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THE IMPACT OF *ARABIDOPSIS* RESEARCH ON PLANT BIOTECHNOLOGY

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ABSTRACT

Arabidopsis thaliana, a small annual weed belonging to the mustard family, has become a widely used model in plant genetic research. It has a small genome, short life cycle, and is easy to mutagenize. Identification of genes based on phenotype alone, often a rather difficult part of molecular genetic research, is easiest in this plant. Laboratories working on the "model" plant *Arabidopsis thaliana* have created a network for sharing resources and ideas, so progress has been rapid. The importance of this plant to biotechnology is that genes isolated from *Arabidopsis* can be used to find their homologs in crop plants. Likewise, fundamental mechanisms can be understood in a model plant, and applied in crop plants.

keywords: plant biotechnology, *Arabidopsis thaliana*, genetics, mutants, transgenic plants

INTRODUCTION

Arabidopsis thaliana (L.) Heynh., a member of the mustard family or cruciferae, seems at first an unlikely object for biotechnology. It is not a crop plant, and is not known to produce any unusually important secondary products. It is a weed, but certainly not one that threatens crops badly enough to make it worth studying as a target for new herbicides. Yet, some of the most advanced recent work in plant molecular genetics, both applied and basic, has been done using this plant. The reason for this is twofold: First, *Arabidopsis* has a number of characteristics that make it one of the best plants for genetic studies (Meyerowitz, 1989). Second, a large number of researchers have become convinced that the first reason is correct. As a result, a large

base of mutants, genetic mapping, gene sequences and libraries has become established in a remarkably short time (Koncz et al., 1992).

The plant is small (Fig. 1), fast-growing, and self-fertile, with a generation time (from germination to the first seeds) under continuous light of about six weeks. By two months, a single plant will have produced thousands of seeds. Large numbers of plants can be grown in a small space, several thousand seedlings can be screened in a single standard Petri dish, and as many as 100 plants can be grown in a small pot. Examination of an *Arabidopsis* root under the microscope shows another important property: organs and tissues are composed of fewer cells than in a larger plant (Fig. 2). The embryo in a seed, like the root in Fig. 2, contains few cells, as compared to larger plants. If seeds are mutagenized, e.g. by treatment with x-rays or a chemical mutagen, chances are high for hitting a cell whose progeny will eventually give rise, through meiosis, to a megaspore or microspore. Furthermore, the flowers self-fertilize unless a cross is deliberately made, further enhancing the chances of obtaining homozygous, recessive mutants, in which both the male and female nucleus derive from the same, original mutated nucleus in the seed that was mutagenized. The size of the haploid nuclear genome is about 70 million DNA base pairs (Fig. 3), about five times that of yeast, and considerably smaller than other higher plants.

This, together with the short generation time, minimize the work needed for mapping and construction of strains carrying any desired set of markers. Apparently such a small genome is enough to specify an organism that is as truly a flowering plant as is, for example, barley, with 25-fold more DNA. Part of the explanation is that the *Arabidopsis* genome contains little repetitive DNA, and gene families tend to be small.

ORGANIZATION OF THE GENOME

The haploid nuclear genome consists of five chromosomes. More than 100 mutants have been placed on a genetic linkage map (Koornneef et al., 1983); such a map in *Arabidopsis* or any other organism is based on the frequencies at which pairs of mutations are found together in the progeny when individual mutants are crossed. In parallel, there is a map consisting of almost as many RFLP (restriction fragment length polymorphism) markers (Chang et al., 1988). Such markers are not visible phenotypes like colors or growth forms, but, rather, consist of specific regions on the genome whose sizes vary between individuals. Each region can be detected with a specific DNA probe, and followed in this way from parents to progeny just like any other genetic marker. New sets of markers and techniques (Jarvis et al., 1994; Fabri and Schaffner, 1994; Bell and Ecker, 1994) will short-cut the mapping process; the classical and RFLP maps have been integrated (Hauge et al., 1993).

