The New *RGA* Locus Encodes a Negative Regulator of Gibberellin Response in *Arabidopsis thaliana*

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ABSTRACT

We have identified a new locus involved in gibberellin (GA) signal transduction by screening for suppressors of the *Arabidopsis thaliana* GA biosynthetic mutant *ga1-3*. The locus is named *RGA* for repressor of *ga1-3*. Based on the recessive phenotype of the digenic *rga/ga1-3* mutant, the wild-type gene product of *RGA* is probably a negative regulator of GA responses. Our screen for suppressors of *ga1-3* identified 17 mutant alleles of *RGA* as well as 10 new mutant alleles at the previously identified *SPY* locus. The digenic (double homozygous) *rga/ga1-3* mutants are able to partially repress several defects of *ga1-3* including stem growth, leaf abaxial trichome initiation, flowering time, and apical dominance. The phenotype of the trigenic mutant (triple homozygous) *rga/spy/ga1-3* shows that *rga* and *spy* have additive effects regulating flowering time, abaxial leaf trichome initiation and apical dominance. This trigenic mutant is similar to wild type with respect to each of these developmental events. Because *rga/spy/ga1-3* is almost insensitive to GA for hypocotyl growth and its bolting stem is taller than the wild-type plant, the combined effects of the *rga* and *spy* mutations appear to allow GA-independent stem growth. Our studies indicate that *RGA* lies on a separate branch of the GA signal transduction pathway from *SPY*, which leads us to propose a modified model of the GA response pathway.

GIBBERELLINS (GAs) are a family of diterpenoid compounds, some of which are bioactive hormones that control a wide variety of growth and developmental responses including seed germination, stem elongation and flower development. Changes in both GA concentration and/or tissue sensitivity can mediate these events (DAVIES 1995). However, the molecular mechanisms by which the GA signal is transduced into morphological and biochemical changes in plants are largely unknown.

Most studies of GA receptors and GA action have focused on the cereal aleurone system (JONES and JACOBSEN 1991; HOOLEY 1994; DAVIES 1995). Several results suggest that GA receptors are located on the external face of the aleurone plasma membrane (HOOLEY *et al.* 1991; GILROY and JONES 1994). However, the specific GA binding proteins have not been purified.

Another approach to examine GA perception and response has been to identify mutants affecting these processes. GA-response mutants isolated from barley, maize, garden pea, rice, tomato and *Arabidopsis thaliana* fall into two phenotypic categories: elongated slender mutants and GA-unresponsive dwarf mutants (reviewed in TAKAHASHI *et al.* 1991; HOOLEY 1994; ROSS 1994; SWAIN and OLSZEWSKI 1996). The slender mutants show constitutive activation of their GA response, and the dwarf mutants are deficient in their GA perception or signal transduction.

The recessive slender mutants, *la cry*^{s} in pea (POTTS *et al.* 1985) and *sln* in barley (STODDART and LLOYD 1986), behave as if saturated with GAs and are unresponsive to either exogenously added GA or GA biosynthesis inhibitors that retard stem elongation in wild-type plants (POTTS *et al.* 1985). A second class of recessive slender mutants, which consists of the *spy* mutant of Arabidopsis and the *pro* mutant of tomato, is still responsive to applied GA (JONES 1987; JACOBSEN and OLSZEWSKI 1993), suggesting that their gene products may negatively regulate a branch of the GA signal transduction pathway (JACOBSEN and OLSZEWSKI 1993). A new slender pea mutant (*sln*) was isolated recently (REID *et al.* 1992). Instead of affecting GA signal transduction, this mutant appears to block deactivation reactions of GA, including 2β-hydroxylation, which allows the accumulation of GA_{20}, the precursor of bioactive GA_1, in developing seeds (ROSS *et al.* 1995).

The GA-unresponsive dwarf mutants, which include *D8, D9* and *Mpl1* in maize (PHINNEY 1956; FUJIOKA *et al.* 1988; HARBERD and FREELING 1989; WINKLER and FREELING 1994) and *gai* in Arabidopsis (KOORNNEEF *et al.* 1985; TALÓN *et al.* 1990), are semi-dominant mutants whose phenotype resembles leaky GA biosynthesis dwarf mutants. However, the former mutants do not respond to exogenous GA treatment for stem elongation and accumulate high levels of bioactive GA_1.

The two GA-response Arabidopsis mutants, *gai* and

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spy, have been characterized in detail (JACOBSEN and OLSZEWSKI 1993; PENG and HARBERD 1993; CAROL *et al.* 1995; WILSON and SOMERVILLE 1995; JACOBSEN *et al.* 1996). Although the *gai* mutant is insensitive to exogenous GA, germination of *gai* seeds can be prevented by paclobutrazol, an inhibitor of GA biosynthetic enzymes (A. L. SILVERSTONE and T-p. SUN, unpublished data). This suggests that *gai* has a reduced response to GA, and the "GA-unresponsive" phenotype is probably due to saturating levels of endogenous GAs in the mutant. Intragenic *gai* suppressor mutants that revert the dwarf phenotype to wild-type were isolated, and they appear to result from loss-of-function mutations at the *GAI* locus (PENG and HARBERD 1993). This result suggests that the *GAI* locus encodes a redundant component of the GA signal transduction pathway (PENG and HARBERD 1993).

The original *spy* mutants were isolated based on their ability to germinate in the presence of 120 μM paclobutrazol, a GA biosynthesis inhibitor (JACOBSEN and OLSZEWSKI 1993). They resemble wild-type plants that have been treated with exogenous GA. Epistasis studies indicate that *SPY* is downstream of, and on the same pathway as, *GAI* (JACOBSEN *et al.* 1996). Because *spy* mutants partially suppress all the phenotypic defects of the GA biosynthetic mutant, *ga1-2*, *SPY* is likely located early in a GA response pathway before it branches to control individual developmental events (JACOBSEN and OLSZEWSKI 1993). The recessive phenotype of the *spy* mutants indicates that SPY negatively regulates the GA signal transduction pathway (JACOBSEN and OLSZEWSKI 1993).

To help elucidate the GA signal transduction pathway, our laboratory has set out to isolate new Arabidopsis mutants affecting the GA response pathway. Our goal was to identify other loci involved in transduction of the GA signal to its cellular sites of action. We also employed a different selection screen than that used to isolate the original *spy* mutants (JACOBSEN and OLSZEWSKI 1993). Our approach was to select for suppressors of mutants in the *GA1* gene that encodes *ent*-kaurene synthase A. This enzyme catalyzes the first committed step in the GA biosynthetic pathway (SUN and KAMIYA 1994). The *ga1-3* mutant contains a 5-kb deletion at the *GA1* locus (SUN *et al.* 1992) and exhibits a non-germinating, male-sterile dwarf phenotype, which can be converted to wild type by repeated application of GA (KOORNNEEF and VAN DER VEEN 1980). By directly selecting for extragenic suppressor mutants of *ga1-3* from an EMS-mutagenized population of *ga1-3* seeds, we have isolated a new class of GA-response mutants that maps at a new locus, designated *RGA* for repressor of *ga1-3*. Ten new alleles of *spy* were also isolated in our suppressor screen. We have analyzed the physiological characteristics of the digenic mutants *rga/ga1-3* and *spy/ga1-3*, and the trigenic mutant *rga/spy/ga1-3* in comparison to the *ga1-3* mutant and the wild-type ecotype Landsberg *erecta* (Ler). Our results indicate that the *RGA* locus regulates a separate branch in the GA signal transduction pathway from the one defined by *GAI* and *SPY*.

MATERIALS AND METHODS

Mutagenesis and selection of suppressor mutants of *ga1-3*: Fifty-six thousand seeds of the *ga1-3* deletion mutant were mutagenized with EMS as described previously (KOORNNEEF and VAN DER VEEN 1980), treated with 100 μM GA_3 to allow for germination and rinsed thoroughly before planting. M_1 plants were allowed to self-pollinate, and their seeds were collected in 25 separate pools. Twenty thousand M_2 seeds from each pool were pretreated with 100 μM GA_3 to permit germination, and M_2 plants that grew taller and/or displayed normal flower development without further GA treatment were identified and their seeds collected. The mutant phenotype was verified in the M_3 plants. The suppressor mutants were then backcrossed once with the wild-type Ler before measurement of mutant phenotypes.

