

DISSERTATION SUMMARY

Genetic analysis of a *Medicago truncatula* mutation causing variegated leaf phenotype

Katalin Kontár

Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

In plants, as in animals, normal cellular differentiation depends on coordinated interactions between the nuclear and organellar genomes. A genetic dissection of plant variegation mutants is a powerful means to gain insight into these poorly understood interactions.

The leaves of the variegation mutant plants typically have green and white sectors. Whereas the cells in the green sectors contain normal chloroplasts, the cells in the white sectors have plastids that lack chlorophyll and/or carotenoid pigments. This indicates that these cells are perturbed in the nuclear-chloroplast communication that is manifested in pigment-deficient plastids (Wetzel et al. 1994).

A common mechanism of variegation is the induction of defective chloroplasts by mutation in nuclear genes for organelle proteins. This is sometimes due to transposable element activity, in which case the green and white cells have different genotypes. In other cases, the two types of cells have the same (mutant) genotype, indicating that the gene defined by the mutation codes for a product that is required for organelle biogenesis in some, but not all, cells of the mutant (Aluru et al. 2001).

The *Medicago truncatula* (*M.t.*) variegated mutant, identified in our group, has green and white stripes on its leaves. The mutant individual has other morphological differences compared to the wild type as well: they are usually dwarf and have small and narrow leaves the arrangement of leaves also differs. In some cases revertant lateral shoots with full-green leaves appear on mutant plants. Our aim is to identify the mutant gene by map-based cloning. For genetic mapping we constructed an F2 segregating population. In this population 36 individuals out of 148 have the variegated phenotype. Based on the segregation ratio the mutation resulted in a recessive allele of a nuclear gene.

We determined the position of the mutant gene on the genetic map of *M.t.*, which consists of eight linkage groups (LG). We chose two to three microsatellite markers from each LG to seek for molecular marker showing linkage with the

mutant gene. The genotypes of the mutant plants were determined for these markers and analyzed by color-map (Kiss et al. 1998). This resulted in the identification of a microsatellite marker closely linked to the mutation on the LG2.

Having this result we determined the genotype of more length-polymorphic and CAPS markers on the LG2 in the population segregating the mutation to assign more precise position of the mutation. Our aim was to determine two bordering molecular markers with which we could identify BAC clones for chromosome-walking.

We identified a gene-specific marker showing tight linkage to the mutation with no recombination detected in the mapping population. Based on available sequence data of *M.t.* we could identify the BAC clone that carries this gene-specific marker at one end of the clone. A new SSR marker was generated at the other end of the BAC clone (in 115 kbp distance), which also did not show recombination with the mutation.

Using different homology searches we found that this *M.t.* BAC clone carries a 2500 bp long sequence (between the two BAC-end markers), which is highly homologous to the *Arabidopsis thaliana* *IMMUTANS* gene. Based on published data it is known that a mutation in that gene also leads to variegated phenotype. Further experiments are in progress to determine whether the *M.t.* ortholog of the *IMMUTANS* gene was mutated in our variegated mutant.

References

- Aluru MR, Bae H, Wu D, Rodermeil SR (2001) The *Arabidopsis immutans* mutation affects plastid differentiation and the morphogenesis of white and green sectors in variegated plants. *Plant Phys* 127:67-77.
- Kiss GB, Kereszt A, Kiss P, Endre G (1998) Colormapping: a non-mathematical procedure for genetic mapping. *Acta Biol Hun* 49:125-142.
- Wetzel CM, Jiang CZ, Meehan LJ, Voytas DF, Rodermeil SR (1994) Nuclear-organelle interactions: the *immutans* variegation mutant of *Arabidopsis* is plastid autonomous and impaired in carotenoid biosynthesis. *Plant J* 6:161-175.