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Gibberellin Deficiency and Response Mutations Suppress the Stem Elongation Phenotype of Phytochrome-Deficient Mutants of Arabidopsis[1]

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Plant growth and development are regulated by numerous internal and external factors. Among these, gibberellin (GA) (an endogenous plant growth regulator) and phytochrome (a photoreceptor) often influence the same processes. For example, in plants grown in the light *Arabidopsis thaliana* hypocotyl elongation is reduced by GA deficiency and increased by phytochrome deficiency. Here we describe experiments in which the phenotypes of Arabidopsis plants doubly homozygous for GA-related and phytochrome-related mutations were examined. The double mutants were studied at various stages in the plant life cycle, including the seed germination, young seedling, adult, and reproductive phases of development. The results of these experiments are complex, but indicate that a fully functional GA system is necessary for full expression of the elongated phenotypes conferred by phytochrome deficiency.

Plants possess a number of regulatory photoreceptors that control plant growth and development in response to changes in the light environment (Kendrick and Kronenberg, 1994). The best characterized of these receptors are the phytochromes (Quail, 1991). The phytochromes are dimeric chromoproteins composed of two nuclear-encoded apoprotein monomers, to each of which is attached a linear tetrapyrrole chromophore. The phytochromes are soluble proteins, and are found in the cytoplasm of plant cells in one of two distinct, interconvertible forms known as Pr and Pfr. Interconversion of Pr and Pfr occurs following absorption of a photon, with the absorption maxima of Pr and Pfr being in the red and far-red wavelengths, respectively. Arabidopsis contains several different phytochromes, the apoprotein components of which are encoded by a small family of divergent genes called *PHYA, PHYB, PHYC, PHYD,* and *PHYE* (Sharrock and Quail, 1989; Clack et al., 1994; Cowl et al., 1994). The *PHYA* gene encodes the apoprotein component of phytochrome A, *PHYB* that of phytochrome B, and so on (Quail, 1991; Quail et al., 1994).

The in planta functions of some of these different phytochromes have been analyzed through the isolation and characterization of mutants that display an elongated hypocotyl in the light. These mutants have a reduced capacity for the light-mediated inhibition of hypocotyl elongation that is normally exhibited by wild-type plants, and in some cases display this phenotype because of deficiencies in phytochrome action (reviewed in Whitelam and Harberd, 1994). For example, the *hy1, hy2,* and *hy6* mutants of Arabidopsis are thought to display an elongated hypocotyl because of a depletion in the levels of the phytochrome chromophore. Because the different phytochrome species are thought to share the same chromophore, it is therefore likely that the *hy1, hy2,* and *hy6* mutants are functionally deficient for all phytochrome species (Chory et al., 1989; Parks et al., 1989; Parks and Quail, 1991). Other elongated hypocotyl mutants are specifically deficient for individual phytochrome species. Thus, *phyA* mutants, which are specifically deficient for phytochrome A, display an elongated hypocotyl in continuous far-red light (although not in continuous white or red light) and do not exhibit an obvious adult mutant phenotype when grown in standard greenhouse conditions (Dehesh et al., 1993; Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993). *phyB* mutants are specifically deficient for phytochrome B and display an elongated hypocotyl in continuous white light and red light, but not in continuous far-red light (Reed et al., 1993; Bradley et al., 1995). When grown in standard greenhouse conditions, adult *phyB* mutants are slender, have increased apical dominance, and are pale green. Phytochromes A and B have distinct, though overlapping, roles in plant development, with phytochrome B playing the predominant role in the development of plants in white light (Johnson et al., 1994; Reed et al., 1994).

In addition to the photoreceptors, which regulate plant development in response to cues from the external light environment, plants possess a number of endogenous growth regulators, known as the phytohormones. The GAs are one class of essential phytohormones that have profound and diverse effects on plant growth and development (Hooley, 1994). GA-related dwarf mutants are known in a number of plant species and can be subclassified as GA response mutants or GA biosynthesis mutants (Ross, 1994). In Arabidopsis the GA biosynthesis mutants typically display impaired seed germination, a dark-green dwarf growth habit, and reduced apical dominance and floral fertility. The phenotype of these GA biosynthesis mutants can be corrected by an application of exogenous bioactive GAs (Koornneef and van der Veen, 1980; Talon et al., 1990a; Wilson and Somerville, 1995). Mutations conferring

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these phenotypes have been identified at five distinct genetic loci (*GA1*, *GA2*, *GA3*, *GA4*, and *GA5*; Koornneef and van der Veen, 1980), and the phenotype appears to be attributable to GA deficiency resulting from lesions in genes encoding the enzymes that are involved in GA biosynthesis. Thus, *GA1* encodes *ent*-kaurene synthetase A, an enzyme that is involved in the early stages of GA biosynthesis (Sun and Kamiya, 1994), and *GA4* encodes a 2-oxoglutarate-dependent dioxygenase, which is likely to be a GA 3β-hydroxylase involved in the final step in the synthesis of bioactive GAs (Chiang et al., 1995).

