Genetic and physiological analysis of flowering time in the C24 line of *Arabidopsis thaliana*

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Abstract

The C24 strain of Arabidopsis thaliana contains a FRIGIDA (FRI) allele that causes late flowering and an FLC allele which suppresses the late-flowering phenotype of FRI. Crosses of C24 to plants containing an allele of FLC that delays flowering results in extremely late-flowering progeny. Crosses of C24 to Landsberg erecta (Ler), which contains an allele of FLC that suppresses the late-flowering phenotype, results in progeny which have a flowering time similar to C24. As is the case for other ecotypes that are late-flowering due to FRI, the C24 strain exhibits a photoperiod response and flowering is promoted by cold treatment and by low red/far-red light ratios.

Introduction

Agrobacterium-mediated transformation of Arabidopsis thaliana is a useful tool to generate insertion mutations, to identify genes by complementation, and to study gene regulation. Many Agrobacterium-based methods of Arabidopsis transformation have been developed including root, cotyledon, in planta, and embryonic transformation procedures (e.g., Valvekens, 1988; Barghchi et al., 1994; Katavic et al., 1994; Sangwan et al., 1991). One parameter that effects transformation efficiency for many procedures is the Arabidopsis genotype. Valvekens (1988), Sangwan (1991), and Barghchi (1994) report that the C24 strain is efficiently transformed and regenerated. The C24 strain has therefore been used in a variety of molecular genetic studies such as promoter trapping (Kertbundit et al., 1991; Topping et al., 1994).

The length of time required for a particular genotype of Arabidopsis to initiate flowering can be an important consideration in the choice of strain for molecular and genetic studies. Genes involved in the regulation of flowering time have been identified by examining naturally occurring variation. In crosses of early-flowering ecotypes to late-flowering ecotypes, the dominant FRIGIDA (FRI) gene was identified as a major regulator of flowering time (Napp-Zinn, 1985; Burn et al., 1993; Lee et al., 1993; Clarke and Dean, 1994). Lee et al. (1994) and Koornneef et al. (1994) reported that Landsberg erecta (Ler) contains an allele of another flowering gene, FLC, that suppresses the late-flowering phenotype of FRI. FLC alleles from other ecotypes (e.g., FLC alleles from Columbia (Col) and Sf-2) act in a semidominant manner with FRI to cause late flowering. The suppressor allele of FLC had only been found in Ler.

In this study, the flowering behavior of C24 was genetically analyzed. C24 contains the unique combination of a FRI allele that delays flowering and an FLC allele that suppresses the late-flowering phenotype of FRI. Although C24 has been reported to have been derived from the Col ecotype, microsatellite analysis indicates that C24 and Col are polymorphic, and thus do not share a common heritage.

Materials and Methods

Plant lines

The C24 line originated from Dr. Keith Lindsey and was provided by Patrick Masson. FLC-Col introgressed into Ler (FLC-Col line) was provided by Maarten Koornneef and described by Lee et al. (1994). Col ecotypes were obtained from the Arabidopsis Biological Resource Center at Ohio State University.

Growth conditions

Unless otherwise stated, plants were grown as described (Lee et al., 1993) with continuous illumination of approximately 100 micromol m-2 sec-1 of cool-white fluorescent light at 22-23C. Growth and light conditions for the photoperiod and vernalization experiment were as described in Lee et al. 1995 (Lee and Amasino, 1995) . The flowering response was measured as the number of leaves in the rosette when the flowering stalk reached 1 cm in height as previously described (Lee et al. 1994).

Molecular techniques

DNA for microsatellite analysis was isolated according to Edwards et al. (1991). The genotype in the region around FLC and FRI was determined by microsatellite analysis according to Bell and Ecker (1994) using loci nga249 and nga8, respectively. FLC is closely linked to the nga249 locus (Lee et al. 1994). Polymerase chain reaction (PCR) conditions were as described (Bell and Ecker, 1994) except that 20 microlitre reactions were prepared and 35 PCR cycles were used instead of 40. PCR products were visualized by staining with ethidium bromide after electrophoresis in a 7% non-denaturing acrylamide gel.

