Plant Physiol. (1996) 111: 27–37

Highly Branched Phenotype of the Petunia *dad1–1* Mutant Is Reversed by Grafting[1]

Carolyn Napoli*

Environmental Horticulture, University of California, Davis, California 95616

The recessive *dad1–1* allele conditions a highly branched growth habit resulting from a proliferation of first- and second-order branches. Unlike the wild-type parent, which has lateral branching delayed until the third or fourth leaf node distal to the cotyledons, *dad1–1* initiates lateral branching from each cotyledon axil. In addition to initiating lateral branching sooner than the wild type, *dad1–1* sustains branching through more nodes on the main shoot axis than the wild type. In keeping with a propensity for branching at basal nodes, *dad1–1* produces second-order branches at the proximal-most nodes on first-order branches and small shoots from accessory buds at basal nodes on the main shoot axis. Additional traits associated with the mutation are late flowering, adventitious root formation, shortened internodes, and mild leaf chlorosis. Graft studies show that a *dad1–1* scion, when grafted onto wild-type stock, is converted to a phenotype resembling the wild type. Furthermore, a small wild-type interstock fragment inserted between a mutant root stock and a mutant scion is sufficient to convert the *dad1–1* scion from mutant to a near wild-type appearance. The recessive *dad1–1* phenotype combines traits associated with cytokinin overexpression, auxin overexpression, and gibberellin limitation, which suggests a complex interaction of hormones in establishing the mutant phenotype.

The shoot system of flowering plants is assembled progressively from modular components that are derived from shoot apical meristems. The vegetative module of a typical flowering plant consists of an internode, a leaf-bearing node, and an axillary bud in the leaf axil (Steeves and Sussex, 1989). The total number of shoot apical meristems increases when axillary buds develop into lateral branches. In most flowering plant species, axillary bud development is delayed, relative to development of leaf primordia, and is first observed in the axil of the second or third leaf below the apex (Esau, 1977). Although axillary buds develop outside the immediate vicinity of the apical meristem, the shoot apex can control bud growth and development in many plant species. Apical dominance and correlative inhibition are physiological terms referring to the inhibitory influence exerted by growing shoot apices. Despite decades of research on apical dominance, no precise regulatory mechanism is agreed upon, although a role for plant hormones is recognized (Cline, 1991, 1994).

A number of experimental strategies aimed at studying apical dominance have made use of genetic variation. One approach relies on the introduction of foreign genes (transgenes) as the means to induce genetic variation. Several laboratories have used this approach to test the roles of auxin and cytokinin in apical dominance by introducing auxin and cytokinin biosynthetic genes from *Agrobacterium tumefaciens* and *Pseudomonas syringae* pv *savastanoi* into tobacco, petunia (*Petunia hybrida*), and Arabidopsis (Klee et al., 1987; Smigocki and Owens, 1988; Medford et al., 1989; Romano et al., 1991; Smigocki, 1991; Sitbon et al., 1992). The engineered increases in endogenous hormone levels produce a series of pleiotropic effects, but the experiments confirm that both cytokinin and auxin are important factors in either promotion (cytokinin) or suppression (auxin) of axillary bud growth. Furthermore, the important factor determining the extent of axillary bud growth appears to be the ratio of auxin to cytokinin rather than absolute levels of either hormone (Klee and Estelle, 1991).

The usefulness of the transgenic approach for producing genetic variability is further illustrated in work by Romano et al. (1993). Because auxin overexpression results in increased ethylene concentration, it is difficult to distinguish auxin-induced from ethylene-induced changes in plant growth and development. To uncouple auxin and ethylene effects, transgenic plants overproducing auxin were crossed to transgenic plants inhibited in ethylene biosynthesis. The important new finding from this experiment is that auxin overproduction alone is responsible for increased apical dominance.

The *rolA*, *rolB*, and *rolC* genes from *Agrobacterium rhizogenes* are additional sources of transgene-based genetic diversity (Schmulling et al., 1988; Estruch et al., 1991). Transgenic plants expressing all three genes display the hairy-root syndrome, but single genes under the control of the cauliflower mosaic virus 35S promoter produce less drastic and more interesting effects on plant growth and development. Of the three genes, 35S-*rolC* gene expression in tobacco produces increased branching, as well as shortened internodes, thin and lanceolate leaves, reduced chlorophyll content, smaller, male-sterile flowers, and increased nodes to flowering (Schmulling et al., 1988; Nilsson et al., 1993). Tobacco containing the *rolC* transgene had reduced levels of isopentyladenosine and GA_{19}, whereas the IAA pools remained relatively unchanged (Nilsson et al., 1993). In this instance, a reduction in cytokinin is associated with increased branching.

A different genetic approach toward understanding apical dominance is the induction of mutations in endogenous genes. Auxin-biosynthetic mutants, auxin-response mutants, and cytokinin-response mutants have been reviewed

[1] Supported by National Science Foundation grant IBN-9119117.

* E-mail canapoli@ucdavis.edu; fax 1–916–752–1819.

by Rothenberg and Ecker (1993). The auxin mutants demonstrate that this hormone is involved in the expression of apical dominance. Additional mutants demonstrating increased lateral branching have been reported, including the Arabidopsis *AMP1* mutant and the *Pisum sativum rms-2* mutant. The *AMP1* mutant has decreased apical dominance as well as altered embryonic development, constitutive photomorphogenesis, and increased cytokinin content (Chaudhury et al., 1993). The *rms-2* mutant has increased branching at basal and aerial nodes, reduced internode length, and elevated levels of free IAA (Beveridge et al., 1994). Most significantly, graft experiments show that apical dominance is almost fully restored to *rms-2* by grafting a mutant scion onto wild-type stock (Beveridge et al., 1994).

I have taken a mutagenesis-based approach to study the role of apical dominance in establishing plant architecture in *P. hybrida* (Napoli and Ruehle, 1996). Mutations have been identified that affect axillary bud growth during the vegetative phase of growth (*decreased apical dominance* mutants) and axillary meristem potential during sympodial branching (*sympodial* mutant). Sympodial branching is a type of axillary branching that establishes the reproductive shoot axis in petunia. Nine recessive mutations define three *Dad* (*Decreased apical dominance*) loci controlling lateral branching during the vegetative phase in petunia (Napoli and Ruehle, 1996). Two of the nine mutants are allelic and define the *Dad1* locus. Because both *dad1* mutants have the same apparent phenotype, only the *dad1–1* allele has been selected for this study. This paper compares in detail the phenotypic differences between wild-type V26 and the *dad1–1* mutant and presents graft analyses that address the role of the root system in controlling apical dominance.

MATERIALS AND METHODS

Petunia hybrida Genetic Stocks and Growth Conditions

Dad1–1 was induced with ethyl methanesulfonate in V26, an inbred genetic stock of *Petunia hybrida* (Napoli and Ruehle, 1996). Growth conditions for petunia were standardized in a temperature-controlled greenhouse environment (Gem III system, Q-COM, Irvine, CA). Greenhouse temperatures ranged between 28°C (day) and 18°C (night). Experiments to compare branching and flowering were performed during months when the photoperiod was greater than 12 h to induce flowering and maximum growth. Root medium and fertilizer composition were provided by Napoli and Ruehle (1996).

