J Plant Growth Regul (1993) 12:207–226

Journal of
Plant
Growth
Regulation

Plant Hormone Mutants

James B. Reid

Department of Plant Science, University of Tasmania, Hobart, Tasmania 7001, Australia

Received June 14, 1993; accepted June 21, 1993

Abstract. The techniques used for the production and identification of plant hormone mutants are described. The properties used to classify these mutants into the broad synthesis and response categories are discussed, and the genetic considerations needed to allow their effective use in plant hormone research examined. A brief outline of significant work on gibberellin (GA), abscisic acid (ABA), auxin, ethylene, cytokinin and phytochrome mutants is provided. The molecular action of these genes is discussed where available and recent rapid advances made in *Arabidopsis* highlighted. Suggestions for future emphasis are made, particularly relating to an examination of the tissue and ontogenetic specificity of the plant hormone genes.

One of the major developments in the study of plant hormones over the last 5 years has been the increased use of single-gene mutants and the exploration of their molecular action. Many reviews on particular aspects of this topic have been published recently (e.g., Klee and Estelle 1991; Koornneef 1991; Kendrick and Nagatani 1991; Reid and Ross 1993). The aim of the present review is therefore to provide an overview and highlight new directions that this research might follow. The pitfalls that may reduce the potential power of the mutant approach will also be examined. No attempt has been made to discuss all the reported phytohormone mutants, especially where mutants from many species show similar responses [e.g., the gibberellin (GA) and abscisic acid (ABA) mutants].

Identification of Mutants

The first mutants implicated with changes in plant hormones possessed distinctive phenotypes, readily identifiable with the proposed effects of the hormone. For example, the dwarf and wilty phenotypes were associated with the GAs and ABA, respectively (Phinney 1956, 1961; Tal and Nevo 1973). Subsequent work with the dwarf and wilty mutants has shown the power of mutants as tools for unravelling the biosynthetic pathways for these groups of plant hormones, as well as their role in the control of certain developmental processes (see below). This approach to identifying hormone mutants is still likely to be fruitful, since in excess of 300 phenotypically distinct single-gene mutations have been reported in each of several major crop species (e.g., maize, tomato, pea). This reservoir of genetic variation is still largely untapped by either developmental biologists or hormone physiologists. To select mutants of potential interest, a precise idea of the expected phenotype is required. Although this information is available for the GAs and ABA, it is not always clear for the other hormones (e.g., cytokinins and auxins), despite a voluminous literature on the supposed actions of these hormones. Equally intriguing to the concept of hormonal control of plant development is the biochemical basis for the many major developmental mutants that show no relationship to the known groups of plant hormones. Graft transmissibility has even been demonstrated for many of these developmental mutants (e.g., flowering and branching; Murfet and Reid 1993).

The more usual approach to the identification of hormone mutants is to carry out mutagenesis studies using chemical mutagens, ionizing radiation, or insertional mutagenesis. The success of this technique depends on the quality of the selection system designed to identify the useful products from the vast bulk of nonuseful products. A lack of knowledge of the expected phenotype in the intact plant is again often limiting. The more specific the screening conditions, the better the chances are that the mutants isolated will be of direct relevance to the area of interest. A good example is a positive selection regime, whereby only the desired mutants survive. Such a technique was elegantly designed and

used by Koornneef et al. (1982) to select for ABA-deficient genotypes in *Arabidopsis thaliana*. They predicted that among nongerminating GA-deficient plants, any ABA-deficient mutant might be expected to germinate, based upon the known interactions between GA and ABA in controlling seed dormancy. A mutant, *aba*, with the predicted phenotype was isolated and shown to possess reduced levels of ABA. Another example where the use of carefully defined screening environments has allowed highly specific mutations to be selected has been the isolation of photomorphogenic mutants. For example, screening in FR light has successfully identified phyA-deficient mutants (Parks and Quail 1993), whereas growth in darkness has identified mutants with constitutive photomorphogenic responses (e.g., Chory et al. 1989b).

A common, but nevertheless successful technique, has been to search for mutants capable of growth on a medium that is either devoid of a necessary hormone or has abnormally high and usually toxic levels of a hormone. This technique has been successful in isolating mutants resistant to high levels of ABA in *Arabidopsis* (Koornneef et al. 1984; Nambara et al. 1992) and auxin in *Nicotiana plumbaginifolia* and *Arabidopsis* (e.g., Blonstein et al. 1988; Estelle and Sommerville 1987; Wilson et al. 1990). However, some of the putative auxin mutants isolated do not appear to operate by directly blocking hormone synthesis or response as was originally hoped. Rather their action may be quite indirect, influencing hormone response by altering the uptake or transport of the hormone to a site where it can elicit a biological response. Such mutants may be of general interest if these responses are of relevance to the development of the whole plant, but in cases where this has not been established, the usefulness of such mutants is debatable.

A particularly useful method of producing mutants is insertional mutagenesis since it allows the production of mutants that are readily amenable to molecular techniques. Insertional mutagenesis involves the production of mutants by the insertion of a known DNA sequence into genes, disrupting their function. This can be done by either the use of transposons (e.g., Phinney et al. 1986; McCarty et al. 1989) or the T-DNA from *Agrobacterium tumefaciens* (Feldmann et al. 1989; Kieber et al. 1993). In both cases the sequence of the inserted DNA is known and hence probes may be used to reisolate it. This also allows the recovery of the DNA flanking the insertion that will include the gene of interest. However, the process may not be entirely straightforward since multiple copies of the transposon may be inserted causing difficulties in determining which insert is responsible for the mutant phenotype (e.g., Phinney et al. 1986). Further, the lack of suitable transposons or susceptibility to infection by *Agrobacterium* limit the species amenable to this technique. However, it has proved feasible in a number of species, and in *Arabidopsis* it has allowed several hormone genes to be cloned (e.g., Kieber et al. 1993).

The isolation and cloning of hormone genes is no doubt one of the major aims of working with hormone mutants. At least in *Arabidopsis* this identification is becoming a reality even where the mutant has not been produced by insertional mutagenesis. Hormone genes have been isolated successfully as a result of very detailed linkage relationships (e.g., Girandat et al. 1992), which may involve chromosome walking, as well as by genomic subtraction. This latter technique requires that the mutation of interest is a result of a large deletion (e.g., Sun et al. 1992). Such studies are rapidly extending our knowledge of how hormones operate, particularly by providing a detailed molecular understanding of the elements in the hormone response pathway (e.g., McCarty et al. 1991; Kieber et al. 1993).

Classification of Mutants

Broadly speaking, phytohormone mutants may be split into two groups: those that influence hormone levels (hereafter called *synthesis mutants*) and those that influence hormone response (hereafter called *response mutants*). The most common type of synthesis mutants are those that block steps leading to the synthesis of the active hormone, such as the GA_1-deficient dwarfs in peas and maize (Ingram et al. 1984, 1986; Phinney 1984; Fujioka et al. 1988a; Reid 1986b; Swain and Reid 1992) and the ABA-deficient wilty mutants in tomato (Tal and Nevo 1973; Neill and Horgan 1985). Overproducing mutants would also be expected and has been reported for cytokinins in the moss, *Physcomitrella patens* (Wang et al. 1981), for GA_1 in peas (Reid et al. 1992) and for ethylene in *Arabidopsis* (Guzmann and Ecker 1990). However, the frequency of overproducing mutants is low and previous suggestions regarding this type of mutant have sometimes not been confirmed (e.g., *yg-6* in tomato; Koornneef et al. 1985a; Perez et al. 1974). Mutants with low hormone levels due to increased conjugation or catabolism of the active hormone would also belong to this broad synthesis category; however, to date no mutants have been proven to operate via these mechanisms in higher plants.

The majority of hormone mutants described belong to the response category. This is not surprising since by definition only a change in the observed

response to the hormone is required. Such a change may occur due to quite indirect effects (e.g., changes in general plant vigor), as well as in the direct sequence of events leading from hormone reception to the phenotypic response under examination. The most sought after and potentially most useful subgroups of response mutants are those that influence the level or affinity of a receptor molecule. Such mutants might be expected to possess phenotypes identical to the hormone synthesis mutants and show pleiotropic effects for all responses to a particular hormone group if it is assumed that a single receptor is involved. Mutants possessing these basic requirements have been described in several species (e.g., *la cry*^{s} slender in peas, Potts et al. 1985; *D8* dwarf in maize, Fujioka et al. 1988b; Harberd and Freeling 1989; *sln* slender in barley, Chandler 1988; Lanahan and Ho 1988; and *etr* ethylene-insensitive mutant in *Arabidopsis*, Bleeker et al. 1988). However, none of these mutants has yet been proven to alter hormone reception, rather than subsequent steps in the response pathway.

It has been suggested that the broad-response category should be subdivided into other groups; for example, mutants influencing the level of the receptor (receptivity mutants), the affinity of the receptor for the active hormone (affinity mutants), and the size of the response produced (response capacity mutants) (Firn 1986). While the object of many research projects is to determine which of these aspects of response a mutant may influence, no reliable way is available to allow this subdivision to be made without a detailed knowledge of the molecular action of the mutation. Response curves do not provide the necessary information (Reid and Ross 1988b) and nor does a detailed study of the pleiotropic effects of the genes. A division of response mutants into "insensitive" types which appear like hormone-deficient mutants but do not respond to the application of a particular hormone, and "constitutive response" types where the response appears saturated even in the presence of a hormone-synthesis inhibitor is, however, possible for both the GA response and ethylene response mutants (e.g., Potts et al. 1985; Guzman and Ecker 1990).

Genetic Considerations

Many early studies using mutants used poorly defined genetic material and consequently the results have proved misleading. The development of near-isogenic lines is a necessity if reliable comparisons are to be made between a mutant and its wild-type allele, especially at the molecular level. It is frequently not sufficient just to use the mutant line and its parental variety; backcrossing to the parental variety is necessary to confirm the stability and single-gene nature of the mutant. The routine production of mutants from one parental variety in mutagenesis programs today has overcome this problem to a large extent (e.g., Koornneef et al. 1980; Guzman and Ecker 1990). However, where the production of isogenic lines is required because the origin of the mutant is less well established (even though it may be of commercial significance) a large input of both time and effort is required. For inbreeding species, a minimum of six, and preferably 12, generations of single plant selection from heterozygous plants is the simplest method, whereas for outbreeding species, repeated backcrossing to the parental variety is the normal technique. This latter technique is perhaps more desirable, since backcrossing to the same variety with a range of mutants can yield an array of lines differing by single mutations, whereas the former technique yields only the single valid comparison for each allelic pair. If isogenic lines are not available, it is still possible to obtain meaningful physiological information by comparing a single gene difference under circumstances where the remainder of the genetic background is randomized. This can be done quite rapidly by using a range of F_2 or F_3 plants from a cross between the parental and mutant lines, although closely linked genes may still cause significant problems.

Frequently, little or no use has been made of the genetic interactions between physiologically related genes (i.e., combinations of mutant genes have not been produced). If the full physiological and biochemical potential is to be gained from mutants such recombinants must be produced (e.g., Koornneef et al. 1982, 1989; Reid 1986a; Kieber et al. 1993). For example, if two mutants are blocking steps in the same biosynthetic pathway, the phenotype of the double mutant will indicate whether or not the genes are leaky (Reid 1986a). Further, a gene blocking early in a pathway will allow examination of the action of a gene operating at a later step in the pathway by the use of feeding experiments, unencumbered by effects of endogenous compounds in the pathway. Therefore, the need for stable isotope-labeled compounds to determine metabolic conversions may be avoided and it may be easier to keep concentrations of metabolites at physiological levels. Interactions between mutant genes acting on different aspects of the same pathway (e.g., hormone synthesis and response mutants) may also provide constructive insights into gene action. For example, by combining the GA-deficient *na* gene in peas with the long-thin, slender gene combination, *la cry*^{s}, it was possible to show

that the *la cry*^{s} gene combination was epistatic to gene *na*, implying that GA levels do not influence elongation in plants possessing both genes *la* and *cry*^{s} (Ingram and Reid 1987; Potts et al. 1985). In addition, the phenotype of mutant combinations may be used in some instances to infer the sequence of action of response mutants (Kieber et al. 1993; Reid 1986a). Such information may be of importance if a range of response mutants is being scanned to try and identify possible receptor mutants for further study.

Genetic manipulations may also be invaluable in determining the hormone interactions between maternal and embryonic tissues (e.g., Ozga et al. 1992; Koornneef et al. 1989). This interaction has been the subject of much speculation and experimentation. By crossing appropriate hormone synthesis mutants it is possible to place seeds possessing a particular hormone (e.g., ABA, Karssen et al. 1983, GA, Swain et al. 1993) onto maternal tissue deficient in this hormone. Such genetically contrived situations have demonstrated the importance of embryonic ABA (compared with maternal ABA) in the control of seed dormancy in *Arabidopsis* and seed GA levels to seed development in peas.