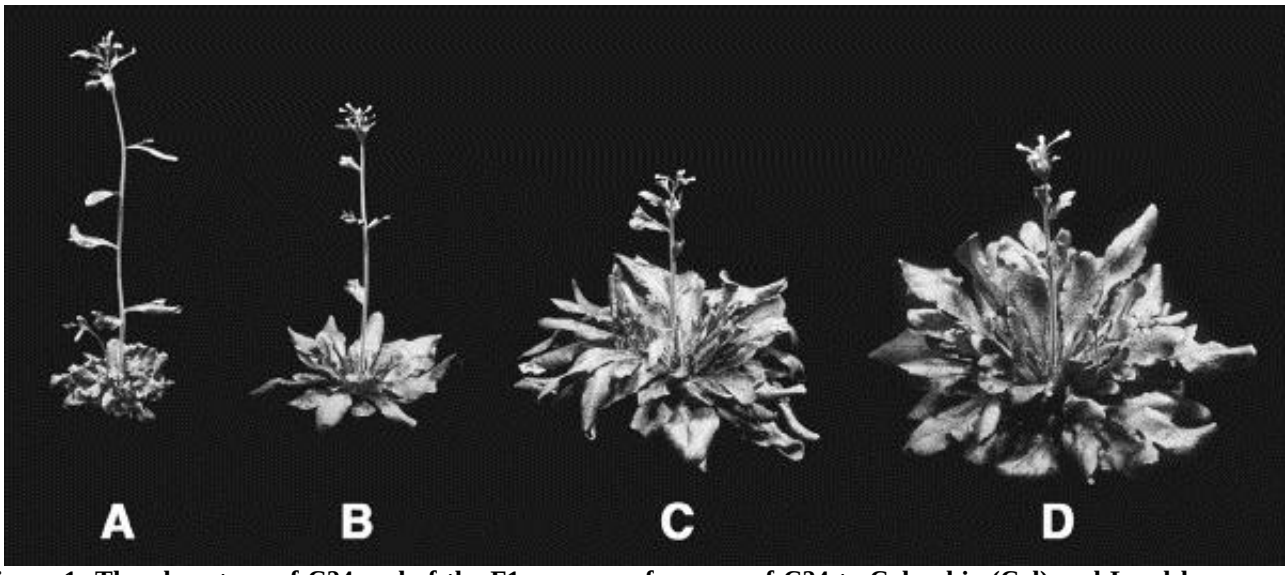

Figure 1: The phenotype of C24 and of the F1 progeny of crosses of C24 to Columbia (Col) and Landsberg erecta (Ler). A) C24. B) F1 of C24 crossed to Ler. C) F1 of C24 crossed to Col. D) F1 of C24 crossed to the Ler line containing the FLC allele from Col.

Results

Late-flowering progeny in the cross of C24 to Col

When the C24 strain was crossed to the Columbia ecotype (Col), the resulting F1 plants were much later flowering than either parent (Figures 1A and 1C).

In the F2 population resulting from self-pollination, more than 1/4 of the plants were later flowering than the latest parent (C24) (Figure 2) indicating that at least two genes are segregating for flowering time.