To confirm that the suppressor mutants were not wild-type contaminants, we devised a PCR test involving two pairs of primers to determine the genotype at the *GA1* locus. A pair of primers were designed to detect the presence of wild-type *GA1* sequence missing in the *ga1-3* deletion mutant (primer 9, 5′-TTTGGCCCAACACACAAACCTT-3′ and primer 10, 5′-AAGCTTCGAACTCAAGGTTCTA-3′). Primers 9 and 10 will amplify a 1.2-kb DNA fragment using wild-type Ler DNA, but will not amplify *ga1*-3 DNA, because the primer sequences are located within the deleted region in the *ga1-3* mutant. A second pair of primers that flanks the 5-kb deletion of the *ga1-3* allele (primer 25, 5′-TGTATGCACGTTAACGATCAAT-3′ and primer 34, 5′-TTTCTTCATACCACCTGCGTTC-3′) will amplify a 0.8-kb DNA fragment using *ga1-3* DNA. Under standard PCR conditions, wild-type *GA1* DNA will not be amplified efficiently by primers 25 and 34 as the product would be ~6 kb. Because we crossed our class II mutants with *spy-4/ga1-2*, a third pair of primers was used to distinguish the *ga1-2* allele from the *ga1-3* allele (primer 10 and primer 14, 5′-ATCGATCAATGCAACCCAAGCT-3′). This primer pair will amplify a 0.6-kb DNA fragment from Ler or *ga1-2* DNA, but will not amplify anything from *ga1-3* DNA. The typical PCR was carried out in a 10 μl volume with 2 ng of genomic DNA, 1.5 mM $MgCl_2$, 250 μM dNTP, 40 ng/μl of each of the primers, and 1 U AmpliTaq polymerase (Perkin Elmer). The PCR thermocycler settings are 40 cycles of 94°, 30 sec; 55°, 30 sec; 72°, 1–2 min, followed by one cycle of 72°, 10 min.

Germination test: Approximately 250 seeds of each genotype were washed with sterile water, cold-treated for 3 days and then sown in petri plates on moist filter paper. The seeds were incubated in continuous light, 250 μE, at 22°. The germination percentage was measured after 7 days.

Plant growth conditions and measurements: Seeds were stratified for 3 days in the cold. Because *ga1-3* and *rga/ga1-3* mutants require GA treatment for germination, they were incubated with 100 μM GA_3 during stratification, and the seeds were rinsed thoroughly with water before planting. The plants were grown at 22° under 16 hr light and 8 hr dark cycles. For the GA responsiveness growth curve, seedlings were grown at 22° under continuous light, 250 μE, for 7 days on media containing Murashige-Skoog salts (GIBCO BRL) with the various GA_3 concentrations. Bolting stem, pedicel and silique length measurements were made with a ruler, and hypocotyl length was determined by a caliper.

Cell length measurements: Epidermal peels were made of the first few internodes of the bolting stem, or impressions of hypocotyls were made using QuickTite superglue (Loctite). The tissues were then stained with 0.05% toluidine blue. Cell lengths were measured using a Diaplan compound microscope (Leitz) at 125× magnification, equipped with a camera lucida (Leitz) and calibrated with a stage micrometer.

Mapping of the *RGA* locus: The *rga-2/ga1-3* mutant, which is in the Ler ecotype, was crossed to a wild-type, Columbia (Col) ecotype, plant. The F_1 progeny was allowed to self, and

we selected for the digenic *rga-2/ga1-3* mutants in the F_2 generation of this cross because we have not identified the phenotype of *rga* in the wild-type *GA1* background. The *GA1* locus maps to position 16.6 of chromosome IV. The cleaved amplified polymorphic sequence (CAPS) technique was employed for mapping the *RGA* locus (KONIECZNY and AUSUBEL 1993).

RESULTS

Isolation of *ga1-3* suppressor mutants: To dissect further the GA signal transduction pathway, we initiated a screen to isolate new mutants of Arabidopsis that would be affected in other loci involved in GA responses. Considering the pleiotropic nature of GA's effects, we chose to isolate tissue- or response-specific mutants, in addition to mutants affecting all GA responses, to identify both common elements and individual branches of the GA signal transduction pathway. By selecting directly for suppression of stem growth retardation or flowering defects (absence of white petals and male sterility) instead of restoration of the nongermination phenotype of the *ga1-3* mutant, we hoped to identify new genes that are tissue- or response-specific and differ from the previously characterized *SPY* locus. Our screen would also avoid the selection of GA-independent germination mutants involved in abscisic acid biosynthesis (KOORNNEEF *et al.* 1982; LÉON-KLOOSTERZIEL *et al.* 1996).

Whereas previous screens utilized a wild-type genetic background to identify elongated mutants (JACOBSEN and OLSZEWSKI 1993) or GA-insensitive dwarf mutants (KOORNNEEF *et al.* 1985), we carried out our screens with the *ga1-3* mutant. Since this mutant has extremely low levels of GAs (ZEEVAART and TALÓN 1992), it might be more sensitive to GA-independent growth. Because the *ga1-3* allele contains a 5-kb intragenic deletion, the isolation of intragenic suppressor mutants was avoided (SUN *et al.* 1992). We mutagenized *ga1-3* with EMS and screened for mutants in the M_2 generation that would suppress all or part of the *ga1-3* phenotype (male sterile, extreme dwarf). The seeds in each generation were pretreated with 100 μM GA_3 to allow for germination. Putative mutants were then rescreened in the M_3 generation to confirm the suppressor phenotype. To eliminate any wild-type contaminants, we designed two sets of PCR primers, one pair that would only amplify the *GA1* allele and a second pair that would only amplify the *ga1-3* allele.

Two phenotypic classes of suppressor mutants of *ga1-3* were identified. Class I mutants are still male-sterile, but are able to bolt and demonstrate stem elongation in the absence of GA (Figure 1A). GA application restores fertility and further stimulates stem growth (Figure 1E). Class II mutants partially suppressed all defects of *ga1-3* including the requirement for GA treatment to induce germination, stem elongation, and male fertility (Figure 1A). Stem growth of class II mutants is also responsive to GA application. Mutants from the same seed lot were considered siblings. We found at least 17 independent class I mutants and 10 independent class II mutants among the M_2 plants screened.

The *rga* mutations are recessive and allelic: We named the class I mutants *rga*, for repressor of the *ga1-3* mutant, and numbered them *rga-1* through *rga-17*. To determine whether the class I mutants were dominant or recessive, we backcrossed eight digenic lines (*rga-1/ga1-3, rga-2/ga1-3, rga-3/ga1-3, rga-5/ga1-3, rga-9/ga1-3, rga-10/ga1-3, rga-11/ga1-3* and *rga-12/ga1-3*) to *ga1-3*, using *ga1-3* as the pollen donor. The F_1 progeny of each cross were phenotypically identical to *ga1-3*. This indicates that these mutations are all recessive. In the F_2 population from the backcross of *rga-2/ga1-3* with *ga1-3*, 50 of 198 plants exhibited the class I phenotype [χ^2 (3:1) = 0.0068, $P > 0.9$], confirming that *rga-2/ga1-3* contains a recessive mutation at a single locus. *rga-1/ga1-3* through *rga-17/ga1-3* were tested for allelism by crossing each with *rga-2/ga1-3*, except *rga-3/ga1-3* was crossed to *rga-5/ga1-3* while *rga-4/ga1-3* and *rga-7/ga1-3* were crossed to *rga-12/ga1-3*. The F_1 generation of all crosses had the class I phenotype. Thus, all 17 *rga* mutants appear to be allelic.

We performed similar analyses with the class II mutants. Because of the phenotypic similarities between our class II mutants and the previously identified *spy* mutant, we crossed two of the class II lines with *spy-4/ga1-2* (JACOBSEN *et al.* 1996). Interestingly, the F_1 seeds from these crosses were often viviparous. All F_1 plants were phenotypically similar to the parental lines. We used primers specific for the different *ga1* alleles to identify the presence of both the *ga1-3* and the *ga1-2* alleles in the F_1 progeny, confirming that the cross was successful. Therefore, these two new class II mutants we isolated were each allelic to *spy*, and they were designated *spy-13/ga1-3* and *spy-14/ga1-3*. The rest of the class II mutants were crossed to *spy-13/ga1-3*. All the F_1 plants had the class II phenotype. Thus, all the class II mutants appear to be allelic to *spy*, and our alleles are subsequently numbered *spy-8/ga1-3* through *spy-17/ga1-3*.