Mutants may also be valuable in identifying common regulatory elements controlling unrelated developmental processes. This stems from the one gene:one primary action hypothesis (Beadle and Tatum 1941). A good example of this use of mutants is found in the genes conferring the slender phenotypes in peas and barley. In both species, the mutant phenotype resembles a wild-type plant treated with saturating levels of GA_1 or GA_3 (Chandler 1988; Lanahan and Ho 1988; Potts et al. 1985). This constitutive response occurs for all GA-mediated responses recorded. In peas this includes internode elongation, rate of leaf expansion, node of first flower, flower development, leaf shape and color, and pod development (see Potts et al. 1985; Reid et al. 1983). However, the biochemical basis of these responses is not known in peas. In barley, in addition to stem elongation, well-characterized biochemical responses to GA are known in the aleurone layer of germinating seeds. In the slender mutant, *sln*, of barley, constitutive production and secretion of α-amylase, protease, and nuclease occur (Chandler 1988; Lanahan and Ho 1988). These results suggest the existance of an unknown step(s) common to the regulation of these diverse GA responses. Clearly, the regulatory pathways must diverge at some point and mutations proving this are known in barley. For example, mutations can influence α-amylase production but not the other systems (Ho et al. 1980). The common regulatory step(s) may include the hypothesized GA receptor and any number of other unknown steps. However, the ABA antagonism of GA-induced responses in barley still occurs in the aleurone of the slender mutant indicating a clear separation of these two processes (Chandler 1988; Lanahan and Ho 1988).

Selected Examples

GA Mutants

Synthesis. The first clearly identified phytohormone mutants were the GA-deficient dwarfs of maize (Phinney 1956, 1961). These mutants and similar dwarfs in peas, (Ingram et al. 1984, Reid and Ross 1993) are among the best-characterized hormone mutants. They were essential to the full understanding of the GA biosynthetic pathways in these species (e.g., Ingram et al. 1984; Phinney and Spray 1982; Spray et al. 1984) and were central to the development of the view that GA_1 is the primary native GA controlling stem elongation in species possessing the early 13-hydroxylation pathway for GA biosynthesis (e.g., Fujioka et al. 1988a; Ingram et al. 1986; Phinney 1984; Ross et al. 1992). They have been reviewed frequently (e.g., MacMillan and Phinney 1987; Reid and Ross 1993), and hence the coverage here is restricted to an overview and recent additions to this story.

The GA synthesis loci in maize are d_1, d_2, d_3, d_5, and an_1 and in peas are *le*, *na*, *lh*, *ls*, and *sln*. Each gene appears to block at least one step in the GA biosynthetic pathway leading to changes in the level of GA_1 (Fig. 1). In peas, none of these genes appears to result in a complete block, since either double mutants (e.g., *na ls*) are shorter than the single mutant types (Reid 1986a), more severe alleles (e.g., *le*^{d}) can be found (Ross and Reid 1987; Ross et al. 1989), or low levels of GAs after the blocks identified (e.g., Swain and Reid 1992). This is also true for the dwarf mutants of maize since direct measurements of GA levels using stable-isotope internal standards show "leakiness" in maize shoots (Fujioka et al. 1988a). Shoot elongation can be shown to correlate closely with endogenous GA_1 levels in both species (Fujioka et al. 1988a; Ingram et al. 1986; Ross et al. 1992; Swain and Reid 1992) but not with the overall level of GAs present in the plant (Ingram et al. 1984; Fujioka et al. 1988a; Phinney and Spray 1982; Ross et al. 1992). The most thoroughly examined loci are the *le* locus in peas and the d_1 locus in maize, which both block the 3β-hydroxylation of GA_{20} to GA_1 (Fig. 1). The nature of this block has been demonstrated both by feeding experiments with [^{3}H, ^{13}C]GA_{20} (Ingram et

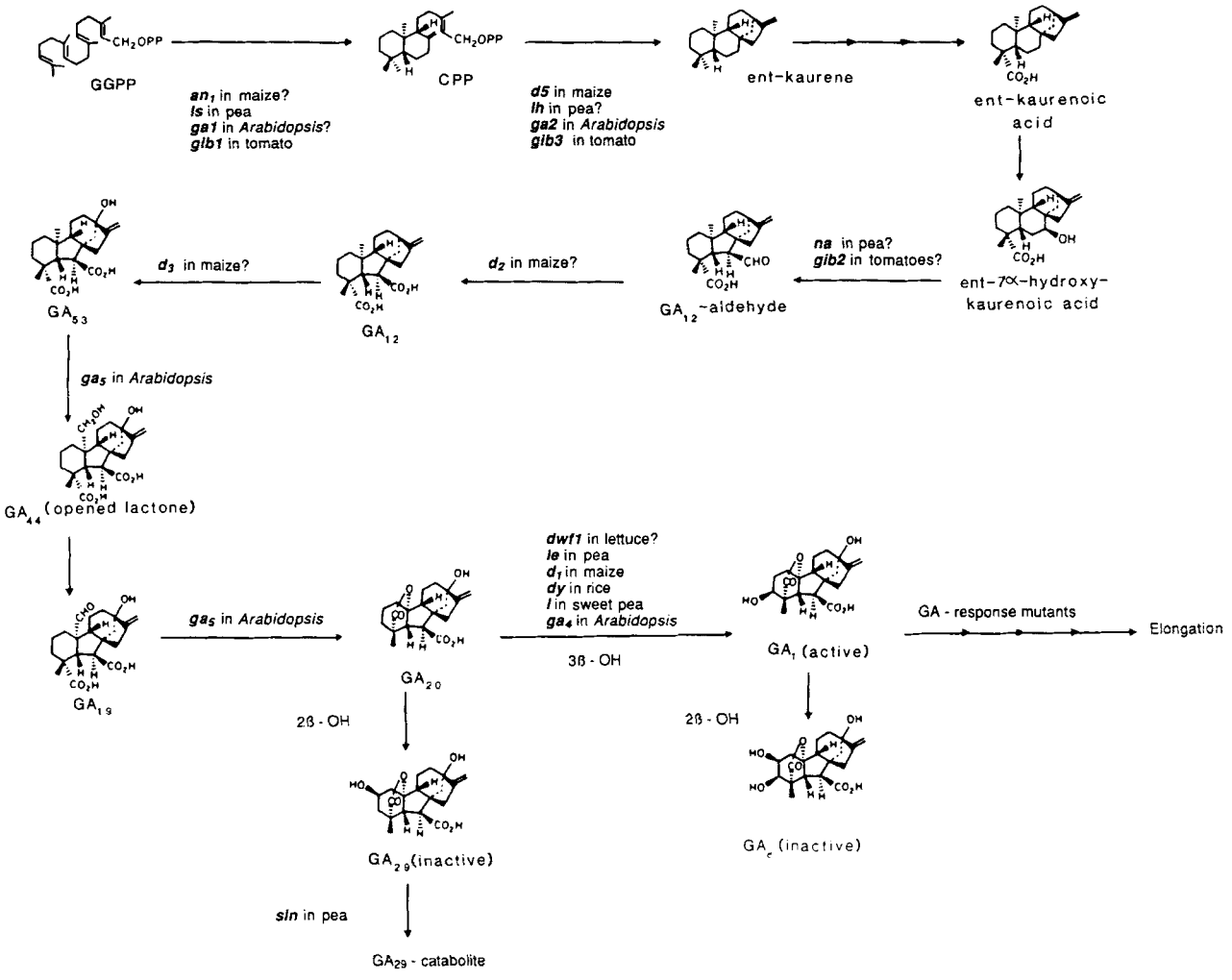

Fig. 1. The early 13-hydroxylation pathway of GA biosynthesis and possible sites of action of certain GA-synthesis mutants. The sites of action of mutants indicated by *?* are either not well defined or are based on application data only.

al. 1984; Spray et al. 1984) and the measurement of endogenous GA levels by GC-SIM (Fujioka et al. 1988a; Ross et al. 1989, 1992; Smith et al. 1992).

Recent work in peas has shown that a new elongated mutant, *sln* (slender), overproduces GA_1 in the young shoot (Reid et al. 1992). This overproduction is due to a blockage in the catabolism of GA_{20} to GA_{29}-catabolite in the developing seed (Reid et al. 1992; Ross et al. 1993). Consequently a large amount of GA_{20} is carried over in the dry seed, which upon germination is 3β-hydroxylated to GA_1. Recent results suggest that *Sln* may be a regulatory gene (Ross, unpublished results), and its tissue specificity deserves examination. Another mutation in peas, lh^i, which blocks GA synthesis in both the shoot and developing seeds has provided direct evidence that the GAs are important for seed development, since abortion of seeds on homozygous lh^i plants is overcome if pollinated with wild-type *Lh* pollen. As expected GA levels in the developing seeds are restored by this treatment (Swain et al. 1993).

Although the significance of GA_1 for the control of internode elongation was originally based on results from dwarf mutants of maize and peas (for reviews, see Phinney 1984; Reid 1986b), substantial evidence suggests that this mechanism is common to many monocots and herbaceous dicots. This includes evidence from GA_1-deficient mutants in rice (Suzuki et al. 1981), *Brassica rapa* (Rood et al. 1989), tomato (Bensen and Zeevaart 1990), lettuce (Waycott et al. 1991), and sweet pea (Ross et al. 1990), and studies with a GA synthesis inhibitor in wheat (Lenton et al. 1987). However, in some of these species GAs belonging to the non-13-hydroxylation pathway have also been identified in

extracts from shoots (e.g., GA_{15}, GA_{24}, GA_9, GA_4). The levels are substantially below the levels of comparable 13-hydroxylated GAs (e.g., Fujioka et al. 1988b), and the biological significance of the pathway may be negligible. However, the presence of GA_3, produced from GA_{20} via GA_5 (Fujioka et al. 1990), is likely to be of significance to elongation where its level approaches or exceeds that of GA_1 (e.g., maize, wheat, and barley, Croker et al. 1990; Hedden and Croker 1992; *Brassica,* Rood et al. 1990).

In other species, the non-13-hydroxylation pathway may form the major GA-biosynthetic pathway present, and the 3β-hydroxylated GA_4 may be the active molecule *per se,* although it cannot be ruled out that it possesses activity only through conversion to GA_1 (e.g., *Arabidopsis,* Zeevaart and Talon 1992).

The genes involved with GA biosynthesis have been targeted for cloning but the only published account is for the *Arabidopsis GA1* locus. This was achieved by genomic subtraction using the deletion mutation *ga1-3* (Sun et al. 1992). The cloned sequences correspond to a 5.0 kb deletion in the *ga1-3* genome. However, the point at which the *ga1* mutations blocks GA biosynthesis is still open to question, although it appears most likely to be between geranyl–geranyl pyrophosphate and *ent*-kaurene (Barendse et al. 1986; Zeevaart and Talon 1992) and hence the A activity of *ent*-kaurene synthetase would be the likely target enzyme, since *ga2* blocks the B activity of *ent*-kaurene synthetase (Zeevaart and Talon 1992).

Response. GA-response mutants are more frequent than GA synthesis types and are probably the most common group of phytohormone mutants. For example, more than 20 non-allelic GA nonresponding mutants have been mapped in maize (Fujioka et al. 1988b). This group of mutants showed an altered response to biologically active GAs, such as GA_1 and GA_3. Insensitivity to applied GAs is a very general test and hence the category is broad and includes mutants that have quite indirect effects on GA response, such as gene *lm* in peas [which results in abnormal root development, Reid and Ross (1988a)] through to putative GA receptor mutants, such as D_8 in maize (Fujioka et al. 1988b; Harberd and Freeling 1989) and the slender mutants in pea (*la cry*^{s}) (Potts et al. 1985) and barley (*sln*) (Chandler 1988; Lanahan and Ho 1988). The sequence of events from GA reception to elongation is not known in any species but the judicious use of the GA-response mutants should allow such steps to be elucidated (see Reid 1990). For example, microtubule and microfibril orientation have been reported to be modified by GA and are of importance for cell elongation (e.g., Mita and Katsumi 1986). The identification of mutants influencing these processes would clearly be of significance. Likewise, debate exists over the changes induced by GA to cell wall characteristics (Cosgrove and Sovonick-Dunford 1989). The examination of the cell wall properties of the short GA-response mutants, *lka* and *lkb* in pea, showed that the osmotic pressure and turgor pressure were elevated compared with wild-type or dwarf GA-deficient *ls* plants. The wall yield threshold was also substantially elevated in both *lka* and *lkb* plants relative to the wild-type (Behringer et al. 1990). The genes *lka* and *lkb* therefore seem to affect principally the chemorheological properties of the cell wall. These two mutants also possess reduced IAA levels in expanding internodes but whether this is the cause or a consequence of the altered elongation requires further examination (McKay et al., unpublished results). These examples are sufficient to show that response mutants may provide the tools to help unravel hormone action by allowing the identification of the elements in the response pathway. They also serve to indicate the interaction between hormones since a hormone response mutant for one hormone (in this case GA) may be a synthesis mutant for another group (IAA).

