

Analysis of TFL1 mutants in Arabidopsis

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Abstract- In Arabidopsis (Flowering Plant) shoot apical meristem (SAM) is converted into inflorescence meristem. Study has been revealed that there are various genes which control this transition. Two types of inflorescence are found in flowering plants: Determinate and indeterminate. Terminal Flower 1 (TFL1) gene is one of those genes which play key role for the maintenance of inflorescence meristem. Tfl1 gene regulates flowering time and maintains the fate of inflorescence meristem. Arabidopsis genome contains six TFL1 like genes. TFL1 belongs to a small gene family. In this study a comparison between TFL1 wild type and TFL1 mutant is conducted according to which TFL1 is able to convert the indeterminate inflorescence to determinate. TFL1 is believed to be a controller or more specifically a regulator of inflorescence architecture and flowering time. TFL1 is believed to be a negative regulator of flowering time so if mutations are introduced in it the flowering time will be accelerated.

I. INTRODUCTION

Post-embryonic life of flowering plants is divided into two distinct phases: an initial vegetative phase, during which leaves with associated lateral shoots or paraclades are produced, and a subsequent reproductive phase, during which flowers are produced. The transition between the two phases is caused by a complex process termed floral induction, which is controlled by both endogenous and environmental signals. In Arabidopsis, a facultative long-day plant, flowering is promoted by long photoperiods, vernalization (transient exposure to cold), and higher growth temperatures (Napp-Zinn, 1985; Martínez Zapater and Somerville, 1990; Koornneef et al., 1995). In angiosperms, the floral transition marks the progression from vegetative to reproductive growth, and thus represents a major change in plant development. This transition is regulated by both inhibitory and promotive graft-transmissible factors that are produced in the leaves and roots and transported to the shoot apical meristem (Evans, 1960; Lang et al., 1977); the arrival of these substances at the shoot apex is correlated with the establishment of the inflorescence meristem and its new developmental patterns. Single gene mutations that affect either the signaling process or the response of the apical meristem have been described in several plant species. For example, in pea the production of floral signals involves the sterile nodes (Sn) and day neutral (Dne) gene products, whereas the vegetative (veg) and late flowering (Lf) gene products influence the sensitivity, or responsiveness, of the apical meristem to these signals (see Murfet, 1990; Poethig, 1990). The signal/response mechanism that regulates the floral transition provides a situation well suited for a molecular analysis of shoot apical meristem function.

TERMINAL FLOWER1 (TFL1) controls flowering time and inflorescence architecture ([Shannon and Meeks-Wagner, 1991](#); [Alvarez et al., 1992](#)). As mutations in *TFL1* accelerate flowering time, *TFL1* is believed to be a negative regulator of flowering time ([Simon et al., 1996](#)). In addition, the inflorescences of *tfl1* mutants are converted from indeterminate into determinate; they produce reduced numbers of flower buds and possess terminal flowers at the shoot apices ([Shannon and Meeks-Wagner, 1991](#); [Alvarez et al., 1992](#); [Bradley et al., 1997](#)). In contrast with the loss-of-function phenotypes, overexpression of *TFL1* delayed flowering and prevented the IM-to-FM transition, resulting in the extension of the IM stage, the production of bract-like leaves, and the proliferation of secondary flowers ([Ratcliffe et al., 1998](#); [Hanzawa et al., 2005](#)). Thus, *TFL1* is a negative regulator of the phase changes of the SAM from vegetative to reproductive and from IM to FM.

Expression of *TFL1* is restricted to the inner cells of mature shoot meristems. *TFL1* mRNA is very low during the vegetative phase, but its levels are strongly upregulated at the switch to flowering ([Simon et al., 1996](#); [Bradley et al., 1997](#); [Ratcliffe et al., 1999](#)). *TFL1* expression does not extend into the outer cell layers, the epidermis of the shoot meristem, or into primordia, yet it controls the identity of all of these cells. First, *TFL1* determines the identity of primordia made. Second, in *tfl1* mutants, all cells, even the epidermis, ectopically express flowering genes ([Weigel et al., 1992](#); [Bowman et al., 1993](#); [Gustafson-Brown et al., 1994](#); [Bradley et al., 1997](#); [Liljegren et al., 1999](#)).