To determine that *RGA* was a distinct locus from *SPY*, we crossed *rga-2/ga1-3* with four different *spy/ga1-3* lines (*spy-8/ga1-3, spy-9/ga1-3, spy-12/ga1-3* and *spy-14/ga1-3*). In the F_1 generation, all the plants had a *ga1-3* phenotype. This showed that indeed they were discrete loci. From the F_2 generation of two separate crosses, we isolated the trigenic mutants *rga-2/spy-8/ga1-3* and *rga-2/spy-9/ga1-3* based on the phenotypes described below (Figure 1, B and D). In addition, the trigenic mutants are dramatically taller than either *rga/ga1-3* or *spy/ga1-3*.

The overall appearances of *rga/ga1-3, spy/ga1-3*, and *rga/spy/ga1-3* mutants are each readily distinguishable from the other two as well as from *ga1-3* and Ler. The *ga1-3* mutants are dark green compared to wild type and have compact leaves (Figure 1, A and C). *rga/ga1-3* and *spy/ga1-3* are intermediate in leaf color and size between Ler and *ga1-3* with *rga/ga1-3* being darker green than *spy/ga1-3* (Figure 1, A and C). The trigenic *rga/spy/ga1-3* has lighter colored leaves (Figure 1, B and

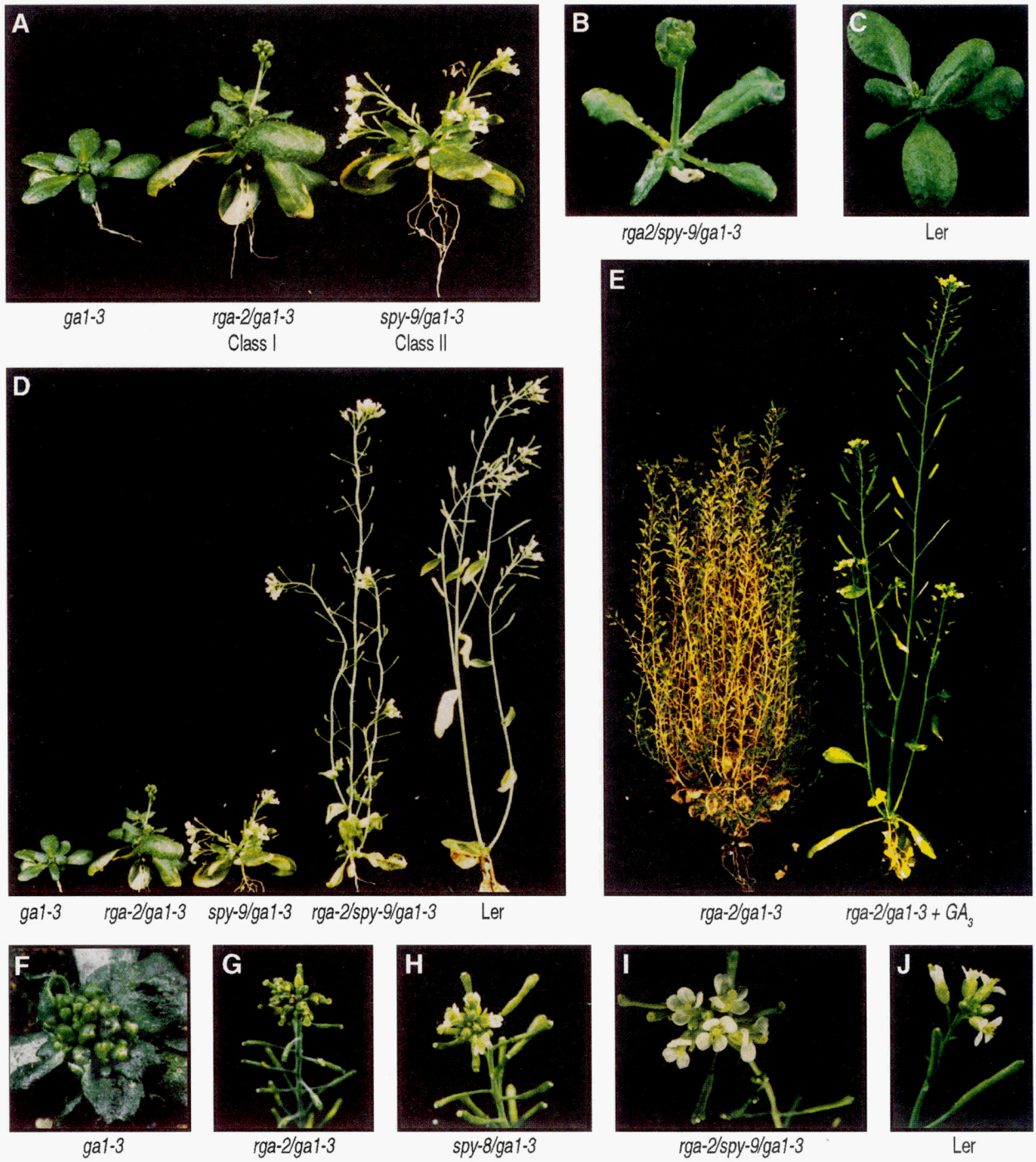

FIGURE 1.—Phenotype of the *rga/ga1-3*, *spy/ga1-3*, and *rga/spy/ga1-3* mutants in comparison to wild-type Ler and *ga1-3* mutants. (A and D) 35-day-old plants as labeled. (B) 19-day-old *rga/spy/ga1-3* mutant, note the lower left rosette leaf is folded and the cauline leaf is curled over the flower bud. (C) 19-day-old Ler plant. (E) Untreated 91-day-old *rga-2/ga1-3* mutant and 47-day-old *rga-2/ga1-3* mutant sprayed once with 100 μM GA_3. (F–J) Inflorescences of labeled plants.

C) and is taller than Ler. The leaves of *rga/spy/ga1-3* are also crinkly and folded inward as compared to Ler (Figure 1, B and C). This is similar in appearance to the leaves of *spy* in the wild-type *GA1* background (JACOBSEN and OLSZEWSKI 1993; WILSON and SOMERVILLE 1995).

We have initiated mapping of the RGA locus employing CAPS markers (KONIECZNY and AUSUBEL 1993). We analyzed 45 F_2 recombinant progeny using one set of CAPS primers for each chromosome: NCC1 (I), GPA1 (II), BGL1 (III), AG (IV) and DFR (V). The *RGA* locus shows linkage with the BGL1 marker (map position 71.8) on chromosome III and appears to be located ~35 cM south of BGL1.

To help determine the function of RGA, we have measured a number of the phenotypic characteristics with respect to *rga/ga1-3*, *spy/ga1-3*, and *rga/spy/ga1-3* in comparison to *ga1-3* and wild-type Ler. These include

TABLE 1

Germination percentage of Ler, *ga1-3* and GA-response mutants

Plant	Germination (%)
Ler	97.1
ga1-3	0–5.3
rga/ga1-3 mutants	
rga-1/ga1-3	7.9
rga-2/ga1-3	1.2
rga-3/ga1-3	4.9
spy/ga1-3 mutants	
spy-8/ga1-3	96.2
spy-9/ga1-3	96.8
rga/spy/ga1-3 mutants	
rga-2/spy-8/ga1-3	97.2
rga-2/spy-9/ga1-3	93.1

The germination percentage was determined for 160–250 seeds for each plant line.

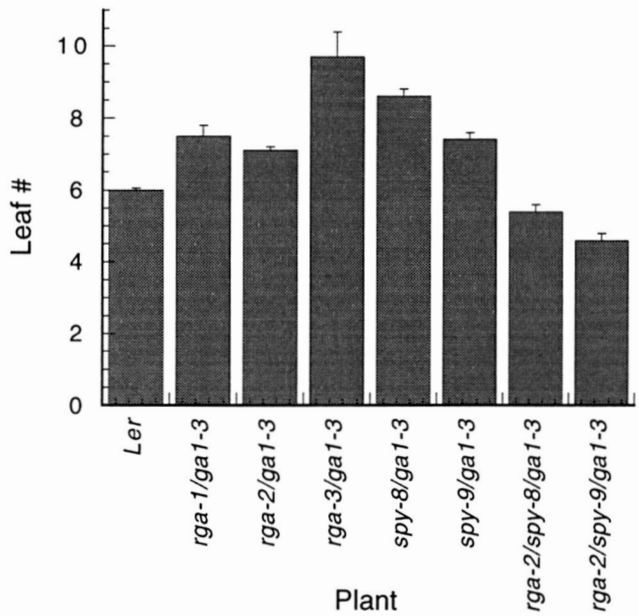

FIGURE 2.—Abaxial trichome appearance in *rga/ga1-3*, *spy/ga1-3*, and *rga/spy/ga1-3* mutants in comparison to wild-type Ler. The *ga1-3* mutant does not produce any trichomes on the abaxial surface of leaves. The first leaf where abaxial trichomes are present was measured for 10 plants from each group. The values plotted are means ± SE.

germination percentage, leaf abaxial trichome initiation, stem growth, flowering time, fertility, apical dominance and hypocotyl response to GA treatment. For all the measurements except germination and hypocotyl response to GA, the *ga1-3* and *rga/ga1-3* seeds were treated with GA_3 to allow for germination, and then the GA_3 was washed away before the seeds were planted.

