Proceedings of the XLIX Italian Society of Agricultural Genetics Annual Congress
Potenza, Italy – 12/15 September, 2005
ISBN **88-900622-6-6**

Poster Abstract - B.02

ISOLATION AND CHARACTERIZATION OF NOVEL ALFALFA MOB1-LIKE GENES

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cell cycle, immuno-localization, two-dimensional electrophoresis, cross-complementation

A family of structurally related proteins (Mob, Mps-one-binder), which includes highly conserved cell cycle-regulated proteins that likely function as protein kinase activating subunits, has been demonstrated to be important for both mitosis completion and cytokinesis in budding yeast. Its founding member, Mob1, was identified in *Saccharomyces cerevisiae*, where it is an essential component of the mitotic exit network (MEN), a complex signal transduction cascade that co-ordinates mitotic exit with cytokinesis.

Mob1-related proteins have been recently found also in animal and plant cells. The study of a spontaneous lethal mutation in a *Drosophila* Mob gene has recently implicated the Mob protein family in the control of animal cell proliferation and apoptosis. Moreover, the identification of the animal Dbf2 homologous proteins NDR (nuclear Dbf2-related), interacting with Mob1-related proteins, and the determination of the human and *Xenopus laevis* Mob proteins structures, suggest that Mob proteins act as kinase activating subunits also in higher eukaryotes.

Plant genomes (e.g. *Arabidopsis thaliana* and *Oryza sativa*) contain uncharacterized Mob1-related genes. Here we report the isolation and characterization of Mob1-like genes in alfalfa (*Medicago sativa*), by using: (i) immunochemical techniques for protein identification and immunolocalization; (ii) a complementation test in yeast cells for functional analysis. (i) One Mob1-like gene and one gene containing a Mob1-domain were cloned in alfalfa and named MsMob1-A and MsMob1-B. Polyclonal antibodies specifically raised against alfalfa Mob1 proteins recognized at least two distinct proteins of about 25 and 47 kDa, which are likely products of the two genes. Two-dimensional PAGE analysis revealed the presence of different isoforms whose characterization by peptide-mass fingerprinting is currently in progress. The subcellular localization of these proteins is consistent with a role of Mob1-like proteins in plant cell proliferation and cytokinesis. (ii) To investigate whether alfalfa Mob1-A is the functional orthologue of budding yeast Mob1, we expressed MsMob1-A in a temperature-sensitive *mob1* yeast mutant. In spite of the high similarity between alfalfa Mob1-A and *S. cerevisiae* Mob1 in their primary sequence, expression of Mob1-A could not rescue the lethality of *mob1* mutant cells at the restrictive temperature, suggesting that interaction of Mob1 proteins with their effectors might be species-specific.