Figure 7.35 *Arabidopsis thaliana*, small annual herb from family Brassicaceae, whose genome is most completely known among the angiosperms, is aptly known as the guinea-pig of plant kingdom.

Arabidopsis Genome

Insignificant small crucifer, *Arabidopsis thaliana* (Figure 7.35), often ignored in the field, holds great promise for opening new frontiers of phylogenetic analysis. With its small genome size of 114.5 mbp (as compared to 165 mbp in *Drosophila melanogaster* and 3000 mbp in humans), the species is the most completely known genetically among all flowering plants. During the last 8 to 10 years, *Arabidopsis thaliana* has become universally recognized as a model plant for such studies. Although it is a non-commercial member of the mustard family, it is favored among basic scientists because it develops, reproduces, and responds to stress and disease in much the same way as many crop plants. The choice of *Arabidopsis* as a genetic tool has been forced by the following attributes:

1. Small genome (114.5 Mb / 125 Mb total).
2. Extensive genetic and physical maps of all 5 chromosomes.
3. A rapid life cycle (about 6 weeks from germination to mature seed).
4. Prolific seed production and easy cultivation in restricted space.
5. Efficient transformation methods utilizing *Agrobacterium tumefaciens*.
6. A large number of mutant lines and genomic resources ·
7. Multinational research community of academic, government and industry laboratories.
8. Easy and inexpensive to grow.
9. Compared to other plants, it lacks the repeated, less-informative DNA sequences that complicate genome analysis.

The *Arabidopsis* Genome Initiative (AGI) is an international collaboration to sequence the genome of the model plant *Arabidopsis thaliana*. Begun in 1996 with the goal of completing the genome sequence by 2004, the genome sequencing was completed at the end of 2000. Comprehensive information on *Arabidopsis* genome is available on the internet via The **Arabidopsis Information Resource (TAIR)**, which provides a comprehensive resource for the scientific community working with *Arabidopsis thaliana*. TAIR is a collaboration between the Carnegie Institution of Washington Department of Plant Biology, Stanford, California, and the National Center for Genome Resources (NCGR), Santa Fe, New Mexico. Funding is provided by the National Science Foundation.

Important studies on *Arabidopsis thaliana* have been devoted to genetic control of development. Transgenic plants of this species have been created that either overexpress or underexpress cyclin B. Overexpression of cyclin B results in accelerated rate of cell division; underexpression results in decelerated rate. Plants with faster rate of cell division contain more cells and are somewhat larger than their wild type counterparts, but otherwise they look completely normal. Likewise, plants with the decreased rate of cell division have less than half the normal number of cells, but they grow at almost the same rate and reach almost the same size as wild-type plants, because as

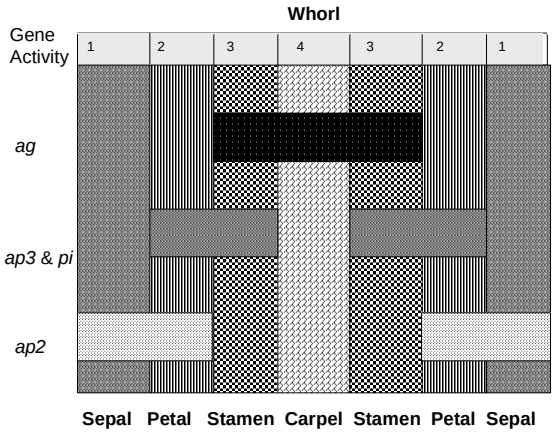

Figure 7.36 Graphic representation of control of floral development in *Arabidopsis thaliana* by the overlapping action of four genes. Gene *ap2* is expressed in the outer two whorls (sepals and petals), *ap3* and *pi* are expressed in the middle two whorls (petals and stamens) and *ag* in the inner two (stamens and carpels). Each whorl has a unique combination of active genes.

the number of cells decrease, the individual cells get larger. The plants thus have ability to adjust to abnormal growth conditions, as opposed to animals which frequently develop proliferative cancer cells.

Table 7.2 Floral development in mutants of *Arabidopsis thaliana*.

