Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – D.43

IDENTIFICATION OF *ACL5* AS A TARGET OF HD-ZIP III TRANSCRIPTION FACTORS

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HD-ZIP III transcription factors, spermine, target genes, vascular development

The *Arabidopsis* HD-ZIP III transcription factor (TF) family consists of five highly related proteins involved in several developmental processes including vascular development. To gain more insights on the role of the HD-ZIP III TFs in the regulation of plant development, we searched for their target genes. A screening of the *Arabidopsis* genome database identified 390 genes containing the BS-III element, a 11 bp pseudo-palindromic sequence recognized by the HD-ZIP III proteins *in vitro*. Among these genes, *ACAULIS5* (*ACL5*) encoding a spermine synthase has been chosen for further investigation based on its putative regulatory role in vascular development. The loss-of-function *acl5-1* mutant is dwarf and characterized by the formation of an increased number of veins and vascular elements in leaves and stems (Hanzawa et al., 1997, 2000; Clay et al., 2005). Further phenotypic analysis of *acl5-1* revealed that it produces more lateral root primordia than wild-type plants, indicating a negative role of spermine or related molecules in this process.

As a first step to investigate whether *ACL5* is indeed a target of HD-Zip III TFs, *in vitro* DNA binding assays have been performed. EMSA experiments demonstrated that the HD-Zip III domain specifically recognizes an *ACL5* promoter region comprising the BS-III element but not a derivative region carrying mutations in BS-III. Next, to investigate whether one or more HD-Zip III TF regulates *ACL5* expression through the BS-III element *in vivo*, transgenic plants expressing the GUS reporter gene under the control of either the *ACL5* promoter or a derivative mutated in the BS-III element have been generated and characterized. Histochemical analysis revealed that an intact BS-III element is essential for GUS expression at the very early phases of vascular development in all the organs examined (leaf primordia, primary root an lateral roots). To assess the relevance of spermine production in procambial cells for proper vascular development and lateral root formation, the *acl5-1* mutant was complemented with the *ACL5* gene driven by its own promoter or a derivative promoter mutated in the BS-III element. The wild-type construct rescued all aspects of the *acl5* mutant phenotype. In contrast, plants expressing *ACL5* under the mutated promoter retain some aspects of the *acl5* phenotype such as increased number of veins in leaves and more lateral roots. Together the data demonstrate that *ACL5* expression and thus spermine synthesis is regulated through the action of one or more HD-Zip III TF at the early stages of vascular development and lateral root formation.