and Basile, D. V. 1984. Hormonal regulation of morphogenesis in Streptocarpus and its relevance to evolutionary history of the Gesneriaceae. Amer. J. Bot. 71:52-64.

Sachs, T. 1986. Cellular interactions in tissue and organ development. Symp. Soc. Exp. Biol. 40:181-210.

Sachs, T. 1988. Ontogeny and phylogeny: phytohormones as indicators of labile changes. In: Gottlieb, L. D. and Jain, S. K. (eds.) Plant Evolutionary Biology. Chapman and Hall, London.

Salisbury, F. B. and Marinos, N. G. 1985. The ecological role of plant growth substances. In: Pharis, R. P. and Reid, D. M. (eds.) Hormonal Regulation of Development III. Role of Environmental Factors. Springer-Verlag, New York.

Scandalios, J. G. and Baum, J. A. 1982. Regulatory gene variation in higher plants. Adv. Genet. 21:347-370.

Sen, S. P. 1985. The molecular basis of hormone action. In: Purohit, S. S. (ed.) Hormonal Regulation of Plant Growth and Development. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht.

Sinnott, E. W. 1935. Evidence for the existence of genes controlling shape. Genetics 20:12-21.

Stebbins, G. L. 1986. Gene action and morphogenesis in plants. In: Gustafson, J. P., Stebbins, G. L., and Ayala, F. J. (eds.) Genetics, Development, and Evolution. Plenum Press, New York.

Stebbins, G. L. and Basile, D. V. 1986. Phyletic phenocopies: a useful technique for probing the genetic and developmental basis of evolutionary change. Evolution 40:422-425.

Stoddart, J. L. 1982. Gibberellin perception and its primary consequences: the current status. In: Wareing, P. F. (ed.) Plant Growth Substances 1982. Academic Press, New York.

Taiz, L. 1984. Plant cell expansion: regulation of cell wall mechanical properties. Ann. Rev. Plant Physiol. 35:585-657.

Theologis, A. 1986. Rapid gene regulation by auxin. Ann. Rev. Plant Physiol.
  37:407-438.
Thimann, K. V. 1977. Hormone Action in the Whole Life of Plants. Univ. of
  Massachusetts Press, Amherst.
Trewavas, A. 1986. Resource allocation under poor growth conditions. A major role
  for growth substances in developmental plasticity. Symp. Soc. Exp. Biol. 40:31-76.
Wallenstein, A. and Albert, L. S. 1963. Plant morphology: its control in
  Proserpinaca by photoperiod, temperature and gibberellic acid. Science
  140:998-1000.
Wardlaw, C. W. 1965. Organization and Evolution in Plants. Longmans, Green and
  Co., London.
Wareing, P. F. 1977. Growth substances and integration in the whole plant. Symp.
  Soc. Exp. Biol. 31:337-365.
Wareing, P. F. and Phillips, I. D. J. 1978. The Control of Growth and
  Differentiation in Plants. Pergamon Press: New York.
Whaley, W. G. 1939. Inheritance of leaf and flower characters in Tropaeolum. J.
  Hered. 30:335-341.