Int. J. Dev. Biol. 36: 59-66 (1992)

Molecular genetic approaches to plant development

WOUT BOERJAN, BART DEN BOER and MARC VAN MONTAGU*

Laboratorium voor Genetica, Universiteit Gent, Gent, Belgium

ABSTRACT Higher plant morphogenesis has received renewed interest over the past few years. The improvement of molecular genetic approaches to generate tagged developmental mutants, for instance by T-DNA insertion, facilitated the isolation and characterization of the altered genes. Here we present recent progress on flower and root morphogenesis in the small crucifer *Arabidopsis thaliana*. The current model of *Arabidopsis* flower development is presented. We report on *FLOWER1 (Fl1)*, which is a T-DNA-tagged *ap2* allele. Our observations indicate that this *Fl1* mutant has, besides the homeotic Ap2 phenotype, an aberrant seed coat, suggesting that this gene has also a function late in flower development. Furthermore, we present a brief summary about root development and focus on the super root (Sur) mutant, which is an ethyl methanesulfonate-induced mutant that produces excess lateral roots. Root explants of the Sur mutant, that do not develop further than the 4-leaf stage, can be induced to produce normal-looking shoots and flowers by addition of only cytokinin to the medium. The phenotype of Sur and its relation to the action of phytohormones is discussed.

KEY WORDS: *Arabidopsis thaliana, flower development, ß-glucuronidase, phytohormones, root mutants*

Introduction

Until recently, plant developmental biology studies have remained rather descriptive. Although a wealth of information was obtained by a judicious choice of model plants (Stewart, 1978; Johri, 1984; Raghavan, 1986; Steeves and Sussex, 1989; Lyndon, 1990; Poethig *et al.*, 1990; Sachs, 1991), it was clear that molecular biology techniques had a much higher potential to improve and rapidly enlarge our knowledge of plant developmental programs.

Although *in vitro* recombinant techniques have been available for nearly twenty years, it is only recently that transgenic plants have been used to unravel the developmental signals. Why was there such a long delay in the application of molecular techniques in plants compared to animal research? This might be the subject of interesting studies for the philosophy and sociology of science. If it is remarkable that plant morphologists and physiologists were slow in introducing the molecular biology approaches, it is more worrying that there is a clear lack of drive coming from the agricultural and industrial research organizations. Because of these two obstacles, the number of teams pursuing plant molecular developmental biology is still quite limited and progress is rather slow.

In our own group, we have mainly contributed to working out the techniques for plant engineering and identification and cloning of plant genes induced under pathogen attack and environmental stress conditions. This resulted in the construction of disease-resistant plants. The skills acquired and the tools constructed during this research allowed us to tackle more fundamental work and to try to make some contributions to plant developmental molecular biology.

Here we present two aspects of this work, namely studies on root growth and on genes controlling flower development. For both studies, the small crucifer of the mustard family, *Arabidopsis thaliana*, was chosen as model plant. The molecular genetic arguments for using *Arabidopsis* have been stressed many times (Bowman *et al.*, 1988) and resulted in a collective effort to construct a physical map linking the existing restriction fragment length polymorphism and genetic maps (Hwang *et al.*, 1991). Possibly, there will also be a start in the effort to sequence some large stretches of the *Arabidopsis* genome.

There are also several morphological advantages to the choice of *Arabidopsis thaliana*. Its organ structure is much simpler due to the limited number of cell layers. Root and flower development and the cell fate in meristems can be followed well, particularly in longitudinal sections of a transgenic plant expressing a given marker gene. A root can even be analyzed as a single organ mount without sectioning. Since the cells are small, the different cell layers can easily be visualized. As it is possible to examine the whole organ, e.g. flower, in one section, *Arabidopsis* is also the material of choice to study differential gene expression by *in situ* hybridization (Drews *et al.*, 1991; Jack *et al.*, 1992).

In plants, organs are formed throughout their lifetime from stem cell populations called meristems. There is no cell migration and most cells, once layered down, are no longer stimulated and grow only by cell elongation. However, this vision might turn out to be less

***Address for reprints**: Laboratorium voor Genetica, Universiteit Gent, K.L. Ledeganckstraat 35, B-9000 Gent , Belgium). FAX: 32-91-645349

0214-6282/92/$03.00

Printed in Spain

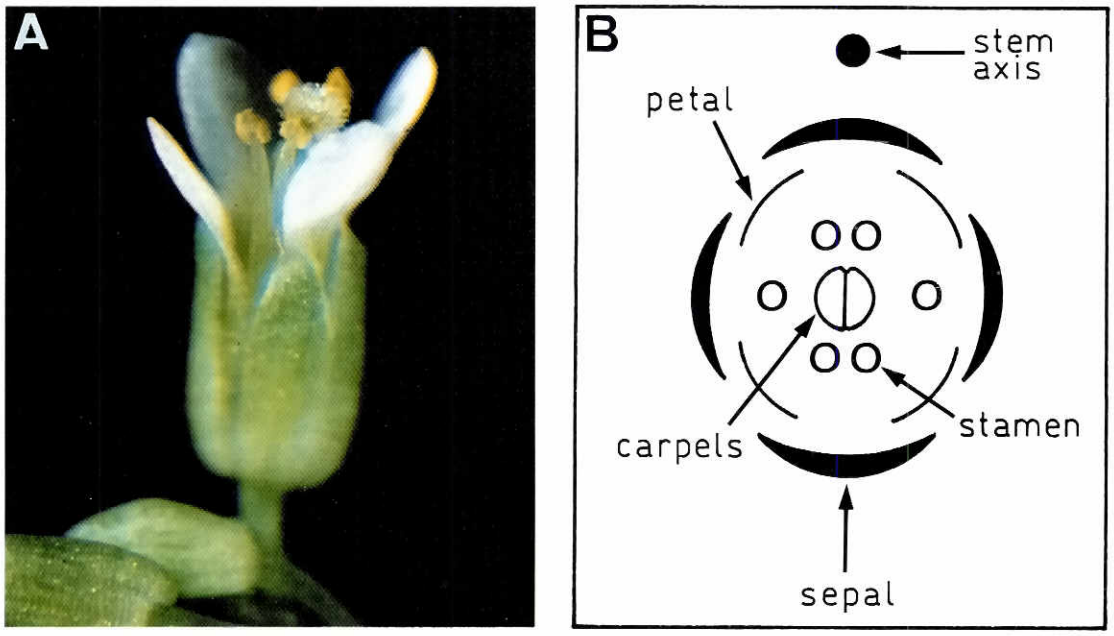

Fig. 1. Wild-type *Arabidopsis thaliana*. **(A)** *Wild-type* Arabidopsis *flower.* **(B)** *Floral diagram of a wild-type* Arabidopsis *flower.*

strict, as can be seen from plants transgenic for the reporter gene ß-glucuronidase under the *cdc2* promoter (P. Ferreira and A. Hemerly, personal communication).