The *gai* mutant of Arabidopsis displays impaired GA responses. *gai* is a semidominant mutation, which confers a phenotype that, in many respects, closely resembles that of GA biosynthesis mutants (Koornneef et al., 1985; Wilson et al., 1992; Peng and Harberd, 1993; Ezura and Harberd, 1995; Putterill et al., 1995). However, the *gai* mutant phenotype is either unresponsive or weakly responsive to exogenous GA treatments (Koornneef et al., 1985; Wilson et al., 1992; Wilson and Somerville, 1995) and is associated with an accumulation of (rather than a deficiency for) the bioactive 3β-hydroxylated GAs (Talon et al., 1990b). It has been suggested that *GAI* may encode a GA-receptor or signal transduction component (as discussed in Peng and Harberd, 1993; Carol et al., 1995; Wilson and Somerville, 1995).

SPINDLY (*SPY*) is another Arabidopsis gene thought to encode a GA-related signal transduction component. *spy* mutants display a resistance to the GA biosynthesis inhibitor paclobutrazol, and adult *spy* mutant plants are paler green and more elongated than wild-type controls (Jacobsen and Olszewski, 1993). Recently, it has been shown that in *spy gai* double-mutant homozygotes, *spy* mutations can suppress the dwarf phenotype conferred by *gai* (Wilson and Somerville, 1995; Jacobsen et al., 1996), suggesting that the GAI and SPY gene products may be involved in the same GA-related signal transduction pathway (Swain and Olszewski, 1996).

It is a striking fact that the phenotypes displayed by GA-related mutants and phytochrome-related mutants or transgenics often have features in common. For example, over-expression of phytochromes A or B in transgenic plants confers a dark-green, dwarfed phenotype, which superficially resembles that of GA-related mutant plants (Boylan and Quail, 1989, 1991; Kay et al., 1989; Keller et al., 1989; Cherry et al., 1991; Wagner et al., 1991; Boylan et al., 1994). Indeed, recent experiments have shown that the levels of bioactive GAs in transgenic tobacco plants, which over-express oat phytochrome A, are substantially lower than that found in nontransgenic controls, and that foliar applications of GA can partially suppress the phenotypic consequences of phytochrome A over-expression (Jordan et al., 1995). On the other hand, phytochrome-deficient mutants display a pale-green, elongated, increased apical dominance phenotype (see Reed et al., 1993), which resembles the phenotype of *spy* mutants (Jacobsen and Olszewski, 1993). In several plant species mutants deficient for phytochrome B-like proteins have been shown to have elevated GA levels, although in some cases this observation is dependent on the stage of growth at which the GA determinations are made (Rood et al., 1990; Beall et al., 1991; Childs et al., 1992; Devlin et al., 1992; López-Juez et al., 1992, 1995). Furthermore, recent studies have shown that in two of these mutants, the *lh* mutant of cucumber and a *phyB* mutant of Arabidopsis, the hypocotyl elongation response to exogenous bioactive GAs is greater than in the wild-type control (López-Juez et al., 1995; Reed et al., 1996). Also, the increased elongation growth of the *lv* mutant of pea is apparently associated with increased GA responsiveness (Weller et al., 1994).