MUTAGENESIS AND SELECTION

Arabidopsis can be conveniently mutagenized by chemical mutagens or by radiation, with the advantages mentioned above: the progeny from a typical mutagenesis experiment can be harvested from a growth chamber rather than a field. The alkylating agent ethylmethane sulfonate (EMS) has been found to be a very effective mutagen, and the treatment conditions are well-characterized (Koornneef et al., 1982). Seeds are treated with EMS, or receive other mutagenic treatments such as radiation. Inside each viable seed there is a multicellular embryo. The DNA in some of the meristematic cells of this embryo may be damaged by the treatment; some of these, now mutant, cells will eventually give rise to pollen grains or ovules that carry the mutation in their gametes. *Arabidopsis* flowers self-fertilize unless a deliberate effort is made to transfer pollen from a different plant. Thus, there is a high chance that sperm and egg nuclei carrying the same mutation will meet, allowing the visible expression of recessive alleles. The first generation obtained by selfing in plants that grow from mutagenized seeds will contain a high proportion of mutants. Seeds of this first generation, referred to as M2, are commercially available.

The frequency of mutations obtained in any particular gene after EMS treatment is 2×10^{-4} (Koornneef et al., 1982). In other words, disregarding the important variability between the different loci, one mutant of the desired type should appear in a sample of about 5000 plants. Mutants have been recovered by screening as few as 2000 plants, either because the frequencies were high for those loci, or because mutations at several distinct loci could result in loss of the same function. This makes it possible to select mutants by visual inspection. It is also practical to use more complex procedures, such as extracting lipids from the leaf of each plant, injecting the extract into the gas chromatograph, and letting the rest of the plant grow for further analysis (Estelle and Somerville, 1986). However, the real advantage of *Arabidopsis* is not the mutation frequency per locus in itself; high frequencies can also be found in other plants. Rather, the compact nature of the treatment and screening makes the work economical and easy to perform.

A case in point is the isolation of auxotrophs. Such nutrient-requiring mutants have been readily available for years in microorganisms, but relatively difficult to obtain in plants. Using a visual screen (fluorescence of the intermediates that accumulate in the mutants), Last et al. (1991) were able to isolate tryptophan auxotrophs, and subsequently elucidate the entire biosynthetic pathway.

Morphogenesis, still not understood in complete molecular detail, has also received contributions from this remarkable model plant. Mutants in nine genes that are specific to formation of the embryo were found (Mayer et al., 1991). There are also

mutants in the structure of the adult plant, for example in leaf size, or changes in the normal rosette growth habit, epidermal trichomes, shape of the pods, and so forth. A gene involved in apical-basal pattern formation in the embryo has been cloned: mutants in the *EMB30* gene lack roots. Though apical-basal patterns are peculiar to plants, the protein encoded is similar to genes known from yeast to humans (Shevell et al., 1994).

Among the most striking and best-understood mutations affecting structure are those mutants in flower form. Mutations in a group of four genes can cause conversions of one flower part to another (Bowman et al., 1991). Such conversions, borrowing from *Drosophila* terminology, are referred to as homeotic. Mutants in the gene *AGAMOUS* have petals where stamens would normally appear in wild type flowers, while instead of the ovary, additional, internal flowers appear. Mutants in the other three genes, *APETALA2*, *APETALA3* and *PISTILLATA*, cause other interconversions. Homologs of these genes are found in the snapdragon (Carpenter and Coen, 1990; Schwarz-Sommer et al., 1990), tomato (Pnueli et al., 1994) and petunia (Halfter et al. 1994). Thus, homeotic selector genes that program floral development are by no means unique to *Arabidopsis*, and will probably be found in all plants including those of agricultural and horticultural importance. A model has been proposed in which each gene has its own field of expression, and interaction with the other genes, in the floral meristem (Bowman et al., 1991; Weigel and Meyerowitz, 1994). The hierarchy of control appears, like the genes themselves, to be conserved between species.