In dicotyledonous plants this seemingly terminal differentiation can be reversed by wounding. What are the molecular signals which guide the transition from a meristem into G_0 or a G_2 cell phase and the reverse? What are the signals which reactivate the cell cycle?

To what degree are somatic plant cells polarized, making their apical plasma membrane different from the basal one? Preparing in this way the unidirectional transfer of signals and gradients of compounds is a likely way for a plant cell to initiate development. We believe that the molecular biologists have the tools to address these questions. A judicious choice of genetic approaches, particularly the generation of T-DNA and transposon mutants and the biochemical analysis will undoubtedly make it possible to put together the first pieces of the development puzzle.

Phyllotaxis and floral development in *Arabidopsis*

Higher plant morphogenesis has received renewed interest over the past few years (Sussex, 1989; Poethig, 1990; Coen, 1991). From these articles it is clear that stem cell populations, which are called meristems, make the plant. They are the source of a variety of structures that arise during plant development and give the plant its characteristic appearance. The primary shoot and root apical meristems are formed during embryonic development. With the exception of the cotyledons and possibly the first leaves none of the organs of the plant are formed during embryogenesis. So in fact most of plant development is post-embryonic. In angiosperms the shoot apical meristem passes through several different phases during the plant's life cycle. At several stages during development of the shoot apical meristem, groups of cells are set aside in the meristem and start a completely new developmental program. Initially, these cells form leaves, the shoot apical meristem is then called vegetative meristem. Later in development, groups of cells are set aside that will form flowers, the meristem is then referred to as inflorescence meristem. Eventually floral organs appear on what is now called the floral meristem. Not only are the structures that these meristems give rise to morphologically different and express a unique set of genes, they also appear in a specific spacial arrangement relative to the shoot axis (Kamalay and Goldberg, 1980; Bernier, 1988; Steeves and Sussex, 1989; Gasser, 1991). Phyllotaxis describes the pattern in which organs are arranged on the growing point of the shoot. It teaches us that plants produce a range of distinct patterns going from vegetative leaves to flowers (Schwabe, 1984).

Phyllotaxis and shoot apical meristems in Arabidopsis

The vegetative meristem of an *Arabidopsis* seedling first produces a pair of opposite leaves which can already be seen in the embryo. The next leaf pair starts on a plane that is perpendicular to the previous one, resulting initially in a so called decussate pattern. Once several leaves are present, new leaves tend to appear one by one leading to a more spiral arrangement (B. den Boer, unpublished data). A key question is: how does the growing point regulate where the primordia will be initiated? This is still not understood. After evocation, the vegetative meristem is transformed into an inflorescence meristem. Cells in the center of this meristem retain the indeterminate growth capacity and localized cell divisions in the flanks generate the floral primordia, which appear in a helical pattern and will develop into flowers. We do not know how the cells in the summit of the inflorescence meristem maintain their stem cell activity. However we do have an idea how many cells of the shoot apical meristem give rise to post-embryonic organs.

Recently a start was made with using sector analysis to generate a fate map for *Arabidopsis*, a plant with an indeterminate inflorescence meristem (Irish, 1991). Cells are marked within the shoot apical meristem with phenotypical observable mutations, for example chlorophyll mutations that lead to white and yellow-green sectors (Poethig, 1987). Subsequently, the distribution pattern of mutant

sectors is scored. Sectors span variable extents of the mature plant body, which demonstrated that the fate of an *Arabidopsis* meristematic cell is not dependent on its lineage. There doesn't seem to be a precise relationship between a cell's position in the meristem and its fate. The first six leaves of an *Arabidopsis* plant are derived from most of the cells that make up a meristem. This suggests that the remainder of the plant, the indeterminate inflorescence with the reiterated pattern of determinate flowers on the stem, is generated from only very few cells in the shoot apical meristem.

Finally, certain cells in the dome of the determinate floral meristem change their division plane. These cells initiate floral organ primordia and give rise to sepals, petals, stamens and the pistil in a whorled (ring of organs) pattern. How the different fates of the organ primordia that build up a flower are specified is still poorly understood, but close to being resolved. In *Drosophila* homeotic genes encode a class of transcription factors that control a cell's positional fate (Ingham, 1988). In flowers, products of homeotic genes acting combinatorially distinguish developmental pathways for floral organs (see below).

It is not unlikely that in fact the same basic principle is involved in specifying where leaves, flowers or e.g. petals appear. In all these cases meristematic cells are altered in their development to the extent that they produce an entirely different structure. Most theories of phyllotaxis are based on the concept of «fields» emanating from young existing primordia positioning the site of formation of the next one (Young, 1978; Schwabe, 1984). Combined with an increase or decrease in extension of an internode this could lead, for instance, to a decussate, helical, or whorled pattern of phyllotaxis.

Genetic control of early floral morphogenesis

Morphogenesis in plants occurs in the absence of cell movement. The plant uses the patterns in which cells are formed by cell division and the direction in which they expand during the enlargement phase of their growth to generate variety in morphology (Fosket, 1990). The ordered array of structures produced by the three different meristems (vegetative, inflorescence and floral) suggests that mechanisms must exist that specify what type of organ is formed during development. Indeed, several mutations exist that disrupt critical steps in morphogenesis which indicates that, for instance, a process such as floral morphogenesis is under genetic control. Recently, several groups have initiated a genetic dissection of the process of flower morphogenesis in *Arabidopsis thaliana* and *Antirrhinum majus*, the snap dragon (Sommer *et al.*, 1990; Coen, 1991; Meyerowitz *et al.*, 1991). We will focus on flower morphogenesis in *Arabidopsis* although very similar mutations also exist in snap dragon. For a comparison of both model plants, see Coen (1991) and Coen and Meyerowitz (1991).