Node-Counting Experiments

Seeds were scattered on the surface of moist rooting medium and maintained on a mist bench until the onset of germination, from 7 to 10 d. Seedlings were transplanted to six-pack containers (model K-806, Kord Products, Bramalea, ON, Canada) at the point when the first true leaf emerged and expanded (approximately 2 weeks from seed sowing). V26 seedlings produced three cotyledons on an intermittent basis. Transplantation was restricted to seedlings with two cotyledons. Seedlings were transplanted to 3.5-inch-square disposable pots (model 10170, McCalif, Cere, CA) approximately 1 month from seed sowing. Plants were maintained in these pots for the duration of an experiment. Leaf node counts started with the first true leaf. Numbers were marked on each leaf with a Sharpie (Sanford, Bellwood, IL) permanent marker. Measurements for lengths and diameters were facilitated by use of a caliper.

Seedling Graft Procedure

All graft procedures employed surface-sterilized seeds to produce axenic seedlings. Manipulations were done in a sterile transfer hood to maintain axenic conditions. Wild-type and mutant seeds were disinfected with 20% bleach for 15 min, followed by three washes in sterile, deionized water, and sown on Murashige and Skoog tissue culture agar (JRH Biosciences, Lenexa, KS) supplemented with 3% Suc. For hypocotyl grafting, the age of seedlings was from 10 to 20 d postgermination. Seedlings were severed within the hypocotyl region, 1 to 2 mm below the cotyledons. Tissue cutting was performed using flame-sterilized, stainless-steel microscissors (Fisher Scientific, catalog no. 08-953-1B). The appropriate combinations of scion and rootstock were selected, and the wound sites were aligned to form a graft union. Removal of the cotyledons from scions was necessary to allow the scions to lie flat on the agar surface in order to align the cut ends of the scion and the stock. The Petri dishes containing the grafted seedlings were sealed with Parafilm (American National Can, Greenwich, CT), set in a near-vertical position relative to gravity, and incubated in a controlled-temperature room (24°C) with 16 h of light. Seedlings were examined on a daily basis for the first 3 d to ensure that stock and scion pieces remained in contact. Graft unions began to heal between the 2nd and 3rd d of alignment. The seedlings were left for an additional 1 to 2 weeks to ensure complete healing in order to promote a strong graft union. Liquid collecting in the bottom portion of Petri dishes was drained when necessary. Adventitious roots were removed from *dad1–1* scions as they developed. After graft union healing, the seedlings were planted in potting medium (C. Napoli, unpublished data) and kept on a mist bench for 1 to 2 weeks before subsequent transfer to the greenhouse. Plants were grown under long days to provide conditions conducive to flowering.

For interstock grafting, the genotype of both the stock and the scion was identical. Hypocotyl interstock grafts were constructed using seedlings from 7 to 14 d postgermination. A seedling was severed within the hypocotyl region directly below the cotyledons and rejoined with an interstock fragment inserted between the two sections of the original seedling. Interstock fragments measured from 2 to 3 mm in length and were obtained from hypocotyl regions of tester seedlings. The incubation and handling of interstock grafts were the same as described above for the hypocotyl grafts.

Vegetative Propagation

Vegetative propagation utilized stem-tip cuttings from vegetative shoots or sympodial shoots. The ends of shoot tips were dipped into Hormodin 1 (Merck, Rahway, NJ), and stem tips were planted into moist root medium. Cut-

tings were held on a mist bench for 2 weeks before transfer to the greenhouse.

RESULTS

Lateral Branching in Wild-Type and Mutant Plants

The recessive *dad1–1* allele conditions a highly branched phenotype that is visibly distinct from the wild type. The difference between mutant and wild type can be discerned as early as 2 to 3 weeks after germination. Wild-type and mutant plants at 5 and 10 weeks postgermination are shown in Figure 1, and the distribution of lateral branches for each genotype is presented graphically in Figure 2. Figure 2A compares the number of lateral branches on wild-type and mutant plants at 10 weeks postgermination, and Figure 2B illustrates the specific nodes that produce lateral branches. For the purposes of this paper, lateral branches are distinguished from short axillary shoots that are no more than 1 cm long and cease growth before significant leaf expansion or internode elongation. Lateral branches are longer than 1 cm, have at least three expanded internodes, and grow in a manner somewhat coordinate with the main shoot, escaping control by the shoot apex.

Wild-type plants produced an average of 3.4 ± 0.48 lateral branches, whereas mutant plants had an average of 9.9 ± 0.99 lateral branches (Fig. 2A). Analysis by the *t* test shows that the difference is significant at $P < 0.001$. The mutant produced more lateral branches by initiating branching sooner and sustaining branching longer (Fig. 2B). The mutant phenotype was very consistent, and additional experiments showed that lateral branching always started at the axils of each cotyledon. The wild-type pattern was variable, and additional experiments with larger populations showed that lateral branching on wild-type plants could initiate occasionally at node 2 (less than 1.5% of plants) and node 3 (approximately 7% of plants).

The zone of lateral branching depicted for both wild type and *dad1–1* in Figure 2B (cotyledonary nodes through node 12) occurred during vegetative growth. After node 12, both wild type and mutant had a zone of slow-growing shoots