Work with GA response mutants has provided results which suggests that GA_1 levels in shoot tissue are regulated by a feedback mechanism. For example, in the dwarf-insensitive mutants *Rht3* (wheat), *D8* (maize), and *gai* (*Arabidopsis*) the levels of GA_1 and GA_{20} are substantially elevated, whereas GA_{19} levels are reduced (Stoddart 1984; Fujioka et al. 1988b; Talon et al. 1990; Hedden and Croker 1992). In contrast, the slender constitutive response mutant in barley, *sln,* possesses reduced levels of GA_1 and GA_{20} and increased levels of GA_{19} (Croker et al. 1990). These observations are consistent with GA_1 action exerting negative control over GA biosynthesis by influencing the activity of GA_{19} 20-oxidase (Hedden and Croker 1992). Thus, in slender plants this enzyme may be downregulated. The elevated levels of GA_{20} in mutants with reduced 3β-hydroxylation capacity, d_1 in maize (Fujioka et al. 1988a), *le* in pea (Ross et al. 1992), and *l* in sweet pea (Ross et al. 1990) may indicate an increase in GA_{19} 20-oxidase activity and hence, also support such a feedback model. However, the short GA response mutants in peas, *lk, lka,* and *lkb* do not accumulate GA_1 (Lawrence et al. 1992). These mutations appear to operate well down the transduction pathway leading from GA_1 reception to elongation and beyond the point at which the feedback occurs. Hence, the feedback

control does not appear to be controlled by the growth rate *per se* (compare with Hedden and Croker 1992).

Only a small group of the GA response mutants possess phenotypes suggesting that they may potentially be involved with reception of the GA_1 signal. These include the dwarf mutants *D8* in maize and *gai* in *Arabidopsis* (Harberd and Freeling 1989; Koornneef et al. 1985b) and the slender constitutive mutants *sln* in barley and *la cry*^{s} in peas (Chandler 1988; Potts et al. 1985). However, none of these mutations has been cloned and therefore their designation as receptor mutations is uncertain. Attempts have been made to identify mutations that suppress GA insensitivity by carrying out mutagenesis on a GA-insensitive *gai* background and screening for the recovery of the GA response. While such suppressors were found, they were all the result of intragenic changes (Peng and Harberd 1993).

ABA Mutants

Synthesis. The well-characterized wilty mutants in tomato (*flc, sit,* and *not,* e.g., Neill and Horgan, 1985; Tal and Nevo 1973), potato, (*droopy,* Quarrie 1982), pea (*wil,* Wang et al. 1984a), and *Arabidopsis* (*aba,* Koornneef et al. 1982) all appear to result from reduced levels of ABA. Likewise, the vivaparous mutants of maize, *vp-2, vp-5, vp-7,* and *vp-9* appear to possess reduced ABA levels due to blocks in carotenoid biosynthesis (Moore and Smith 1985; Neill et al. 1986). The data from these mutants have shown that ABA controls aspects of seed dormancy and germination (e.g., Koornneef et al. 1982, 1989), the water relations of vegetative tissues (e.g., Tal and Nevo 1973), and cold tolerance (Heino et al. 1990). This was possible even in the absence of suitable ABA synthesis inhibitors or a detailed knowledge of the biosynthetic pathway. These results and the phenotypic effects and genetic interactions of these mutants have been reviewed extensively (e.g., Koornneef 1986; Koornneef et al. 1989) and provide an excellent example of the power of the mutational approach to the study of plant development.

Recent work with the ABA-deficient mutants has been successful in elucidating the ABA biosynthetic pathway. Two proposals have been widely examined: first, direct synthesis of ABA by the cyclization and oxidation of farnesyl pyrophosphate (C_{15} pathway), and second, an indirect pathway via the oxidative cleavage of an epoxy carotenoid (C_{40} pathway). Recent work by several groups supports the indirect pathway. For example, the viviparous mutants in maize possess inhibited carotenoid biosynthesis, as well as reduced ABA levels (Moore and Smith 1985; Neill et al. 1986) suggesting that a carotenoid intermediate may be necessary for ABA synthesis. The ABA-deficient mutant, *aba,* in *Arabidopsis,* has a reduced ability to carry out the epoxidation of zeoxanthin (Duckham et al. 1991; Rock and Zeevaart 1991) and hence produce ABA precursors such as violaxanthin and neoxanthin. ABA appears to be derived from all-*trans*-violaxanthin via all-*trans*-neoxanthin and 9′-*cis*-neoxanthin. The 9′-*cis*-neoxanthin is then asymmetrically cleaved to give xanthoxin plus a C25 apo-carotenoid (Parry and Horgan 1992a,b; Parry et al. 1992, Fig. 2). This step may be the control point. Xanthoxin is then metabolized to ABA-aldehyde and ABA. This last step is blocked by the *flc* and *sit* mutations in tomato and *droopy* in potato (Duckham et al. 1989; Parry et al. 1988). This blockage can lead to the accumulation of 2-*trans*-ABA-alcohol in the mutants due to conversion from ABA-aldehyde (Parry and Horgan 1992a). The third ABA-deficient mutant in tomato, *not,* may possess an enzyme with reduced substrate specificity and hence cleaves more all-*trans* than 9′-*cis*-neoxanthin (Parry et al. 1992).

Response. ABA response mutants have been isolated in several species, including *Arabidopsis thaliana* (Koornneef et al. 1984), barley (Ho et al. 1980; Raskin and Ladyman 1988), maize (Robichaud et al. 1980) and the fern, *Ceratopteris* (Hickok 1985). Such mutants are usually selected by their lack of response to applied ABA. Some exhibit insensitivity for a wide range of the ABA responses examined (e.g., *abi1* and *abi2* interfere with water relations, seed dormancy, and seedling growth in *Arabidopsis,* Koornneef et al. 1984; Finkelstein and Somerville 1990). Other mutants have been identified that influence a more specific range of ABA response. For example, *abi3* in *Arabidopsis* only has a marked effect on seed development (Koornneef et al. 1984; Finkelstein and Somerville 1990), whereas the "cool" barley mutant fails to close its stomata after ABA treatment (Raskin and Ladyman 1988). These results suggest that at least some common steps occur for such wide-ranging effects of ABA as its effect on seed dormancy, seedling growth, and stomatal closure. Further, the presence of multiple loci in *Arabidopsis* (Koornneef et al. 1984) argues for a multi-step response pathway.

Recently, the *ABI3* gene has been isolated and sequenced using positional cloning (Giraudat et al.

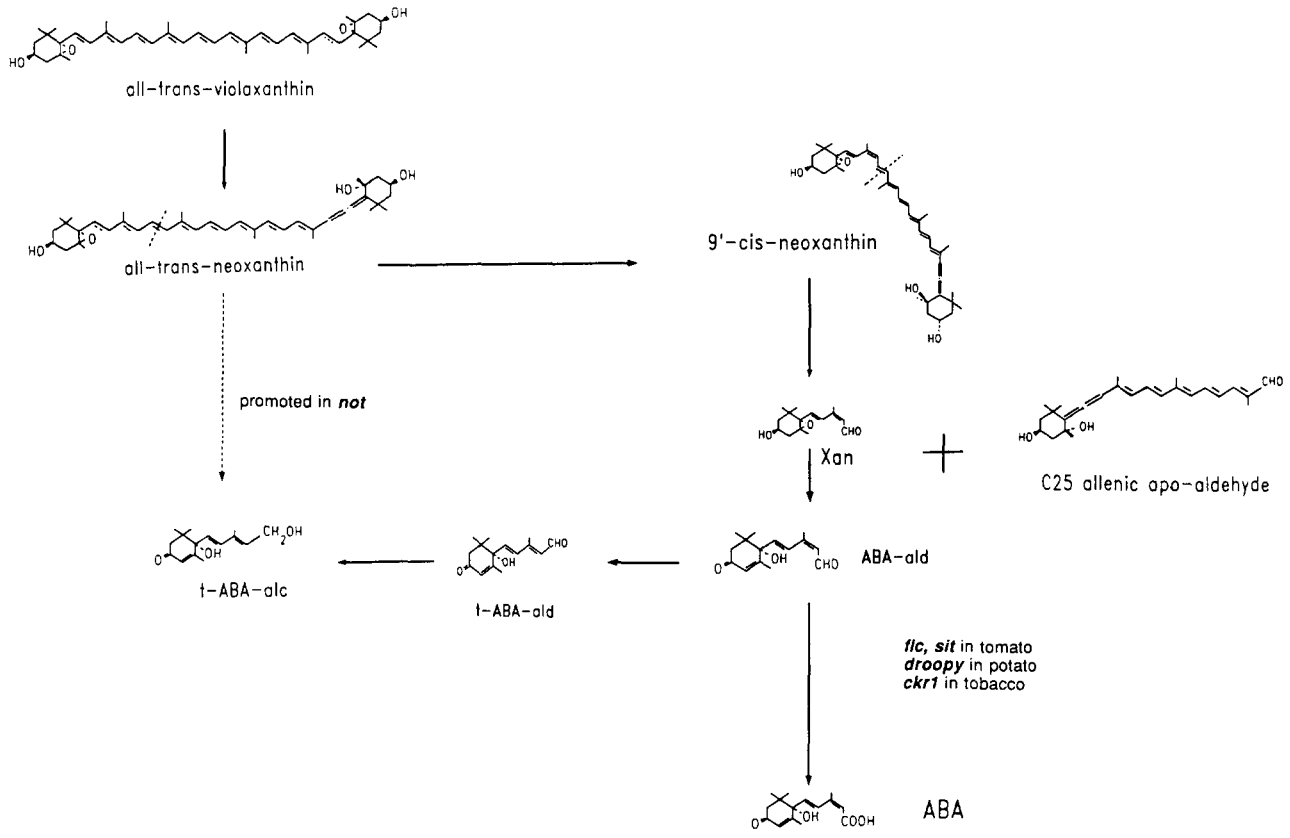

Fig. 2. The proposed pathway for ABA biosynthesis and the possible sites of action of certain ABA synthesis mutants (from Parry et al. 1992; Parry and Horgan 1992a).

1992). An RFLP closely linked to *abi3* was identified, and by analyzing an overlapping set of cosmid clones containing this marker, the *abi3* locus was localized within a 35 kb region (Giraudat et al. 1992). A putative *ABI3* gene was then identified and an allele possessing a premature stop codon (*abi3-4*) was shown to reduce the size of the predicted protein. The ABI3 protein contains a putative nuclear targeting sequence and several regions of the protein have similarities to activation domains of transcription regulators. These results are consistent with the view that the ABI3 protein may participate in ABA-regulated gene expression in the developing seed. The *AIB3* gene also possesses domains that bear high sequence similarity to the *VP1* gene from maize (Giraudat et al. 1992; McCarty et al. 1991) isolated by transposon tagging (McCarty et al. 1989). While developing seeds of the maize mutant *vp1* also possess reduced sensitivity to ABA (resulting in precocious germination, Robichaud et al. 1980), differences in the phenotypes of the *abi3* and *vp1* mutants do exist (Koornneef et al. 1989; Hattori et al. 1992; Finkelstein and Sommerville 1990). Consequently, despite strong similarities between *ABI3* in *Arabidopsis* and *VP1* in maize, it is still open to question whether they are direct functional equivalents. Nevertheless, the work on the identification of elements of the response pathway for ABA is exciting and shows that the elements of hormone response pathways are likely to be known in the near future.

Auxin Mutants

Synthesis. Although extensive searches have been carried out for auxin-synthesis mutants, clear mutants have been notoriously difficult to isolate. This has been explained either because (1) they are lethal, (2) there is duplication of the genes in the pathway, (3) there is duplication of the pathway, or (4) there is a lack of information on the appearance of such mutants. The first reason seems unlikely since most known hormone-synthesis mutants are "leaky" to varying degrees (e.g., Neill and Horgan 1985; Reid 1986a; Ross et al. 1989). The fourth

seems possible given the current lack of evidence for a clear role for IAA in stem elongation in intact plants (Romano et al. 1991) and the lack of a clear phenotype for some untreated auxin response mutants. The second and third require further examination but results from studies of tryptophan mutants (a possible IAA precursor, Law 1987) suggest they must be seriously considered (Last et al. 1991). Such mutants are urgently required as they would not only help to define the endogenous role for auxins, but may also clarify the confusion over the biosynthetic pathway for IAA in higher plants.