The mechanism of action of phytochrome, the photoreversible plant pigment which plays a central role in the control of growth and development, has remained a mystery for decades. With the help of molecular genetics, the action of phytochrome may soon be understood. The first plant mutants having abnormal light responses were the *hy* mutants of *Arabidopsis*, which have "high", or long, hypocotyls (Koornneef et al., 1980). Seedlings of these mutants continue to grow rapidly even in the light, whereas wild type seedlings do so only until they have escaped from the soil. Mutants are now available for at least two members of the phytochrome gene family, *PHYB* (Reed et al., 1993) and *PHYA* (Nagatani et al., 1993; Whitelam et al., 1993). The phytochrome family consists, in this plant, of five genes (Clack et al., 1994). These mutants are being used to sort out the functions of each photoreceptor in the complex response of the plant to light. One of the original *hy* mutants, *hy4*, has now been shown to be defective in a gene encoding a protein similar to the DNA repair enzyme photolyase (Ahmad and Cashmore, 1993). Photolyase fits predictions (Galland and Senger, 1987) of what a blue light receptor might look like: a protein with flavin and other blue-light absorbing chromophores. Photolyases use visible light to help catalyze the splitting of thymidine dimers, but similar proteins might control development by

other photoreactions. Plants that are mutants at *HY4*, of course, are defective in their response to blue light. If *HY4* or one of the other classes of blue-light insensitive mutants (Liscum and Hangarter, 1991) indeed lead to the blue light photoreceptor genes and their transducers (Reymond et al., 1992), this will be another "first" for *Arabidopsis*. Conversely, there are mutants in genes whose products are regulatory molecules; these mutants, while grown in the dark, behave, almost, as if they had seen light (Chory et al., 1990; Deng et al., 1992; Misera et al., 1994). At least one of these, *cop1*, encodes a regulatory protein with the zinc-finger motif that is well known from other organisms (Deng et al., 1992). *DET1*, on the other hand, encodes a novel nuclear-localized protein (Pepper et al., 1994).

Plant growth substances act as internal messengers for external signals such as light, gravity, stress or pathogen attack, and serve to integrate the different parts of the plant into a functioning whole organism. Mutants in hormone response pathways will help elucidate the mechanisms. Mutants in hormone response pathways were isolated soon after the first photoresponse mutants; for example a gibberellin-insensitive mutant (Koornneef et al., 1985). Rapid progress has been made with ethylene, chemically the simplest of plant hormones (Kieber and Ecker, 1993). Abscisic acid-insensitive (*abi*) mutants have been isolated, and the *ABI1* product, as deduced from the sequence, is a protein serine or threonine phosphatase (Meyer et al., 1994). *ABI3* encodes a protein that looks like a transcription factor (Giraudat et al., 1992). The auxin pathway has also been the focus of attention; the auxin-resistance gene *AXR1* encodes a protein with sequence homology to ubiquitin-activating enzyme, though its action may be different (Lyser et al., 1993). More surprises and correlations are in store as the genes corresponding to available hormone-response phenotypes are cloned. Genes encoding signal transducers, for example a G protein α subunit (Ma et al., 1990) have been isolated, and the search has begun for the pathways they belong to.

Until recently, no pathogens of *Arabidopsis* were known or studied. However, Oomycetes (*Peronospora* and *Pythium*), bacteria (*Pseudomonas* and *Xanthomonas*) and other pathogens pose a threat to the plant. Furthermore, they open up opportunities for the study of plant-pathogen interactions and the inheritance of resistance (Davis and Hammerschmidt, 1993; Mauch-Mani and Slusarenko, 1993). Mutants at several loci have been isolated which spontaneously form lesions on leaves that are reminiscent of those occurring in disease (Dietrich et al., 1994). The mutants show some resistance to *Peronospora parasitica*, a fungus causing downy mildew. Mutants at another locus, *RPS2*, conversely, lose the ability to mount a hypersensitive defense response to virulent *Pseudomonas* strains (Yu et al., 1993). Samac and Shah (1994) used antisense RNA expression to reduce the level of the major chitinase in the plant; susceptibility to infection by *Botrytis cinerea* increased only slightly. Plant-pathogen interactions are, of course, one of the most important

facets of plant biotechnology, so this is a promising area. One problem is that most pathogens are not as well-studied genetically as the host plant, *Arabidopsis*.