Mutations in the *LEAFY (LFY)* gene lead to plants that do not switch from the inflorescence meristem to the floral meristem. Instead of flowers this mutant basically produces shoots, although aberrant flowers can be produced late in development (Schulz and Haughn, 1991). Since an early program in development (shoot formation) is repeated later and the shoots can be considered to be transformed flowers, Lfy is a heterochronic as well as a homeotic mutant. *TERMINAL FLOWER (TFL)* is another gene acting in the inflorescence meristem (Shannon and Meeks-Wagner, 1991). In *Arabidopsis* the inflorescence meristem is indeterminate and the cells in the summit will keep on producing lateral meristems that form flowers. In Tfl mutants the cells in the summit somehow lose their stem cell activity and the inflorescence terminates with a flower. It is not clear yet what the molecular functions are of the *LFY* and *TFL* genes.

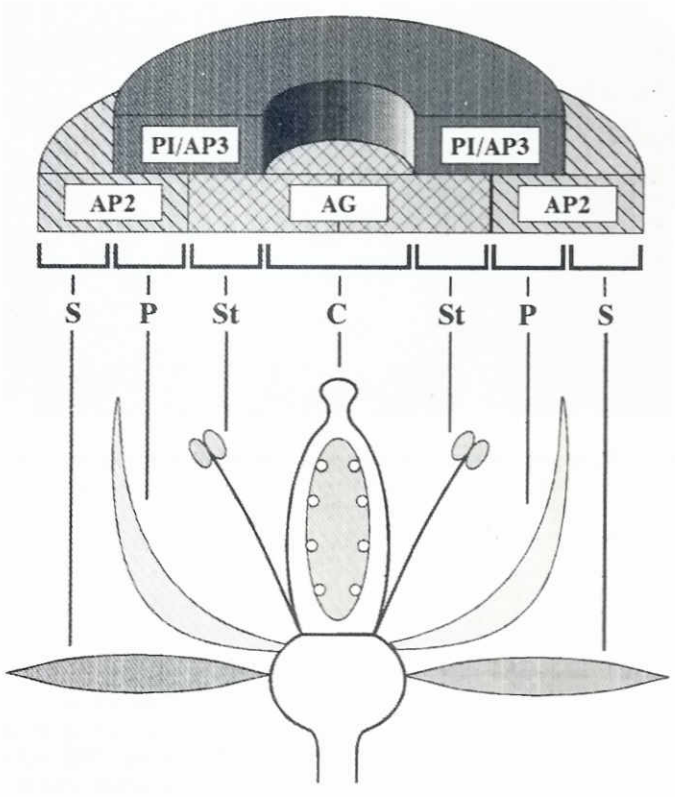

Fig. 2. Model for flower morphogenesis. *The model is based on three classes of homeotic genes:* AP2, PI/AP3, *and* AG, *each of which is predicted to be expressed in two adjacent whorls. A whorl consists of a ring of organs. C, carpel; P, petal; S, sepal; St, stamen (modified from Meyerowitz* et al., *1991).*

Genetic control of specification of whorl identity in flowers

Arabidopsis flowers consist of four whorls (rings) of organs that appear sequentially. Going from outside to inside the flower consists of two whorls of non-reproductive organs. The first whorl or calyx contains four sepals and the second whorl or corolla has four petals in alternating positions with the sepals. The inner two whorls contain the reproductive organs. The androecium consists of six stamens in whorl 3 and the gynoecium of two carpels in whorl 4 (Fig. 1), also referred to as pistil. Mutations that specifically alter floral morphogenesis can affect organ number, organ position and organ identity. These include the so-called homeotic mutants. Floral homeotic mutants are characterized by the transformation of floral organs into other floral organs or into leaves.

Apetala2 (Ap2) is a homeotic mutant with flowers that consist only of reproductive organs (stamens and carpels). Sepals are replaced by female organs (carpels) in whorl 1 and petals by male organs (stamens) in whorl 2 (Kunst *et al.*, 1989). In the extreme *ap2* alleles organs are absent in whorl 2 (Bowman *et al.*, 1991b; Jofuku *et al.*, 1990). This indicates that AP2 not only regulates organ fate in the perianth but also organ number.

Another extreme phenotype is displayed by Agamous (Ag). Ag

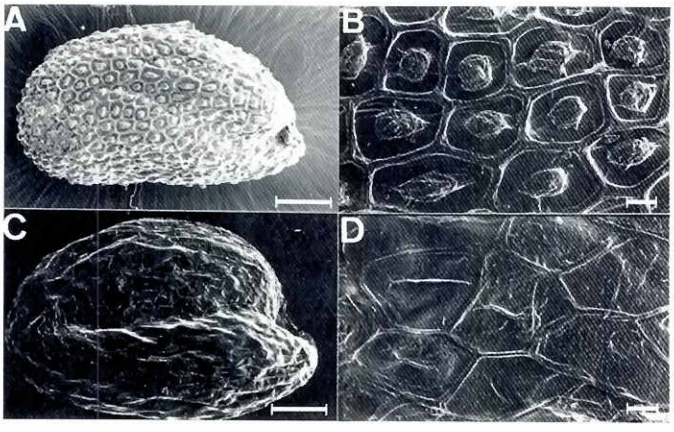

Fig. 3. Scanning electron micrographs of wild-type A. thaliana ecotype C24 and mutant seed coats. (A) *Wild-type seed coat. Bar = 100 μm.* **(B)** *Wild-type seed coat. Bar = 10 μm.* **(C)** Flower1 *(Fl1) seed coat. Bar = 100 μm.* **(D)** *Fl1 seed coat. Bar = 10 μm.*

mutants show the opposite phenotype of *Ap2* mutants. All organs present are non-reproductive organs. Stamens are replaced by petals and the pistil is transformed into sepals. On top of these homeotic changes, the Ag flower is indeterminate. The basic sequence of whorls (sepals, petals, petals) is repeated over and over (Bowman *et al.*, 1989). This indicates that AG regulates organ fate of the reproductive organs and seems to have a «stop» function. In other words, AG controls the determinacy of the floral meristem.

Other homeotic mutants, where organ fate in two adjacent whorls is affected, include Apetala3 (Ap3) and Pistillata (Pi). Both mutants change whorls 2 and 3. In Ap3, petals are transformed into sepals and stamens into carpels. This leads to a flower with two whorls of sepals and two whorls of carpels. Pi has essentially the same phenotype except that organs are absent in whorl 3 (Bowman *et al.*, 1989).