This paper describes experiments in which the relationship between the phytochrome and GA systems in the regulation of plant growth and development is further explored through examination of the phenotype of Arabidopsis plants, which are doubly mutant for mutations affecting both phytochrome and GA action. Two GA-related mutations (*ga1–3* and *gai*, representing GA-deficiency and GA-response mutations, respectively), and two phytochrome-related mutations (*hy1–1* and *phyB-1*, representing general and specific phytochrome-deficiency mutations, respectively), were chosen for our experiments. Here we describe the phenotypes displayed by *ga1–3 hy1*, *ga1–3 phyB-1*, *gai hy1*, and *gai phyB-1* double mutants with respect to seed germination, hypocotyl elongation, stem elongation (adult plant height), chlorophyll content, and floral fertility. In many respects, the phenotype displayed by these double mutants is more similar to that displayed by the GA-related (single) mutants than it is to the phytochrome (single) mutants, suggesting that GA-related mutations are epistatic to phytochrome-related mutations. However, other aspects of the phenotype of these double mutants are more similar to that displayed by phytochrome (single) mutants, indicating that there is no simple relationship between the phytochromes and the GAs in the regulation of plant growth.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The mutant lines *gai*, *ga1–3*, *hy1–1*, and *phyB-1* were obtained from M. Koornneef (Wageningen Agricultural University, The Netherlands) and are all derived from the Landsberg *erecta* laboratory strain. *ga1–3* is thought to be a null mutation, because there is an approximately 5-kb deletion at the *GA1* locus in the *ga1–3* line (Sun et al., 1992; Sun and Kamiya, 1994). *ga1–3* mutants require exogenous GAs for germination, display a male-sterile, dwarf phenotype, and have greatly reduced levels of endogenous bioactive GAs (Sun et al., 1992; Sun and Kamiya, 1994). As described above, the semidominant *gai* mutation confers an impaired GA-response dwarf phenotype and is thought to identify a gene involved in GA reception or signal transduction (discussed in Peng and Harberd, 1993). *hy1–1* has an apparent defect in phytochrome chromophore biosynthesis and therefore is thought to be functionally deficient for all phytochromes (Parks and Quail, 1991). The *phyB-1* mutation is thought to be a null mutation because of the presence of a translational stop codon in the PHYB coding

region (Reed et al., 1993; Bradley et al., 1995). In subsequent sections of this paper *ga1–3*, *hy1–1*, and *phyB-1* mutations are referred to as *ga1*, *hy1*, and *phyB*, respectively. For plants grown in soil, seeds were first chilled (4°C) on wet filter paper in a Petri dish for 4 to 7 d to break dormancy and then sown onto "Arabidopsis Mix" (2 parts Levington's M3 potting compost: 1 part grit/sand). In most cases the plants were grown in standard greenhouse conditions using natural light and a photoperiod at about 23°C. For plant height and chlorophyll content determinations, plants were grown in controlled environment chambers with a photoperiod of 16 h in light and 8 h in darkness at 23°C. To allow *ga1*, *ga1 hy1*, and *ga1 phyB* double mutants to germinate, seeds were chilled on filter paper soaked in a 1×10^{-4} M GA_3 solution.

Selection of Double Mutants

The *ga1 hy1*, *ga1 phyB*, *gai hy1*, and *gai phyB* double mutants were selected from progenies of corresponding crosses. For *ga1 hy1* and *ga1 phyB* double mutants, F_2 individuals displaying elongated hypocotyls and requiring exogenous GA for male fertility were identified and seeds were collected. All F_3 seedlings displayed a requirement for exogenous GA_3 for germination and an elongated hypocotyl (following treatment with GA_3), confirming that these lines are homozygous for *ga1 hy1* or *ga1 phyB*. For the *gai hy1* and *gai phyB* double mutants, F_2 individuals displaying elongated hypocotyls and also conferring semidwarfism were identified. An examination of the self-pollination (F_3) progeny revealed that the F_2 plants were homozygous for *hy1* or *phyB*, but heterozygous for *gai/GAI*. Double-mutant homozygotes were identified in the F_3 populations. The genotypes of these double-mutant lines were confirmed in subsequent generations. All progenies were uniform and did not display segregation for either single mutant phenotype.

Hypocotyl Length and Plant Height Measurement

Hypocotyl length was measured against a ruler. Mature plant height was measured from the base of the plant (aerial parts) to the tip using a cotton thread.

Chlorophyll Content Determinations

Chlorophyll content was determined essentially as described by Chory (1992). Healthy leaves of 21-d-old plants were collected and ground up in an Eppendorf tube, using a plastic pestle. One milliliter of 80% acetone was added and the tube was rapidly wrapped in metal foil and then shaken for 30 min. The debris was then pelleted by centrifugation. The optical densities of the supernatant at 645 and 663 nm were determined (A_{645} and A_{663}) and used to calculate the chlorophyll concentration according to the formula chlorophyll (a + b) = $20.21(A_{645}) + 8.22(A_{663})$.