CLONING OF GENES FOR WHICH A MUTANT PHENOTYPE IS KNOWN

It is not usually sufficient to find a mutant in the function of interest. The objective of most research projects includes identifying the relevant gene. Ideally, it should be possible to do this starting with the mutant phenotype, without any additional information about the gene. In these instances the advantages of using *Arabidopsis* are significant. For example, consider the isolation of *ABI3*, one of three distinct "abscisic acid insensitive" loci (Giraudat et al., 1992). Seeds of *ABI3* mutants have reduced seed dormancy, are relatively insensitive to abscisic acid, and are defective in seed maturation. With the help of the genetic linkage and RFLP maps mentioned above, the region of chromosomal DNA carrying the *ABI3* mutation was localized to a single cosmid (cosmid vectors carry about 40 kb of genomic DNA). In a genetic complementation experiment, a fragment of the 40 kb chromosomal DNA insert from the cosmid was introduced into the *ABI3* mutant and found to restore the wild-type function to the mutant. This fragment was then used (as a DNA hybridization probe) to isolate cDNA clones containing the complete sequence. This experiment would have been slower and more difficult to perform in a plant with a much larger genome, and with fewer available libraries and genetic markers.

Transformation and regeneration of *Arabidopsis*, as for many other dicots, has become a relatively straightforward and routine procedure, using for example, the methods of Valvekens et al. (1988). The plant, once again because it is easy to screen large numbers of progeny, is ideal for basic studies such as a recent one showing stable transgene expression (Dehio and Schell, 1993).

Insertional mutagenesis of *Arabidopsis* with T-DNA (Koncz et al., 1990; Feldmann, 1991) is a powerful genetic tool; stocks of seeds carrying such insertions are available for screening and were used to isolate the *HY4* gene mentioned above (Ahmad and Cashmore, 1993). Another successful application of this method is the cloning of an S18 ribosomal protein gene (Van-Lijsebettens et al., 1994). Mutants at this locus, whose product is expressed specifically in meristems, develop pointed first leaves and suffer from other structural defects. The small genome size of *Arabidopsis* is conducive to cloning by the technique of genomic subtraction. In this method, a region of 1 kb or more that is missing in the mutant genome is amplified by the polymerase chain reaction (PCR) and then mapped to the gene of interest (Straus and Ausubel, 1990).

OUTLOOK: HOW USEFUL WILL *ARABIDOPSIS* BE FOR BIOTECHNOLOGY?

From the brief survey above, it is clear that basic research has benefitted greatly from the focus of many labs on a single model plant. It is important to remember, though, that *Arabidopsis* is not a crop plant. Its small size is not always an advantage, making it difficult to obtain large amounts of any specific tissue. On the whole, these problems are insignificant when weighed against the ability to quickly isolate new genes of interest. In general, the genes of other higher plants share enough homology with *Arabidopsis* to enable one to find the gene of interest in a crop plant, by using the more easily isolated *Arabidopsis* gene as a DNA hybridization probe. Practical applications should follow soon upon the results that we have outlined here. It is worthy of note that this logic has been used in the reverse direction, i.e. from crop plants to model plant. *Arabidopsis* has become such a widely-used model whose genes of importance are found by homology to those cloned from crop plants. A recent example is the ethylene-forming enzyme, central to problems of ripening and post-harvest physiology: the tomato gene was used to find the *Arabidopsis* homolog (Gomez-Lim et al., 1993).

REFERENCES

Ahmad, M. and Cashmore, A.R. (1993). *HY4* gene of *A. thaliana* encodes a protein with characteristics of a blue-light photoreceptor. Nature 366, 162-166.

Bell, C.J. and Ecker, J.R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19, 137-144.

Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. Development 112, 1-20.