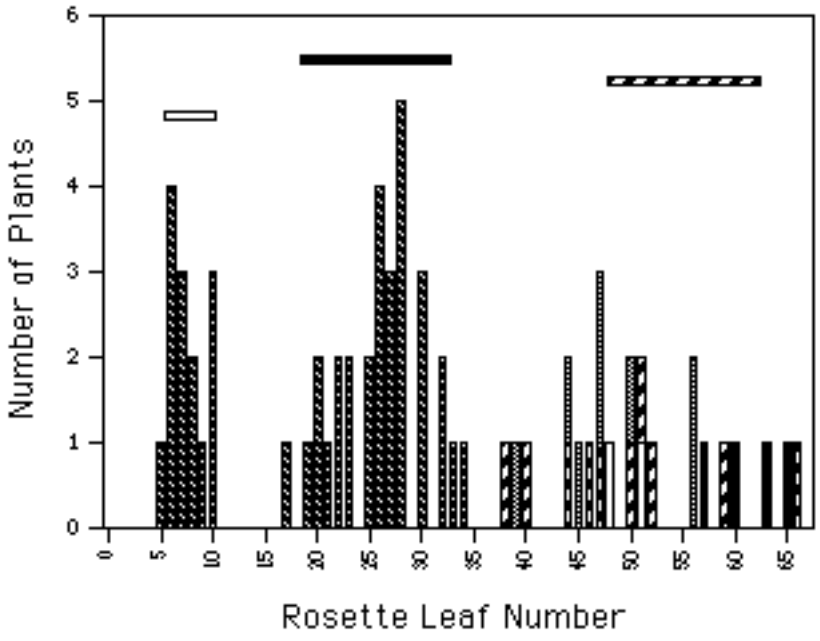

Figure 2. Frequency distribution of rosette leaf number in an F2 population derived from a cross between C24 and Columbia (Col). The genotypes at FLC and FRI of the plants flowering similar to the parental lines were not determined (black columns with white dots). The genotypes of plants that were homozygous for both FRI from C24 (FRI-C24) and FLC from Col (FLC-Col) are shown as filled columns. The genotypes of plants that were homozygous for FRI-C24 and heterozygous for FLC-Col are shown as open columns. The genotypes of plants that were heterozygous for FRI-C24 and homozygous for FLC-Col are shown as dotted columns. The genotypes of plants that were heterozygous for both FRI-C24 and FLC-Col are shown as stripped columns. The horizontal bars represent the leaf number distributions of the C24 line (filled bar), Col (open bar) and F1 (striped bar).

Microsatellite analysis was used to determine which loci contributed to late flowering in the F2 population. Late-flowering co-segregated with Col DNA at microsatellite locus nga249 in a homozygous or heterozygous state and with C24 DNA at microsatellite locus nga8 in a homozygous or heterozygous state. These two loci are located in the region of two previously identified genes that regulate flowering time, FLC and FRI (Lee et al. 1994). These data indicate that one copy of the FRI allele from C24 (FRI-C24) and one copy of the FLC allele from Col (FLC-Col) are required to cause the late flowering observed in the F2 plants. The C24 strain therefore appears to contain a late-flowering allele of FRI and an allele of FLC which suppresses the late-flowering effect of FRI similar to the Ler allele of FLC.

FLC Analysis

To determine whether the C24 strain contains a suppressor allele of FLC, C24 was crossed to two lines: the Ler ecotype which contains an FLC suppressor allele, and the FLC-Col line in which the FLC allele from Col has been introgressed into Ler (Lee et al. 1994). The F1 progeny of C24 crossed to Ler flowered in a similar leaf number range as the C24 parental line (Figures 1B and 3A). In the F2 population, a continuous range of flowering times were observed and only a few plants were slightly later flowering than the C24 parent (Figure 3A). The F1 progeny of C24 crossed to the FLC-Col line flowered with approximately twice as many leaves as the C24 line (Figures 1D and 3B). In the F2 from this cross, the majority of the population flowered after forming more than 40 leaves (Figure 3B). Because the cross of C24 to a Ler line with a late-flowering FLC allele results in later-flowering progeny than C24 crossed to Ler, the FLC allele of C24 appears to be a suppressor of late flowering similar to the FLC allele from Ler.