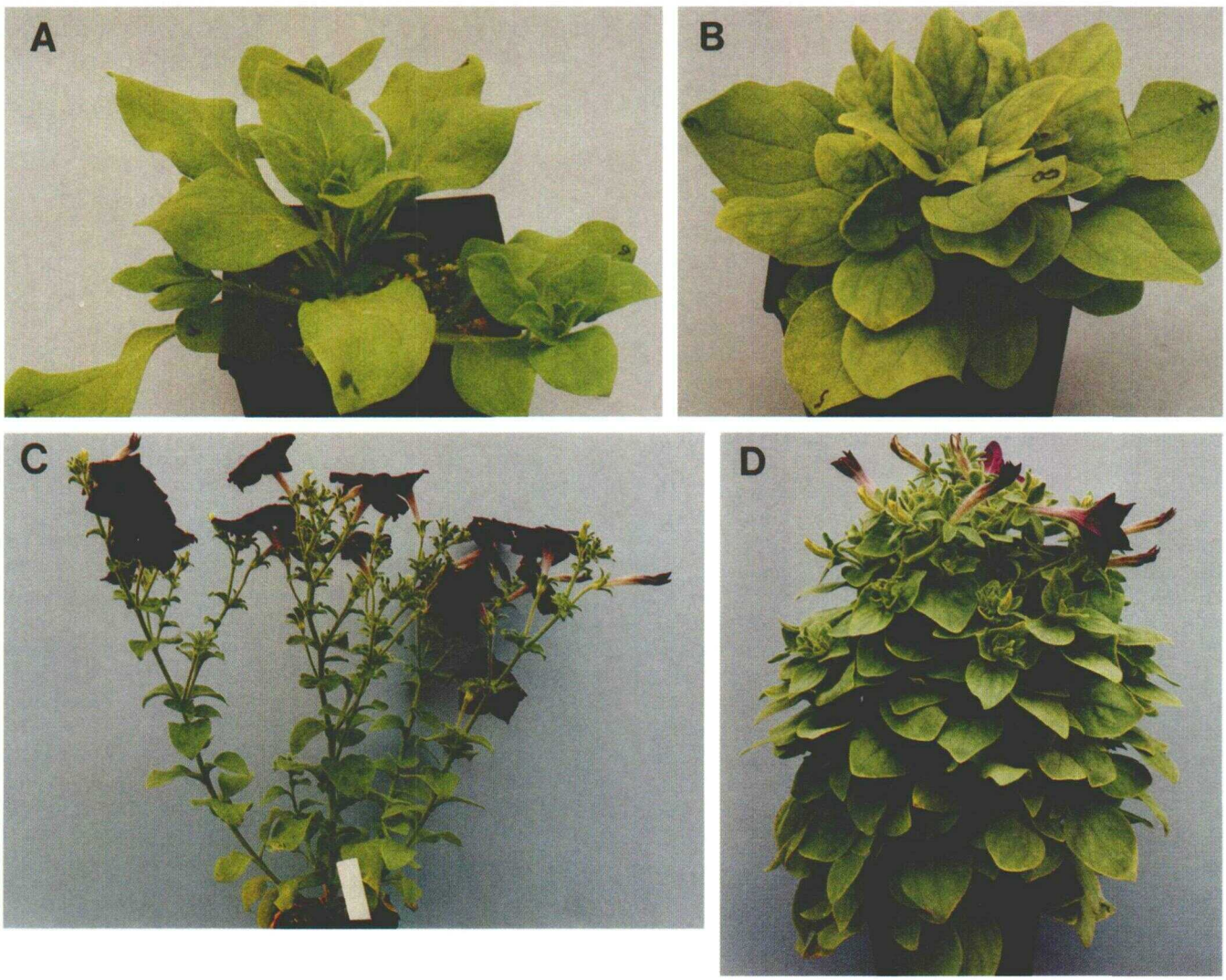

Figure 1. Growth habit differences between wild type and *dad1–1*. A, Wild type at 5 weeks. B, Mutant at 5 weeks. C, Wild type at 10 weeks. D, Mutant at 10 weeks.

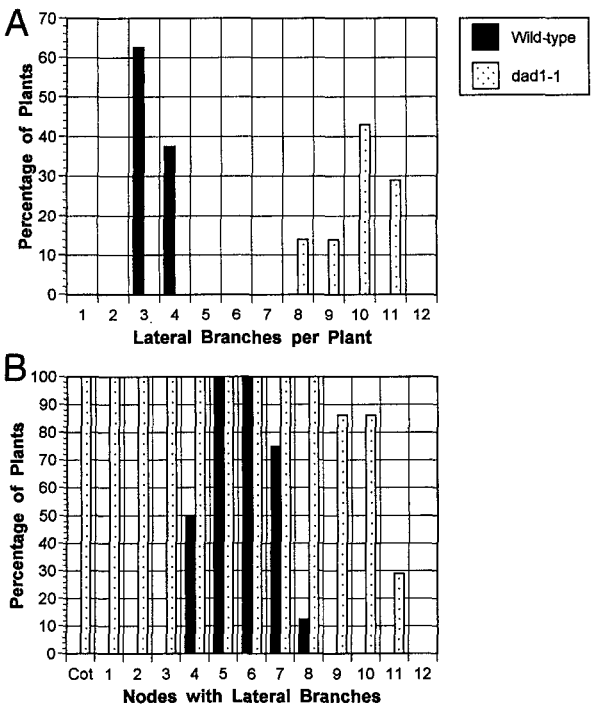

Figure 2. Graphic analyses for lateral branch formation with wild-type and *dad1–1* plants at 10 weeks postgermination. A, Comparison of the total number of lateral branches for each genotype. B, Comparison of the nodes producing lateral branches for each genotype. Population sizes are eight plants for wild type and seven plants for *dad1–1*.

or arrested buds. Lateral branching commenced again in both phenotypes when plants exhibited reproductive maturity. After the production of the first terminal flower, the reproductive shoot system was established by sympodial branching initiated from the axil of the last leaf. The node immediately proximal to the last leaf consistently produced a lateral branch.

Lateral branch outgrowth on wild-type plants could be inhibited by growing plants to maturity within a limited space, such as in six-pack cells that provide less than 75 cm^3 of root medium volume. Under these conditions, axillary buds developed but often did not emerge from leaf axils. Despite the suppression of most basal buds, the distal-most buds on the main stem branch broke in response to reproductive maturity in the manner described. Branching in the mutant could not be completely suppressed by crowding, but branch growth and development was limited. Nonetheless, the distinction between wild type and mutant under these conditions was obvious, as demonstrated in Figure 3A for the wild type and in Figure 3B for the mutant.

First- and Second-Order Branching

dad1–1 displayed first-order lateral branching on the main shoot axis and second-order branching on first-order branches. Second-order branching was not a common wild-type trait and occurred only when apices were removed to release apical dominance. An exception occurred for the last two nodes on wild-type first-order branches, where axillary buds broke as described above in response to reproductive maturity. Typical first-order branches from wild-type and mutant plants are compared in Figure 3C. Each branch shown in Figure 3C originated from a basal node. Whereas second-order branches in wild type did not develop at basal nodes on the first-order branch, second-order branches originated at four lower nodes in the mutant. Second-order branching began on the mutant as early as 5 weeks postgermination and was typically limited to the first three or four nodes at the basal end of the most proximal first-order branches. The earliest formed second-order branches grew vigorously and attained reproductive maturity. The total number of branches presented in Figure 2 for the mutant is a conservative estimate because counting was limited to first-order branches over 2 cm in length and did not include second-order branches.

dad1–1 produced extra axillary buds, or accessory buds, in addition to principal axillary buds. Accessory buds developed from cotyledonary axils as early as 5 weeks postgermination. Accessory bud formation was infrequent and limited to the basal nodes. The shoots derived as accessory buds did not grow with the same vigor as shoots derived from primary axillary buds. An accessory bud is indicated by an arrow in Figure 3D. Accessory buds have not been observed from vegetative nodes on wild-type plants.