Germination: Gibberellins are necessary for germination of Arabidopsis seeds as indicated by the nongerminating phenotype of the *ga1-3* mutant (Table 1). The germination rate of the *ga1-3* mutant can vary from 0 to 5.3% depending on the experiment. Germination percentages of *rga/ga1-3* mutant seeds are as low as that of the control *ga1-3* mutant seeds (Table 1). The slight leakiness seen in the *ga1-3* and *rga/ga1-3* mutants (1.2–7.9% germination) could be due to varying amounts of GA_3 carry over in the seeds, because the parental *ga1-3* and *rga/ga1-3* plants were sprayed with GA_3 to induce seed set. Alternatively, the leakiness could be caused by physical damage to the seed coats. Physical removal of the seed coat will substitute for the GA requirement for germination of *ga1-3* mutants. *ga1-3* or *rga/ga1-3* mutants that have had their seed coats mechanically removed grow into plants that are phenotypically similar to those whose seeds were pretreated with GA_3. The *spy/ga1-3* mutants restore the germination percentage to a wild-type level as has been shown previously (Table 1 and JACOBSEN and OLSZEWSKI 1993). The trigenic mutants *rga/spy/ga1-3* germinate at the same level as *spy/ga1-3*. GA_3 treatment restores *ga1-3* and *rga/ga1-3* seeds to wild-type germination percentages (data not shown).

Trichome initiation: Recently, trichome initiation on Arabidopsis leaf surfaces has been demonstrated to be controlled by GA and day length (CHIEN and SUSSEX 1996). Wild-type plants do not produce trichomes on the abaxial surface of the first-formed rosette leaves. Both long day (LD) conditions and GA application can stimulate wild-type plants to produce abaxial trichomes earlier (CHIEN and SUSSEX 1996). The *ga1-3* mutant does not make abaxial trichomes under LD or short day (SD) conditions (CHIEN and SUSSEX 1996). We found that both *rga* and *spy* mutants in the *ga1-3* background partially restored trichome production one to three leaves later than Ler, depending on the allele (Figure 2). Moreover, the trigenic mutants, *rga/spy/ga1-3*, demonstrated an additive effect between *rga* and *spy*, initiating abaxial trichomes one to two leaves earlier than Ler (Figure 2).

Apical dominance: To examine the role of GA in apical dominance, we counted the number of axillary branches originating from the rosette stem. *ga1-3* has greatly reduced apical dominance and is very "bushy" with an average of 22.9 rosette branches compared to Ler, which has only 2.1 rosette branches (Table 2). Because *ga1-3* stems do not elongate, this branching results in a more ball-shaped plant (Figure 1F). All *rga/ga1-3* mutants and *spy-8/ga1-3* are intermediate between *ga1-3* and Ler, with half the number of rosette branches as *ga1-3*, but still quite a few more than Ler. GA_3 treatment can revert *rga/ga1-3* to wild-type apical dominance (Figure 1E). *spy-9/ga1-3* has almost the same number of branches as Ler. The trigenic *rga-2/spy-8/ga1-3* shows an additive effect of increasing apical dominance, while *rga-2/spy-9/ga1-3* is similar to *spy-9/ga1-3*.

Stem elongation: One of the most well-known effects of GA is to stimulate stem growth, especially in rosette plants. The extreme dwarf *ga1-3* does not bolt at all. We compared the final bolting stem height of the digenic mutants *rga/ga1-3* and *spy/ga1-3* and the trigenic mutant *rga/spy/ga1-3* to the height of Ler (Table 3). The *rga/*

TABLE 2

Apical dominance of Ler, *ga1-3* and GA-response mutants

Plant	No. of rosette branches
Ler	2.1 ± 0.2
ga1-3	22.9 ± 0.9
rga/ga1-3 mutants	
rga-1/ga1-3	10.3 ± 1.1
rga-2/ga1-3	10.4 ± 1.0
rga-3/ga1-3	10.8 ± 0.7
spy/ga1-3 mutants	
spy-8/ga1-3	12.2 ± 0.9
spy-9/ga1-3	3.2 ± 0.2
rga/spy/ga1-3 mutants	
rga-2/spy-8/ga1-3	4.5 ± 0.5
rga-2/spy-9/ga1-3	4.8 ± 0.4

The average values of axillary branches originating from the rosette were determined from 10 plants in each line. Values are means ± SE.

ga1-3 and *spy/ga1-3* mutants exhibit some GA independent growth of the bolting inflorescence stem, and the elongation of the bolting stem of *rga/ga1-3* mutants occurs with or without the GA_3 treatment necessary to induce germination. There is some variation in stem growth between alleles for both loci. However, we do not know if any of these EMS-generated alleles are null mutations. The *rga/ga1-3* mutants range in height from 30 to 50% of the wild-type Ler bolting height (Table 3). The two *spy/ga1-3* mutants are quite a bit shorter, between 20 and 25% wild-type height. However, the trigenic mutant, *rga/spy/ga1-3,* is able to suppress completely the *ga1-3* growth defect, and grows ~20% taller than Ler. The stems of the trigenic mutants are also very wavy compared to Ler (Figure 1D).

GAs are known to control both cell division and cell elongation (DAVIES 1995). To determine whether the stem elongation in the *rga/ga1-3, spy/ga1-3* and *rga/spy/ga1-3* mutants is caused by changes in cell size and/or cell number, we measured the length of epidermal cells from the first few internodes (Table 3). When we measured epidermal cell lengths from other mature internodes in the bolting stem, we found them to be a similar size to those in the first few internodes (data not shown). The epidermal cell length of *rga/ga1-3* is half the length of Ler (Table 3). This correlates well with the overall bolting stem height of *rga/ga1-3* also being half that of Ler. The *spy/ga1-3* mutants have smaller epidermal cells than *rga/ga1-3,* and this correlates with the shorter stature of these plants (Table 3). The trigenic mutant has cells similar in size to *rga/ga1-3* mutants. Dividing the bolting stem height by the average epidermal cell length, we can calculate the total number of cells along the epidermis of the stem. The *rga/ga1-3* and *spy/ga1-3* mutants have a similar cell number to Ler, but *rga/spy/ga1-3* has twice the number of cells as Ler (Table 3). The trigenic mutant does not achieve its taller stature through increasing cell elongation beyond that in the digenic mutants, but rather by producing more cells.

Finally, to understand if other factors, such as total number of internodes can affect the changes in height, we counted the number of siliques initiated on the main stem (Table 3). Whereas *spy-9/ga1-3* produced the same number of siliques as Ler, *spy-8/ga1-3* had 30% more siliques, and the *rga/ga1-3* mutants form 60–90% more siliques than Ler. The trigenic mutants had over twice the number of siliques per stem as wild type. Although *rga/ga1-3* had a large increase in silique number, the cell number in the bolting stem was not affected. A synergistic effect in *rga/spy/ga1-3* resulted in both a greater number of siliques formed and more cells produced.

Flowering time: Flowering time can be measured by both days to flower (chronological age) and the number of leaves formed on the main stem (developmental age). GAs are required for flower induction in Arabi-

TABLE 3

Stem growth characteristics of Ler and GA-response mutants

Plant	Bolting stem height (cm)	Epidermal cell length (μm)	No. of cells/stem	No. of siliques/stem
Ler	20.3 ± 0.4	270.9 ± 11.9	749 ± 36	28 ± 1.3
rga/ga1-3 mutants				
rga-1/ga1-3	9.2 ± 0.2	126.2 ± 3.7	729 ± 27	43.8 ± 1.0
rga-2/ga1-3	10.5 ± 0.4	129.9 ± 5.1	808 ± 44	53 ± 2.4
rga-3/ga1-3	7.1 ± 0.2	132.9 ± 4.7	534 ± 24	44.2 ± 1.6
spy/ga1-3 mutants				
spy-8/ga1-3	5.8 ± 0.3	74.4 ± 1.9	779 ± 45	37.3 ± 2.1
spy-9/ga1-3	4.0 ± 0.1	67.2 ± 2.6	595 ± 27	28.4 ± 1.8
rga/spy/ga1-3 mutants				
rga-2/spy-8/ga1-3	23.7 ± 0.8	127.4 ± 4.6	1860 ± 92	67.4 ± 2.0
rga-2/spy-9/ga1-3	24.0 ± 1.2	133.1 ± 5.2	1803 ± 114	58.8 ± 2.4

The bolting stem height was measured for 10 plants, and the number of siliques on the main stem of each plant counted. The epidermal peels were made from the first one to three internodes, and between 95 and 140 cell lengths were measured for each mutant line. The number of cells per stem was calculated from the division of the bolting stem height by the average cell length. Values are means ± SE.