II. MUTANT TFL1

Mutation in *TFL1* gene of Arabidopsis results in conversion of indeterminate inflorescence to determinate inflorescence. Flowering time of Arabidopsis significantly reduce due to mutation in *TFL1* gene. During the vegetative growth phase of wild-type Arabidopsis, primordia give rise to leaves separated by short internodes and form a compact rosette. In wild type *TFL1* the induction of flowering by appropriate environmental signals, such as long days (LD), results in the apical meristem acquiring an inflorescence identity and generating floral meristem from its periphery. In addition, the shoot elongates (bolts) bearing two or three leaves with secondary inflorescences in axils, above which flowers occur. In mutant *TFL1* Arabidopsis shoot elongates after producing fewer rosette leaves and Limits the development of the normally indeterminate inflorescence by promoting formation of terminal floral meristem. Inflorescence development in mutant *TFL1* Arabidopsis terminates with a compound floral structure consisting of the terminal flower.

Experimental design and Data description

- Introducing mutations in the genome (SOT1)
- Expression analyses of mutant lines
- Comparison of gene expressions in different mutant lines
- Identifying the candidate genes under the effect of mutations through differential gene expression analysis

WT-152	To find the genes under the influence of high TFL1 expression.
WT- <i>tfl</i>	To find the genes, influenced by <i>tfl</i> 1
WT-35STFL	To find the genes under control of higher expression of TFL1.
35STFL-152	Genes under control of SOT1 mutation.
35STFL- <i>tfl</i>	Difference between higher and lower expression of TFL.

We have following data samples:

- Mutant TFL
- Wild type TFL1.
- Over expressed TFL (35STFL)
- 152 → locally developed line derived from 35STFL. It has higher expression of TFL along with another mutation that is yet to be characterized. (SOT1 Mutant X-Rays).

III. RESULTS

- ❑ Dimensions of data: 33602 14.
- ❑ Zero in data: 8416.
- ❑ Zero removed(Dim):25186 23
- ❑ The given data is raw date. We have performed RPKM normalization and after that We have checked the propensity and expression of gene by drawing barplot and log2boxplot.

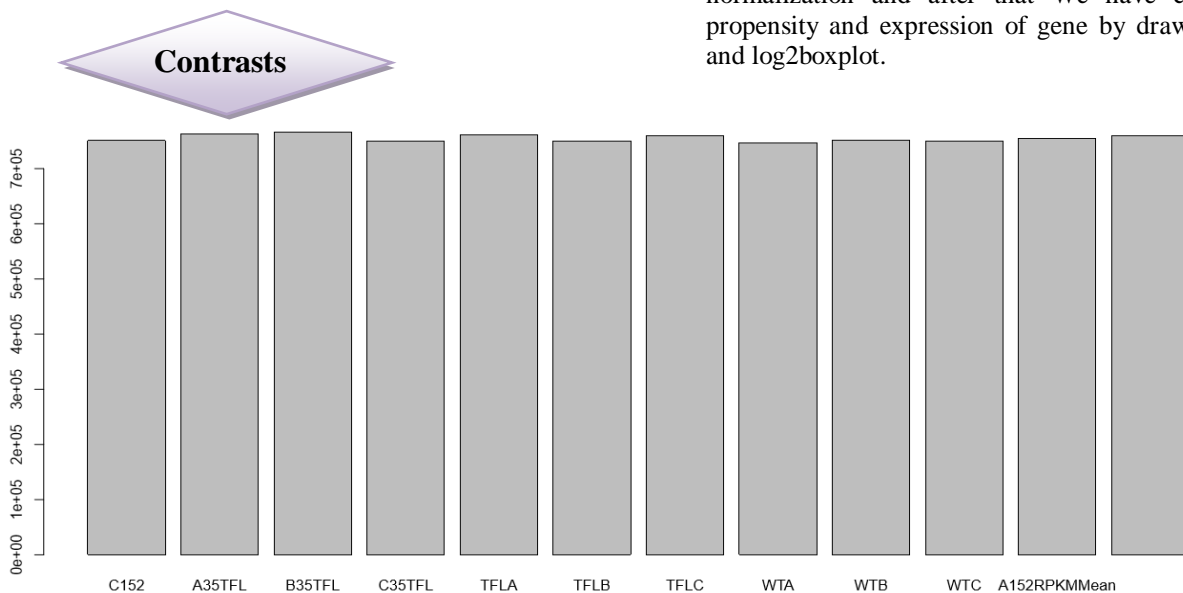


Figure 3. Barplot(rpkm normalized)