Carpenter, R. and Coen, E.S. (1990). Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. Genes Dev. 4, 1483-1493.

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.

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

Clack, T., Mathews, S. and Sharrock, R.A. (1994). The phytochrome apoprotein family in *Arabidopsis* is encoded by five genes: the sequencees and expression of *PHYD* and PHYE. Plant Mol. Biol. 25, 413-427.

Davis, K.R. and Hammerschmidt, R. (1993). *Arabidopsis thaliana* as a model for plant-pathogen interactions. APS Press, St Paul.

Dehio, C. and Schell, J. (1993). Stable expression of a single-copy *rolA* gene in transgenic *Arabidopsis thaliana* plants allows an exhaustive mutagenic analysis of the transgene-associated phenotype. Mol. Gen. Genet. 241, 359-366.

Deng, , X-W, , Matsui, M., Wei, N., Wagner, D., Chu, A.M., Feldmann, K.A. and Quail, P.H. (1992). *COP1*, an *Arabidopsis* photomorphogenic regulatory gene, ecnodes a novel protein with both a Zn-binding motif and a domain homologous to the β-subunit of trimeric G-proteins. Cell 71, 791-801.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A. and Dangl, J.L. (1994). *Arabidopsis* mutants simulating disease resistance response. Cell 77, 565-577.

Estelle, M.A. and Somerville, C.R. (1986). The mutants of *Arabidopsis*. Trends Genet. 2, 89-93.

Fabri, C.O. and Schaffner, A.R. (1994). An *Arabidopsis thaliana* RFLP mapping set to localize mutations to chromosomal regions. Plant J. 5, 149-156.

Feldmann, K.A. (1991). T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. Plant J. 1, 71-82.

Galland, P.A. and Senger, H. (1988). The role of pterins in the photoreception and metabolism of plants. Photochem. Photobiol. 48, 811-820.

Giraudat, J., Hauge, B.M., Valon, C., Smalle, J., Parcy, F. and Goodman, H.M. (1992). Isolation of the *Arabidopsis ABI3* gene by positional cloning. Plant Cell 4, 1251-1261.

Gomez-Lim, M.A., Valdes-Lopez, V., Cruz-Hernandez, A. and Saucedo-Arias, L.J. (1993). Isolation and characterization of a gene involved in ethylene biosynthesis from *Arabidopsis thaliana*. Gene 134, 217-221.

Halfter, U., Ali, N., Stockhaus, J., Ren, L. and Chua, N.-H. (1994). Ectopic expression of a single homeotic gene, the petunia gene *green petal*, is sufficient to convert sepals to petaloid organs. EMBO J. 13, 1443-1449.

Hauge, B.M., Hanley, S.M., Cartinhour, S., Cherry, J.M., Goodman, H.M., Koornneef, M., Stam, P., Chang, C., Kempin, S., Medrano, L. and Meyerowitz, E.M. (1993). An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J. 3, 745-754.

Jarvis, P., Lister, C., Szabo, V. and Dean, C. (1994). Integration of CAPS markers into the RFLP map generated using recombinant inbred lines of *Arabidopsis thaliana*. Plant Mol. Biol. 24, 685-687.

Kieber, J.J. and Ecker, J.R. (1993). Ethylene gas: it's not just for ripening any more! Trends Genet. 9, 356-362.

Koncz, C., Chua, N.-H., and Schell, J. (1992). Methods in *Arabidopsis* Research. World Scientific, Singapore.

Koncz, C., Mayerhofer, R., Koncz-Kalman, Z., Nawrath, C., Redei, G.P. and Schell, J. (1990). Isolation of a gene encoding a novel chloroplast protein by T-DNA tagging in *Arabidopsis thaliana*. EMBO J. 9, 1337-1346.

Koornneef, M., Dellaert, L.W.M., and Van der Veen, J.H. (1982). EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. Mutation Res. 93, 109-123.