However, several poorly defined IAA-deficient or auxin-requiring mutants have been described. Blonstein et al. (1988), using leaf protoplasts from *Nicotiana plumbaginifolia,* identified clones requiring auxin for growth. These auxin auxotropic variants could not be regenerated to complete plants, although grafting to wild-type *N. tabacum* plants did allow limited development. They formed a bush of small shoots suggesting weakened apical dominance, slow expansion of leaves, and small, brown spots on older leaves prior to desiccation of the whole leaf and death without abscission. Recently, an IAA-deficient mutant, *lkb,* has been identified in *Pisum* (McKay et al., unpublished results). The mutant has a short, stout, brittle stem, reduced leaflet area, and lateral branching (Reid and Ross 1989). It has elevated turgor pressures and an increased wall yield threshold (Behringer et al. 1990). However, whether the change in IAA levels is the primary effect of the mutation or a consequence of the altered growth pattern remains to be determined. A tryptophan-synthesis mutant in *Arabidopsis, trp1-1,* may also result in reduced IAA levels (Last and Fink 1988). Plants possessing *trp1-1* are slow growing and possess crinkled leaves, a bushy habit, and at least some infertile flowers. This phenotype of *trp1-1* plants is consistent with reduced IAA levels and is similar to the known auxin-resistant mutant *axr-1* (Last and Fink 1988). However, *Arabidopsis* mutants, which are blocked in the terminal step of tryptophan synthesis (e.g., *trp2* which has impaired tryptophan synthetase β activity, Last et al. 1991), do not result in phenotypes that suggest a deficiency of IAA possibly because of the genetic redundancy for genes controlling aromatic amino acid biosynthesis (Last et al. 1991). Consequently, there is sufficient tryptophan for IAA synthesis. Leaky auxin-synthesis mutants have been identified in the moss *Physcomitrella patens* where they result in reduced caulonemata and gametophore production (Ashton et al. 1979; Ashton and Cove 1990).

One reason why auxin mutants may not have been pursued as actively as other classes of hormone mutants in *Arabidopsis* may be that endogenous auxin levels have been raised and lowered by genetic manipulation in transgenic plants. For example, the auxin gene from the T-DNA of *Agrobacterium* (coding for tryptophan monooxygenase) was incorporated into petunia under the control of a strong, constitutively expressed promoter (the cauliflower mosaic virus 19S promoter) (Klee et al. 1987). A tenfold increase in auxin levels (compared with wild-type plants) resulted in plants with almost complete apical dominance, greater internode length, leaf curling, increased xylem and phloem production, and parthenocarpic fruit (Klee et al. 1987). The reverse situation was created by the transformation of tobacco plants with the *iaaL* gene from *Pseudomonas savastanoi.* This gene encodes for an IAA-lysine synthetase which converts IAA to the inactive conjugate IAA-lysine (Romano et al. 1991). The plants expressing this gene display reduced apical dominance, reduced rooting, and inhibition of vascular differentiation. However, internode elongation was not altered. Using an HPLC-based detection system, IAA levels were reported to be reduced by up to 19-fold, and the phenotypic effects of *iaaL* expression were reversed by crossing to plants that overproduce IAA (containing the *iaaM* gene, Romano et al. 1991). These results appear to confirm an endogenous role for IAA in apical dominance, root growth, and vascular differentiation, but leave a role in shoot elongation in question. With the aid of tissue-specific promoters, such transgenic plants offer prospects to also explore the effects of auxin in specific tissues. Further, Romano et al. (1993) have uncoupled auxin responses from ethylene responses in transgenic plants by incorporating both the auxin-overproducing tryptophan monooxygenase gene and the ethylene synthesis-inhibiting ACC deaminase gene. This has shown that auxin, rather than ethylene, primarily controls apical dominance and leaf epinasity. The effects on elongation were complex and require clarification.

Response. Auxin-response mutants are more common and better described than auxin synthesis mutants. The diageotropic (*dgt*) mutant of tomato appears to be one example, since it shows a reduced response to applied auxin for both hypocotyl elongation and ethylene production (Kelly and Bradford 1986). It was suggested that it may influence the primary site of auxin perception (Kelly and Bradford 1986). Hicks et al. (1989), by using a photoaffinity auxin analogue, showed labeling of a polypeptide doublet in membranes from stems of the parental variety but not from *dgt* plants. They suggest the two polypeptides may be part of the auxin receptor

system. This mutant was originally described as an ethylene-synthesis mutant due to its low production of ethylene after auxin treatment and the ability of applied ethylene to phenotypically normalize certain responses (Zobel 1973). Scott (1988) has shown that the lack of auxin responses in the *dgt* mutant does not impair its GA responses.

Other auxin-response mutants tend to be categorized as auxin resistant (e.g., Blonstein et al. 1988; Estelle and Sommerville 1987; Lincoln et al. 1990; Maher and Martindale 1980; Muller et al. 1985; Wilson 1990), in that they are capable of growth when cultured with elevated auxin levels. The primary action of these mutants at the biochemical level is not clear, although they share certain phenotypic responses (e.g., *axr1*, *axr2*, and *aux1* all possess altered root growth and geotropism, Lincoln et al. 1990; Wilson et al. 1990). Some of these mutants also appear to influence shoot development (e.g., *axr1* and *axr2* alter shoot length) suggesting that they may influence common steps in the sequence of events between auxin levels and development in several tissues (e.g., Estelle and Sommerville 1987; Lincoln et al. 1990; Wilson et al. 1990). However, the *aux1* mutant only influences the roots. Thus the *AUX1* gene may have a specific function in the hormonal regulation of gravitropism (Pickett et al. 1990).

Ethylene Mutants

Synthesis. As with the GAs, applied ethylene has clearly defined effects on intact plants. For example, it influences elongation, increases apical dominance, abscission, leaf senescence, ethylene production, and fruit ripening (see Roberts et al. 1987). These clear effects, combined with a well-defined biosynthetic pathway and the availability of biosynthesis inhibitors would seem an ideal situation for the selection of ethylene mutants. However, unlike the GAs and ABA, this has not been the case until recently. For example, even though many genes influencing fruit ripening have been characterized in tomato, none seems to act by directly altering ethylene synthesis (Grierson et al. 1987; Roberts et al. 1987). However, Oeller et al. (1991) have shown that an antisense ACC synthase gene that blocks ethylene production will inhibit fruit ripening in tomato unless ethylene is supplied confirming the important role ethylene plays in this process. A similar effect has been produced by an antisense gene against the ethylene-forming enzyme (Hamilton et al. 1990; Picton et al. 1993). The reason for the previous lack of mutants is not clear but may stem from the fact that many of the phenotypic responses to ethylene observed in seedlings require elevated levels. The removal or blockage of ethylene production in wild-type plants does not result in dramatic effects. Most mutants might be expected to reduce synthesis rather than elevate ethylene levels even where a branched biosynthetic pathway occurs. A comparison with GA mutants illustrates this point, since GA-deficient mutants are common but overproducers are rare.

However, mutants with elevated ethylene levels have been reported. The *lk* mutant in peas results in a severely dwarfed, GA-insensitive plant that possesses swollen stems and petioles (especially in the dark) and increased apical dominance (Ross and Reid 1986). Thus, this has some similarities to ethylene-treated peas. Further, *lk* plants elongate in response to applied AVG (an inhibitor of ethylene production). Under certain circumstances, *lk* plants produce elevated levels of ethylene (Ross and Reid 1986), but it is not clear if this is the primary action of the gene or a secondary effect resulting from the dramatic effects of the *lk* mutation. The inability to fully restore the wild-type phenotype by AVG argues for a secondary effect. The *Epi* gene in tomato has likewise been shown to result in ethylene overproduction and confers increased stem and petiole thickness, epinasty, and a compact growth habit (Fujino et al. 1988). This mutant possesses elevated ACC levels (the precursor of ethylene) but it does not revert to a phenocopy of the parental variety after treatment with ethylene synthesis or action inhibitors (Fujino et al. 1989). Neither the *lk* or *Epi* mutants have therefore proved to be of major benefit to ethylene research.

Again it has been the combination of a good selection procedure combined with the benefits of a mutagenesis program using *Arabidopsis* that has allowed rapid progress to be made. Guzman and Ecker (1990) used alterations to the ethylene "triple response" to screen for potential ethylene mutants in dark-grown *Arabidopsis*. They identified two allelic mutants (*etol-1* and *etol-2*) which displayed a constitutive triple response (i.e., in the absence of exogenous ethylene). The phenotype included reduced hypocotyl and root elongation, swelling of the hypocotyl and an exaggerated tightening of the apical hook (Guzman and Ecker 1990). Etiolated seedlings of mutant *etol-1* produced 40 times more ethylene than did wild-type seedlings but could be reverted to the wild-type phenotype by the application of inhibitors or ethylene biosynthesis (e.g., AVG or AIB) or ethylene action (e.g., *trans*-cyclooctene or $AgNO_3$). The *etol-1* mutation appears to influence some step, before the conversion

of ACC to ethylene, possibly the rate limiting step, SAM to ACC (Guzman and Ecker 1990). A further two ethylene overproducing loci have been identified by *eto2* and *eto3* (Kieber et al. 1993).

Response. Several well-defined ethylene response mutants have been isolated in *Arabidopsis* using screens based on the ethylene 'triple response.' The first was a dominant ethylene-insensitive mutant, *etr* (Bleecker et al. 1988). The mutant inhibits a wide range of normal ethylene responses. Consequently, the inhibitory effect of ethylene on elongation and the feedback suppression of ethylene synthesis is reduced while the normal enhancement of peroxidase activity, seed germination, and leaf senescence is reduced. The results suggest that *etr* controls a step common to all these responses. *In vivo* binding of ethylene is also reduced by the *etr* mutation suggesting that *etr* may influence the ethylene receptor itself, although identification of the gene product is still required. The *etr* mutation has been mapped, and, by chromosome walking, an 18 kb fragment containing the mutant gene has been isolated. A candidate *ETR* gene that codes for a putative transmembrane protein kinase has been identified (Bleecker 1991).

Guzman and Ecker (1990) also isolated two mutants, *ein1* and *ein2,* which were insensitive to high concentrations of ethylene. [A third locus, *ein3,* has recently been identified (see Kieber et al. 1993).] They were highly elongated, and the apical hook opened readily in the presence of ethylene. They also possessed small increases in ethylene levels possibly due to an alteration in autoinhibitional control as a consequence of defective ethylene reception (Guzman and Ecker 1990). The *ein1-1* mutation is dominant, and, from linkage data, may be allelic with *etr,* whereas the *ein2* mutation is recessive (Guzman and Ecker 1990). In the same study, two non-allelic mutants that lacked apical hooks were selected, *hls1* and *hls2*. The *hls1* mutant did not form a hook even in the presence of 10 μl/l of ethylene although root and hypocotyl extension were inhibited to the same extent as that in wild-type plants. This suggests the hookless mutants have uncoupled components of the triple response and that the *HLS1* gene product may act to antagonize ethylene action for just one of these responses (Guzman and Ecker 1990). All these mutants possess identifiable phenotypes when grown into mature, light-grown plants which, on examination, may provide useful insights into the endogenous role of ethylene during normal plant growth.

Ethylene-response mutants with a constitutive triple response (like the overproducer *eto1*) have recently been isolated and shown to belong to a single locus, *ctr1* (Kieber et al. 1993). These were separated from ethylene overproducing mutants because they were insensitive to ethylene biosynthesis inhibitors. Adult *ctr* plants are smaller, bolt later, and have smaller root systems than wild-type plants and show constitutive expression of ethylene-regulated genes. The gene *ctr1* was epistatic to *ein1* but not to *ein3* suggesting that the *CTR1* gene product operates downstream in the response pathway from the *EIN1* gene product (Kieber et al. 1993). The *CTR1* gene was tagged by a T-DNA insertion. This allowed the flanking sequences to be rescued and hence a cDNA identified. Confirmation came from sequencing *ctr1* mutations produced by chemical mutagenesis and showing that induced point mutations had occurred in the putative gene. The gene *CTR1* encodes a protein with similarity to a Raf-type, serine/threonine protein kinase. In animals, such proteins are thought to mediate signal transmission and ultimately activate gene expression. The role in plants will be fascinating when illuminated.