Additional Phenotypic Traits Associated with the *dad1–1* Mutation

dad1–1 conditions a relatively nonpleiotropic phenotype, especially since the mutation affects a very important physiological process. However, in addition to decreased apical dominance, four traits are associated with the mutation: (a) a delay in the onset of flowering, (b) shortened internodes, (c) a propensity to develop adventitious roots on stem sections of lower branches, and (d) mild leaf chlorosis. The flowering response time, in terms of leaf nodes, is presented for wild-type and mutant plants in Figure 4. Both genotypes produce the first terminal flower over a range of nodes, with the average at 20.7 ± 1.1 for wild type and 26.3 ± 1.7 for the mutant. Analysis by the *t* test showed the difference to be significant at $P < 0.001$. The delay in flowering is seen in Figure 1, C and D, which depict wild-type and mutant plants at the same point in time but at different stages of development.

To test whether the increase in lateral branches imposed physiological constraints that contributed to the late-flowering phenotype, lateral branches were continuously removed from *dad1–1* plants as the buds emerged from leaf axils. These plants are called *dad1–1* (trimmed), and the flowering data are included in Figure 4. Although lateral branch removal shifted the final node to an average of 25.6 ± 1.6, there was no significant difference in the flowering responses for the two *dad1–1* populations ($P = 0.22$ as determined by the *t* test). The major impact of lateral branch removal was an acceleration of flower development. Table I shows the results of an experiment terminated 60 d postgermination in which both *dad1–1* and

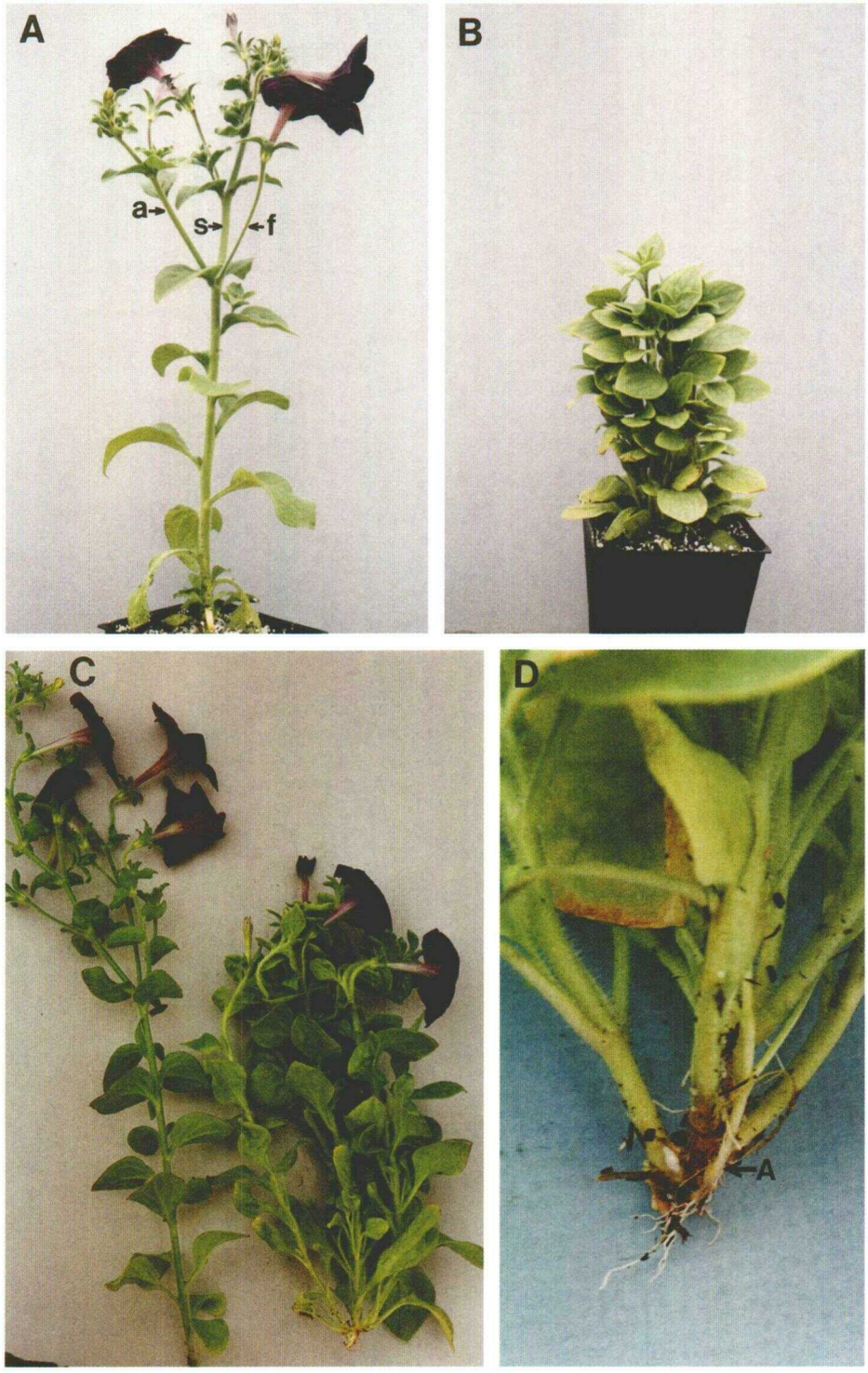

Figure 3. Effect of crowding on branching and a comparison of branches from wild-type and mutant plants. A, Wild type; B, *dad1–1*. Both plants are shown at approximately 8 weeks postgermination. s, Sympodial shoot; a, lateral branch derived from the node immediately proximal to the last leaf; f, first terminal flower. C, Wild-type (left) and mutant (right) first-order branches. D, Basal portion of a first-order branch from the mutant showing an accessory bud (arrow A) and adventitious roots.

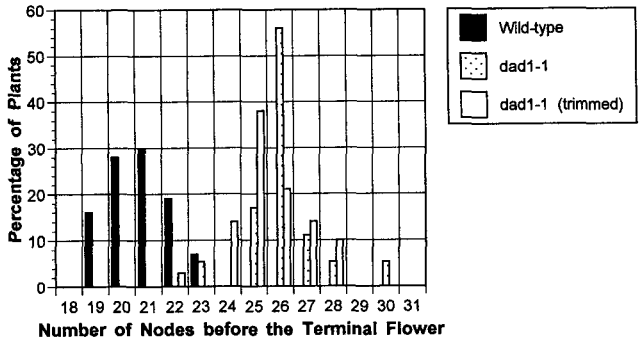

Figure 4. Flowering responses for wild-type and mutant plants. Data are calculated to show the percentage of plants terminating the monopodial axis after a particular leaf node. *dad1–1* (trimmed) plants are described in the text. Population sizes: wild type, 57 plants; *dad1–1*, 18 plants; *dad1–1* (trimmed), 29 plants.

dad1–1 (trimmed) plants had significantly fewer flowers than the wild type. However, *dad1–1* (trimmed) plants had more flowers than untrimmed mutant plants, even though the nodes to flowering were approximately the same for both *dad1–1* populations. Duncan's multiple range test showed that each of the populations was significantly different ($P < 0.05$) from the other populations.