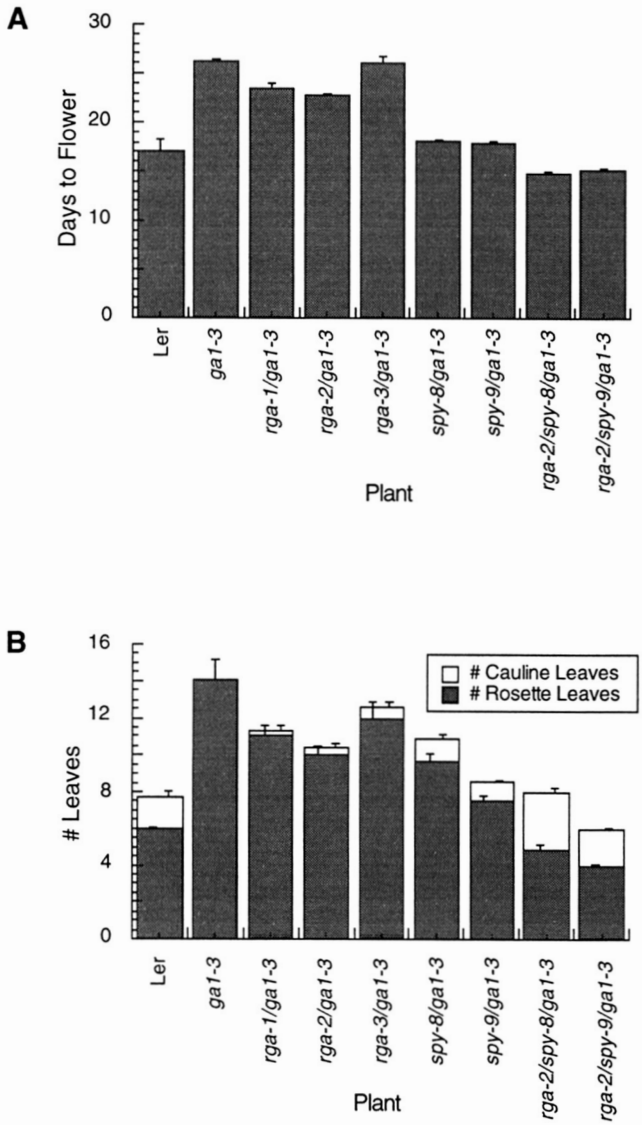

FIGURE 3.—Flowering time of *rga/ga1-3*, *spy/ga1-3*, and *rga/spy/ga1-3* mutants in comparison to wild-type Ler and *ga1-3*. (A) Number of days from germination until floral buds are clearly visible. (B) Number of cauline and rosette leaves produced by the primary inflorescence stem of each plant after bolting. The values plotted are the mean ± SE of 10 plants measured.

dopsis because under SD conditions, the *ga1-3* mutant does not flower (WILSON *et al.* 1992). Under LD conditions, flowering time in *ga1-3* is delayed in comparison to Ler, as *ga1-3* flowers 11 days later, producing 3.5 more leaves than Ler (WILSON *et al.* 1992). The *spy* mutants in Col ecotype flower 6 days earlier than wild type, also making six fewer leaves. Exogenous GA treatment can decrease Col flowering time to almost the same as *spy* (JACOBSEN and OLSZEWSKI 1993). We measured flowering time under LD conditions of our mutant and wild-type plants by both chronological age (Figure 3A) and developmental age (Figure 3B). The two measurements showed similar results. Ler flowers after 17 days and produces 7.7 total leaves (Figure 3, A and B). In contrast, *ga1-3* flowers after 26.3 days and produces almost twice as many leaves as Ler (Figure 3, A and B). All three alleles of *rga/ga1-3* are intermediate between Ler and *ga1-3*. The *spy/ga1-3* alleles flower only slightly slower chronologically or developmentally than Ler (Figure 3, A and B). Moreover, both trigenic mutants show an additive effect and flowered earlier than Ler chronologically (Figure 3A), but *rga-2/spy-8/ga1-3* flowered at the same time as wild type developmentally (Figure 3B).

Silique development and fertility: GAs are involved in stamen, embryo and fruit development (DAVIES 1995). Slender GA-response mutants, including *spy* and *la cry*^{s}, exhibit parthenocarpic fruit development (DE HAAN 1927; JACOBSEN and OLSZEWSKI 1993). The elongation of the pedicel (Table 4), a modified stem, seems to reflect the synergistic control of *rga* and *spy* on bolting stem growth (Table 3). The pedicels of *rga/ga1-3* and *spy/ga1-3* are twice the length of those produced by *ga1-3* and ~30% of the wild-type length (Table 4). The trigenic mutants show an additive effect on pedicel length and grow to be 75% of wild-type length, longer than even the sum of *rga/ga1-3* and *spy/ga1-3* pedicels (Table 4).

Male fertility is restored in *spy/ga1-3* mutants (JACOBSEN and OLSZEWSKI 1993) as shown by the flower shedding pollen (Figure 1H) and the production of viable seeds from self-fertilized flowers (Tables 1 and 4). The petals of *spy/ga1-3* flowers also develop normally (Figure 1H). In contrast, *rga/ga1-3* flowers are phenotypically identical to *ga1-3* (Figure 1, F and G), and the siliques are seedless. Nevertheless, the fertility of *spy/ga1-3* is not identical to wild type. *spy/ga1-3* produced siliques with 12–25% the number of seeds of wild type (Table 4). Unexpectedly, the interaction between *rga* and *spy* has a negative effect on fertility in the trigenic mutants. Although individual fertile *rga/spy/ga1-3* siliques have a seed set similar to *spy/ga1-3*, ~85% of the siliques are empty (Table 4). The smaller size of the *spy/ga1-3* siliques correlates with their reduced seed set. The sterile siliques of *ga1-3* and *rga/ga1-3* are 12% the length of wild-type siliques (Table 4). The trigenic mutants produced siliques the same length as *spy/ga1-3* mutants irrespective of their fertility.

GA response curve for hypocotyl growth: To characterize further the effect of *rga* on GA signal transduction, we measured the hypocotyl responsiveness to GA_3 treatments (Figure 4). The hypocotyls of all the mutants show a linear response to exogenous GA_3 between 0.01 and 5 μM (Figure 4). The saturation for the response occurs between 10 and 50 μM with growth inhibition occurring at 100 μM (data not shown). At saturating concentrations of GA_3, the *ga1-3* and *rga-2/ga1-3* mutants are similar in height (4.7 and 4.2 mm, respectively), whereas *spy-8/ga1-3* is quite a bit shorter (3.2 mm). We found that *rga-2/ga1-3* was as responsive to GA_3 as *ga1-3*, as seen by their similar slopes (Figure 4A). In contrast, the *spy/ga1-3* mutants displayed only $^{1}/_{2}$ the responsiveness (Figure 4B) and the trigenic mutants

TABLE 4

Growth and fecundity of siliques

Plant	Pedicel length (mm)	Silique length (mm)	No. of seeds/silique
Ler	6.0 ± 0.2	10.7 ± 0.1	42.4 ± 1.2
ga1-3	<1	1.4 ± 0.0	Sterile
rga/ga1-3 mutants			
rga-1/ga1-3	2.0 ± 0.1	1.6 ± 0.1	Sterile
rga-2/ga1-3	2.0 ± 0.1	1.7 ± 0.1	Sterile
rga-3/ga1-3	1.8 ± 0.1	1.8 ± 0.1	Sterile
spy/ga1-3 mutants			
spy-8/ga1-3	2.0 ± 0.1	3.8 ± 0.2	5.1 ± 0.7
spy-9/ga1-3	1.6 ± 0.1	4.7 ± 0.1	10.1 ± 1.0
rga/spy/ga1-3 mutants			
rga-2/spy-8/ga1-3	4.5 ± 0.2	4.9 ± 0.2	6.9 ± 1.3 (fertile siliques)[a]
rga-2/spy-9/ga1-3	4.8 ± 0.2	4.7 ± 0.1	4.0 ± 0.6 (fertile siliques)[a]

Pedicel and silique lengths, and seed number are from the average of 30 siliques. Values are means ± SE.
[a] 85% are sterile.

rga-2/spy/ga1-3 showed less than $^1/_3$ the responsiveness to GA treatment compared to that of *ga1-3* (Figure 4C).