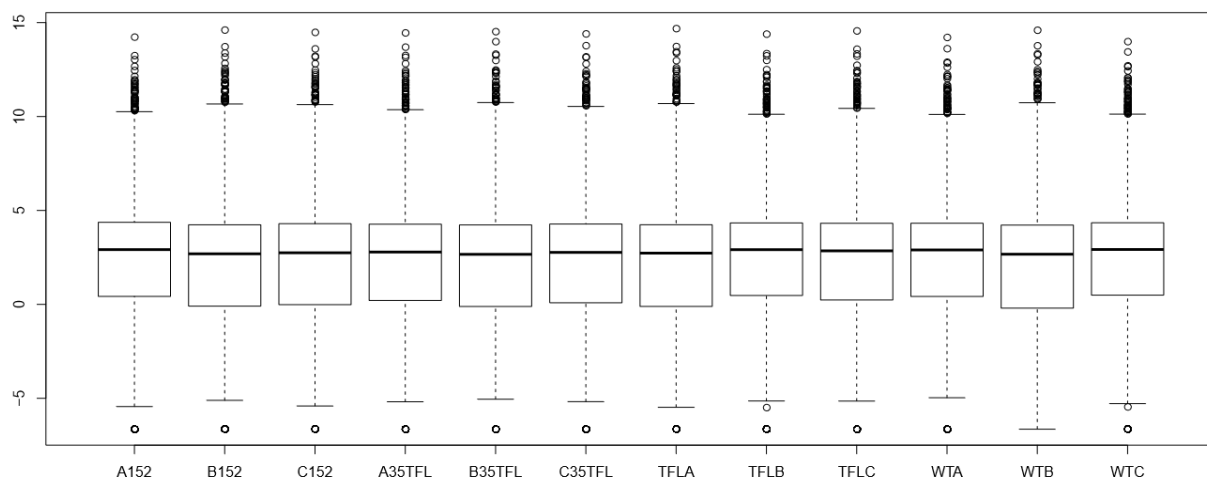


Figure 4. boxplot(rpkm Normalized)

	A152 - WT	A35TFL - WT	TFL - WT
-1	75	53	12
0	24837	24926	25060
1	274	207	114

- ❑ In our data there are 273 up regulated Genes divided into 46 clusters on the basis of function, Regulating different pathways e.g. response to steroid hormone stimulus, brassinosteroid mediated signalling , protein serine/threonine kinase activity
- ❑ 75 down regulated genes divided into 13 clusters on the basis of function, regulating different pathways e.g. DNA unwinding during replication, Nucleic acid-binding, flavonoid biosynthetic process, Glycoside hydrolase, subgroup, catalytic core.

IV. DISCUSSIONS:

Tfl 1 gene is an intriguing repressor which inhibits or slows down inflorescence. Growth of flowering stems or we can say the flowering in wild type Arabidopsis is indeterminate. So a terminal flower is not formed. To produce a terminal flower in Arabidopsis Tfl1 gene is used as an inhibitor of flowering which we can use to convert indeterminate flowering into determinate flowering which is our requirement. Tfl1 gene regulates the transcriptional repression and shows a down regulation effect in the Arabidopsis.

So by looking into these results we can conclude that tfl1 is a repressor gene that can be used in flowering plants to introduce determinate flowering. In future it can be used as a repressor in plants which are the main producers of food items to maximize the yield of the food products produced by them. We can also use it to inhibit the flowering at the base of the plant so that the flower may be able to get more sunlight in the case of those flowering plants which needs sunlight for inflorescence like sunflower. We can also prune the loss due to indeterminate flowering by regulating the expression of tfl1 gene and also we can control the setting of flowers on a plant. Below are some of the examples of determinate flowering which can be achieved by regulating the expression of Tfl 1 gene.



Determinate inflorescence with acropetal maturation



Determinate inflorescence with basipetal maturation



Determinate inflorescence with divergent maturation

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