The first 8 to 9 leaf nodes on a wild-type V26 plant were associated with compact internodes, i.e. a rosette habit. An incremental increase in internode length occurred after the rosette stage, with final internodal lengths of 2 to 3 cm on wild-type plants. *dad1–1* also had a rosette stage, followed by internode elongation, but the internodes failed to achieve wild-type length after the rosette stage. The data presented in Figure 5A show the comparison between internodes on wild-type and mutant plants for the 12 nodes proximal to the last 2 leaf nodes. The internode between the last 2 leaves was compact for both wild type and mutant and is not included in the data. With the exception of internode −6 (counting from the terminal flower), the differences in internode expansion were significant ($P < 0.05$) for internodes −8 through −2. The mutant attained 80 to 48% of wild-type length depending on the shoot module position. In addition to their compact habit, mutant plants had smaller stem diameters. Figure 5B shows the results of comparisons between wild-type and mutant stems. These measurements were taken at three positions on the main stem of mature plants. For each position, the differences from wild type were significant ($P < 0.001$). The diameter of mutant plants was approximately 56% of the diameter of wild-type plants.

Table I. *A comparison of flowering times for wild-type and mutant populations*

The data are presented as the average number of open flowers.

Genotype	Flowers[a]	n[a]
Wild type	5.6 ± 0.7	9
dad1–1	0.3 ± 0.5	7
dad1–1 (trimmed)[b]	2 ± 1	6

[a] *n* = number of plants. [b] *dad1–1*-trimmed plants had axillary buds removed during the experiment.

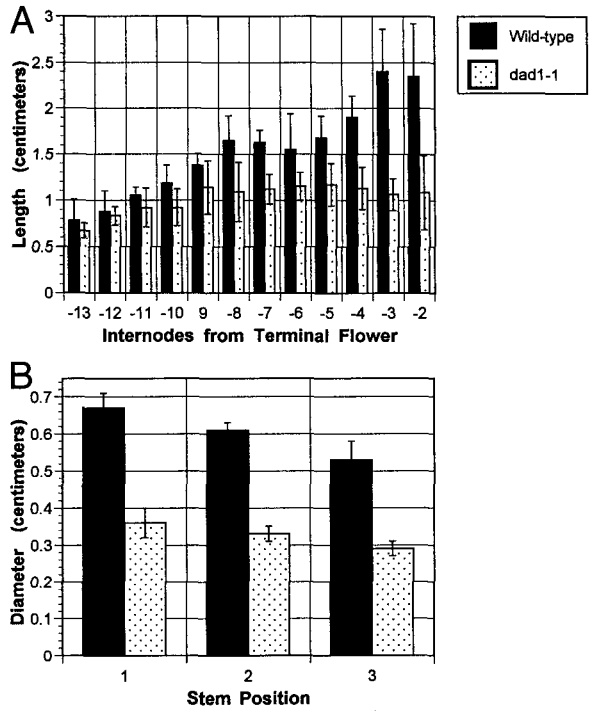

Figure 5. Analysis of the morphological differences between wild-type and mutant plants. A, Differences in internode elongation. B, Stem diameter measurements at three positions on the monopodial axis. The data are presented as the average internode lengths for four wild-type and four mutant plants and the average stem diameters for five wild-type and seven *dad1–1* plants.

The leaves on *dad1–1* mutants had a small degree of interveinal chlorosis. This was not due to an underdeveloped root system. A visual comparison of wild-type and mutant roots suggests that, if anything, the mutant may have slightly more root mass than the wild type (data not presented). Supplemental feeding with 200 $\mu g\ mL^{-1}$ $FeSO_4$ did not alleviate the foliar chlorosis. The mild chlorosis was visible in seedlings and continued throughout the life of the plant.

Finally, *dad1–1* developed adventitious roots around the cotyledonary area and on lower stem segments of basal branches. Adventitious roots developed on the hypocotyl region and cotyledonary branches by the 5th week postgermination, and adventitious roots continued to form as basal first- and second-order lateral branches developed. Figure 3D shows in *dad1–1* a typical first-order branch with basal second-order branches. Adventitious roots can be seen at the proximal end of branches. Although the rooting was not extensive, it was a consistent feature of the mutant phenotype. Adventitious rooting was not observed on stems of wild-type plants.

Graft Studies

A graft technique was developed for petunia seedlings to observe the effect of grafting on early vegetative nodes. Grafting at a hypocotyl position was advantageous because scions have the full complement of vegetative nodes, and

this permitted examination of each phase of plant growth. Due to the small size of petunia seedlings, axenic graft construction on agar proved to be the most efficient method for handling young seedlings. Axenic culture was beneficial, since the agar surface provided a constant moist environment conducive for graft healing, and graft unions healed as soon as the 2nd or 3rd d after alignment.

Two grafting methods were used: (a) hypocotyl grafts in which the two seedlings used for either stock or scion material were severed in the hypocotyl regions and the blunt ends of appropriate combinations were united, and (b) hypocotyl interstock grafts in which one seedling was severed in the hypocotyl region and rejoined with a 2-mm hypocotyl fragment from the same or different genotype. The results of the hypocotyl and hypocotyl interstock experiments are summarized in Tables II and III and examples of grafted plants are shown in Figure 6.

dad1–1 scions were converted to a phenotype resembling wild type when grafted onto wild-type stock (hypocotyl grafting) or in the *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ combination (hypocotyl interstock grafting). *dad1–1* stock or *dad1–1* interstock did not induce a phenotypic change on wild-type scions with either grafting technique (data not shown), and the grafting procedure alone was not responsible for converting mutant to wild type, since self-grafted controls for both hypocotyl and hypocotyl interstock grafts expressed the mutant phenotype. Grafting had no affect on the wild type, because control plants could not be distinguished from nongrafted wild-type plants. The genotypes of all *dad1–1* scions that reverted to a near wild-type appearance were confirmed as mutant by vegetative propagation (see "Materials and Methods"). In addition to confirming the genotype of the scion, results from the vegetative propagation show that no epigenetic effects were induced by grafting.

During the seedling stage, both the wild-type$_{stock}$-*dad1–1*$_{scion}$ (hypocotyl grafts) and the *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ combination have a close resemblance to wild type. Differences between genetic wild-type plants and either wild-type$_{stock}$-*dad1–1*$_{scion}$ or *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants became apparent as plants matured and grew beyond the rosette stage. Figure 6A shows hypocotyl grafts at 4 weeks postgrafting, and Figure 6, B through G, contrasts two points in the development of hypocotyl grafts. Figure 6, H through M, shows hypocotyl interstock grafts at one point in development and grown under crowded conditions to suppress branching in wild-type plants. In each case, wild-type$_{stock}$-*dad1–1*$_{scion}$ plants and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants were consistently slower to mature than wild type but were considerably faster than *dad1–1* controls.