We measured the lengths of the epidermal cells of the hypocotyls at two different GA concentrations, 0.01 and 1 μM (Table 5), to determine whether the more elongated growth of the hypocotyls was caused by cell elongation and/or cell division. At 0.01 μM GA_3, *ga1-3* has the smallest cells, and the shortest hypocotyl. *rga/ga1-3* has epidermal cell lengths a little longer than *ga1-3*, and *spy/ga1-3* has cells a little longer than Ler (Table 5). However, all the mutants and Ler have a similar number of cells per hypocotyl (Table 5). In contrast, the trigenic mutant has epidermal cells six times longer than *ga1-3*, but the number of cells per hypocotyl is similar to *ga1-3* and the digenic mutants (Table 5). In response to 1 μM GA_3, the epidermal cell length increased in the *ga1-3*, *rga/ga1-3*, and *spy/ga1-3* mutants, but the cell number was unchanged (Table 5). Neither exogenous GA treatment nor activation of GA signal transduction affects cell number in the hypocotyl. This influence of GA on cell growth contrasts with the effect in the bolting stem, where we have only tested the GA response mutants, but activation of GA signal transduction affects both cell length and cell number.

DISCUSSION

The *ga1-3* deletion mutant does accumulate a very low level of GAs (ZEEVAART and TALÓN 1992). This may be accomplished by the activity of a homologue of GA1, or by the activity of another terpene cyclase that can substitute for *ent*-kaurene synthase A. Therefore, our suppressor screen might obtain mutants with increased levels of GAs due to mutations that either partially substitute for GA1 function or block inactivation reactions of GAs. However, this type of suppressor will be a phenocopy of the leaky *ga1* alleles, which are semi-dwarf, and whose seed germination percentage and flower fertility are only slightly reduced (KOORNNEEF *et al.* 1983). Both *rga/ga1-3* and *spy/ga1-3* mutants are likely to affect GA signal transduction, rather than having altered GA metabolism, because their phenotypes are quite different from those of leaky *ga1* mutants. The *rga/ga1-3* mutants partially restore stem growth, but still are nongerminating and male-sterile. The *spy/ga1-3* mutants restore all three processes, but the overall phenotype of the plants does not resemble the leaky *ga1* mutants. In addition, application of exogenous GA_3 drastically reduced the fertility of the *spy/ga1-3* mutant. The *spy* mutant is also resistant to the GA biosynthesis inhibitor paclobutrazol (JACOBSEN and OLSZEWSKI 1993). To rule out definitively the possibility that either phenotype results from an increased accumulation of bioactive GAs, we have made initial measurements of GA concentrations in Ler, *ga1-3*, *rga/ga1-3* and *spy/ga1-3*. Preliminary results indicate that the GA levels of *rga/ga1-3* and *spy/ga1-3* are identical to *ga1-3* (data not shown). The phenotype of *rga/ga1-3* is therefore likely to be the result of constitutive activation of GA signal transduction rather than accumulation of bioactive GAs.

We have not identified *rga* in the wild-type *GA1* background, indicating that any phenotype probably will be subtle. An explanation for *rga/GA1* not having an obvious phenotype is discussed in our proposed model for GA signal transduction below.

We have backcrossed several of the *spy/ga1-3* mutants to Ler to generate *spy/GA1* lines. Our *spy* alleles display a range of phenotypes from weak (shorter plants) to strong (taller plants with crinkled leaves and reduced fertility, data not shown). This diverse collection of *spy* mutants will be useful in determining the function of SPY.

In comparing *rga/ga1-3*, *spy/ga1-3*, and *rga/spy/ga1-3* with *ga1-3* and Ler, we found that SPY regulates all known GA-mediated developmental events while RGA only affects a subset of GA responses, *i.e.*, stem elongation, flowering, trichome initiation and apical domi-

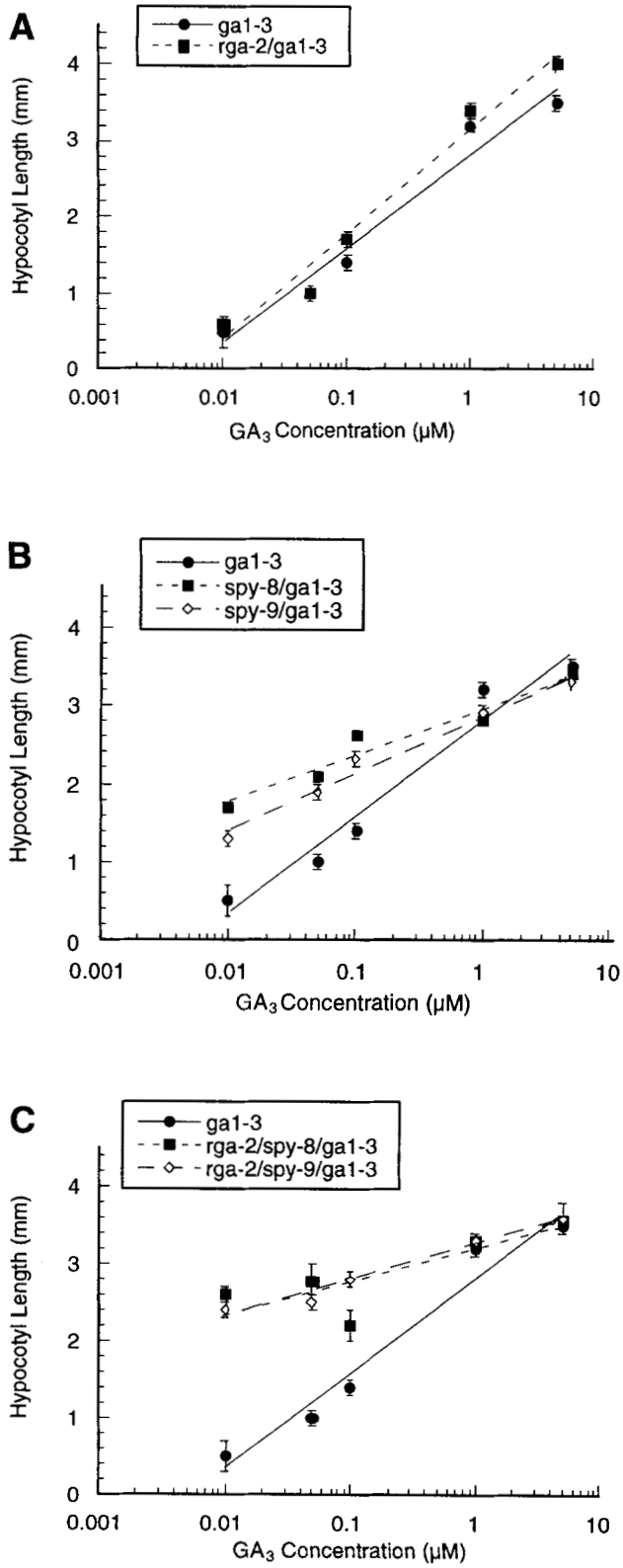

FIGURE 4.—Hypocotyl response to GA concentration. (A) *ga1-3* compared to *rga-2/ga1-3*. (B) *ga1-3* compared to *spy-8/ga1-3* and *spy-9/ga1-3*. (C) *ga1-3* compared to *rga-2/spy-9/ga1-3*. The equations for the linear regressions are as follows: (A–C) *ga1-3*, $y = 2.8 + 1.2 \log(x)$, $R = 0.98$. (A) *rga-2/ga1-3*, $y = 3.1 + 1.4 \log(x)$, $R = 0.99$. (B) *spy-8/ga1-3*, $y = 2.9 + 0.6 \log(x)$, $R = 0.97$ and *spy-9/ga1-3*, $y = 2.9 + 0.7 \log(x)$, $R = 0.99$. (C) *rga-2/spy-8/ga1-3*, $y = 3.2 + 0.4 \log(x)$, $R = 0.80$ and *rga-2/spy-9/ga1-3*, $y = 3.3 + 0.5 \log(x)$, $R = 0.99$. The values plotted are the mean ± SE of 10 plants measured.

nance. Moreover, for the common processes controlled by both RGA and SPY, there is an additive effect in the trigenic mutant *rga/spy/ga1-3*, which is much less responsive to exogenous GA treatment. These results suggest that SPY and RGA affect two separate branches of the GA signal transduction pathway that interact and lead to the proper regulation of shared responses.