RESULTS

The Seed Germination Phenotype Conferred by *ga1* Is Epistatic to That Conferred by *hy1* and *phyB*

The germination ability of seeds homozygous for *ga1*, *gai*, *hy1*, *phyB*, *ga1 hy1*, *ga1 phyB*, *gai hy1*, and *gai phyB* was compared with that of the wild-type control (Landsberg *erecta*, wild type), following chilling in the presence or absence of exogenous GA_3 (Fig. 1). As expected, the *ga1* mutant seed germinated very poorly following chilling in the absence of GA_3, and germination was restored (in this experiment to approximately 70%) following chilling in the presence of GA_3 (Koornneef and Van der Veen, 1980). Germination of the other single mutant lines (*gai*, *hy1*, and *phyB*) was not significantly different from that of wild type (more than 90%). Double mutants containing *ga1* (*ga1 hy1* and *ga1 phyB*) failed to germinate following chilling in the absence of exogenous GA_3, and germination was restored to the level observed in the *ga1* single mutant as a result of GA_3 treatment. Thus, homozygosity for *hy1* or for *phyB* does not suppress the impaired seed germination conferred by *ga1*.

gai and *ga1* Antagonize the Effects of *hy1* and *phyB* on Hypocotyl Elongation

The hypocotyl elongation of seedlings homozygous for *gai*, *hy1*, *phyB*, *gai hy1*, and *gai phyB*, grown in white light, was compared with that of the wild type. As expected, the *hy1* and *phyB* mutants exhibited hypocotyls that were more than twice the length of those of the wild type (Koornneef et al., 1980), and *gai* mutant hypocotyls were approximately 60% of the length of the wild type (Fig. 2). The hypocotyl length of the *gai hy1* and *gai phyB* double mutants was

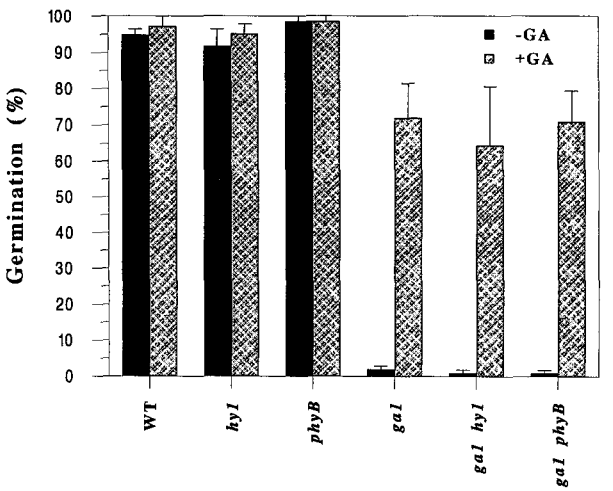

Figure 1. The effect of exogenous GA on the germination of *ga1 hy1* and *ga1 phyB* double mutants, parental controls, and the wild type (WT). Seeds were chilled on filter paper soaked either with water or 10^{-4} M GA_3 for 7 d at 4°C and then sown on soil without further GA treatment. The number of germinated and nongerminated seeds was recorded 7 d after sowing. The percentage germination is shown. Results are presented as mean (error bars represent SE) of three separate experiments (n = 24–48 for each experiment).

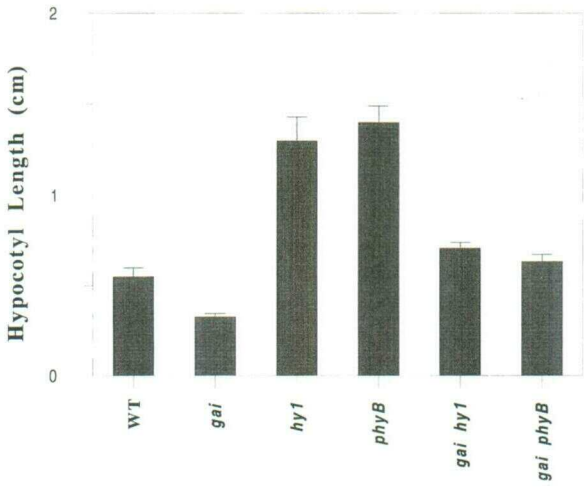

Figure 2. Comparison of hypocotyl lengths of 14-d-old *gai hy1* and *gai phyB* double-mutant seedlings, parental controls, and the wild type (WT) (n = 10–18). Results are presented as means with error bars representing SE.

intermediate between that of *gai* and that of *hy1* or *phyB* and was close to that of the wild-type control (Fig. 2). Additional experiments showed that *gai* hypocotyl elongation is unaffected by exposure to 10^{-4} M GA_3, a GA concentration above the saturation point for stimulation of *ga1* hypocotyl elongation (data not shown; Reed et al., 1996). Thus, hypocotyl elongation of *gai hy1* and *gai phyB* double mutants is greater than can be elicited by GA in the *gai* single mutant.