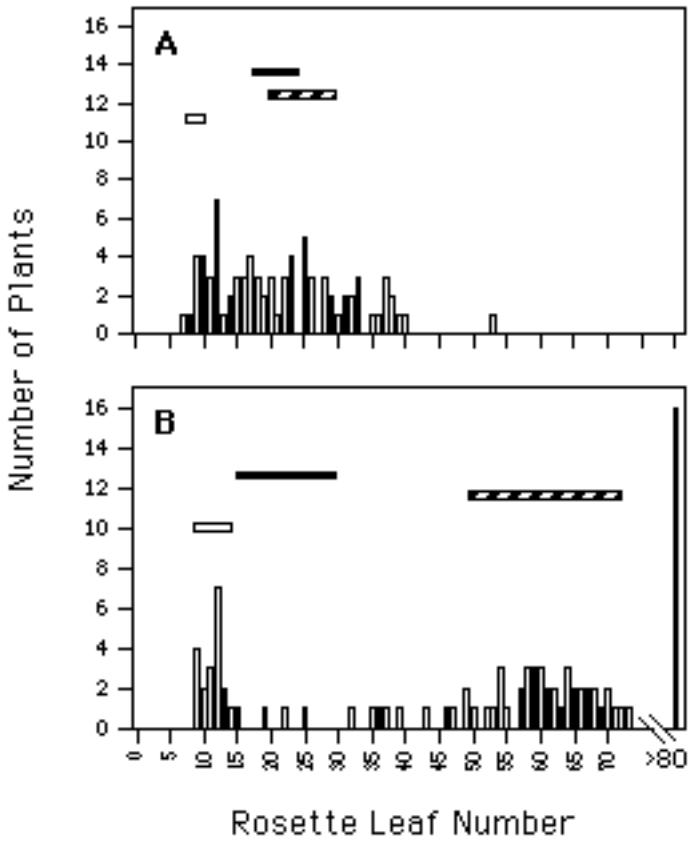

Figure 3. Frequency distribution of rosette leaf number in F2 populations derived from crosses between A) C24 and Landsberg erecta (Ler) and B) C24 and a Ler line containing the FLC allele from Columbia (FLC-Col). The horizontal bars represent the leaf number distributions of the C24 line (filled bar), Ler (open bar), the FLC-Col line (dotted bar) and F1 (striped bar).

FRI Analysis

An allelism test was performed to determine whether the late-flowering locus from C24, which mapped close to FRI by microsatellite analysis, was a FRI allele. Because the late-flowering gene in C24 and FRI are both dominant, the F1 of C24 crossed to a Col line containing the FRI allele from Sf-2 (Lee et al. 1994) was then crossed to Col. The testcross progeny therefore contain at least one copy of the late-flowering FLC allele (from Col). Among 257 testcross progeny, no plants were early flowering,

indicating that no recombination occurred between FRI and the late-flowering locus in C24. Thus, the late-flowering locus in C24 is likely to be allelic with FRI. It is possible that these genes are not allelic, but the absence of recombinants indicates that at a probability of 99% the genes must be linked by fewer than 4 cM.

Flowering behavior of C24 under different environmental conditions

The effect of various light conditions and cold treatment on the flowering behavior of the C24 line and F1 plants derived from crosses of C24 to Col and Ler was examined (Figure 4).