Koornneef, M., Elgersma, A., Hanhart, C.J., van Loenen-Martinet, E.P., van Rijn, L., Zeevaart, J.A.D. (1985). A gibberellin insensitive mutant of *Arabidopsis thaliana*. Physiol. Plant. 65, 33-39.

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

Koornneef, M., van Eden, J., Hanhart, C.J., Stan, P., Braaksma, F.J. and Feenstra, W.J. (1983). Linkage map of *Arabidopsis thaliana*. J. Hered 74, 265-272.

Last, R.L., Bissinger, P.H., Mahoney, D.J., Radwanski, E.R. and Fink, G.R. (1991). Tryptophan mutants in *Arabidopsis*: the consequences of duplicated tryptophan synthase β genes. Plant Cell 3, 345-358.

Leyser, H.M.O.A., Lincoln, C.A.A., Timpte, C.A., Lammer, D.A., Turner, J.A. and Estelle, M. (1993). *Arabidopsis* auxin-resistance gene *AXR1* encodes a protein related to ubiquitin-activating enzyme E1. Nature 364, 161-164.

Liscum, E. and Hangarter, R.P. (1991). *Arabidopsis* mutants lacking blue light-dependent inhibition of hypocotyl elongation. Plant Cell 3, 685-694.

Ma, H., Yanofsky, M.F. and Meyerowitz, E.M. (1990). Molecular cloning and characterization of *GPA1*, a G protein α subunit gene from *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 87, 3821-3825.

Mauch-Mani, B. and Slusarenko, J.A. (1993). *Arabidopsis* as a model host for studying plant-pathogen interactions. Trends Microbiol. 1, 265-270.

Mayer, U., Torres Ruiz, R.A., Berleth, T., Misera, S. and Jurgens, G. (1991). Mutations affecting body organization in the *Arabidopsis* embryo. Nature 353, 402-407.

Meyer, K., Leube, M.P. and Grill, E. (1994). A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science 264, 1452-1455.

Meyerowitz, E.M. (1989). *Arabidopsis*, a useful weed. Cell 56, 263-269.

Misera, S., Muller, AJ, Weiland-Heidecker, U. and Jurgens, G. (1994). The *FUSCA* genes of *Arabidopsis*: negative regulators of light responses. Mol. Gen. Genet. 244, 242-252.

Nagatani, A., Reed, J.W. and Chory, J. (1993). Isolation and initial characterization of *Arabidopsis* mutants that are deficient in phytochrome A. Plant Physiol 102, 269-277.

Pepper, A., Delaney, T., Washburn, T., Poole, D. and Chory, J. (1994). *DET1*, a negative regulator of light-mediated development and gene expression in *Arabidopsis*, encodes a novel nuclear-localized protein. Cell 78, 109-116.

Pnueli, L., Hareven, D., Rounsley, S.D., Yanofsky, M.F. and Lifschitz, E. (1994). Isolation of the tomato *AGAMOUS* gene *TAG1* and analysis of its homeotic role in transgenic plants. Plant Cell 6, 163-173.

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

Reymond, P., Short, T.W., Briggs, W.R. and Poff, K.L. (1992). Light-induced phosphorylation of a membrane protein plays an early role in signal transduction for phototropism in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 89, 4718-4721.

Samac, D.A. and Shah, D.M. (1994). Effect of chitinase antisense RNA expression on disease susceptibility of *Arabisopsis* plants. Plant Mol. Biol. 25, 587-596.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H. and Sommer, H. (1990). Genetic control of flower development: homeotic genes in *Antirrhinum majus*. Science 250, 931-936.

Shevell, D.E., Leu, W.M., Gillmor, C.S., Xia, G., Feldmann, K.A. and Chua, N.-H. (1994). *EMB30* is essential for normal cell division, cell expansion and cell adhesion in *Arabidopsis* and encodes a protein that has similarity to Sec7. Cell 77, 1051-1062.

Straus, D. and Ausubel, F.M. (1990). Genomic subtraction for cloning DNA corresponding to deletion mutations. Proc. Natl. Acad. Sci. USA 87, 1889-1893.

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.