ga1 phyB and *ga1 hy1* double mutants displayed hypocotyl lengths that were intermediate between those of *ga1* and *hy1* or *phyB* (data not shown). Because the *ga1* containing double mutants needed exogenous GA_3 to achieve germination, we cannot be sure that the hypocotyl growth observed was not due to residual GA_3. However, these experiments suggest that, at the stage of hypocotyl elongation, a normally functioning GA system is required for full expression of the phenotype due to phytochrome deficiency. Similar conclusions were obtained following experiments using the *lh* mutant of cucumber and a *phyB* mutant of Arabidopsis (López-Juez et al., 1995; Reed et al., 1996).

GA-Related and Phytochrome-Related Mutations Have Additive Effects on Young Seedling Leaf Expansion

The cotyledons of the *hy1*, *phyB*, *ga1*, and *gai* mutants are all smaller than that of the wild type (data not shown; see also Neff and van Volkenburgh, 1994). The cotyledons of all of the double mutants, *ga1 hy1*, *ga1 phyB*, *gai hy1*, and *gai phyB*, were much smaller than those of the wild type and were also smaller than those of any of their parental controls (data not shown). This result suggests that the GA

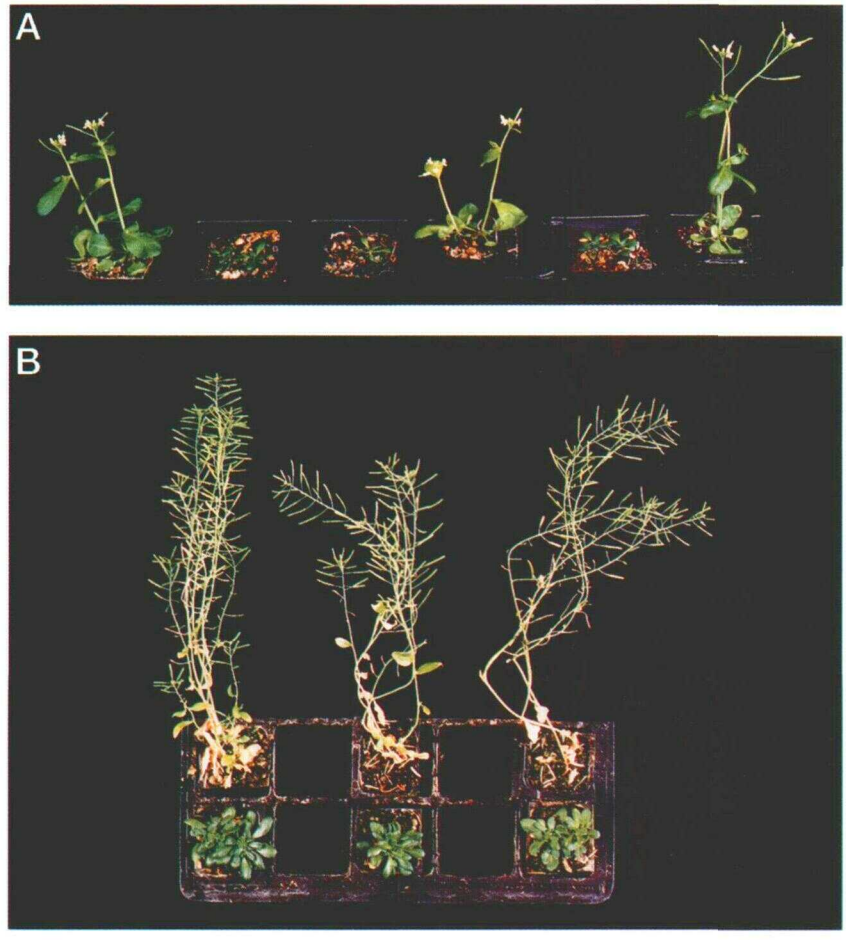

Figure 3. Comparison of wild-type, *ga1*, *ga1 hy1*, and *ga1 phyB* plants. A, Thirty-five-day-old plants (side view), left-to-right: WT, *ga1*, *ga1 hy1*, *hy1*, *ga1 phyB*, and *phyB*. B, Sixty-five-day-old plants. Top row, left-to-right: wild type, *hy1*, and *phyB*. Bottom row, left-to-right: *ga1*, *ga1 hy1*, and *ga1 phyB*.

system and the phytochrome system have additive effects on the control of cotyledon expansion.