Cytokinin Mutants

Synthesis. No single-gene cytokinin synthesis mutants have been proven in intact higher plants. This is possibly because the expected phenotype is unclear. For example, Medford et al. (1989) have introduced a chimeric, heat-inducible promotor-isopentenyl transferase gene into tobacco and *Arabidopsis*. They found elevated cytokinin levels even without heat shock, and these were associated with the release of axillary buds, and reduced stem length and root development. After heat shock, substantial further increases in the cytokinin content were found (e.g., 52-fold increase in zeatin), but no further alterations in growth and differentiation occurred. Unfortunately, these results do little to clarify the role cytokinin levels play in controlling the pattern of differentiation in whole plants.

Differences in both the metabolism and sensitivity to cytokinins have been demonstrated at the tissue culture level. Mok et al. (1980) have evidence of a single gene difference in *Phaseolus vulgaris* which controls the cytokinin dependency of hypocotyl callus cultures. Variation in the metabolism of cytokinins between callus cultures from *P. vulgaris* and *P. lunatus* has also been demonstrated (Turner et al. 1985). In *Nicotiana tabacum,* the dominant genes *Hl-1* and *Hl-2* result in a cytokinin-autotropic phe-

notype for tissue cultures (i.e., they do not require cytokinins for growth as do cultures from the leaf of wild-type plants, Meins and Foster 1986). Plants possessing *Hl-1* do not overproduce *trans*-zeatin riboside or certain other cytokinins even though gene *Hl-1* is suggested to have ontogenetic functions similar to the isopentyl transferase (*ipt*) locus of the Ti plasmid (Hansen et al. 1985, 1987). This may indicate that *Hl-1* enhances the sensitivity of certain plant tissues to cytokinins. The mutant however does not markedly alter overall plant growth.

Cytokinin mutants are well characterised in mosses. The cytokinin-overproducing mutants of *Physcomitrella patens* possess a 100-fold increase in isopentanyladenine and zeatin levels (Wang et al. 1981). They possess increased numbers of gametophores and resemble wild-type plants treated with cytokinin (Wang et al. 1984b). This clearly demonstrates that cytokinins play a key role in controlling gametophore production. Somatic hybridization has indicated that the genes at at least three loci can lead to the overproduction of gametophores and cytokinin (see Wang 1987). Cytokinin-deficient mutants may also occur. They do not form gametophores but can be induced to do so by adding cytokinin (Wang et al. 1984b). A mutant with the appropriate phenotype for a cytokinin-insensitive mutant has also been found. It produces normal levels of cytokinins and is similar to the wild-type except that it lacks gametophores (Wang et al. 1984b, 1987). Such elegant studies stress the biological significance of the cytokinins in mosses.

Response. Su and Howell (1992) have isolated a cytokinin-resistant mutant, *ckr1*, in *Arabidopsis* by identifying the features of seedlings most sensitive to cytokinin. Root growth was most sensitive, and, in particular, primary root growth was inhibited and root hair elongation stimulated. The mutant plants had a similar stature and growth habit to wild-type plants but were slightly more chlorotic, with cup-shaped leaves (Su and Howell 1992). A cytokinin-resistant mutant isolated from *Nicotiana plumbaginifolia* (Blonstein et al. 1991) appears to block ABA synthesis (Parry et al. 1991), illustrating the difficulty in selecting for mutants with specific effects.

Phytochrome Mutants

Phytochrome mutants have been included in this review for two reasons. First, alterations in phytochrome levels or function frequently results in modified responses to the phytohormones with the consequence that the same mutant may be classified as both a hormone and a phytochrome mutant. The *lv* mutant in peas is a good example. It was initially characterized as a GA-hypersensitive mutant due to its enhanced response to applied GA_1 (Reid and Ross 1988b). Further work showed that it could be classified as a phytochrome mutant since it lacked the normal end-of-day FR response and exhibited only a weak response to R light compared with the isogenic parental line (Nagatani et al. 1990; Weller and Reid 1993). The *yg-6* mutant in tomatoes and *ein* in *Brassica* are other examples (Koornneef et al. 1985a; Perez et al. 1974; Rood et al. 1990; Devlin et al. 1992). Second, phytochrome mutants may be classified into synthesis and response types in the same way as the phytohormone groups. This latter group includes the so-called transduction mutants, as well as "response" mutants in the sense of Adamse et al. (1988c). Transduction mutants are suggested to act in the direct sequence of events leading from the photoreceptor to the physiological response, whereas response mutants influence a single-specific response (Adamse et al. 1988c). As with the phytohormone mutants indirect effects can also impinge on the size of a specific response. Consequently, unless the biochemical/molecular effects have been determined it may be impossible to distinguish true response mutants from more general indirect effects.

Synthesis. Early work on phytochrome synthesis mutants focussed on the *au* mutant in tomato which results in a long hypocotyl, a marked reduction of chlorophyll levels, and strongly reduced chloroplast development, anthocyanin synthesis, seed germination (Koornneef et al. 1985a), and photoregulation of chlorophyll a/b-binding protein (*cab*) synthesis (Sharrock et al. 1988). Both spectrophotometric (Koornneef et al. 1985a) and immunological (Parks et al. 1987) analyses have shown that the phytochrome content of dark-grown *au* plants is reduced to below 5% of the wild-type. In the light, a reduction of approximately 50% was observed (Adamse et al. 1988b). These results have been interpreted to suggest that the *au* mutation prevents the accumulation of light-labile phytochrome (phyA) (Parks et al. 1987) but does not influence the accumulation of light-stable phytochrome (phyB) (López-Juez et al. 1990b). However, it appears that mRNA from the *phyA* gene is produced in dark-grown *au* plants and is functional *in vitro* (Sharrock et al. 1988). Consequently, the cause of the block in the production of functional phyA in *au* is currently unknown.

In *Arabidopsis*, a number of mutants isolated be-

cause of their long hypocotyls, have been shown to possess altered phytochrome responses (e.g., Koornneef et al. 1980). A family of five genes, *PHYA, PHYB, PHYC, PHYD,* and *PHYE,* have been shown to code for the phytochrome apoprotein in this species (Sharrock and Quail 1989). The mutants *hy1, hy2,* and *hy6* are deficient in spectrophotometrically detectable phytochrome in etiolated seedlings even though they accumulate the phyA apoprotein (Chory et al. 1989a; Koornneef et al. 1980; Parks et al. 1989). This appears to be due to an impairment in the synthesis of the tetrapyrrole chromophore (Parks and Quail 1991; Quail 1991). These mutants may therefore be deficient in the other photochemically active forms of phytochrome as well. By comparison, mutants *hy3* and *hy5* possess normal levels of phytochrome in etiolated seedlings (Chory et al. 1989a; Koornneef et al. 1980; Parks et al. 1989). The *hy3* mutation results in reduced phyB levels (Nagatani et al. 1991a; Somers et al. 1991) due to a mutation in the *PHYB* gene (i.e., the *HY3* locus encodes phyB, Reed et al. 1993). Plants possessing *hy3* are not strongly inhibited by continuous red light (Koornneef et al. 1980) and lack normal end-of-day FR responses and shade avoidance responses. They appear as if they possess a constitutive far-red elongation response (Nagatani et al. 1991a; Whitelam and Smith 1991). They also flower earlier than wild-type plants (Goto et al. 1991; Reed et al. 1993). The *lh* mutant in cucumber and the *ein* mutant in *Brassica* have a similar phenotype to *hy3* and also appear to be deficient in phyB (López-Juez et al. 1990a; 1992; Devlin et al. 1992). These results suggest that phyB is necessary for perception of red light and the normal responses regulated by the photoequilibrium established by the R:FR ratio (McCormac et al. 1992).

A new mutant, *hy8,* has been selected because of its long hypocotyl in FR light. Two alleles at this locus lack phyA when examined by immunoblot or spectrophotometric analyses, while a third appears to lack biologically active phy A. All three possess normal levels of phyB and phyC (Parks and Quail 1993). These results suggest that phyA is responsible for the FR high-irradiance response of etiolated seedlings, but does not play a major role in prolonged red or white light. Hence, the *hy8* and *hy3* mutants show reciprocal responses to extended R and FR light and indicate that the different molecular species of phytochrome have distinct photosensory roles in regulating seedling development. These mutants have also provided information regarding the active form of phytochrome. It has long been assumed that P_{fr} was the active form of phytochrome. This was confirmed by the *au, hy1,* and *hy2* mutants, which possess reduced P_r levels in the dark but grow long in darkness like the wild-type plants. If P_r was active, then the mutants should be shorter than the wild-type in the dark (Quail et al. 1987).

Response. The *lv* mutant in pea (Nagatani et al. 1990) has similar phenotypic characteristics to the *lh* mutant in cucumber and *hy3* in *Arabidopsis.* All three mutants possess elongated stems when grown in white or red light, slightly lower chlorophyll levels, and lack the normal end-of-day FR response (e.g., Adamse et al. 1987, 1988a; Reed et al. 1993; Nagatani et al. 1990). However, *lv* plants appeared to possess normal levels of phytochrome (both type I and II) in both the light and dark when examined spectrophotometrically or immunologically (Nagatani et al. 1990). This suggested that *lv* plants possessed a modified response to phyB because of a block in the transduction pathway (Nagatani et al. 1990; Weller and Reid 1993). However, other alleles of the *lv* locus have recently been shown to be deficient in phyB (Weller et al. unpublished results).

There has recently been substantial debate about the role of GAs in the phytochrome response. Plants possessing *lv* show an enhanced responsiveness to GA_1 (Reid and Ross 1988b). Recent results in which GA levels have been determined by GC-SIM (Weller et al., unpublished results) have confirmed previous bioassay results (Reid and Ross 1988b) which indicated that there was no alteration in the level of GA_1 in *lv* pea plants. Hence, the enhanced elongation in *lv* plants is caused by changes in the GA-responsiveness of the tissue rather than by changes in GA levels. This is consistent with results that show that darkness and end-of-day FR light also promote elongation in pea by altering the GA responsiveness of tissue rather than by increasing GA_1 levels (Ross and Reid 1989; Reid et al. 1990; Weller et al., unpublished results). Earlier reports that light influenced growth by regulating the conversion of GA_{20} to GA_1 in peas (e.g., Campbell and Bonner 1986), therefore, do not stand up to scrutiny. A similar view that changes in responsiveness to GA are involved in the enhanced elongation of phytochrome mutants is reported for the *lh* mutant in cucumber (López-Juez et al. 1993) and is supported by changes in the responsiveness to GA in light- and dark-grown rice seedlings (Nick and Furuya 1993). This contrasts with the results presented for *ein* in *Brassica* and ma_3^R in *Sorghum.* While the *ein* mutant in *Brassica* has a similar photomorphogenic phenotype to *lv* in peas and *lh* in cucumber, consistent with the observed deficiency in a light-stable phyB-like species of phytochrome (Devlin et al. 1992), it is reported to possess ele-

vated levels of GA_1 and GA_3 (Rood et al. 1990). In *Sorghum*, the ma_3^R mutation results in abnormal phytochrome physiology (Childs et al. 1991). The phenotypic effects are similar to *lv* and include increased elongation, a reduction in leaf area, reduced phytochrome control of anthocyanin production, reduced branching, earlier flowering, and a reduced photoperiod response (Pao and Morgan 1986). This mutant is also reported to possess elevated GA_1 levels (Beall et al. 1991). These results suggest a clear difference between pea and cucumber, on the one hand, and *Sorghum* and *Brassica*, on the other. However, detailed response data for GA_1 and GA_3 are required for the latter genera before it is assumed that phytochrome acts in these species through modifying GA levels rather than by modifying GA responsiveness as in peas and cucumber.

The *hp* mutant in tomato, which results in high levels of anthocyanins (Adamse et al. 1988b), reduced plant height in red and yellow light (Mochizuki and Kamimura 1985) and elevated chlorophyll levels in fruit tissue (Adamse et al. 1988c), may be a hypersensitive phytochrome mutant, since its phenotype is in many respects the reverse of the phytochrome-deficient *au* mutant. The *lw* mutant in peas has similarities to *hp* in tomato in that it also appears to possess a hypersensitive phytochrome response (Weller and Reid 1993). It is shorter, more sensitive to low-fluence red and white light, has enhanced anthocyanin and chlorophyll accumulation, delayed flowering, and a larger photoperiod response (Weller and Reid 1993; Weller, unpublished results). The phenotype of *lw* is also similar to transgenic plants overexpressing the *phyA* gene (Nagatani et al. 1991b). The function of *Lw* is unknown, but, in support of the hypothesis that phytochrome action modulates the GA response in pea, *lw* plants show a reduced response to GA_1 but no change in GA_1 levels (Jolly et al. 1987; Weller et al., unpublished results).