The four homeotic mutants have in common that in each of them the fate of the floral organs is changed in two adjacent whorls.

Flowers of the triple mutants Ap2/Ap3/Ag and Ap2/Pi/Ag have essentially a shoot-like phenotype with this difference, that the leaves are arranged in whorls (Bowman *et al.*, 1991b). This suggests that leaves are the «ground stage» of the floral organ primordia and subsequent expression of the homeotic genes specifies the different floral organs. It also indicates that the four whorl identity genes are necessary and sufficient for the specification of all floral organ primordia.

Genetic experiments have let to a model by which the four floral homeotic genes *(AP2, AG, AP3,* and *PI)* act alone and in combination to specify the identities of two floral organs (Bowman *et al.*, 1991b). A modified version of this model is shown in Fig. 2. Although the model explains basically where and how the different floral organs arise, several aspects of flower development are still unclear. How are the male and female gametophytic pathways established? Which genes control the specification of the different cell types in the reproductive organs? Expression of some of the whorl identity genes is crucial not only during the early stages of flower development but also later when cell types are specified in the floral organs. Ag mutants lack reproductive organs which would enable us to see whether there is any effect in the absence of *AG* gene product on cell specification in these organs. However, *in situ* hybridization experiments on wild-type plants indicate that *AG* expression late in development is restricted to a small number of specific cell types. Expression is detected in the endothelium and nectaries of the stamens and in the endothelium (the cell layer that surrounds the embryo sac in the carpels) as well as in the stigmatic papillae (Bowman *et al.*, 1991a). Ap2 mutants do develop reproductive organs. This allows one to verify whether absence of the *AP2* gene product has an effect late in development of these organs. Our observations on FLOWER1 (FL1), which is a T-DNA tagged *ap2* allele (Jofuku *et al.*, 1991), indicate that the structure of the seed coat is affected (Figure 3). What the exact nature is of the aberrant seed coat is still unclear. The seed coat is derived from the integuments, which surround the embryo sac. This suggests that the *AP2* gene product is present in this tissue. *In situ* hybridization experiments using an AP2 probe show the presence of *AP2* RNA in unfertilized ovules of wild-type pistils (den Boer *et al.*, in preparation).

Two homeotic genes that control floral organ identity *(AP2* and *AG)* are thus not only expressed before and during organ primordium formation but also much later in flower development. At least for one of them *(AP2)* it has been shown that absence of the wild-type gene product leads to a mutant phenotype late in reproductive organ development, indicating not only that the gene is expressed during this stage but, moreover, that it has a function late in flower development.

Root development in *Arabidopsis*

Description of the primary Arabidopsis root

The origin of the *Arabidopsis thaliana* root is established during the first cell divisions of the zygote. The zygote is a polarized cell which divides to form a smaller apical cell that gives rise to the embryo except for the root and a basal cell that will develop into the root and the suspensor, which supplies the young embryo with nutrients from the mother plant (Jürgens *et al.*, 1991).

The development of the *Arabidopsis* root is relatively simple as compared to that of the shoot, since the basic morphology is reiterated and development of lateral roots is not initiated at the apical meristem. Hence, the structure is maintained during most of the *Arabidopsis* life span.

The root consists of a limited number of cell types. Along the radial axis, the root consists of an outer epidermis, which can develop root hairs; a cortex, which is made of parenchyma cells; an endodermis (the innermost layer of the cortex), which has thickened primary cell walls, the Casparian strips, which prevent inward flow of water and solutes through the apoplast. The central portion of the root is occupied by the vascular cylinder. It consists of an outer pericycle that retains meristematic activity. This cell layer initiates the development of lateral roots which force their way through the endodermis and the cortex. Lateral roots are usually initiated opposite to xylem elements (Steeves and Sussex, 1989) and not near to the root apex. Furthermore, the vascular cylinder consists of the xylem and phloem elements.

The longitudinal root axis is usually divided into different zones: the meristematic zone at the root apex, where cell division occurs, the elongation zone, and the differentiation zone. A group of cells in the center of the root apex has a very low mitotic activity. This group of cells, whose function is still unclear, is termed the quiescent center (Clowes, 1976). The root apex is protected by the