The seedling rosette leaves of the *ga1* and *gai* mutants are smaller and narrower than those of the wild type, *phyB* and *hy1* (Koornneef and van der Veen, 1980; Figs. 3A and 4A). The early seedling rosette leaves of the *ga1 hy1*, *ga1 phyB*, *gai hy1*, and *gai phyB* double mutants were much smaller than those of the *hy1* and *phyB* parental controls, and slightly less expanded than those of the *ga1* and *gai* controls (Figs. 3A and 4A). At later stages of development, the mature rosette leaves of the double mutants had expanded to sizes that were indistinguishable from that of the *ga1* or *gai* controls (Figs. 3B and 4B). Thus, homozygosity for *hy1* or *phyB* does not suppress the adult leaf size phenotype conferred by *ga1* or *gai*. At earlier stages of leaf development the combined effects of phytochrome system and GA system deficiencies appear to result in smaller and narrower leaves than are seen in the parental controls.

ga1 and *gai* Are Fully and Partially Epistatic to *hy1* and *phyB*, Respectively, in Controlling Stem Elongation Growth

In our experiments mature *phyB* mutant plants were taller than *hy1* and wild-type plants. Wild type, *hy1*, and *phyB* were all more than twice the height of *gai*, whereas *ga1* bolt stems never elongated significantly (Figs. 3B and 5). Although there were clear differences between the *gai phyB* and *gai* young seedlings (*gai phyB* had longer petioles and bolted earlier than *gai*, Fig. 4A), the mature *gai phyB* plants were only 30% taller than the *gai* plants and only one-half of the height of the *phyB* plants (Fig. 5). Furthermore, the mature *gai hy1* plants were approximately 18% taller than the mature *gai* plants (Fig. 5). Thus, *gai* substantially suppresses the effects of *hy1* and *phyB* on mature plant height.

The mature plant phenotype of the *ga1 hy1* and *ga1 phyB* mutants was indistinguishable from that of the *ga1* mutant control plants. All of these genotypes confer very short bolt stems (Fig. 3B). Thus, *ga1* is epistatic to *hy1* and *phyB* in its effects on mature plant height.

hy1 Is Epistatic to *ga1* and *gai* in Regulating Chlorophyll Accumulation

Previous experiments have shown that the chlorophyll content of the *hy1* mutant is less than that of the wild type (Chory et al., 1989). This reduced chlorophyll content is manifest in the yellow-green appearance of *hy1* mutant plants (Figs. 3A and 4A). Cotyledons of *ga1 hy1* and *gai hy1* double mutants are also yellow-green and are closer in coloration to those of the *hy1* parent than they are to the dark-green cotyledons that are characteristic of the *ga1* or *gai* parent (data not shown). At later stages of develop-

