#### Poster Abstract - C.35

# **BIOTECHNOLOGICAL STRATEGIES TO ALFALFA IMPROVEMENT**

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We tried to face some aspects of alfalfa improvement: the increase of seed size to allow precision sowing, the delay of leaf senescence to reduce yield losses.

### STRATEGIES TO IMPROVE SEED SIZE:

The ectopic expression of the transcription factor Aintegumenta (ANT), produces hyperplasia of organ and seeds in tobacco transgenic plants because ANT affects the duration of cell proliferation during organogenesis, but does not affect organ morphology (Mitsukami and Fisher, 2000).

In order to obtain alfalfa seeds of suitable size for precision sowing we expressed the ANT gene under the control of two seed specific promoters : USP and LeB4, that are expressed in different periods of seed development.

Plants of highly regenerable genotypes of alfalfa and *M. truncatula* were transformed with the ANT harbouring constructs and the transgenic plants obtained were analysed by Southern. Expression and phenotypic analysis are currently in progress.

## STRATEGIES TO CONTROL SENESCENCE:

Manipulation of senescence programme in alfalfa was performed expressing the IPT gene, that encodes an enzyme catalysing the rate limiting step in cytokinin biosynthesis, under a senescence specific promoter (SAG12). This strategy was shown to delay leaf senescence in model plants (Gan and Amasino, 1995).

The SAG12-IPT construct was used to transform the genotype Regen sy 27 of alfalfa via *Agrobacterium tumefaciens*.

Transgenic plants were verified by Southern analysis and expression of the transgene was checked by RT-PCR.

A preliminary evaluation was carried out growing leaf pieces of transgenic and control plants in vitro, leaves expressing SAG12-IPT construct displayed a stay-green phenotype.

Field evaluation is currently in progress.

Literature:

Gan S., Amasino R.M., Science, 1995, 22; 270(5244):1986-8.

Mizukami Y., Fischer R.L., Proc Natl Acad Sci, U S A, 2000, 18;97(2):942-7.