Common responses regulated by *RGA* and *SPY*: We found an additive effect between *spy* and *rga* in the *ga1-3* background with respect to trichome initiation, flowering time, apical dominance and stem elongation. *SPY* and *RGA* most likely encode negative regulators of the GA signal transduction pathway because all mutant alleles are recessive.

Each mutant locus alone in the *ga1-3* background caused a partial reversion to the wild-type phenotype. Thus, *rga/ga1-3* and *spy/ga1-3* were intermediate between *ga1-3* and Ler for abaxial leaf trichome initiation, flowering time and apical dominance. However, with respect to these developmental processes the trigenic mutant *rga/spy/ga1-3* was very similar to Ler.

We examined stem elongation in three different tissues: hypocotyls, bolting inflorescences, and pedicels. The hypocotyl was also used to measure the responsiveness of the different mutants to GAs to test if there was a change in sensitivity of tissues to GA. The *rga-2/ga1-3* mutant demonstrated a GA responsiveness similar to *ga1-3* as indicated by the similar slopes of their hypocotyl GA response curves (Figure 4A). The other two alleles of *rga/ga1-3* also had hypocotyl GA response curves similar to *ga1-3* (data not shown). However, the two *spy/ga1-3* alleles were less responsive than *ga1-3* (Figure 4B), and the trigenic mutants were least responsive (Figure 4C). At low GA_3 concentrations, the hypocotyl of *spy/ga1-3* mutants are longer than *ga1-3* and *rga/ga1-3* mutants, but at saturating GA_3 concentrations, the *spy/ga1-3* mutants are shorter. Our results indicating that *spy/ga1-3* is less responsive to GA contrast with a previous report that *spy-1/ga1-2* has the same slope for GA_3 response as *ga1-2*. (JACOBSEN and OLSZEWSKI 1993). More recently, the *spy-1* mutant was found to contain a second tightly linked mutation *hy2* (JACOBSEN *et al.* 1996), which may affect GA responsiveness. *hy2* mutants are blocked in phytochrome chromophore biosynthesis, and, therefore, lack active phytochrome (PARKS and QUAIL 1991). The digenic *ga1-3/hy3* mutant is more responsive to exogenous GA than *ga1-3*, indicating that phytochrome B regulates GA sensitivity of the cells (REED *et al.* 1996). Therefore, the GA response curve of the *spy-1/hy2/ga1-2* mutant might represent a balance between decreased sensitivity to GA_3 caused by the *spy* mutation (Figure 4) and increased sensitivity caused by *hy2*.

To determine if cell size and/or cell number changed

TABLE 5

Hypocotyl growth characteristics of *ga1-3* and GA-response mutants

Plant	0.01 μM GA_3			1 μM GA_3		
	Hypocotyl height (mm)	Epidermal cell length (μm)	No. of cells/ hypocotyl	Hypocotyl height (mm)	Epidermal cell length (μm)	No. of cells/ hypocotyl
Ler	1.8 ± 0.1	144.5 ± 9.4	12.5 ± 1.1	3.8 ± 0.2	271.2 ± 10.9	14.0 ± 0.9
ga1-3	0.5 ± 0.2	42.4 ± 1.9	11.8 ± 4.7	3.2 ± 0.1	245 ± 14.9	13.1 ± 0.9
rga/ga1-3 mutant						
rga-2/ga1-3	0.6 ± 0.1	55.2 ± 3.0	10.9 ± 1.9	3.4 ± 0.1	303.9 ± 14.4	11.2 ± 0.9
spy/ga1-3 mutant						
spy-8/ga1-3	1.7 ± 0.1	164.7 ± 8.0	10.3 ± 0.8	2.8 ± 0.1	256.2 ± 13.5	10.9 ± 0.7
rga/spy/ga1-3 mutant						
rga-2/spy-8/ga1-3	2.6 ± 0.1	251.4 ± 11.9	10.3 ± 0.6	3.3 ± 0.1	272.4 ± 12.9	12.1 ± 0.7

Hypocotyl heights were determined from 10 plants for each line. Epidermal cell lengths were measured for between 19 and 53 cells. Cells per hypocotyl was calculated by the division of the hypocotyl height by the average cell length. Values are means ± SE.

in response to the GA_3 treatment, the length and number of epidermal cells were measured in hypocotyls that grew in the presence of 0.01 and 1 μM GA_3. We found that cell elongation was the primary mechanism of hypocotyl growth response to GA (Table 5). The cell number was similar between mutants, and there was little change in response to GA_3 treatment. The trigenic mutant *rga/spy/ga1-3* had very long cells that were almost insensitive to GA_3 in the medium; both its cell and hypocotyl lengths corresponded to the length of the *ga1-3* and *rga/ga1-3* mutants treated with optimal concentrations of GA. This suggests that the trigenic mutant has a constitutively activated GA response, whereas each of the digenic mutants only exhibit a partially activated GA response.

In general, we found that the bolting stems of the *rga/ga1-3* mutants were taller than the stems of the *spy/ga1-3* mutants (Table 3), indicating that *rga* may affect the stem growth process more than *spy*. However, because allelic strength can affect the bolting height, identification and testing of null mutations of each locus will be necessary to confirm these relationships. Longer epidermal cells are the major factor causing the *rga/ga1-3* digenic mutants to be taller than *spy/ga1-3* because the number of cells per stem is similar (Table 3). The trigenic mutant has cells the same size as the digenic *rga/ga1-3* mutants, but there is an increase in cell number that appears to be the cause of the much taller stature of the trigenic mutant.

To assess the developmental age of the inflorescence meristem before it senesced and whether a longer-lived meristem would be able to produce more nodes, we counted the number of siliques produced on the main stem. The trigenic mutants made 110–140% more siliques than Ler (Table 3). Although the trigenic mutants bolted earlier, they grew more slowly. Ler achieved its final height in 32 days, but it took *rga-2/spy-8/ga1-3* 55 days and *rga-2/spy-9/ga1-3* 61 days to cease growing. Because the inflorescence meristem of the trigenic mutant senesces later, it can produce twice the number of siliques as wild-type plants. Thus, a constitutively active GA response seems to lead to delayed senescence of the meristem.

Our results indicated that GA-mediated growth of hypocotyls is accomplished only by changes in cell elongation and that of bolting stems occurs by changes in both cell size and number. This difference between hypocotyls and bolting stems could be accounted for by the nature of these two tissues. The hypocotyl is formed early during embryo development and no longer possesses a meristematic region. In contrast, the bolting stem is created much later by the inflorescence meristem of the young plant, and the final height of the stem is determined by both the number of cells produced by the meristem and their elongation.

The pedicel is a modified stem, and differences in length between genotypes reflect the results observed with the bolting stem. It is 30% of the wild-type length in the *rga/ga1-3* and *spy/ga1-3* mutants, but is restored to ~75% of the wild-type length in the trigenic mutant (Table 4). Similar to bolting stem height, this additive affect lends credence to the notion that *RGA* and *SPY* regulate separate branches of the GA signal transduction pathway.

***SPY*-regulated responses:** *spy* mutants are able to supersede the GA requirement for germination (Table 1, JACOBSEN and OLSZEWSKI 1993), but *rga* mutants cannot, indicating that *RGA* is either on a different branch of the GA response pathway or functions after a step that does not control germination. The low level of seed germination seen in *rga/ga1-3* and *ga1-3* mutants can vary between experiments (0–10%). This probably reflects a small percentage of seeds whose coats have been physically damaged. The germination percentage is higher in seeds that have been sterilized (data not shown). Since the sterilization procedure uses harsh chemicals (95% ethanol and 5% hypochlorite) as well as vortexing, we would expect an increase in mechani-

cal damage to the seed coats and a corresponding increase in germination.