Genotype	Whorl			
	1	2	3	4
wildtype	sepals	petals	stamens	carpels
ap2/ap2	carpels	stamens	stamens	carpels
ap3/ap3	sepals	sepals	carpels	carpels
pi/pi	sepals	sepals	carpels	carpels
ag/ag	sepals	petals	petals	sepals

The studies on genetic control of flower development in *Arabidopsis* have revealed interesting results. During floral development (as in other tetracyclic plants), each whorl of the floral parts (sepals, petals, stamens and carpels) arises from a separate whorl of initials. Three types of mutations result in three different phenotypes, one lacking sepals and petals, the second lacking petals and stamens and the third lacking stamens and carpels. Crosses between homozygous organisms have resulted in identification of four genetic groups (Table 7.2). Mutations in the gene *ap2* (*apetala-2*) result in phenotype without sepals and petals. The phenotype lacking petals and stamens is caused by mutation in either of two genes, *ap3* (*apetala-3*) or *pi* (*pistillata*). The genotype lacking stamens and carpels is caused by mutations in the gene *ag* (*agamous*). Each of these genes has been cloned and sequenced. They are all transcription factors, members of **MAD box** family of transcription factors, each containing a sequence of 58 amino acids.

An interesting finding from this study is that mutation in any of the genes eliminates two floral organs belonging to adjacent whorls. The pattern suggests that *ap2* is necessary for sepals and petals, *ap3* and *pi* are both necessary for stamens and *ag* necessary for stamens and carpels. As mutant phenotypes are caused by loss-of-function in alleles, it may be inferred that *ap2* is expressed in whorls 1 and 2, *ap3* and *pi* expressed in whorls 2 and 3, and *ag* is expressed in whorls 3 and 4. The floral development in this plant is thus controlled by combinational effect of these four genes. Sepals develop from tissue in which *ap2* is active; petals by combination of *ap2, ap3* and *pi,* stamens by combination of *ap3, pi* and *ag;* and carpels where only gene *ag* is expressed. This is graphically represented in figure 7.36.

It is pertinent to remember that *ap2* expression and *ag* expression are mutually exclusive. In presence of *ap2* transcription factor *ag* is repressed, and in the presence of *ag* transcription factor, *ap2* is repressed. Ac-

cordingly, in *ap2* mutants, *ag* expression spreads to whorls 1 and 2, and, in *ag* mutants, *ap2* expression spreads to whorls 3 and 4. This assumption enables us to explain the phenotypes of single and even double mutants. This pattern of gene expression has been assayed by *in situ* hybridization of RNA in floral cells with labelled probes for each of the genes. The results confirm the above assumption of repressive action of concerned genes. It is significant that triple mutation involves all the genes. The phenotype of *ap2 pi ag* triple mutant does not have any normal floral organs. There are concentric whorls of leaves instead.

Gene trees

Molecular systematics presents powerful tools for constructing phylogenetic trees. Commonly used methods over the recent years include studies on chloroplast DNA using restriction site polymorphism (cpRFLP), analysis of chloroplast gene for subunit F of NADP dehydrogenase (ndhF, in the small copy region), for 'a' and 'b' subunits of RNA polymerase II (*rpoA* and *rpoC2*, in a large single copy region), for 'b' subunit of ATP synthase (*AtpB*), ITS region of ribosome, phytochrome B, and granule bound starch synthaseI. An encouraging congruence of results of these diverse studies was met in tribe Stipeae of grasses. In other cases, results from chloroplast phylogeny and nuclear phylogeny did not agree, suggesting caution in relying on any attribute singly for constructing molecular phylogenies. The gene trees constructed from *rbcL* have great utility in angiosperms. Chase et al., (1993) attempted to yield the phylogeny of all seed plants using 499 *rbcL* sequences. The analysis proved a few sequences to be pseudogenes, and entire families were represented by single sequences. The data set have been reanalyzed by other authors to yield parsimonious trees (Rice et al., 1997). *RbcL* data has supported that Caryophyllidae is monophyletic. It has also supported the union of family pairs Asclepiadaceae-Apocynaceae, Araliaceae-Apiaceae, and Brassicaceae-Capparaceae. The data also supported the polyphyletic nature of Saxifragaceae and Caprifoliaceae.