Alfalfa improvement through biotechnology: a focus on senescence and seed size.

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The production of a successful alfalfa variety implies breeding for morpho-physiological characters leading to improved vigour, forage quality and seed yield characteristics. (Rotili et al., 1999).

This paper reports on two biotechnological approaches focused at improving characters having a strong impact on the final forage yield and quality such as senescence and seed size.

1-Senescence. In all forage crops the senescence of leaves leads to remarkable losses of forage and protein yield. Molecular understanding of senescence in both crops and model species has led to the identification of Senescence Associated Genes (SAGs) whose function is understood in some cases and offers a chance to manipulate this process (Lim and Nam, 2005). The delay of leaf senescence has been successfully obtained in several crops mainly by a regulated over-production of cytokinins in leaves according to the strategy reported by Gan and Amasino (1995). They used the SAG12 promoter to express the bacterial ipt gene in senescing leaves; since cytokinins inhibit senescence and therefore the SAG12 promoter activity, an autoregulatory loop is then activated. SAG12-ipt plants exhibit a stay-green phenotype with no abnormalities.

In this work a successful delay of leaf senescence in *Medicago sativa* was obtained following transformation of alfalfa with the SAG12-IPT construct. Several independent transformants were obtained as determined by Southern analysis and all of them expressed the transgene as measured by RT-PCR. *In vitro* and *in vivo* analyses showed that SAG12-IPT plants exhibited a stay-green phenotype that has the potential to improve the quantity and quality of alfalfa forage.

2-Seed size. Better conditions of lucerne meadow establishment and reduction of interference effects among plants can be achieved by increased seed size. In addition, several reports show a positive correlation between seed size and the seedling vigour. The use of precision sowing can be also implemented by the availability of larger seeds. Only very few examples are known in model species that report on increased organ size in plants. In particular Mizukami and Fisher (2000) succeeded in increasing the size of all *Arabidopsis* organs by over-expression of the transcription factor Aintegumenta (ANT).

In this work the organ size regulator of *Arabidopsis* Aintegumenta was expressed under seed specific promoters (USP and LegP) with the aim to obtain an increase in size restricted to seeds. Transgenic plants were obtained in tobacco as model plant and in alfalfa. The transgene expression was shown in tobacco capsules of USP-ANT plants. The phenotype of these plants is currently under evaluation.

References

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