Table II. *A summary of all graft combinations using the hypocotyl graft technique*

Genetic Identity Stock	Genetic Identity Scion	Phenotype Scion	n[a]
Wild type	Wild type	Wild type	50
dad1–1	*dad1–1*	*dad1–1*	9
dad1–1	Wild type	Wild type	18
Wild type	*dad1–1*	"Wild type"[b]	43

[a] n = the number of successful grafts produced for the specified combination. [b] The use of the term "Wild type" implies a partial restoration of phenotype.

Table III. *Summary of hypocotyl interstock graft experiments*

Genetic Identity Stock	Genetic Identity Interstock	Genetic Identity Scion	Phenotype Scion	n[a]
Wild type	Wild type	Wild type	Wild type	4
dad1–1	*dad1–1*	*dad1–1*	*dad1–1*	3
Wild type	*dad1–1*	Wild type	Wild type	6
dad1–1	Wild type	*dad1–1*	"Wild type"[b]	9

[a] n = the number of successful grafts produced for the specified combination. [b] The use of the term "Wild type" implies a partial restoration of phenotype.

Table IV displays the results of three separate graft experiments and shows the difference among controls and grafted plants for the occurrence of first-order branches and flowers on the reproductive axis derived from the main shoot. *dad1–1* controls were not included in the data sets due to their retarded development in comparison to wild-type and near-wild-type phenotypes. Analysis by the *t* test and Duncan's multiple range test show that the populations are not significantly different from the others with respect to branching ($P < 0.05$); however, the data are significantly different with regard to the number of flowers. This supports the visual observation that although wild-type controls, wild-type$_{stock}$-*dad1–1*$_{scion}$ plants, and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants had the same general type of growth habit, the *dad1–1* scions on these grafted plants were slower to express reproductive maturity than wild-type controls.

Morphological differences were analyzed for hypocotyl interstock-grafted plants and controls (Table IV, experiment 3) to support the observation that the wild-type interstock converted the *dad1–1* scion from a compact phenotype to a near-wild-type appearance. The graphs shown in Figure 7 display differences among wild-type controls, *dad1–1* controls, and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants. The characteristics used to analyze differences were the internode length in the four nodes proximal to the last two leaves (Fig. 7A), the length of the expanded internode in the first anthoclade (first sympodial subunit) on the reproductive shoot (Fig. 7B), and stem diameter (Fig. 7C). Duncan's multiple range test on the data presented in Figure 7, A and B, showed no significant difference for wild-type controls and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants, but both sets of data were significantly different from the data from the *dad1–1* controls ($P < 0.05$). Although *dad1–1* controls appeared to be different from wild-type controls and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants, the variability in the data for stem diameters (Fig. 7C) was too great to demonstrate a significant difference among the plants.

The conversion of the *dad1–1* phenotype to near wild type was not dependent on grafting in the hypocotyl region. The same type of phenotypic reversal was observed in grafts constructed by splicing seedlings in the epicotyl region and making the combination wild-type$_{stock}$-*dad1–1*$_{scion}$ (data not presented). In addition, grafting was accom-

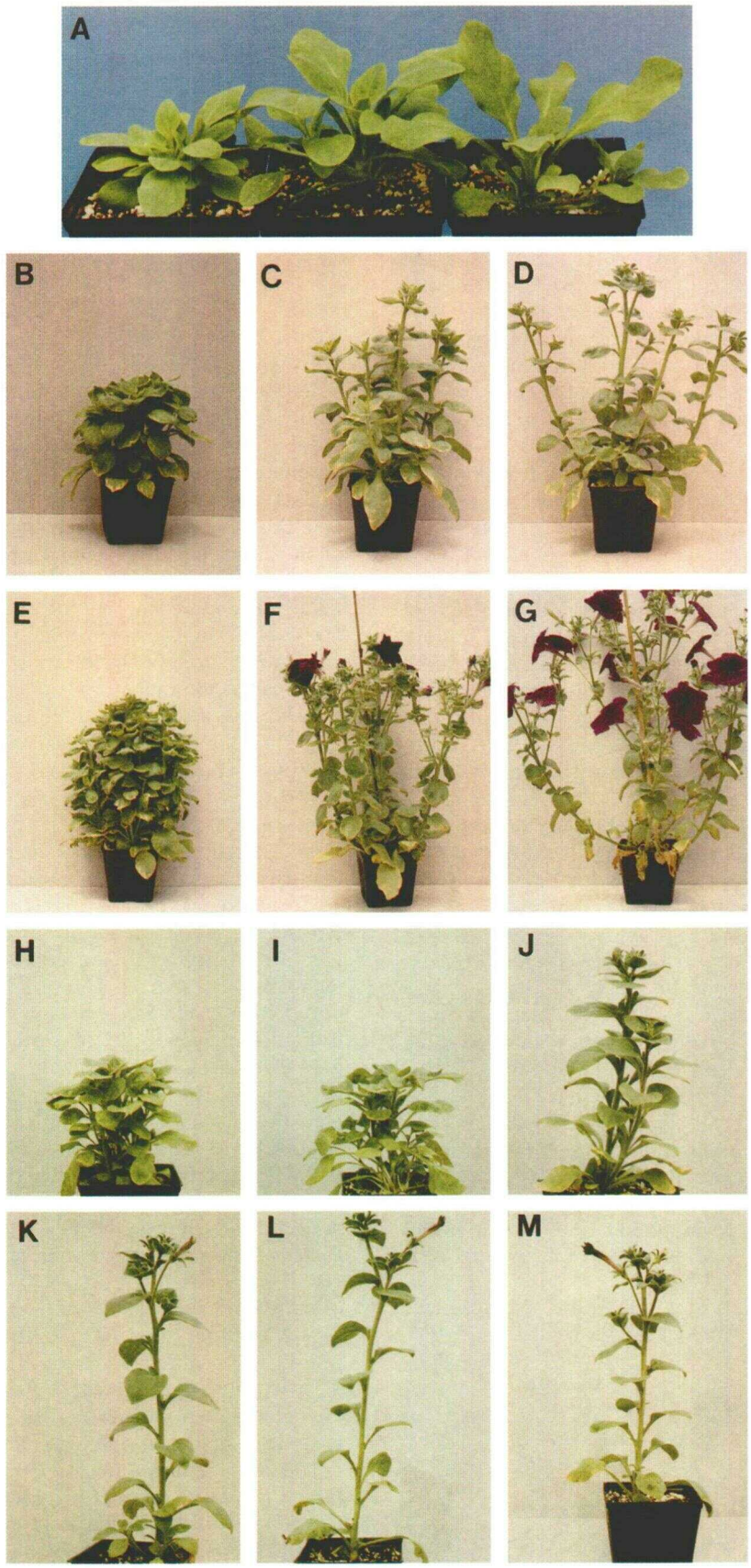

Figure 6. (Legend appears on facing page.)

