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Researches carried out at the section of Perugia of the Institute of Plant Genetic of Italian National Research Council face three main topics of alfalfa breeding: Forage and seed production, Forage quality and Mode of reproduction. All the research tasks are performed applying innovative strategies of breeding, recurring to genomic approaches, molecular markers and genetic transformation.

In details forage and seed production are trying to be accomplished through the modification of the organ size. Organ size control is of great importance from an agronomic point of view. Although plant growth is greatly influenced by environmental factors it seems that the intrinsic size of a plant is given by internal developmental factors (Mizukami and Fisher, 2000). Recent evidences proved that it is possible to escape such control to obtain plant organs of bigger size, in fact ectopic expression of the transcription factor Aintegumenta in Arabidopsis and tobacco resulted in an enlargement of all shoot organs without altering their morphology (Mizukami and Fisher, 2000). The possibility to alter organ size is also important in alfalfa breeding both for forage yield and for sowing. The obvious advantages of getting bigger alfalfa leaves apart, an increase in seed size would allow the use of precision sowing in order to optimise the number of plants in the field (Rotili et al., 1999). For this purpose the Aintegumenta cDNA was cloned under constitutive and seed-specific promoters, the constructs realised were used to transform alfalfa and putative transgenics are currently analysed.

Another trait that hampers the maximization of alfalfa leaf yields is leaf senescence starting from the basal leaves whose loss is not compensated by the growth of new apical ones. A delay in leaf senescence was obtained by ectopic expression of the isopentenyl phosphotransferase (ipt) gene, leading to an increase of cytokinins in the leaves, under a senescence specific promoter in several crops (Gan and Amasino, 1995; McCabe et al., 2001). The construct SAG12-ipt, kindly provided by Prof. Amasino, was used to transform alfalfa and transgeneic plants are currently characterised.

The production of bloat-free alfalfa plants is a long term aim of a research carried out at this experimental station. At this purpose experiments on the model systems *Lotus corniculatus* have been performing for several years and the role of transactivator genes and environmental conditions on the expression of structural genes of the pathway have been investigated. Transgenic *Lotus* and alfalfa for exogenous regulators and for an *Arabidopsis thaliana* structural gene of the pathway have been produced. Such transgenes resulted very efficient in affecting the pathway in Lotus but very poor if any effect showed on alfalfa. It suggests that for the synthesis of Condensed tannins in alfalfa leaves, the expression some late gene of the pathway (probably those involved in polymerisation and vacuole compartmentalisation) is mandatory.

The *Lotus* system seems a very suitable model for analysing this metabolic pathway because at different with Arabidopsis harbours two alternative biosynthetic branches: the one originating from cathechin and that originating from epicatechin. Studies through HPLC analyses are in progress to determine if such branches are alternative or complementary and how transformations modified tannins composition. Cloning in Lotus of the genes coding for ANR and LAR the enzyme for the synthesis of epicatechin and catechin, respectively, are in progress.

A genomic approach is also utilised to gain knowledge on trait related to forage quality working on the model species *Medicago truncatula*. Our approach consists on the selection of mutants with an

increased accumulation of anti-bloating compound in leaves and with altered accumulation of saponins. Condensed tannins (CT), the most important anti-bloating factors of forages, are expressed in *Medicago* spp only in seeds. By an activation tagging approach we aim to select *Medicago truncatula* mutants which accumulate CT in leaves. At the moment, two strategies which differ for the insertional elements are being evaluated. The first employs a T-DNA construct with 4 copies of a 35S enhancer while the second is based on the tobacco Tnt1 and Tto retrotransposons. Leaves of transgenic plants will be analysed by a CT specific colorimetric test based on the DMACA dye . Plants positive to the DMACA test will be further analysed by HPLC. The DNA sequence flanking the insertional element will be cloned and re-introduced in alfalfa for a functional prove. Saponins are antinutritional compounds which play an important role in plant –pathogen interaction. The screening of the mutant collection will be performed by an haemolytic test according to Tava et al (1992). Plants showing an altered level of saponin accumulation will be further analysed in order to identify the molecular determinants of the altered accumulation level.
A complementary strategy to improve forage quality consist on the modification of forage proteins. Zeins, the main storage proteins of maize that accumulate in the endoplasmic reticulum of the endosperm cells, are particularly interesting at this purpose because they are rich in the essential sulphur amino acids. Over-expression of certain zein genes in plants such as alfalfa would be expected to improve the nutritional characteristics of this crop. Recently, significant accumulation values have been reached but still far from those considered useful for nutritional purposes. At the moment we are investigating whether targeting to cytosol and chloroplast instead of the endoplasmic reticulum may yield higher β-zein accumulation in transgenic plants.

With the long term of transferring the apomictic mode of reproduction (i.e. clonal reproduction through seeds) in alfalfa we are trying to identify the relevant genes in the tropical grass *Paspalum simplex* through a comparative mapping strategy. To date, the related locus has been identified on both genetic and physical bases. Comparative mapping with model species (rice and *Arabidopsis*) and partial sequencing reveals the presence, on this locus of several genes that can be putatively involved in apomixis. Further studies are in progress to test these genes functionally through reverse genetics and expression analysis.