The fascinating group of mutants that show constitutive photomorphogenic responses can also be considered to be phytochrome transduction chain mutants. These mutants include the *det* and *cop* mutants in *Arabidopsis* (Chory et al. 1989b; Deng et al. 1991), and the *lip1* mutant in pea (Frances et al. 1992). These mutants show certain characteristics of light-grown plants even when grown in complete darkness (e.g., leaf expansion, reduced elongation, mRNA production by light-regulated genes). The wild-type gene product can be conceived as an inhibitor of de-etiolation which is prevented from acting in the presence of P_{fr} (i.e., on exposure to light) (Chory et al. 1989b). The *COP1* gene has been sequenced and shows some similarity to a transcription repressor in yeast suggesting a possible role at the end of the response pathway (Deng et al. 1992).

Phytochrome-sensitivity mutants that influence only one or a few phytochrome responses are also known. For example, the photoperiod response genes *sn, dne, ppd,* and *hr* in pea (see Murfet and Reid 1993), result in the loss of the phytochrome-controlled long-day response (Reid and Murfet 1977). Although the response to light quality may appear to differ in these genotypes (Reid 1982), these mutants probably do not directly influence phytochrome levels or the transduction chain since they appear essentially normal for other phytochrome-controlled processes. As expected, *lv* is partially epistatic to the dominant genes at these loci because it acts at an earlier step in the response pathway leading from phytochrome to the photoperiod response. Since these mutants influence a specific phytochrome response, they would be categorized as "response" mutants to separate them from the transduction mutants in the terminology of Adamse et al. (1988c).

Conclusions

Rapid advances have been made over the last 3 years in the molecular analysis of hormone genes isolated principally from *Arabidopsis*. These advances are just beginning to provide details on the elements in the hormone-response pathway (e.g., Kieber et al. 1993) as well as identification of the control of hormone biosynthesis at the molecular level (e.g., Sun et al. 1992). Early results suggest some of these response elements may be similar across such divergent groups of organisms as plants and animals. Studies with phytochrome mutants have shown the full potential of genetic techniques to build up an integrated picture of the control of development from the molecular level to the whole plant response. The next few years hold real potential that similar studies on plant hormone mutants will allow the control of plant development by hormones to be similarly integrated. This approach will be strengthened by the innovative design of selection techniques to isolate mutants with specific desired functions.

One of the most striking features of the mutants examined to date has been the similarity of phenotypes for similar mutants in widely divergent species (e.g., ABA, GA, or phyB deficiencies). This similarity argues for similar control systems across different higher plant species. At the biochemical level, the site of action of mutants is also similar. For example, of 20 well-defined mutants that block GA_1 synthesis, six block the conversion of GA_{20} to

GA_1, while eight appear to block the two steps between geranylgeranyl pyrophosphate and kaurene (Fig. 1). The limited number of steps for which multiple mutants have been isolated may suggest that these are the key steps regulating the biosynthesis of the active hormone. Such steps and the genes controlling them deserve special attention.

The mutants that have been studied at the biochemical level are those that produce specific, and often marked phenotypic effects. Many mutants that influence hormone biosynthesis may be missed using the selection criteria outlined earlier if they do not result in obvious phenotypic effects. Such mutants may not influence steps that are limiting the production of the biologically active hormone, or the hormone itself may not markedly alter differentiation in the whole plant (e.g., Medford et al. 1989). However, these mutants are still of potential interest and in the future, direct scanning for them using carefully designed selection procedures may provide valuable data.

Virtually all hormone analyses, including those on mutants, have used tissue that either varies ontogenetically or contains a range of cell types. Consequently, site-specific alterations in hormone levels have rarely been examined. Even relatively crude analyses (e.g., Swain and Reid 1992; Swain et al. 1993) have shown that hormone synthesis genes may show marked tissue specificity. Whether this implies that these genes are regulators of structural genes or that gene families exist that control hormone production in different tissues has not been determined. However, such age- and tissue-specific questions deserve detailed examination. The production of transgenic plants with tissue-specific promotors attached to hormone-synthesis genes may answer many of these questions and overcome many of the deficiencies with the plant hormone concept.

Finally, at a more classical level, the detailed pleiotropic effects of the presently known phytohormone mutants deserve further attention. Each group of plant hormone has been suggested to have an enormous range of effects, but work with mutants has normally concentrated on only one or a few of these effects. Much evidence, albeit possibly negative, may still be gained by the judicious use of the presently known mutants. They still provide one of the most promising tools to study hormone physiology in higher plants.

Acknowledgments. I wish to thank Dr. J. J. Ross, S. Swain, and J. L. Weller for helpful discussions and comments on the manuscript, and Mrs. B. Thomson and A. Poole for technical assistance. The support of the Australian Research Council is gratefully acknowledged.

References

Adamse P, Jaspers PAPM, Bakker JA, Kendrick RE, Koornneef M (1988a) Photophysiology and phytochrome content of long-hypocotyl mutant and wild-type cucumber seedlings. Plant Physiol 87:264–268

Adamse P, Jaspers PAPM, Bakker JA, Wesselius JC, Heeringa GH, Kendrick RE, Koornneef M (1988b) Photophysiology of a tomato mutant deficient in labile phytochrome. J Plant Physiol 133:436–440

Adamse P, Jaspers PAPM, Kendrick RE, Koornneef M (1987) Photomorphogenetic responses of a long-hypocotyl mutant of *Cucumis sativus* L. J Plant Physiol 127:481–491

Adamse P, Kendrick RE, Koornneef M (1988c) Photomorphogenetic mutants of higher plants. Photochem Photobiol 48:833–841

Ashton NW, Cove DJ (1990) Mutants as tools for the analytical dissection of cell differentiation in *Physcomitrella patens* gametophytes. In: Chopra RN, Bhatla SC (eds) Bryophyte development: Physiology and biochemistry. CRC Press, Boca Raton, pp 17–31

Ashton NW, Cove DJ, Featherstone DR (1979) The isolation and physiological analysis of mutants of the moss, *Physcomitrella patens*, which overproduce gametophores. Planta 144:437–442

Barendse GWM, Kepczynski J, Karssen CM, Koornneef M (1986) The role of endogenous gibberellins during fruit and seed development: Studies on gibberellin-deficient genotypes of *Arabidopsis thaliana*. Physiol Plant 67:315–319

Beadle GW, Tatum EL (1941) Genetic control of biochemical reactions in *Neurospora*. Proc Natl Acad Sci USA 27: 499–506

Beall FD, Morgan PW, Mander LN, Miller FR, Babb KH (1991) Genetic regulation of development in *Sorghum bicolor*. V. The ma_3^R allele results in gibberellin enrichment. Plant Physiol 95:116–125

Behringer FJ, Cosgrove DJ, Reid JB, Davies PJ (1990) The physical basis for altered stem elongation rates in internode length mutants of *Pisum*. Plant Physiol 94:166–173

Bensen RJ, Zeevaart JAD (1990) Comparison of *ent*-kaurene synthetase A and B activities in cell-free extracts from young tomato fruits of wild-type and *gib-1*, *gib-2*, and *gib-3* tomato plants. J Plant Growth Regul 9:237–242

Bleeker AB, Estelle MA, Sommerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241:1086–1089

Bleeker AB (1991) Genetic analysis of ethylene response in *Arabidopsis thaliana*. In: Jenkins GI, Schuch W (eds) Molecular biology of plant development. The Company of Biologists Limited, Cambridge, pp 149–158

Blonstein AD, Parry AD, Horgan R, King PJ (1991) A cytokinin-resistant mutant of *Nicotiana plumbaginifolia* is wilty. Planta 183:244–250

Blonstein AD, Vahala T, Koornneef M, King PJ (1988) Plants regenerated from auxin-auxotrophic variants are inviable. Mol Gen Genet 215:58–64

Campbell BR, Bonner BA (1986) Evidence for phytochrome regulation of gibberellin A_{20} 3β-hydroxylation in shoots of dwarf (*lele*) *Pisum sativum* L. Plant Physiol 82:909–915

Chandler PM (1988) Hormonal regulation of gene expression in the "slender" mutant of barley (*Hordeum vulgare* L.). Planta 175:115–120

Childs KL, Pratt LH, Morgan PW (1991) Genetic regulation of development in *Sorghum bicolor*. VI. The ma_3^R allele re-

sults in abnormal phytochrome physiology. Plant Physiol 97:714–719

Chory J, Peto CA, Ashbaugh M, Saganich R, Pratt L, Ausubel F (1989a) Different roles for phytochrome in etiolated and green plants deduced from characterization of *Arabidopsis thaliana* mutants. Plant Cell 1:867–880

Chory J, Peto CA, Feinbaum R, Pratt L, Ausubel F (1989b) *Arabidopsis thaliana* mutant that develops as a light-grown plant in the absence of light. Cell 58:991–999

Cosgrove DJ, Sovonick-Dunford SA (1989) Mechanism of gibberellin-dependent stem elongation in peas. Plant Physiol 89:184–191

Croker SJ, Hedden P, Lenton JR, Stoddart JL (1990) Comparison of gibberellin in normal and slender barley seedlings. Plant Physiol 94:194–200

Deng X-W, Caspar T, Quail PH (1991) *cop*1: A regulatory locus involved in light-controlled development and gene expression in *Arabidopsis*. Genes Dev 5:1172–1182

Deng X-W, Matsui M, Wei N, Wagner D, Chu AM, Feldman D, Quail PH (1992) COP1, an *Arabidopsis* regulatory gene, encodes a protein with both a zinc-binding motif and a G_β homologous domain. Cell 71:791–801

Devlin PF, Rood SB, Somers DE, Quail PH, Whitelam GC (1992) Photophysiology of the elongated internode (*ein*) mutant of *Brassica rapa: ein* mutant lacks a detectable phytochrome B-like polypeptide. Plant Physiol 100:1442–1447

Duckham SC, Linforth RST, Taylor IB (1991) Abscisic-acid-deficient mutants at the *aba* gene locus of *Arabidopsis thaliana* are impaired in the epoxidation of zeaxanthin. Plant Cell Environ 14:601–606

Duckham SC, Taylor IB, Linforth RST, Al-Naieb RJ, Marples BA, Bowman WR (1989) The metabolism of *cis*-ABA-aldehyde by the wilty mutants of potato, pea and *Arabidopsis thaliana*. J Exp Bot 217:901–909

Estelle MA, Sommerville CR (1987) Auxin resistant mutants of *Arabidopsis thaliana* with an altered morphology. Mol Gen Genet 206:200–206

Feldmann KA, Marks MD, Christianson ML, Quatrano RS (1989) A dwarf mutant of *Arabidopsis* generated by T-DNA insertion mutagenesis. Science 243:1351–1354

Finkelstein RR, Somerville CR (1990) Three classes of abscisic acid (ABA)-insensitive mutations of *Arabidopsis* define genes that control overlapping subsets of ABA responses. Plant Physiol 94:1172–1179

Firn RD (1986) Growth substance sensitivity: The need for clearer ideas, precise terms and purposeful experiments. Physiol Plant 67:267–272

Frances S, White MJ, Edgerton MD, Jones AM, Elliott RC, Thompson WF (1992) Initial characterization of a pea mutant with light-independent photomorphogenesis. Plant Cell 4:1519–1530

Fujino DW, Burger DW, Bradford KJ (1989) Ineffectiveness of ethylene biosynthetic and action inhibitors to phenotypically revert the *Epinastic* mutant of tomato (*Lycopersicon esculentum* Mill.). J Plant Growth Regul 8:53–61

Fujino DW, Burger DW, Yang SF, Bradford KJ (1988) Characterization of an ethylene overproducing mutant of tomato (*Lycopersicon esculentum* Mill. cultivar VFN8). Plant Physiol 88:774–779

Fujioka S, Yamane H, Spray CR, Gaskin P, MacMillan J, Phinney BO, Takahashi N (1988a) Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, *dwarf-1*, *dwarf-2*, *dwarf-3*, and *dwarf-5* seedlings of *Zea mays* L. Plant Physiol 88:1367–1372

Fujioka S, Yamane H, Spray CR, Katsumi M, Phinney BO, Gaskin P, MacMillan J, Takahashi N (1988b) The dominant non-gibberellin-responding dwarf mutant (*D8*) of maize accumulates native gibberellins. Proc Natl Acad Sci USA 85:9031–9035

Fujioka S, Yamane H, Spray CR, Phinney BO, Gaskin P, MacMillan J, Takahashi N (1990) Gibberellin A_3 is biosynthesized from gibberellin A_{20} via gibberellin A_5 in shoots of *Zea mays* L. Plant Physiol 94:127–131

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation of the *Arabidopsis AB13* gene by positional cloning. Plant Cell 4:1251–1261