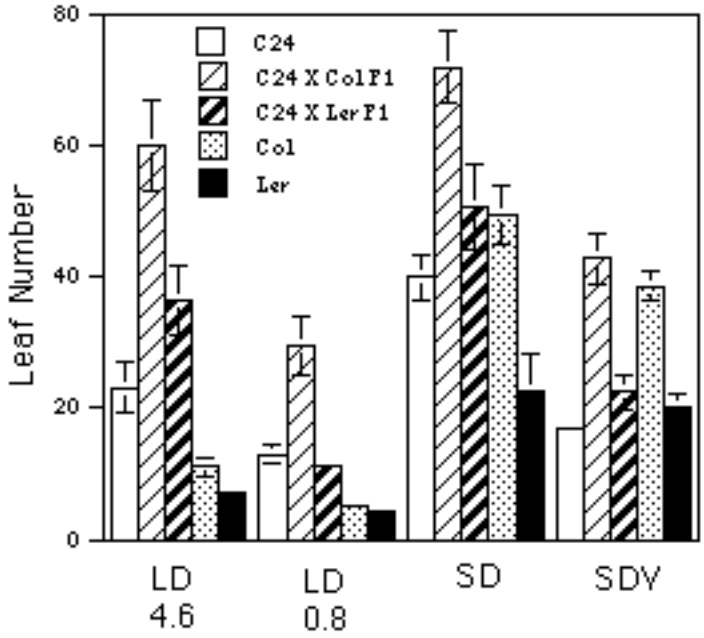

Figure 4. Average rosette leaf number of the C24 line and crosses of C24 to Columbia (Col) and Landsberg erecta (Ler) under different environmental conditions. C24 (open columns), the F1 of C24 crossed to Col (thin striped columns), the F1 of C24 crossed to Ler (thick striped columns), Col (dotted columns), and Ler (filled columns) were grown under long-day photoperiods with varying amounts of red/far-red light, short-day photoperiods, and short-day photoperiods with cold treatment. Long-day photoperiods (LD) consisted of 20 hrs light with a red/far-red of 4.6 and 0.8 (Lee et al. 1995). The short-day photoperiod (SD) consisted of 8 hrs light with a red/far-red ratio of 1.3. Plants were cold treated for 40 days in short days as described by Lee et al. (1994) and then grown under short-day photoperiod (SDV) with a red/far-red light ratio of 1.3. The averages and standard deviations of the values from 10 plants are presented.

All genotypes exhibit a photoperiod response. C24 is late flowering under long-day photoperiods and even later flowering under short-day photoperiods. The time to flowering of C24 and F1 plants derived from C24 is greatly reduced by low red/far-red light ratios and cold treatment. F1 plants from the cross of C24 to Col are later flowering than the C24 parent under all conditions due to the presence of FLC-Col. These results are similar to the interactions of the FRI allele from Sf-2 with the FLC alleles from Col and Ler (Lee et al., 1994; Lee and Amasino, 1995).

Microsatellite Analysis of C24 and Col

C24 has been assumed to be derived from a Col ecotype. Five microsatellite markers (nga8, nga63, nga162, nga168, and nga249) were analyzed to determine if C24 was related to any of the five Col

strains maintained by the Arabidopsis Biological Resource Center: Col-0, Col-1, Col-2, Col-3, and Col-4. All five Col strains gave identical PCR products for all five microsatellite markers tested. C24 and the Col strains produced the same PCR products for only one marker, nga63. Four markers (nga8, nga162, nga168, and nga249) gave a unique PCR product for C24. Microsatellite analysis therefore indicates that C24 and Col are not genetically similar.

Discussion

These results demonstrate that the C24 strain contains an allele of FRI that causes late flowering and an FLC allele which suppresses the extreme late-flowering phenotype of FRI. The C24 strain is late flowering under long-day photoperiods and even later flowering under short-day photoperiods, demonstrating that C24 exhibits a photoperiod response. Flowering time in C24 can be reduced by cold treatment and by growth under low red/far-red light ratios.

The origin of the C24 strain is not known. Microsatellite analysis demonstrates that C24 is not genetically similar to any of the commonly used Col lines. C24 contains a late-flowering FRI allele similar to many late-flowering ecotypes (Napp-Zinn, 1985; Burn et al., 1993; Lee et al., 1993; Clarke and Dean, 1994). The relatively early-flowering C24 strain may have been derived from a late-flowering ecotype by acquisition of an allele of FLC that suppresses the late-flowering phenotype of FRI. FLC suppressor alleles can be readily derived through mutagenesis of late-flowering FLC alleles (Michaels et al. unpublished). The recessive glabrous trait of C24 indicates that this line may have been subjected to mutagenesis. This mutagenesis may have created the suppressor allele of FLC present in C24, or this allele may have originated naturally.