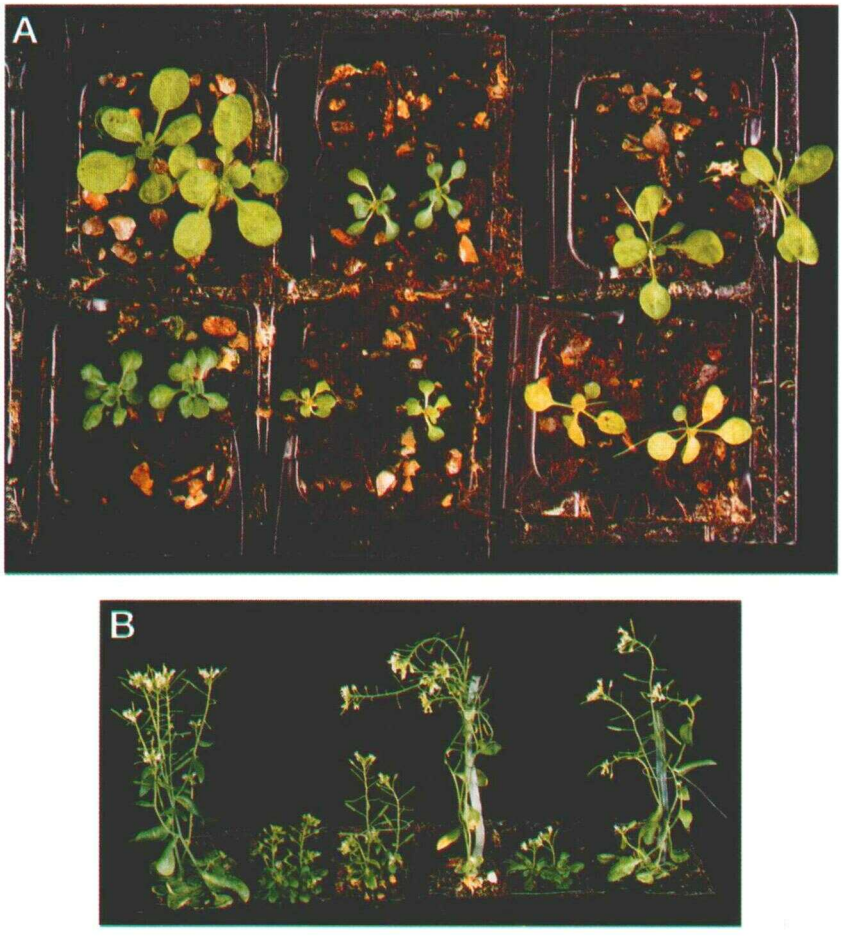

Figure 4. Comparison of wild-type, *gai*, *gai hy1*, and *gai phyB* plants. A, Twenty-one-day-old plants. Top row, left to right: wild type, *gai phyB*, and *phyB*. Bottom row, left to right: *gai*, *gai hy1*, and *hy1*. B, Forty-nine-day-old plants, left to right: wild type, *gai*, *gai phyB*, *phyB*, *gai hy1*, and *hy1*.

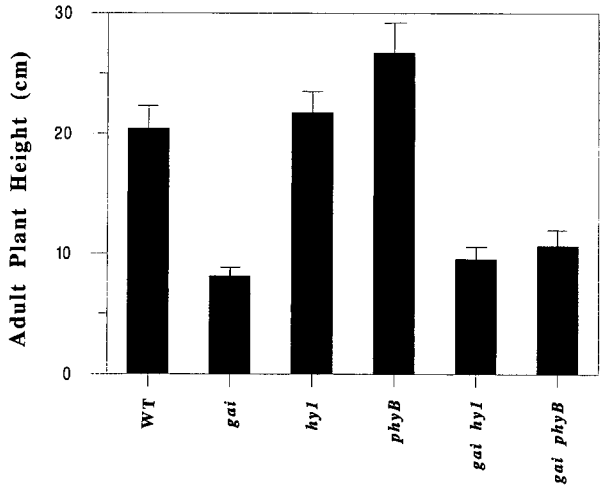

Figure 5. Comparison of adult plant heights (65–80 d old, after cessation of growth) of *gai hy1* and *gai phyB* double mutants, together with parental and wild-type (WT) controls. Results are presented as means, with error bars representing SE (n = 30–35).

ment, the young seedling rosette leaves of *ga1 hy1* and *gai hy1* were still visibly paler than those of *ga1* and *gai* controls. To confirm these observations, chlorophyll content determinations were performed on 21-d-old plants. These experiments showed that *ga1 hy1* and *gai hy1* plants contained significantly less chlorophyll than did *ga1* or *gai* controls (Fig. 6). Thus, in the regulation of chlorophyll accumulation in 21-d-old plants, the effects of a deficiency in phytochrome action appear to be epistatic over the effects of a deficiency in GA action. However, it is possible that the presumed deficiency in tetrapyrrole biosynthesis in the *hy1* mutant may have effects additional to the ones on phytochrome function, and that the yellow-green color is attributable to a direct effect on chlorophyll biosynthesis, rather than to reduced phytochrome function.

The *phyB* mutant also has a lower chlorophyll content than the wild type, although not as low as in *hy1*. *ga1 phyB* and *gai phyB* double mutants have a significantly lower chlorophyll content than the *gai* control (see Fig. 6), indicating that a deficiency for phytochrome B can significantly reduce the high chlorophyll contents conferred by *ga1* or *gai*.

ga1 Is Epistatic to *hy1* and *phyB* in Its Effects on Floral Fertility

ga1 mutant flowers are infertile because of impaired anther development. This sterility can be overcome by exogenous GA treatments (Koornneef and Van der Veen, 1980). *gai*, *hy1*, and *phyB* flowers are relatively fertile. In our experiments the *ga1 hy1* and *ga1 phyB* mutants were as infertile as the *ga1* controls (Fig. 3B; note the lack of fruit-bearing inflorescence stems in all plants homozygous for *ga1*). Thus, *ga1* is completely epistatic to *hy1* and *phyB* in its effects on floral fertility. As expected, the fertility of the *ga1 hy1* and *ga1 phyB* mutants was restored by exogenous GA treatments (data not shown).