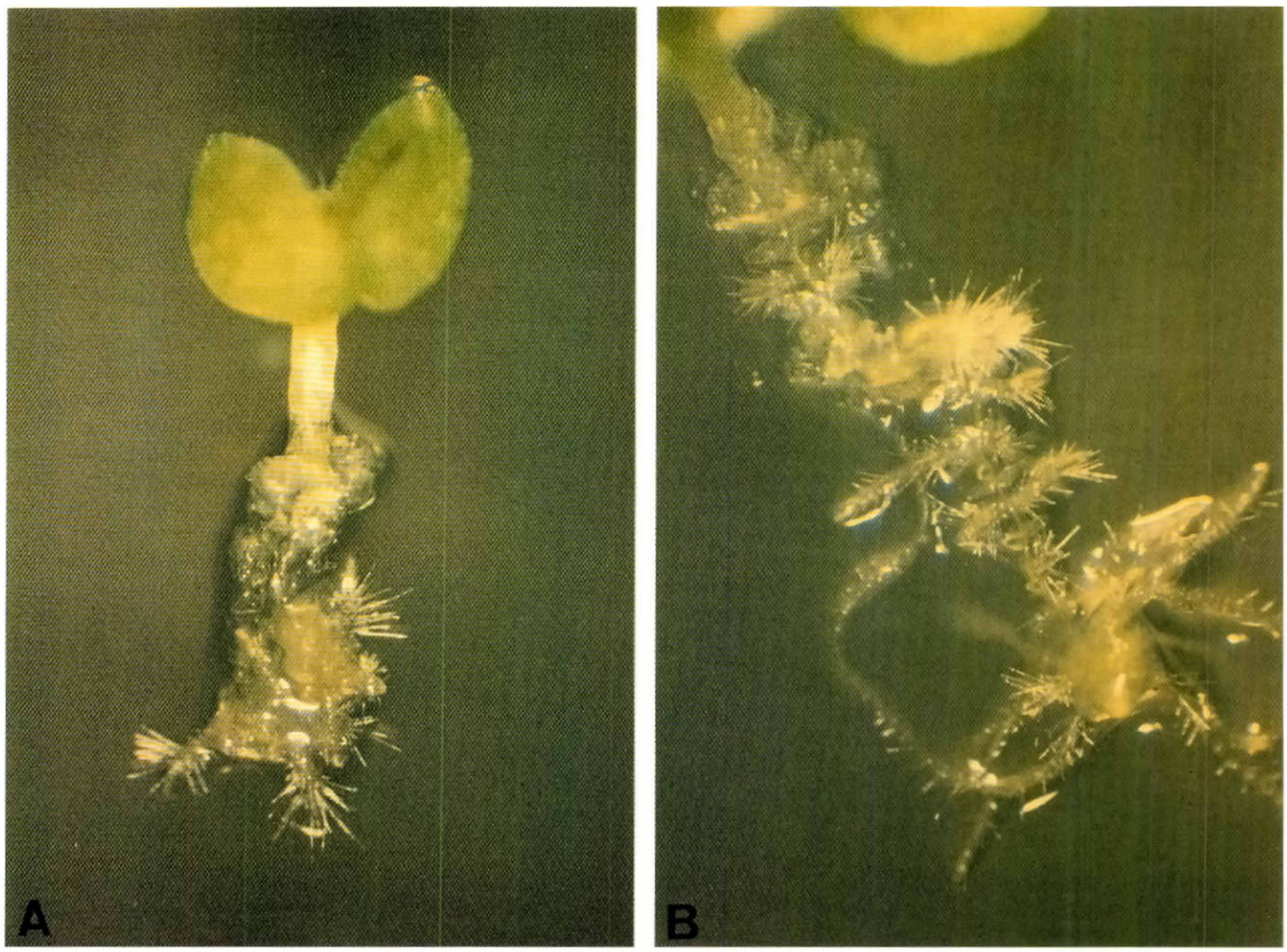

Fig. 4. Phenotype of the Sur mutant nine (A) and fifteen (B) days after germination.

root cap, a group of short-living parenchyma cells which also function in graviperception. In contrast to the apical shoot meristem, very little is known about the organization of the *Arabidopsis* root meristem and the fate of the stem cells.

Root development is influenced by environmental stimuli, like gravity, light, aeration, a range of stress conditions (for a review, see Feldman, 1984; Schiefelbein and Benfey, 1991), and by developmental processes in which phytohormones play an important role (Torrey, 1976).

Phytohormones and root development

It has been known for some time that undifferentiated plant tissue can be induced to produce roots or shoots, depending on the ratio of exogenously applied auxin to cytokinin. Analogous results were obtained by analyzing *Agrobacterium tumefaciens*-induced crown galls on plants. These neoplastic proliferations are caused by the introduction of a piece of the bacterial tumor-inducing (Ti) plasmid, the T-DNA, into the plant cell. This T-DNA consists of several genes among which are genes that lead to auxin and cytokinin overproduction upon introduction into the plant cell (Gheysen *et al.*, 1985). Insertion of transposon Tn5 into the T-DNA of *Agrobacterium tumefaciens* leads to crown gall tumors with altered morphology (Leemans *et al.*, 1982; Joos *et al.*, 1983). Depending on the auxin to cytokinin ratio in the tumor, the phenotype is rooty, shooty, or undifferentiated (Akiyoshi *et al.*, 1983). Upon infection by *Agrobacterium rhizogenes*, a range of plant species induces a »hairy root« phenotype that is caused by the introduction of a piece of the bacterial root-inducing (Ri) plasmid into the plant cell. This T-DNA bears genes, the *rol* genes, which also influence plant development by changing the hormone metabolism (Spena *et al.*, 1987; Schmlling *et al.*, 1988). *RolB*, for instance, codes for an indole-ß-glucosidase (Estruch *et al.*, 1991b) and *rolC* for a cytokinin-ß-glucosidase (Estruch *et al.*, 1991a); thus, these bacterial gene products are able to hydrolyse certain hormone conjugates into free and active phytohormones.

Recently, molecular genetic approaches made it possible to generate transgenic plants which express such bacterial genes under the control of constitutive or inducible promoters (Klee *et al.*, 1987; Medford *et al.*, 1989; Klee and Estelle, 1991). In this way, the effect of altered hormone levels on development can be studied

throughout the whole plant and throughout development. Consistent with earlier observations that exogenously applied IAA can initiate lateral and adventitious roots (Hartman and Kester, 1975; Wightman *et al.*, 1980), transgenic plants overproducing IAA show a range of phenotypes, including increased adventitious root formation (Klee *et al.*, 1987).

Diversity of Arabidopsis *root mutants*

Much of our knowledge about root development has to come from the study of mutants. Several *Arabidopsis* root mutants have been isolated. Among them, pattern mutants such as doppelwurzel, möve, monopteros, and wurzellos (Jürgens *et al.*, 1991); a set of root hair mutants which have defects in the initiation or elongation of root hairs (Schiefelbein and Somerville, 1990); mutants with alterations in root tip rotation patterns (Okada and Shimura, 1990); mutants with an altered root gravitropism (Bell and Maher, 1990); and a series of auxin-resistant mutants with root growth abnormalities including altered root gravitropism (Maher and Martindale, 1980; Estelle and Somerville, 1987; Lincoln *et al.*, 1990; Wilson *et al.*, 1990). Schiefelbein and Benfey (1991) presented a summary of root morphology mutants in *Arabidopsis* and other species. *Arabidopsis* mutants are of particular interest because the mutations can easily be mapped on the *Arabidopsis* chromosomes. *Arabidopsis thaliana* is the model of choice to isolate mutant genes due to its small genome and low content of interspersed repetitive DNA (Leutwiler *et al.*, 1984; Pruitt and Meyerowitz, 1986), the availability of a genetic linkage map (Koornneef, 1987) and two restriction fragment length polymorphism maps (Chang *et al.*, 1988; Nam *et al.*, 1989), the development of a yeast artificial chromosome library (Hwang *et al.*, 1991) and the simplicity of transformation (Valvekens *et al.*, 1988).