Van Lijsebettens, M., Vanderhaeghen, R., De Block, M., Bauw, G., Villarroel, R., and Van Montagu, M. (1994). An S18 ribosomal protein gene copy at the *Arabidopsis PFL* locus affects plant development by its specific expression in meristems. EMBO J. 13, 3378-3388.

Weigel, D. and Meyerowitz, E.M. (1994). The ABCs of floral homeotic genes. Cell 78, 203-209.

Whitelam, G.C., Johnson, E., Peng, J., Carol, P., Anderson, M.L., Cowl, J.S. and Harberd, N.P. (1993). Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. Plant Cell 5, 757-768.

Yu, G.L., Katagiri, F. and Ausubel, F.M. (1993). *Arabidopsis* mutations at the RPS2 locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene*avrRpt2*. Mol. Plant Microbe Interact. 6, 434-443.

Fig. 1. A flowering *Arabidopsis thaliana* seedling, with a tobacco leaf shown for scale.

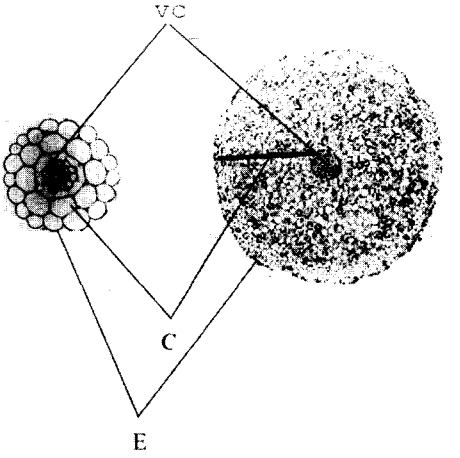

Fig. 2. Comparison of the anatomy of young roots of *Arabidopsis* and a typical dicot. Tissues from outside inwards: E, epidermis; C, cortex; VC, vascular cylinder. Note the relatively small number of cells that make up an organ in *Arabidopsis* (left), while many more cells are required to construct the same tissues in other plants (right).

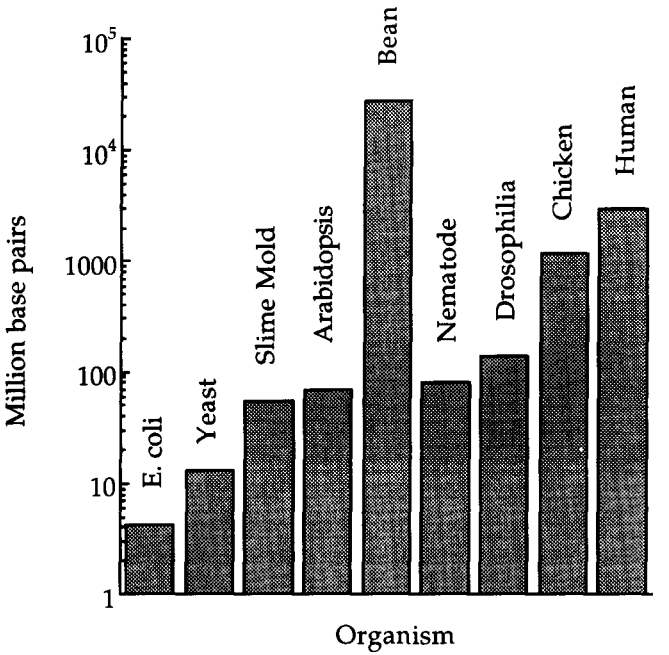

Fig. 3. Haploid genome size of *Arabidopsis*, compared with other well-studied organisms. A very wide range of genome sizes is found in flowering plants, some of which have more DNA per cell than the human genome, as illustrated. Genome size and genome complexity do not always go together, particularly in the highest eukaryotes. Nevertheless, it is the small genome size of *Arabidopsis* which helps in mapping the genome and screening libraries, and complementation of mutants. *A. thaliana* falls between the slime mold *Dictyostelium discoideum* and the nematode *Caenorhabditis elegans*.