DISCUSSION

This paper describes the experiments we used to investigate the possible relationship between GA action and phytochrome action using GA-related and phytochrome-related mutants of Arabidopsis. The results of these epistasis experiments are complex, and suggest that the relationship between GA action and phytochrome action may be different at different developmental stages. For seed germination, mature plant height, and floral fertility the effects of the GA deficiency conferred by *ga1* were absolutely epistatic to any effect of the phytochrome deficiencies conferred by *phyB* or *hy1*. Thus, in these cases GA deficiency completely masks the effect of phytochrome deficiency. For hypocotyl elongation GA deficiency (or reduced GA responses) and phytochrome deficiency elicit opposite effects. Thus, light-grown, phytochrome-deficient mutant hypocotyls are taller than the wild type, whereas light-grown, GA-deficient (*ga1*), or reduced GA response (*gai*) mutant hypocotyls are shorter than the wild type. The *gai hy1* and *gai phyB* double-mutant hypocotyls were intermediate in length. The observation that *gai hy1* and *gai phyB* hypocotyls are longer than the *gai* hypocotyls treated with saturating concentrations of GA_3 indicates that, for hypocotyl elongation, phytochrome effects are mediated by factors additional to the GA system. For cotyledon expansion, the effects of GA deficiency (or reduced GA responses) and phytochrome deficiency were additive. *ga1*, *gai*, *hy1*, and *phyB* all individually confer a reduction in cotyledon expansion, and pairwise combinations of GA-related and phytochrome-related mutations resulted in a more severe reduction in cotyledon expansion than was seen in any of the single mutants. Finally, the chlorophyll content of the *ga1 hy1* and *gai hy1* double mutants, at the cotyledon and young seedling leaf stages, was more similar to that of *hy1* single mutants than to that of *ga1* and *gai* single mutants.

The *hy1* and *phyB* mutations differ with respect to their effects on the phytochrome system, in that *hy1* confers a

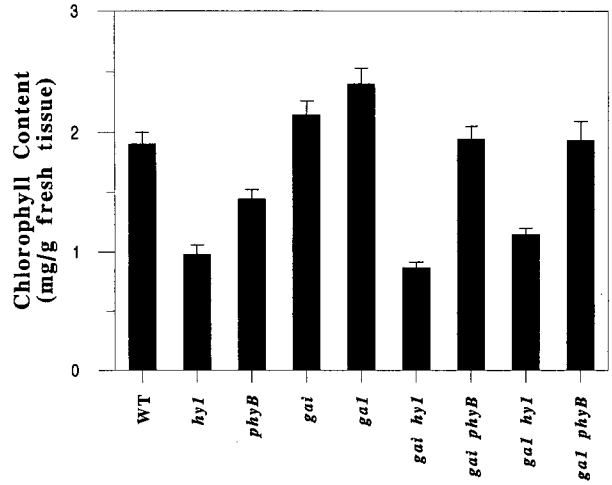

Figure 6. Chlorophyll contents of *ga1 hy1*, *ga1 phyB*, *gai hy1*, and *gai phyB* double mutants, together with parental and wild-type (WT) controls. Results are presented as means (with error bars representing SE) of three separate experiments.

global phytochrome deficiency, whereas *phyB* confers a specific deficiency for phytochrome B. For most of the aspects of the phenotype studied in this paper, the effects conferred by *hy1* were similar to those conferred by *phyB*, confirming that phytochrome B plays a predominant role in the regulation of growth and development of plants grown in white light (Reed et al., 1993).

Recent work has identified Arabidopsis mutations (e.g. *spy* alleles) that suppress the effects of *ga1* and/or *gai* mutations on a phenotype (Jacobsen and Olszewski, 1993; Carol et al., 1995; Wilson and Somerville, 1995; Jacobsen et al., 1996). Despite certain similarities in the phenotypes that they confer, such as a tendency toward enhanced elongation growth, it is clear that the phytochrome-deficient mutations and the *spy* mutations belong to fundamentally different categories. For example, *spy* alleles can suppress the GA requirement of *ga1* mutants for germination (Jacobsen and Olszewski, 1993). As shown above, neither *hy1* nor *phyB* can suppress this effect of *ga1*.

It is clear that our results are too complex to permit the construction of a simple information flow pathway linking the phytochrome and GA systems. It is entirely possible that phytochrome and GA systems influence physiological processes via independent pathways. However, the most obvious property of adult phytochrome-deficient, GA-deficient double mutants is that they exhibit the dwarfism characteristic of GA deficiency, rather than the elongated growth characteristic of phytochrome deficiency. It is clear that a functional GA system is necessary if the full phenotypic effects of phytochrome deficiency are to be expressed.

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