Isolation of the super root (Sur) mutant

In the Laboratorium voor Genetica (Gent, Belgium), an *Arabidopsis* mutant with aberrant root development has been isolated in the course of a mutagenesis experiment which was aimed at the identification of genes involved in the control of tissue-specific gene expression. As an approach to unraveling the mechanisms of developmentally and tissue-specifically regulated gene expression, a genetic selection scheme was developed to isolate *Arabidopsis thaliana* mutants which deregulate *in trans* the expression pattern of the ribulose-1,5-bisphosphate carboxylase small subunit gene *(rbcS)*. The selection scheme involves the transformation of *Arabidopsis* plants with a T-DNA that contains three marker genes: the neomycin phosphotransferase II *(nptII)* under the control of the constitutive cauliflower mosaic virus 35S promoter and the hygromycin phosphotransferase *(hpt)* and ß-glucuronidase *(gus)* genes, both under control of identical copies of the promoter of *ats1A* (Krebbers et al., 1988), one of the four *Arabidopsis rbcS* genes. The tissue-specific expression pattern of the chimeric *ats1A-gus* construct can easily be visualized by histochemistry. The colorless substrate 5-bromo-4-chloro-3-indolyl-a-d-glucuronide *(X-gluc)* is hydrolyzed by ß-glucuronidase, the chromogenic group dimerizes and precipitates as a blue color in cells expressing the gene (Jefferson, 1987). The *ats1A-gus* gene was expressed in all green tissues. In roots, GUS activity was restricted to the root tip and the few pericyclic cells undergoing cell division as a first step in the development of lateral root meristems. The basis for the expression of *ats1A-gus* in meristems is still unknown. However, several other genes have been shown to be expressed in root tips (Bäumlein *et al.*, 1991; Boerjan, unpublished results). This unexpected expression might be a consequence of the undifferentiated state of the apical root cells.

When such transformed plants were grown on media containing 150 mg/l hygromycin, root formation was completely inhibited. After ethyl methanesulfonate mutagenesis, M2 seeds were selected on hygromycin-containing medium and scored for root formation. Since the transgenic plants contain the *gus* gene under control of the same regulated promoter as was used to select the mutants, mutations which affect the expression *in trans* would activate both reporter genes simultaneously and can be discriminated from mutants *in cis* (W. Boerjan, unpublished data).

One class of mutants, called *SUPER ROOT* (Sur), isolated in this selection expressed the *gus* gene in roots in the same cell types as in the wild-type-transformed plant: thus, in root tips and pericyclic cells undergoing cell division to produce lateral roots. However, the overall root morphology had changed. The mutants showed a substantial increase in the number of lateral roots (Fig. 4), hence, an increase in zones where the *gus* and *hpt* genes were expressed. These lateral roots themselves also developed lateral roots. The Sur mutants do not develop more than 4 to 6 leaves. The roots of these Sur mutants quickly develop chloroplasts and can be propagated independently of phytohormones. Moreover, (i) these Sur mutant roots can be induced to develop shoots and flowers when only cytokinin is supplied to the growth medium whereas wild-type roots need a cultivation period on auxin-containing media prior to cytokinin-containing media in order to develop shoots; (ii) the phenotype of the Sur mutant can be mimicked by transferring wild-type-transformed seedlings temporarily to auxin-containing media; (iii) the *ats1A-gus* gene is expressed in roots of wild-type-transformed seedlings when these roots are incubated on auxin-containing media.

The phenotype of the Sur mutants becomes visible about seven days after germination. The first symptoms are an extensive peeling off of epidermal and cortical cells from the hypocotyl and the development of adventitious roots by the hypocotyl. Interestingly, a similar observation was made by Karlin-Neumann *et al.* (1991) when cultivating *Arabidopsis* plants, transformed with a chimeric *tms2* gene on medium supplemented with naphthaleneacetamide (NAM). The *tms2* gene (derived from *Agrobacterium tumefaciens*) codes for an indole-3-acetamide hydrolase that can convert NAM to naphthaleneacetic acid (NAA), an active auxin. It is interesting to note that *Nicotiana tabacum* plants, infected with *Agrobacterium rhizogenes* develop hairy-root disease and that these roots can also be propagated without exogenous phytohormone supplementation. All these data together strongly suggest that these Sur mutants have impaired hormone housekeeping, which can be caused by, for example, enhanced auxin biosynthesis, enhanced auxin conjugate hydrolysis, enhanced sensitivity to auxins, cytokinin depletion, a lack of an inhibitor of lateral root development or an accumulation of a downstream compound in the signal transduction cascade that leads to lateral root development, etc.

Work is in progress to analyze the endogenous phytohormone content and to map the sur mutation on the *Arabidopsis* chromosome. Once the mutation is mapped, the mutant can be complemented with large cosmids which overlap the mutation. Identification of the mutant gene will contribute to the elucidation of the signal transduction pathways that lead to root development and provide insight into the complex field of phytohormone-regulated gene expression.

Acknowledgments

This work was supported by grants from the Services of the Primer Minister (U.I.A.P. 120C0187) and the Commission of European Commission BRIDGE (BIOT-CT90-0207 (SMA)). W.B. and B.d.B. were indebted to the «Instituut voor Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw» and the Commission of the European Communities for predoctoral fellowships, respectively.

References

AKIYOSHI, D.E., MORRIS, R.O., HINZ, R., MISCHKE, B.S., KOSUGE, T., GARFINKEL, D.J., GORDON, M.P. and NESTER, E.W. (1983). Cytokinin/auxin balance in crown gall tumors is regulated by specific loci in the T-DNA. *Proc. Natl. Acad. Sci. USA 80:* 407-411.

BÄUMLEIN, H., BOERJAN, W., NAGY, I., BASSÜNER, R., VAN MONTAGU, M. and INZÉ, D. (1991). A novel seed protein gene from *Vicia faba* is developmentally regulated in transgenic tobacco and *Arabidopsis* plants. *Mol. Gen. Genet. 225:* 459-467.

BELL, C.J. and MAHER, E.P. (1990). Mutants of *Arabidopsis thaliana* with abnormal gravitropic responses. *Mol. Gen. Genet. 220:* 289-293.

BERNIER, G. (1988). The control of floral evocation and morphogenesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 39:* 175-219.

BOWMAN, J.L., SMYTH, D.R. and MEYEROWITZ, E.M. (1989). Genes directing flower development in *Arabidopsis*. *Plant Cell 1:* 37-52.