Table IV. *Results of hypocotyl and hypocotyl interstock grafting*

Graft Combination	*n*[a]	Branches[b]	Flowers[c]
Experiment 1[d]			
Wild-type$_{stock}$-*dad1–1*$_{scion}$	3	7.0 ± 1.4	1.33 ± 0.5
Wild-type controls[e]	3	5.7 ± 0.5	3.0 ± 0
Experiment 2[d]			
Wild-type$_{stock}$-*dad1–1*$_{scion}$	2	5.5 ± 1.5	3.5 ± 0.5
dad1–1$_{stock}$wild-type$_{interstock}$-*dad1–1*$_{scion}$	3	6.7 ± 1.9	1.3 ± 1.2
Wild-type controls[e]	3	4.0 ± 0.8	5 ± 0
Experiment 3[g]			
dad1–1$_{stock}$wild-type$_{interstock}$-*dad1–1*$_{scion}$	6	1.8 ± 1.6	2.5 ± 0.8
Wild-type controls[h]	11	1.4 ± 1.3	7.2 ± 2.2

[a] *n* = number of plants. [b] Primary lateral branches. [c] Open flowers. [d] Plants grown under noncrowded conditions. [e] Wild-type controls and number of plants: *dad1–1*$_{stock}$-wild-type$_{scion}$ (2). [f] Wild-type controls and number of plants: wild-type$_{stock}$-wild-type$_{scion}$ (1). [g] Plants grown under crowded conditions to suppress branching. [h] Wild-type controls and number of plants: nongrafted wild-type (3).

plished using mature greenhouse-grown plants. However, due to the differences in stem diameters, the only successful combinations were wild-type$_{stock}$-*dad1–1*$_{scion}$. In each of three instances, wild-type stock converted a *dad1–1* scion to a near-wild-type appearance.

dad1–1 scions expressed foliar chlorosis regardless of the genetic identity of the rootstock. Whereas a nongrafted mutant or a mutant self-graft expressed foliar chlorosis in all leaves as well as the shoot apex, *dad1–1* scions in the combinations of wild-type$_{stock}$-*dad1–1*$_{scion}$ or *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ expressed chlorosis only at the shoot apex and only during the vegetative phase of growth. Nongrafted *dad1–1* mutants showed foliar chlorosis during the reproductive phase of growth.

There were instances when a *dad1–1* scion did not respond to the presence of wild-type rootstock or the wild-type interstock. In each case, adventitious roots had developed and established a root system from the *dad1–1* scion above the graft union. The phenotype of the scion changed gradually to a near-wild-type appearance in the new growth after removal of the scion roots. *dad1–1* scions consistently produced a low but constant number of adventitious roots during graft healing on agar and after transfer to potting medium. Adventitious root formation was reduced but not eliminated on *dad1–1* scions expressing a near-wild-type phenotype.

DISCUSSION

In *P. hybrida*, a functional *Dad1* locus is required for the expression of apical dominance during early vegetative growth. In the absence of a functional DAD1 gene product, a proliferation of first- and second-order branching occurs at the earliest nodes. In addition to initiating lateral branching sooner than wild type, the mutant continued lateral branching past the range for wild-type branching. Increased branching in the acropetal direction could result from an extended phase of vegetative growth that delays reproductive maturity, since the recessive *dad1–1* allele conditions a late-flowering phenotype. Although removal of lateral branches did not affect the total number of nodes on the main shoot axis, the process did accelerate flower development once floral induction was initiated.

Grafting was carried out to determine if a wild-type stock could modulate the expression of the mutant phenotype. The surprising result was that, not only could a wild-type stock reverse many of the phenotypic effects conditioned by the recessive *dad1–1* allele, but a small wild-type interstock was sufficient to produce the same effect. Interstock grafting is used in nursery production to control tree stature, and in experiments by Hartmann et al. (1990), dwarfing in apple trees was the outcome of the use of a dwarfing interstock. The results of interstock grafting presented here are the opposite of those described by Hartmann et al. (1990) in that a "dwarf" (*dad1–1*) interstock did not promote dwarfing in the wild-type scion.

The differences between the partial wild-type appearance of *dad1–1* scions and genetic wild type are subtle and can be detected only in side-by-side comparisons with wild-type controls. The significant features of the near-wild-type appearance of wild-type$_{stock}$-*dad1–1*$_{scion}$ and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants are (a) the reduced number of nodes producing lateral branches, (b) suppression of second-order branching, (c) earlier flowering, (d) some loss of the compact habit, (e) reduction in adventitious root formation, and (f) reduction of foliar chlorosis. The reduction of adventitious roots may be a consequence of the suppression of lateral branches, which serve as a site for adventitious root formation.

Grafting experiments cannot determine whether the graft effect is due to the transmission from the wild type of

Figure 6. (Figure appears on facing page.) Graft results. A, Hypocotyl grafts at 4 weeks postgrafting. From left to right: *dad1–1* self-graft, *dad1–1*$_{scion}$-wild-type$_{stock}$, and wild-type self-graft. B, C, and D, Hypocotyl grafts at 8 weeks postgrafting. B through G, *dad1–1* self-graft (B, 8 weeks; E, 11 weeks); wild-type$_{stock}$-*dad1–1*$_{scion}$ (C, 8 weeks; F, 11 weeks); wild-type self-graft (D, 8 weeks; G, 11 weeks). H through M, Interstock grafting at 7 weeks postgrafting. H, *dad1–1* nongrafted. I, *dad1–1*$_{stock}$*dad1–1*$_{interstock}$*dad1–1*$_{scion}$. J, *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$. K, Wild-type nongrafted. L, Wild-type$_{stock}$wild-type$_{interstock}$wild-stock$_{scion}$. M, Wild-type$_{stock}$-*dad1–1*$_{interstock}$-wild-type$_{scion}$.