The presence of a late-flowering allele of FRI and suppressor allele of FLC in C24 should be considered in the choice of ecotypes with which C24 will be crossed. Crosses of C24 to plants which contain a late-flowering allele of FLC (like Col) will result in late-flowering plants. Crosses of C24 to plants derived from Landsberg, which contain a suppressor allele of FLC, will result in plants which flower in a reasonable amount of time.

Acknowledgments

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References

Barghchi, M., K. Turgut, R. Scott and J. Draper. 1994. High-frequency transformation from cultured cotyledons of Arabidopsis thaliana ecotypes "C24" and "Landsberg erecta". Plant Growth Regulation 14: 61-67.

Bell, C.J. and J.R. Ecker. 1994. Assignment of 30 Microsatellite loci to the linkage map of Arabidopsis. Genomics 19(1): 137-144.

Burn, J.E., D.R. Smyth, W.J. Peacock and E.S. Dennis. 1993. Genes conferring late flowering in Arabidopsis thaliana. Genetica 90(2-3): 147-155.

Clarke, J.H. and C. Dean. 1994. Mapping FRI, a locus controlling flowering time and vernalization response in Arabidopsis thaliana. Mol. Gen. Genet. 242: 81-89.

Edwards, K., C. Johnstone and C. Thompson. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Research 19(6): 1349.

Katavic, V., G.W. Haughn, D. Reed, M. Martin and L. Lunst. 1994. In planta transformation of AArabidopsis thaliana. Mol. Gen. Genet. 245: 363-370.

Kertbundit, S., H. De Greve, F. Deboeck, M. Van Montagu and J. Hernalsteens. 1991. In vivo random B-glucuronidase gene fusions in AArabidopsis thaliana. Proc. Natl. Acad. Sci. USA 88: 5212-5216.

Koornneef, M., H. Blankestijn-de Vries, C. Hanhart, W. Soppe and T. Peeters. 1994. The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg erecta wild-type. Plant J. 6(6): 911-919.

Lee, I. and R.M. Amasino. 1995. Effect of vernalization, photoperiod and light quality on the flowering phenotype of Arabidopsis plants containing the FRIGIDA gene. Plant Physiol. 108: 157-162.

Lee, I., S.D. Michaels, A.S. Masshardt and R.M. Amasino. 1994. The late- flowering phenotype of FRIGIDA and LUMINIDEPENDENS is suppressed in the Landsberg erecta strain of Arabidopsis. Plant J. 6(6): 903-909.

Lee, I., A. Bleecker and R. Amasino. 1993. Analysis of naturally occurring late flowering in AArabidopsis thaliana. Mol. Gen. Genet. 237(1-2): 171-176.

Napp-Zinn, K. (1985). Arabidopsis thaliana. In: CRC Handbook of Flowering. (ed. A.H. Halevy), pp. 492-503. CRC Press, Inc., Boca Raton, FL.

Sangwan, R.S., Y. Bourgeois and B.S. Sangwan-Norreel. 1991. Genetic transformation of Arabidopsis thaliana zygotic embryos and identification of critical parameters influencing transformation efficiency. Mol. Gen. Genet. 230(3): 475-485.

Topping, J., F. Agyeman, B. Henricot, and K. Lindsey. 1994. Identification of molecular markers of embryogenesis in Arabidopsis thaliana by promoter trapping. Plant J. 5(6): 895-903.

Valvekens, D., M. Van Montagu, and M. Van Lijsebettens. 1988. Agrobacterium tumefaciens-mediated transformation of Arabidopsis root explants using kanamycin selection. Proc. Natl. Acad. Sci. USA 87: 5536-5540.

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