JACOBSEN and OLSZEWSKI (1993) reported that *spy* mutants can produce parthenocarpic siliques, which suggests that the elongation of the silique proper is affected by *SPY*. Silique length has been previously reported to be loosely correlated with seed set (BARENDSE *et al.* 1986). However, silique growth was thought to be at least partially dependent on the GAs supplied by the seeds (BARENDSE *et al.* 1986). Unfertilized siliques do not elongate and often senesce early. In *spy/ga1-3* mutants, the siliques are 36–44% of wild-type length, but the seed set is only 12–25% of wild type (Table 4). In the trigenic mutants, the siliques are similar in length to the *spy/ga1-3* mutants, but ~85% of the siliques are empty (Table 4). This suggests that *SPY* is important for silique growth independent of whether fertilization and subsequent embryo development has occurred. *RGA*, on the other hand, does not appear to regulate development of the silique proper as the *rga/ga1-3* siliques are identical in length to *ga1-3*. However, the *spy/ga1-3* siliques all have seeds, and we have not compared the lengths of siliques produced by emasculated flowers from the mutants.

An updated model of the GA signal transduction pathway: Previous results using the *spy* mutant suggested the presence of more than one GA signal transduction pathway in Arabidopsis (JACOBSEN and OLSZEWSKI 1993). Our results in combination with the work already performed on *gai* and *spy* have led us to propose a modified model of GA signal transduction (Figure 5). Because *spy* is completely epistatic to *gai*, *SPY* has been placed downstream of *GAI* on the same pathway (JACOBSEN *et al.* 1996). However, since *spy* mutants are still GA-responsive, *SPY* may regulate only one branch of the GA signal transduction pathway (JACOBSEN and OLSZEWSKI 1993). Identification of *RGA* allows us to define a new branch of the GA response pathway (Figure 5) that is different from the one defined by *GAI* and *SPY*. In our epistasis analysis, we found that *rga* and *spy* mutants often have additive effects in the *ga1-3* mutant background. This indicates that they probably act on separate pathways. Interestingly, the two pathways seem to converge to regulate several common responses. Therefore, we propose that *RGA* and *SPY* regulate two branches of the GA response pathway. As predicted in our model, the *rga/spy/ga1-3* mutants display GA-independent growth and revert many aspects of *ga1-3* to either a wild-type or a GA-treated wild-type phenotype. We do not know if both branches of the pathway have the same or different GA receptors.

SPY seems to affect many different GA-regulated developmental processes, including germination, silique growth, flower development, stem elongation, apical dominance, flower initiation, and trichome initiation. Therefore, we place it as a regulator of branch "A" after *GAI*, but before the branch divides to control specific events (Figure 5). If *GAI* were located before the separation of the two branches, the *spy/gai* digenic mutant would only show partial epistasis, which is not the case. Thus, the A branch, defined by *SPY* and *GAI* is discrete from the "B" branch, defined by *RGA*. *RGA*, in contrast, appears to regulate a subset of the same developmental events as *SPY*. Thus, branches A and B converge to control the "Common Responses" in an additive fashion (Figure 5).

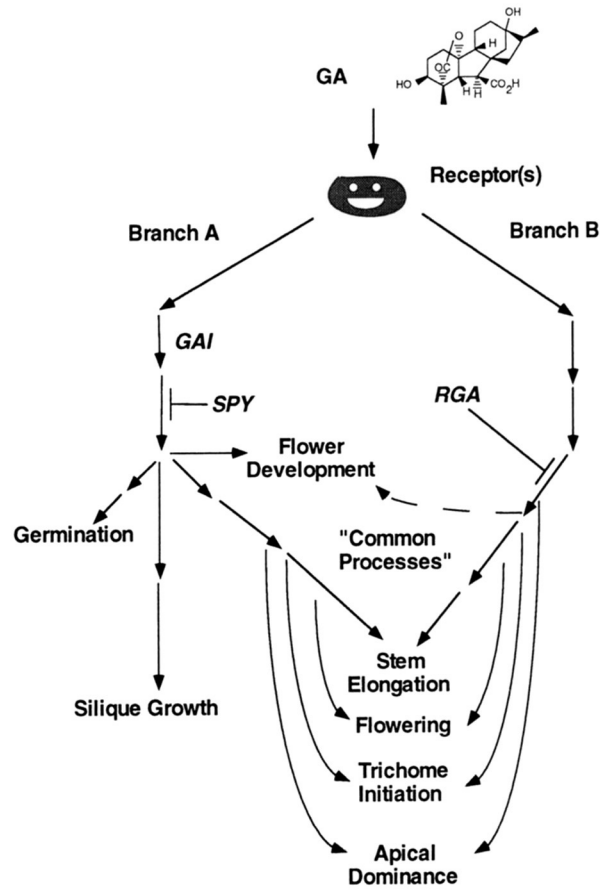

FIGURE 5.—Proposed model for the GA signal transduction pathway. *SPY* and *GAI* define branch A that regulates numerous GA-mediated responses as indicated. *RGA* defines a second branch, B. The common processes would require derepression of both branches of the pathway for a full response to GA.

Too little GA results in male-sterile flowers as evident in the *ga1-3* mutant (Figure 1F), and too much GA, such as an overdose of GA in wild-type plants, also results in male sterility. Although *rga* does not appear to affect flower development in the *ga1-3* background, it has a strong effect in the *spy/ga1-3* background. Therefore, we have the dashed line in the model showing *RGA* modulating flower development. The low fertility and flower morphology of the trigenic *rga/spy/ga1-3* mutant resembles the infertility of *spy/GA1* or a wild-type Arabidopsis plant treated with exogenous GA_3 (JACOBSEN and OLSZEWSKI 1993). The *rga/spy/ga1-3* trigenic mutant may have a constitutive GA response that mimics the GA overdose response in wild-type flowers.

It is interesting that Arabidopsis employs this

branched signal transduction pathway that converges at the end rather than a simpler linear one. One possibility is that the two branches may serve to fine tune the plant's response to GA. The *rga/GA1* mutant may not have a drastic phenotype because the A branch is down-regulated to compensate for the activation of the B branch in this mutant.

An alternative model for GA response places *RGA* downstream of *SPY* on the same pathway. The additive effects could result from the combined effect of leaky mutations in both *spy* and *rga* loci. However, several pieces of evidence favor the branched pathway model. The phenotype of *gai* indicates that it is deficient, but not lacking, in GA response. As mentioned earlier, *spy* is completely epistatic to *gai* (JACOBSEN *et al.* 1996). If *gai* affects both branches, the digenic *gai/spy* mutant would have been shorter than the single *spy* mutant. Examining the phenotype of the trigenic *rga/gai/ga1-3* and the tetragenic *rga/spy/gai/ga1-3* mutants will help determine if our model is accurate. In addition, we have isolated several *rga* deletion alleles generated by fast-neutron bombardment, and their phenotype is similar to the EMS alleles (A. L. SILVERSTONE and T-p. SUN, unpublished data).

The *rga/spy/ga1-3* phenotype resembles the *la cry*^{s} phenotype in peas and the *sln* mutant in barley. Products of the wild-type *La* and *Cry* alleles in pea and *Sln* allele in barley have been suggested to act as repressors of the GA receptor or elsewhere in a GA signal transduction pathway to suppress the growth rate of the plant (POTTS *et al.* 1985; STODDART 1990). Individually, the *la* or *cry*^{s} alleles have only small effects on stem growth in pea. However, in either the wild-type or various GA biosynthetic mutant backgrounds, *la cry*^{s} show a slender phenotype that is unaffected by exogenous GAs or inhibitors of GA biosynthesis (POTTS *et al.* 1985).

Although *SPY* has been recently cloned using a T-DNA tagged *spy-4* allele, the deduced amino acid sequence of the protein does not provide many clues as to its function, except to show 10 tetratricopeptide repeats that are likely to be important for protein-protein interaction (JACOBSEN *et al.* 1996). Similarly, a transposon-tagged allele of *GAI* gene was cloned recently, but the protein it encodes does not show homology to other known proteins (N. HARBERD, personal communication).

Our cloning and functional analysis of *RGA*, now in progress, should provide insight into the mechanisms controlling GA response. *RGA* also gives us a handle to elucidate further the second branch of the GA signal transduction pathway that was only hinted at by previously isolated GA-responsive slender mutants. Because too little response to GA will result in incomplete development, yet too much response will cause developmental defects such as male-sterile flowers, the duplication of GA signal transduction pathways may exist to enable finer manipulation of GA response. Studies on the two branches of this pathway may reveal why so much of the regulation appears to be duplicated and how both branches of the pathway are controlled to mediate proper responses to GA

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