Goto N, Kumagai T, Koornneef M (1991) Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol Plant 83:209–215

Grierson D, Purton ME, Knapp JE, Bathgate B (1987) Tomato ripening mutants. In: Thomas H, Grierson D (eds) Developmental mutants in higher plants. Cambridge University Press, Cambridge, pp 73–94

Guzman P, Ecker JR (1990) Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell 2:513–523

Hamilton AJ, Lycett GW, Grierson D (1990) Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature 346:284–287

Hansen CE, Meins F, Aebi R (1987) Hormonal regulation of zeatin-riboside accumulation by cultured tobacco cells. Planta 172:520–525

Hansen CE, Meins F, Milani A (1985) Clonal and physiological variation in the cytokinin content of tobacco-cell lines differing in cytokinin requirement and capacity for neoplastic growth. Differentiation 29:1–6

Harberd NP, Freeling M (1989) Genetics of dominant gibberellin-insensitive dwarfism in maize. Genetics 121:827–838

Hattori T, Vasil V, Rosenkrans L, Hannah LC, McCarty DR, Vasil IK (1992) The *Viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. Genes Dev 6:609–618

Hedden P, Croker SJ (1992) Regulation of gibberellin biosynthesis in maize seedlings. In: Karssen CM, van Loon LC, Vreugdenhil D (eds) Progress in plant growth regulation. Kluwer Academic Publishers, Netherlands pp 534–544

Heino P, Sanderson G, Lang V, Nordin K, Palva ET (1990) Abscisic acid deficiency prevents development of freezing tolerance in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Gen 79:801–806

Hickok LG (1985) Abscisic acid resistant mutant in the fern *Cerotopteris:* Characterization and genetic analysis. Can J Bot 63:1582–1585

Hicks GR, Rayle DL, Lomax TL (1989) The *diageotropica* mutant of tomato lacks high specific activity auxin binding sites. Science 245:52–54

Ho T-HD, Shih SC, Kleinhofs A (1980) Screening for barley mutants with altered hormone sensitivity in their aleurone layers. Plant Physiol 66:153–157

Ingram TJ, Reid JB (1987) Internode length in *Pisum:* Biochemical expression of the *le* and *na* mutations in the slender phenotype. J Plant Growth Regul 5:235–243

Ingram TJ, Reid JB, MacMillan J (1986) The quantitative rela-

tionship between gibberellin A_1 and internode elongation in *Pisum sativum* L. Planta 168:414–420

Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis CL, MacMillan J (1984) Internode length in *Pisum*. The *Le* gene controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta 160:455–463

Jolly CJ, Reid JB, Ross JJ (1987) Internode length in *Pisum*. Action of gene *lw*. Physiol Plant 69:489–498

Karssen CM, Binkhorst-van der Swan DLC, Breckland ARE, Koornneef M (1983) Induction of dormancy during seed development by endogenous abscisic acid: Studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. Planta 157:158–165

Kelly MO, Bradford KJ (1986) Insensitivity of the *diageotropica* tomato mutant to auxin. Plant Physiol 82:713–717

Kendrick RE, Nagatani A (1991) Phytochrome mutants. Plant J 1:133–139

Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) *CTR1*, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the Raf family of protein kinases. Cell 72:427–441

Klee H, Estelle M (1991) Molecular genetic approaches to plant hormone biology. Annu Rev Plant Physiol Plant Mol Biol 42:529–551

Klee H, Horsch R, Rogers S (1987) *Agrobacterium* mediated plant transformation and its further applications to plant biology. Ann Rev Plant Physiol 38:467–486

Koornneef M (1986) Genetic aspects of abscisic acid. In: Blonstein AD, King PJ (eds) Plant gene research. A genetic approach to plant biochemistry. Springer-Verlag, Wien, pp 35–54

Koornneef M (1991) Isolation of higher plant developmental mutants. In: Jenkins GI, Schuch W (eds) Molecular biology of plant development. The Company of Biologists Limited, Cambridge, pp 1–19

Koornneef M, Cone JW, Dekens EG, O'Herne-Roberts EG, Spruit CJP, Kendrick RE (1985a) Photomorphogenic responses of long hypocotyl mutants of tomato. J Plant Physiol 120:153–165

Koornneef M, Elgersma A, Hanhart CJ, Van Loenen Martinet EP, Van Rijn L, Zeevaart JAD (1985b) A gibberellin insensitive mutant of *Arabidopsis thaliana*. Physiol Plant 65:33–39

Koornneef M, Hanhart CJ, Hilhorst HWM, Karssen CM (1989) In vivo inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana*. Plant Physiol 90:463–469

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in nongerminating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 61:385–393

Koornneef M, Rauling G, Karssen CM (1984) The isolation and characterization of abscisic acid insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol 100:147–160

Lanahan MB, Ho T-HD (1988) Slender barley: A constitutive gibberellin-response mutant. Planta 175:107–114

Last RL, Bissinger PH, Mahoney DJ, Radwanski ER, Fink GR (1991) Tryptophan mutants in *Arabidopsis:* The consequences of duplicated tryptophan synthase β genes. Plant Cell 3:345–358

Last RL, Fink GR (1988) Tryptophan-requiring mutants of the plant *Arabidopsis thaliana*. Science 240:305–310

Law DM (1987) Gibberellin-enhanced indole-3-acetic acid biosynthesis: D-tryptophan as the precursor of indole-3-acetic acid. Physiol Plant 70:626–632

Lawrence NL, Ross JJ, Mander LN, Reid JB (1992) Internode length in *Pisum*. Mutants *lk, lka,* and *lkb* do not accumulate gibberellins. J Plant Growth Regulation 11:35–37

Lenton JR, Hedden P, Gale MD (1987) Gibberellin insensitivity and depletion in wheat-consequences for development. In: Hoad GV, Lenton JR, Jackson MB, Atkin BK (eds) Hormone action in plant development—a critical appraisal. Butterworths, London, pp 145–160

Lincoln C, Britton JH, Estelle M (1990) Growth and development of the *axr1* mutants of *Arabidopsis*. Plant Cell 2: 1071–80

López-Juez E, Buurmeijer WF, Heeringa GH, Kendrick RE, Wesselius JC (1990a) Response of light-grown wild-type and long hypocotyl mutant cucumber plants to end-of-day far-red light. Photochem Photobiol 52:143–149

López-Juez E, Kobayashi M, Kamiya Y, Kendrick RE (1993) A possible involvement of gibberellins in the action of phytochrome-B: A study using the cucumber *lh* mutant. Plant Cell Physiol 34:S71

López-Juez E, Nagatani A, Buurmeijer WF, Peters JL, Furuya M, Kendrick RE, Wesselius JC (1990b) Response of light-grown wild-type and *aurea*-mutant tomato plants to end-of-day far-red light. J Photochem Photobiol B Biol 4:391–405

López-Juez E, Nagatani A, Tomizawa K-I, Deak M, Kern R, Kendrick RE, Furuya M (1992) The cucumber long hypocotyl mutant lacks a light-stable PHYB-like phytochrome. Plant Cell 4:241–251

McCarty DR, Carson CB, Stinard PS, Robertson DS (1989) Molecular analysis of *viviparous-1:* An abscisic acid-insensitive mutant of maize. Plant Cell 1:523–532

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil LK (1991) The *viviparous-1* developmental gene of maize encodes a novel transcription activator. Cell 66:895–905

McCormac AC, Whitelam GC, Boylan MT, Quail PH, Smith H (1992) Contrasting responses of etiolated and light-adapted seedlings to red:far-red ratio: A comparison of wild type, mutant and transgenic plants has revealed differential functions of members of the phytochrome family. J Plant Physiol 140:707–714

MacMillan J, Phinney BO (1987) Biochemical genetics and the regulation of stem elongation by gibberellins. In: Cosgrove DJ, Knievel DP (eds) Physiology of cell expansion during plant growth. American Society of Plant Physiology, Rockville, pp 156–171

Maher EP, Martindale SJB (1980) Mutants of *Arabidopsis thaliana* with altered response to auxin and gravity. Biochem Genet 18:1041–1053

Medford JI, Horgan R, El-Sawi Z, Klee HJ (1989) Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. Plant Cell 1:403–413

Meins F, Foster R (1986) A cytokinin mutant derived from cultured tobacco cells. Dev Genet 7:159–165

Mita T, Katsumi M (1986) Gibberellin control of microtubule arrangement in the mesocotyl epidermal cells of the d_5 mutant of *Zea mays* L. Plant Cell Physiol 27:651–659

Mochizuki T, Kamimura S (1985) Photoselective method for se-

lection of *hp* at the cotyledon stage. Tomato Genet Coop Rpt 35:12–13

Mok MC, Mok DWS, Armstrong DJ, Rabakoarihanta A, Kim S-G (1980) Cytokinin autonomy in tissue cultures of *Phaseolus:* A genotype-specific and heritable trait. Genetics 94:675–686

Moore R, Smith JD (1985) Graviresponsiveness and abscisic-acid content of roots of carotenoid-deficient mutants of *Zea mays* L. Planta 164:126–128

Muller J-E, Goujaud J, Caboche M (1985) Isolation *in vitro* of naphthaleneacetic acid-tolerant mutants of *Nicotiana tabaccum*, which are impaired in root morphogenesis. Mol Gen Genet 199:194–200

Murfet IC, Reid JB (1993) Developmental mutants. In: Casey R, Davies DR (eds) Peas—genetics, molecular biology and biotechnology. CAB International, Wallingford, UK, pp. 165–216

Nagatani A, Chory J, Furuya M (1991a) Phytochrome B is not detectable in the hy3 mutant of *Arabidopsis*, which is deficient in responding to end-of-day far red light treatments. Plant Cell Physiol 32:1119–1122

Nagatani A, Kay SA, Deak M, Chua N-H, Furuya M (1991b) Rice type I phytochrome regulates hypocotyl elongation in transgenic tobacco seedlings. Proc Natl Acad Sci USA 88:5207–5211

Nagatani A, Reid JB, Ross JJ, Dunnewijk A, Furuya M (1990) Internode length in *Pisum*. The response to light quality, and phytochrome type I and II levels in *lv* plants. J Plant Physiol 135:667–674

Nambara E, Satoshi N, McCourt P (1992) A mutant of *Arabidopsis* which is defective in seed development and storage protein accumulation is a new *abi3* allele. Plant J 2:435–441

Neill SJ, Horgan R (1985) Abscisic acid production and water relations in wilty tomato mutants subjected to water deficiency. J Exp Bot 36:1222–1231

Neill SJ, Horgan R, Parry AD (1986) The carotenoid and abscisic acid content of *viviparous* kernels and seedlings of *Zea mays* L. Planta 169:87–96

Nick P, Furuya M (1993) Phytochrome dependent decrease of gibberellin-sensitivity. A case study of cell extension growth in the mesocotyl of *japonica* and *indica* type rice cultivars. Plant Growth Regulation 12:195–206

Oeller PW, Min-Wong L, Taylor LP, Pike DA, Theologis A (1991) Reversible inhibition of tomato fruit senescence by antisense RNA. Science 254:437–439

Ozga JA, Brenner ML, Reinecke DM (1992) Seed effects on gibberellin metabolism in pea pericarp. Plant Physiol 100: 88–94

Pao C-I, Morgan PW (1986) Genetic regulation of development in *Sorghum bicolor*. I. Role of the maturity genes. Plant Physiol 82:575–580

Parks BM, Jones AM, Adamse P, Koornneef M, Kendrick RE, Quail PH (1987) The *aurea* mutant of tomato is deficient in spectrophotometrically and immunochemically detectable phytochrome. Plant Mol Biol 9:97–107

Parks BM, Quail PH (1991) Phytochrome-deficient *hy1* and *hy2* long hypocotyl mutants of *Arabidopsis* are defective in phytochrome chromophore biosynthesis. Plant Cell 3: 1177–1186

Parks BM, Quail PH (1993) *hy8*, a new class of *Arabidopsis* long hypocotyl mutants deficient in functional phytochrome A. Plant Cell 5:39–48

Parks BM, Shanklin J, Koornneef M, Kendrick RE, Quail PH (1989) Immunochemically detectable phytochrome is present at normal levels but is photochemically nonfunctional in the *hy1* and *hy2* long hypocotyl mutants of *Arabidopsis*. Plant Mol Biol 12:425–437

Parry AD, Blonstein AD, Babiano MJ, King PJ, Horgan R (1991) Abscisic-acid metabolism in a wilty mutant of *Nicotiana plumbaginifolia*. Planta 183:237–243