BOWMAN, J.L., DREWS, G.N. and MEYEROWITZ, E.M. (1991a). Expression of the Arabidopsis floral homeotic gene *AGAMOUS* is restricted to specific cell types late in flower development. *Plant Cell 3:* 749-758.

BOWMAN, J.L., SMYTH, D.R. and MEYEROWITZ, E.M. (1991b). Genetic interactions among floral homeotic genes of Arabidopsis. *Development 112:* 1-20.

BOWMAN, J.L., YANOFSKY, M.F. and MEYEROWITZ, E.M. (1988). *Arabidopsis thaliana*, a review. *Oxford Surv. Plant Mol. Cell Biol. 5B:* 57-87.

CHANG, C., BOWMAN, J.L., DEJOHN, A.W., LANDER, E.S. and MEYEROWITZ, E.M. (1988). Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA 85:* 6856-6860.

CLOWES, F.A.L. (1976). The root apex. In *Cell Division in Higher Plants* (Ed. M.M. Yeoman). Academic Press, London, pp. 253-284.

COEN, E.S. (1991). The role of homeotic genes in flower development and evolution. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:* 241-279.

COEN, E.S. and MEYEROWITZ, E.M. (1991). The war of the whorls: genetic interactions controlling flower development. *Nature 353:* 31-37.

DREWS, G.N., BOWMAN, J.L. and MEYEROWITZ, E.M. (1991). Negative regulation of the *Arabidopsis* homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell 65:* 991-1002.

ESTELLE, M.A. and SOMERVILLE, C. (1987). Auxin-resistant mutants of *Arabidopsis thaliana* with an altered morphology. *Mol. Gen. Genet. 206:* 200-206.

ESTRUCH, J.J., CHRIQUI, D., GROSSMANN, K., SCHELL, J. and SPENA, A. (1991a). The plant oncogene *rolC* is responsible for the release of cytokinins from glucoside conjugates. *EMBO J. 10:* 2889-2895.

ESTRUCH, J.J., SCHELL, J. and SPENA, A. (1991b). The protein encoded by the *rolB* plant oncogene hydrolyses indole glucosides. *EMBO J. 10:* 3125-3128.

FELDMAN, L.J. (1984). Regulation of root development. *Annu. Rev. Plant Physiol. 35:* 223-242.

FOSKET, D.E. (1990). Cell division in plant development. *Sem. Dev. Biol. 1:* 357-366.

GASSER, C.S. (1991). Molecular studies on the differentiation of floral organs. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:* 621-649.

GHEYSEN, G., DHAESE, P., VAN MONTAGU, M. and SCHELL, J. (1985). DNA flux across genetic barriers: the crown gall phenomenon. In *Genetic Flux in Plants* (Advances in Plant Gene Research, Vol. 2) (Eds. B. Hohn and E.S. Dennis). Springer Verlag, Vienna, pp. 11-47.

HARTMAN, H.E. and KESTER, D.E. (1975). *Plant Propagation – Principles and Practice*. Englewood Cliffs, Prentice-Hall.

HWANG, I., KOHCHI, T., HAUGE, B.M., GOODMAN, H.M., SCHMIDT, R., CNOPS, G., DEAN, C., GIBSON, S., IBA, K., LEMIEUX, B., ARONDEL, V., DANHOFF, L. and SOMERVILLE, C. (1991). Identification and map position of YAC clones comprising one third of the *Arabidopsis* genome. *Plant J. 1:* 367-374.

INGHAM, P.W. (1988). The molecular genetics of embryonic pattern formation in *Drosophila*. *Nature 335:* 25-34.

IRISH, V.F. (1991). Cell lineage in plant development. *Curr. Opin. Genet. Dev. 1:* 169-173.

JACK, T., BROCKMAN, L.L., and MEYEROWITZ, E.M. (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. *Cell 68:* 683-697.

JEFFERSON, R.A. (1987). Assaying chimeric genes in plants: the *GUS* gene fusion system. *Plant Mol. Biol. Rep. 5:* 387-405.

JOFUKU, K.D., DEN BOER, B., VAN MONTAGU, M. and OKAMURO, J.K. (1990). Cloning of the homeotic regulatory gene locus *apetala-2* by insertional mutagenesis in *Arabidopsis thaliana*. Fourth International Conference on *Arabidopsis* Research, Vienna (Austria), p. 112.

JOFUKU, K.D., DEN BOER, B., VAN MONTAGU, M. and OKAMURO, J.K. (1991). Regulation of floral development in *Arabidopsis thaliana*. *J. Cell. Biochem. (Suppl.) 15A:* 34.

JOHRI, B.M. (1984). *Embryology of Angiosperms*. Springer Verlag, Berlin.

JOOS, H., INZÉ, D., CAPLAN, A., SORMANN, M., VAN MONTAGU, M. and SCHELL, J. (1983). Genetic analysis of T-DNA transcripts in nopaline crown galls. *Cell 32:* 1057-1067.

JÜRGENS, G., MAYER, U., TORRES RUIZ, R.A., BERLETH, T. and MISÉRA, S. (1991). Genetic analysis of pattern formation in the *Arabidopsis* embryo. *Development (Suppl.) 1:* 27-38.

KAMALAY, J.C. and GOLDBERG, R.B. (1980). Regulation of structural gene expression in tobacco. *Cell 19:* 935-946.

KARLIN-NEUMANN, G.A., BRUSSLAN, J.A. and TOBIN, E.M. (1991). Phytochrome control of the *tms2* gene in transgenic *Arabidopsis*: a strategy for selecting mutants in the signal transduction pathway. *Plant Cell 3:* 573-582.

KLEE, H. and ESTELLE, M. (1991). Molecular genetic approaches to plant hormone biology. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:* 529-551.

KLEE, H.J., HORSCH, R.B., HINCHEE, M.A., HEIN, M.B. and HOFFMAN, N.L. (1987). The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. *Genes Dev. 1:* 86-96.

KOORNNEEF, M. (1987). Genetic map of *Arabidopsis thaliana*. In *Genetic Maps*, Vol. 4 (Ed. S.J. O'Brien). Cold Spring Harbor Laboratory, New York, pp. 742-745.

KREBBERS, E., SEURINCK, J., HERDIES, L., CASHMORE, A.R. and TIMKO, M.P. (1988). Four genes in two diverged subfamilies encode the ribulose-1,5-bisphosphate carboxylase small subunit polypeptides of *Arabidopsis thaliana*. *Plant Mol. Biol. 11:* 745-759.

