

## ROLE OF A NOVEL *KNOX1* TRANSCRIPTION FACTOR OF *MEDICAGO TRUNCATULA* IN THE DETERMINATION OF LEAF MORPHOLOGY

IANNELLI M.A.\* , DI GIACOMO E.\* , IAFRATE S.\* , RODRIGUES-POUSADA R.A.\*\* ,  
PENG J.\*\*\* , CHEN R.\*\*\* , FRUGIS G.\*

\*) Istituto di Biologia e Biotecnologia Agraria (IBBA), Operative Unit of Rome, Consiglio Nazionale delle Ricerche (CNR), Via Salaria Km 29,300, 00015 Monterotondo Scalo (Italy)  
\*\*) Dipartimento di Biologia di Base ed Applicata, University of L'Aquila, Via Vetoio, 67010 Coppito (Italy)  
\*\*\*) Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK 73401 (US)

*Transcription factors, M. truncatula, leaf development, functional genomics, alternative splicing*

In higher plants, *KNOX1* homeobox transcription factors play a critical role in compound leaf development. The presence of *KNOX1* in developing leaves characterizes compound-leaved versus simple-leaved species, where *KNOX1* genes are expressed only in the shoot apical meristem (SAM) and permanently down-regulated during leaf development. It has been recently shown that modifications of *KNOX1* gene expression, through changes of cis-regulatory properties, represent the main evolutionary step of leaf morphology complexity.

In simple-leaved species, *KNOX1* are essential for proper formation and morphogenetic activity of the SAM, whereas in most of compound-leaved species they exert their role in leaf dissection through the maintenance of a transient indeterminacy and morphogenetic activity at the marginal blastozone. In the inverted repeat-lacking clade (IRLC) of legumes, an alternative pathway, involving the Arabidopsis *LEAFY* (*LFY*) orthologs - *UNIFOLIATA* (*UNI*) of pea and *SINGLE LEAFLET1* (*SGL1*) of *M. truncatula* - was proposed to act in the formation of compound leaves.

In order to explore the role of *KNOX1* transcription factors in the development of trifoliolate compound leaves in *Medicago truncatula*, a model species for forage legumes, six *MtKNOX*s have been identified and characterized in our lab.

Here we present the molecular and functional characterization of *MtKNOX6*, a homologue of Arabidopsis *SHOOTMERISTEMLESS* (*STM*) that is located in a separate branch of the phylogenetic tree with respect to the strictly *STM*-like proteins. *MtKNOX6* expresses in developing leaves, in the SAM and during inflorescence development, is regulated through alternative splicing and the translated proteins retain the ability to functionally interact with the BEL protein PNY/BLH9 to enter the nucleus. Transgenic lines that overexpressed *MtKNOX6* in the heterologous system *Arabidopsis thaliana* did not phenocopy Arabidopsis plants that overexpressed *STM*, that suggesting different biochemical and molecular properties of *MtKNOX6* with respect to *STM*. Also, overexpression of either *STM* or *MtKNOX6* in *M. truncatula* did not trigger the same developmental alterations, that confirming the data obtained in Arabidopsis. Two *mtknnox6* knock-out lines were isolated in collaboration with The Samuel Roberts Noble Foundation (US) and their characterization is in progress.