

THE DELAYED TERMINAL FLOWER PHENOTYPE IS CAUSED BY A CONDITIONAL MUTATION IN THE CENTRORADIALIS GENE

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Racemose inflorescences can be divided into two classes, determinate and indeterminate, based on the presence or the absence of a terminal flower. The *centroradialis* mutation converts the normally indeterminate raceme of *Antirrhinum majus* into the indeterminate one. The *cen* gene has been cloned by Bradley et al. (1996) and found to encode a phosphatidylethanolamine binding protein. The number of lateral flowers made before the terminal flower is more or less constant in all the described *cen* mutant alleles.

Here we describe a new allele of *centroradialis*, *cen-2*, obtained from an X-ray mutagenesis experiment. In the progeny of the *cen-2* plants, a few WT plants appeared, suggesting that the *cen-2* allele was unstable. In the progenies of the putative revertants, the proportion of *cen*-looking plants varied continuously from about one fourth to the quasi-totality of the plants. When checked again one month later, secondary inflorescences of a large proportion of the plants scored as mutant did show a WT phenotype and some of the plants scored as WT presented a mutant phenotype. One plant formed a terminal flower after 37 lateral flowers. This phenotype was named Delayed Terminal Flower or DTF. Using crossings, we showed genetically that the DTF phenotype is due to heteroallelism between the *cen-2* mutant allele and a new allele occurring from the reversion.

When the cDNA probe for *cen* became available, we could also confirm molecularly that the DTF phenotype is indeed due to heterozygosity between the *cen-2* allele and a reverted *cen* allele we named *cen-2r*(DTF) and that the wild-type plants are homozygous for *cen-2r*(DTF). The variable phenotype observed for the DTF plants suggested that the phenotype is influenced by the environment. We have therefore compared the flowering behaviour of DTF plants under different growth conditions. Under poorly inductive conditions, DTF plants did not produce a terminal flower before they died or aborted their inflorescence. A terminal flower was made under more favourable conditions, with a drop from around 40 lateral flowers to 11 under the best conditions. These experiments clearly show that the DTF plants present a conditional *centroradialis* phenotype, looking WT under poorly inducing conditions, *cen* under very good conditions and DTF in all intermediate conditions.

In order to determine at the molecular level the nature of the mutation, we have sequenced 6 kb of the *cen* locus from the wt line 164 and the homologous region from the *cen-2r*(DTF) homozygous plant. Many differences were detected in the form of deletions, insertions and base changes. The more striking difference is a 1.9 kb insertion in the *cen-2r*(DTF) allele 580 bp upstream of the ATG. This insertion is flanked by a 16 bp target site duplication suggesting that the insert is an unknown retrotransposon. We also found a 3 bp addition in the coding region of the gene. In order to find out which modification was responsible for the phenotype, we have sequenced the *centroradialis* allele of Snowman from which the X-rayed population was derived and we found that the 3 bp insertion was the only difference between the Snowman and the *cen-2r*(DTF) allele and is therefore responsible for the DTF phenotype. We showed that this was the footprint of a new CACTA class transposon that results in the addition of an isoleucine between Asp148 and Gly149 of the CEN protein. It is proposed that this insertion modifies the affinity for the ligand by disrupting the hydrogen bonds of the amino acids of the ligand binding cavity.

References:

Bradley D., Carpenter R., Copsey L., Vincent C., Rothstein S. and Coen E. (1996) Control of inflorescence architecture in *Antirrhinum*. *Nature*, **379**:791-797.