Parry AD, Griffiths A, Horgan R (1992) Abscisic acid biosynthesis in roots. II. The effects of water-stress in wild-type and abscisic-acid-deficient mutant (*notabilis*) plants of *Lycopersicon esculentum*. Planta 187:192–197

Parry AD, Horgan R (1992a) Abscisic acid biosynthesis in higher plants. In: Karssen CM, van Loon LC, Vreugdenhil D (eds) Progress in plant growth regulation. Kluwer Academic Publishers, Netherlands, pp 160–172

Parry AD, Horgan R (1992b) Abscisic acid biosynthesis in roots. I. The identification of potential abscisic acid precursors, and other carotenoids. Planta 187:185–191

Parry AD, Neill SJ, Horgan R (1988) Xanthoxin levels and metabolism in the wild-type and wilty mutants of tomato. Planta 173:397–404

Peng J, Harberd NP (1993) Derivative alleles of the *Arabidopsis* gibberellin-insensitive (*gai*) mutation confer a wild-type phenotype. Plant Cell 5:351–360

Perez AT, Marsh HV, Lachman WH (1974) Physiology of the *yellow-green 6* gene in tomato. Plant Physiol 53:192–197

Phinney BO (1956) Growth response of single-gene dwarf mutants in maize to gibberellic acid. Proc Natl Acad Sci USA 42:186–189

Phinney BO (1961) Dwarfing genes in *Zea mays* and their relation to the gibberellins. In: Klein RM (ed) Plant growth regulation. Iowa State College Press, Ames, pp 489–501

Phinney BO (1984) Gibberellin A_1, dwarfism and the control of shoot elongation in higher plants. In: Crozier A, Hillman JR (eds) The biosynthesis and metabolism of plant hormones. Society of Experimental Biology Seminar Series 23. Cambridge University Press, Cambridge, pp 17–41

Phinney BO, Freeling M, Robertson DS, Spray CR, Silverthorne J (1986) Dwarf mutants of maize—the gibberellin pathway and its molecular future. In: Bopp M (ed) Plant growth substances, 1985. Springer-Verlag, Berlin, Heidelberg, pp 55–64

Phinney BO, Spray C (1982) Chemical genetics and the gibberellin pathway in *Zea mays* L. In: Wareing PF (ed) Plant growth substances, 1982. London Academic Press, London, pp 101–110

Pickett FB, Wilson AK, Estelle M (1990) The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. Plant Physiol 94:1462–1466

Picton S, Barton SL, Bouzayen M, Hamilton AJ, Grierson D (1993) Altered fruit ripening and leaf senescence in tomato expressing an antisense ethylene-forming enzyme transgene. Plant J 3:469–481

Potts WC, Reid JB, Murfet IC (1985) Internode length in *Pisum*. Gibberellins and the slender phenotype. Physiol Plant 63: 357–364

Quail PH (1991) Phytochrome: A light-activated molecular switch that regulates plant gene expression. Ann Rev Genet 25:389–409

Quail PH, Gatz C, Hershey HP, Jones AM, Lissemore JL, Parks BM, Sharrock RA, Barker RF, Idler K, Murrey MG, Koornneef M, Kendrick RE (1987) Molecular biology of phytochrome. In: Furuya M (ed) Phytochrome and photoregulation in plants. Academic Press, Tokyo, pp 23–37

Quarrie SA (1982) Droopy: A wilty mutant of potato deficient in abscisic acid. Plant Cell Environ 5:23–26

Raskin I, Ladyman JAR (1988) Isolation and characterization of a barley mutant with abscisic-acid-insensitive stomata. Planta 173:73–78

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell 5:147–157

Reid JB (1982) Flowering in *Pisum:* Effect of gene *Hr* on spectral sensitivity. Crop Sci 22:266–268

Reid JB (1986a) Internode length in *Pisum*. Three further loci, *lh*, *ls* and *lk*. Ann Bot 57:577–592

Reid JB (1986b) Gibberellin mutants. In: Blonstein AD, King PJ (eds) Plant gene research. A genetic approach to plant biochemistry. Springer-Verlag, Wien, pp 1–34

Reid JB (1990) Gibberellin synthesis and sensitivity mutants in *Pisum*. In: Pharis RP, Rood SB (eds) Plant growth substances 1988, Springer-Verlag, New York pp 74–83

Reid JB, Hasan O, Ross JJ (1990) Internode length in *Pisum:* Gibberellins and the response to far-end-rich light. J Plant Physiol 137:46–52

Reid JB, Murfet IC (1977) Flowering in *Pisum:* The effect of light quality on genotype *lf e Sn Hr*. J Exp Bot 28:1357–1364

Reid JB, Murfet IC, Potts WC (1983) Internode length in *Pisum*. II. Additional information on the relationship and action of loci *Le, La, Cry, Na* and *Lm*. J Exp Bot 34:349–364

Reid JB, Ross JJ (1988a) Internode length in *Pisum*. Further studies on the 'micro' gene, *lm*. Physiol Plant 72:547–554

Reid JB, Ross JJ (1988b) Internode length in *Pisum*. A new gene, *lv*, conferring an enhanced response to gibberellin A_1. Physiol Plant 72:595–604

Reid JB, Ross JJ (1989) Internode length in *Pisum*. Two further gibberellin-insensitivity genes, *lka* and *lkb*. Physiol Plant 75:81–88

Reid JB, Ross JJ (1993) A mutant-based approach, using *Pisum sativum*, to understanding plant growth. Int J Plant Sci 154:22–34

Reid JB, Ross JJ, Swain SM (1992) Internode length in *Pisum*. A new slender mutant with elevated levels of C_{19} gibberellins. Planta 188:462–467

Roberts JA, Grierson D, Tucker GA (1987) Genetic variants as aids to examine the significance of ethylene in development. In: Hoad GV, Lenton JR, Jackson MB, Atkin BK (eds) Hormone action in plant development—a critical appraisal. Butterworths, London, pp 107–118

Robichaud CS, Wong J, Sussex IM (1980) Control of *in vitro* growth of *viviparous* embryo mutants of maize by abscisic acid. Dev Genet 1:325–330

Rock CD, Zeevaart JAD (1991) The *aba* mutant of *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis. Proc Natl Acad Sci USA 88:7496–7499

Romano CP, Cooper ML, Klee HJ (1993) Uncoupling auxin and ethylene effects in transgenic tobacco and *Arabidopsis* plants. Plant Cell 5:181–189

Romano C, Hein M, Klee H (1991) Inactivation of auxin in tobacco transformed with the indoleacetic acid-lysine synthetase gene of *Pseudomonas savastanoi*. Genes Dev. 5: 438–446

Rood SB, Pearce D, Williams PN, Pharis RP (1989) A gibberellin-deficient *Brassica* mutant —*rosette*. Plant Physiol 89: 482–487

Rood SB, Williams PH, Pearce D, Murofushi N, Mander LN, Pharis RP (1990) A mutant gene that increases gibberellin production in *Brassica*. Plant Physiol 93:1168–1174

Ross JJ, Davies NW, Reid JB, Murfet IC (1990) Internode length and leaf growth in *Lathyrus odoratus* L. Effects of genes *l* and *lb* on gibberellin metabolism and levels. Physiol Plant 79:453–458

Ross JJ, Reid JB (1986) Internode length in *Pisum*. The involvement of ethylene with the gibberellin-insensitive erectoides phenotype. Physiol Plant 67:673–679

Ross JJ, Reid JB (1987) Internode length in *Pisum*. A new allele at the *le* locus. Ann Bot 59:107–109

Ross JJ, Reid JB (1989) Internode length in *Pisum*. Biochemical expression of the *le* gene in darkness. Physiol Plant 76: 164–172

Ross JJ, Reid JB, Dungey HS (1992) Ontogenetic variation in levels of gibberellin A_1 in *Pisum*. Implications for the control of stem elongation. Planta 186:166–171

Ross JJ, Reid JB, Gaskin P, MacMillan J (1989) Internode length in *Pisum*. Estimation of GA_1 levels in genotypes *Le, le* and le^d. Physiol Plant 76:173–176

Ross JJ, Reid JB, Swain SM (1993) Control of stem elongation by gibberellin A_1: Evidence from genetic studies including the slender mutant, *sln*. Aust J Plant Physiol 20:585–595

Scott IM (1988) Effects of gibberellin on shoot development in the *dgt* mutant of tomato. Ann Bot 61:389–392

Sharrock RA, Parks BM, Koornneef M, Quail PH (1988) Molecular analysis of the phytochrome deficiency in an *aurea* mutant of tomato. Mol Gen Genet 213:9–14

Sharrock RA, Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana:* Structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev 3:1745–1757

Smith VA, Knott CJ, Gaskin P, Reid JB (1992) The distribution of gibberellins in vegetative tissues of *Pisum sativum* L. Biological and biochemical consequences of the *le* mutation I. Plant Physiol 99:368–371

Somers DE, Sharrock RA, Tepperman JM, Quail PH (1991) The *hy3* long hypocotyl mutant of *Arabidopsis* is deficient in phytochrome B. Plant Cell 3:1263–1274

Spray C, Phinney BO, Gaskin P, Gilmour SJ, MacMillan J (1984) Internode length in *Zea mays* L. The *dwarf-1* mutant controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta 160:464–468

Stoddart JL (1984) Growth and gibberellin-A_1 metabolism in normal and gibberellin-insensitive (*Rht 3*) wheat (*Triticum aestivum* L.) seedlings. Planta 161:432–438

Su W, Howell SH (1992) A single genetic locus, *ckr1*, defines *Arabidopsis* mutants in which root growth is resistant to low concentrations of cytokinin. Plant Physiol 99:1569–1574

Sun T, Goodman HM, Ausubel FM (1992) Cloning the *Arabidopsis GA1* locus by genomic subtraction. Plant Cell 4: 119–128

Suzuki Y, Kurogochi S, Murofushi N, Ota Y, Takahashi N (1981) Seasonal changes of GA_1, GA_{19} and abscisic acid in three rice cultivars. Plant Cell Physiol 22:1085–1093

Swain SM, Reid JB (1992) Internode length in *Pisum*. A new allele at the *Lh* locus. Physiol Plant 86:124–130

Swain SM, Reid JB, Ross JJ (1993) Seed development in *Pisum*. The lh^i allele reduces gibberellin levels in developing seeds, and increases seed abortion. Planta 191:482–488

Tal M, Nevo Y (1973) Abnormal stomatal behavior and root resistance, and hormonal imbalance in three wilty mutants of tomato. Biochem Genet 8:291–300

Talon M, Koornneef M, Zeevaart JAD (1990) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc Natl Acad Sci USA 87:7983–7986

Turner JE, Mok MC, Mok DWS (1985) Zeatin metabolism in fruits of *Phaseolus*. Comparison between embryos, seed coat and pod tissues. Plant Physiol 79:321–322

Wang TL (1987) Cytokinin mutants. In: Horgan R, Jeffcoat B (eds) Cytokinins—plant hormones in search of a role, Monograph 14. British Plant Growth Regulator Group, Bristol, pp 55–67

Wang TL, Donkin ME, Martin ES (1984a) The physiology of a wilty pea: Abscisic acid production under water stress. J Exp Bot 351:1222–1232

Wang TL, Futers TS, McGeory F, Cove DJ (1984b) Moss mutants and the analysis of cytokinin metabolism. In: Crozier A, Hillman JR (eds) The biosynthesis and metabolism of plant hormones. Society of Experimental Biology Seminar Series 23. Cambridge University Press, Cambridge, pp 135–164

Wang TL, Horgan R, Cove DJ (1981) Cytokinins from the moss *Physcomitrella patens*. Plant Physiol 68:735–738

Waycott W, Smith VA, Gaskin P, MacMillan J, Taiz L (1991) The endogenous gibberellins of dwarf mutants of lettuce. Plant Physiol 95:1169–1173

Weller JL, Reid JB (1993) Photoperiodism and photocontrol of stem elongation in two photomorphogenic mutants of *Pisum sativum* L. Planta 189:15–23

Whitelam GC, Smith H (1991) Retention of phytochrome-mediated shade avoidance responses in phytochrome-deficient mutants of *Arabidopsis*, cucumber, and tomato. J Plant Physiol 139:119–125

Wilson AK, Pickett FB, Turner JC, Estelle M (1990) A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. Mol Gen Genet 222:377–383

Zeevaart JAD, Talon M (1992) Gibberellin mutants in *Arabidopsis thaliana*. In: Karssen CM, van Loon LC, Vreugenhil D (eds) Progress in plant growth regulation. Kluwer Academic Publishers, Netherlands, pp 34–42

Zobel RW (1973) Some physiological characteristics of the ethylene-requiring tomato mutant *diagetropica*. Plant Physiol 52:383–389