KUNST, L., KLENZ, J.E., MARTINEZ-ZAPATER, J. and HAUGHN, G.W. (1989). *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana*. *Plant Cell 1:* 1195-1208.

LEEMANS, J., DEBLAERE, R., WILLMITZER, L., DE GREVE, H., HERNALSTEENS, J.-P., VAN MONTAGU, M. and SCHELL, J. (1982). Genetic identification of functions of TL-DNA transcripts in octopine crown galls. *EMBO J. 1:* 147-152.

LEUTWILER, L.S., HOUGH-EVANS, B.R. and MEYEROWITZ, E.M. (1984). The DNA of *Arabidopsis thaliana*. *Mol. Gen. Genet. 194:* 15-23.

LINCOLN, C., BRITTON, J.H. and ESTELLE, M. (1990). Growth and development of the axr1 mutants of *Arabidopsis*. *Plant Cell 2:* 1071-1080.

LYNDON, R.F. (1990). *Plant Development, The Cellular Basis*. London, Unwin Hyman.

MAHER, E.P. and MARTINDALE, S.J.B. (1980). Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity. *Biochem. Genet. 18:* 1041-1053.

MEDFORD, J.I., HORGAN, R., EL-SAWI, Z. and KLEE, H.J. (1989). Alterations of endogenous cytokinins in transgenic plants using a chimeric isopentenyl transferase gene. *Plant Cell 1:* 403-413.

MEYEROWITZ, E.M., BOWMAN, J.L., BROCKMAN, L.L., DREWS, G.N., JACK, T., SIEBURTH, L.E. and WEIGEL, D. (1991). A genetic and molecular model for flower development in *Arabidopsis thaliana*. *Development (Suppl.) 1:* 157-167.

NAM, H.-G., GIRAUDAT, J., DEN BOER, B., MOONAN, F., LOOS, W.D.B., HAUGE, B.M. and GOODMAN, H.M. (1989). Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana*. *Plant Cell 1:* 699-705.

OKADA, K. and SHIMURA, Y. (1990). Reversible root tip rotation in *Arabidopsis* seedlings induced by obstacle-touching stimulus. *Science 250:* 274-276.

POETHIG, R.S. (1987). Clonal analysis of cell lineage patterns in plant development. *Am. J. Bot. 74:* 581-594.

POETHIG, R.S. (1990). Phase change and the regulation of shoot morphogenesis in plants. *Science 250:* 923-930.

POETHIG, R.S., McDANIEL, C.N. and COE, E.H. (1990). The cell lineage of the maize shoot. In *Genetics of Pattern Formation and Growth Control* (Ed. A.P. Mahowald). Wiley-Liss Inc., New York, pp. 197-208.

PRUITT, R.E. and MEYEROWITZ, E.M. (1986). Characterization of the genome of *Arabidopsis thaliana*. *J. Mol. Biol. 187:* 169-183.

RAGHAVAN, V. (1986). *Embryogenesis in Angiosperms*. Cambridge University Press, Cambridge.

SACHS, T. (1991). *Pattern Formation in Plant Tissues*. Cambridge University Press, New York.

SCHIEFELBEIN, J.W. and BENFEY, P.N. (1991). The development of plant roots: new approaches to underground problems. *Plant Cell 3:* 1147-1154.

SCHIEFELBEIN, J.W. and SOMERVILLE, C. (1990). Genetic control of root hair development in *Arabidopsis thaliana*. *Plant Cell 2:* 235-243.

SCHÜMLLING, T., SCHELL, J. and SPENA, A. (1988). Single genes from *Agrobacterium rhizogenes* influence plant development. *EMBO J. 7:* 2621-2629.

SCHULTZ, E.A. and HAUGHN, G.W. (1991). *LEAFY*, a homeotic gene that regulates inflorescence development in *Arabidopsis*. *Plant Cell 3:* 771-781.

SCHWABE, W.W. (1984). Phyllotaxis. In *Positional Controls in Plant Development* (Eds. P.W. Barlow and D.J. Carr). Cambridge University Press, Cambridge, pp. 403-440.

SHANNON, S. and MEEKS-WAGNER, D.R. (1991). A mutation in the *Arabidopsis TFL1* gene affects inflorescence meristem development. *Plant Cell 3:* 877-892.

SOMMER, H., BELTRÁN, J.-P., HUIJSER, P., PAPE, H., LÖNNIG, W.-E., SAEDLER, H. and SCHWARZ-SOMMER, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus:* the protein shows homology to transcription factors. *EMBO J. 9:* 605-613.

SPENA, A., SCHÜMLLING, T., KONCZ, C. and SCHELL, J.S. (1987). Independent and synergistic activity of *rol A, B* and *C* loci in stimulating abnormal growth in plants. *EMBO J. 6:* 3891-3899.

STEEVES, T.A., and SUSSEX, I.M. (1989). *Patterns in Plant Development*, 2nd ed. Cambridge University Press, Cambridge.

STEWART, R.N. (1978). Ontogeny of the primary body in chimeral forms of higher plants. In *The Clonal Base of Development* (36th Symposium of the Society for Developmental Biology) (Eds. S. Subtelny and I.M. Sussex). Academic Press, New York, pp. 131-160.

SUSSEX, I.M. (1989). Developmental programming of the shoot meristem. *Cell 56:* 225-229.

TORREY, J.G. (1976). Root hormones and plant growth. *Annu. Rev. Plant Physiol. 27:* 435-459.

VALVEKENS, D., VAN MONTAGU, M. and VAN LIJSEBETTENS, M. (1988). *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis* root explants using kanamycin selection. *Proc. Natl. Acad. Sci. USA 85:* 5536-5540.

WIGHTMAN, F., SCHNEIDER, E.A. and THIMANN, K.V. (1980). Hormonal factors controlling the initiation and development of lateral roots. II. Effects of exogenous growth factors on lateral root formation in pea roots. *Physiol. Plant. 49:* 304-314.

WILSON, A.K., PICKETT, F.B., TURNER, J.C. and ESTELLE, M. (1990). A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Mol. Gen. Genet. 222:* 377-383.

YOUNG, D.A. (1978). On the diffusion theory of phyllotaxis. *J. Theor. Biol. 71:* 421-432.