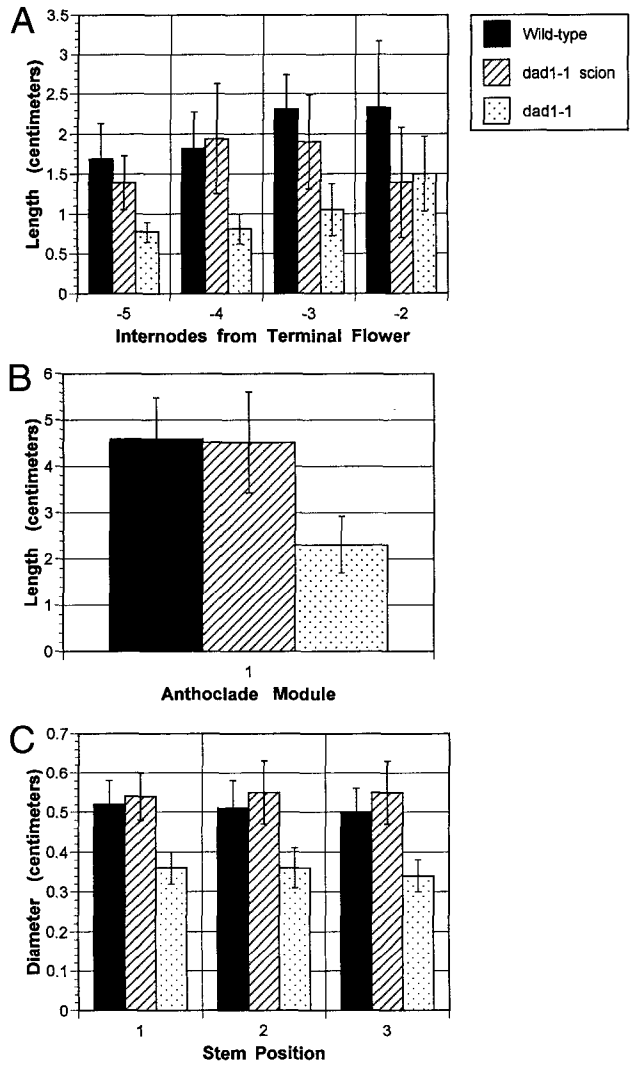

Figure 7. Measurements to demonstrate morphological differences among wild-type, *dad1–1*, and *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants. A, Comparison of internode lengths in four internodes proximal to the last two leaf nodes. B, Comparison of lengths of the expanded internode in the first anthoclade subunits of the sympodial axis. C, Stem diameters at three positions on the vegetative, monopodial axis. Data are presented as the average of 11 wild-type control plants, 9 *dad1–1* control plants, and 6 *dad1–1*$_{stock}$-wild-type$_{interstock}$-*dad1–1*$_{scion}$ plants.

a mobile substance that suppresses branching or whether the wild-type interstock tissue metabolizes a substance transmitted from mutant roots. Interstock grafting shows that the mutant phenotype can be partially converted to wild type as long as the interstock is placed between the *dad1–1* scion and *dad1–1* roots. However, the presence of the wild-type interstock is negated whenever a root system develops on the mutant scion.

Given the importance of roots for the expression of the mutant phenotype and given that increased levels of cytokinin should induce axillary buds to break dormancy, it is tempting to speculate that increased cytokinin is associated with the mutant phenotype. However, foliar chlorosis is inconsistent with increased levels of cytokinin. Moreover, the physical appearance of the *dad1–1* mutant suggests perturbations in other hormones, such as auxin overexpression and GA limitation. These effects can be seen as adventitious root formation (auxin effect) and the reduction in stature (GA effect).

The *dad1–1* mutant has similarities with two other genetic alterations that modify apical dominance, the transgene-induced mutant of tobacco expressing the 35S-*rolC* gene fusion (Schmulling et al., 1988; Estruch et al., 1991) and the *rms*-2 mutant in pea (Beveridge et al., 1994). The common features between *dad1–1* and the *rolC*-affected tobacco plants include reduced apical dominance, reduced internode length, increased nodes to flowering, and leaf chlorosis. The *rms*-2 mutant and the *dad1–1* mutant have three features in common: increased branching, reduction in internode length, and graft responsiveness. Of the two mutants, the petunia mutant has more features in common with the transgene-induced tobacco mutant. Because "RolC activity leads to major alterations in the metabolism of cytokinins and gibberellins" (Nilsson et al., 1993), it may be that the *dad1–1* allele produces similar effects in petunia. These effects may include a decreased level of cytokinin.

In summary, the *dad1–1* mutant is altered in the control of apical dominance for the earliest nodes on the plant, and the presence of the growing shoot has no discernible negative affect on axillary bud and accessory bud growth at this early stage of development. Graft experiments show that the expression of the mutant phenotype requires a mutant root system that is contiguous with the shoot system. The mutant phenotype combines traits associated with auxin overexpression, cytokinin overexpression, and GA limitation, which suggests a complex interaction of hormones in establishing the *dad1–1* phenotype. Clearly, it is essential that further experimentation on the *dad1–1* will involve an analysis of hormone concentrations.

ACKNOWLEDGMENTS

I thank Archie Tang for his help in setting up some initial graft experiments, Drs. Hongzhan Huang and Ken Tourjee for their help with the statistical analyses, and Drs. Richard Jorgensen, Fredrick Bliss, and Neelima Sinha for critical reading of the manuscript. I am grateful to Dr. Morris Cline for comments on the manuscript and for helpful discussions of apical dominance.

Received October 11, 1995; accepted January 30, 1996.

LITERATURE CITED

Beveridge CA, Ross JJ, Murfet IC (1994) Branching mutant *rms*-2 in *Pisum sativum*. Plant Physiol **104:** 953–959

Chaudhury AM, Letham S, Craig S, Dennis E (1993) *amp1*—a mutant with high cytokinin levels and altered embryonic pattern, faster vegetative growth, constitutive photomorphogenesis and precocious flowering. Plant J **4:** 907–916

Cline MG (1994) The role of hormones in apical dominance. New approaches to an old problem in plant development. Physiol Plant **90:** 230–237

Cline MG (1991) Apical dominance. Bot Rev **57:** 318–358

Esau K (1977) Anatomy of Seed Plants. John Wiley and Sons, New York

Estruch JJ, Chriqui D, Grossmann K, Schell J, Spena A (1991) The plant oncogene *rolC* is responsible for the release of cytokinins from glucoside conjugates. EMBO J **10:** 2889–2895

Hartmann HT, Kester DE, Davies FT (1990) Plant Propagation, Principles and Practices. Regents/Prentice Hall, Englewood Cliffs, NJ, pp 336–337

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

Klee H, Horsch R, Hinchee MA, Hein MB, Hoffmann NL (1987) The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. Genes Dev **1:** 86–96

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

Napoli C, Ruehle J (1996) New mutations affecting meristem growth and potential in *Petunia hybrida* Vilm. J Hered (in press)

Nilsson O, Moritz T, Imbault N, Sandberg G, Olsson O (1993) Hormonal characterization of transgenic tobacco plants expressing the *rolC* gene of *Agrobacterium rhizogenes* T_L-DNA. Plant Physiol **102:** 363–371

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

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

Rothenberg M, Ecker JR (1993) Mutant analysis as an experimental approach towards understanding plant hormone action. Dev Biol **4:** 3–13

Schmulling T, Schell J, Spena A (1988) Single genes from *Agrobacterium rhizogenes* influence plant development. EMBO J **7:** 2621–2629

Sitbon F, Hennion S, Sunberg B, Little CHA, Olson O, Sandberg G (1992) Transgenic tobacco plants coexpressing the *Agrobacterium tumefaciens iaaM* and *iaaH* genes display altered growth and indoleacetic acid metabolism. Plant Physiol **99:** 1062–1069

Smigocki AC (1991) Cytokinin content and tissue distribution in plants transformed by an reconstructed isopentenyl transferase gene. Plant Mol Biol **16:** 105–115

Smigocki AC, Owens LD (1988) Cytokinin gene fused with a strong promoter enhances shoot organogenesis and zeatin levels in transformed plant cell. Proc Natl Acad Sci USA **85:** 5131–5135

Steeves TA, Sussex IM (1989) Patterns in Plant Development. Cambridge University Press, Cambridge, UK