Proceedings of the 58th Italian Society of Agricultural Genetics Annual Congress
Alghero, Italy – 15/18 September, 2014
ISBN **978-88-904570-4-3**

Poster Communication Abstract – 6.02

STUDIES ON THE REGULATORY REGIONS OF A *DHFR/TS* GENE OF *ARABIDOPSIS THALIANA* CONTROLLING ITS EXPRESSION IN ROOT MERISTEMS

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gene promoters, plant meristems, Arabidopsis thaliana, dihydrofolate reductase, thymidylate synthase

In plants, like in protozoa, dihydrofolate reductase and thymidylate synthase belong to a bifunctional protein allowing a concerted regulation of these enzymes, which are important for cell proliferation and DNA endoreduplication. Analyses of expression by *In situ* hybridization and promoter activity of a carrot *DHFR-TS* gene have confermed a proliferation-dependent pattern of expression of plant *DRTS* genes (1), but expression of *DHFR-TS* genes appears to occur also in differentiated tissues. Studies conducted in our laboratory have indeed revealed distinct patterns of activity of the promoters of the three *DHFR-TS* genes of Arabidopsis (2). Surprisingly, only the *AtDRTS2* promoter showed a clear meristematic activity, whereas the activity of the *AtDRTS1* promoter was limited to vascular tissues and the promoter of the *AtDRTS3* gene showed partial activity in the shoot meristem, in the root columella and in the root central cylinder. Nevertheless, recent analyses by RT-PCR are suggesting a meristematic expression also for the *AtDRTS1* gene. Functional studies conducted on the *AtDRTS2* promoter have revealed the presence of various regulatory regions involved in the control of its expression. In particular, the inactivation of an HEX site decreased *AtDRTS2* promoter activity in meristematic cells whereas the inactivation of an adjacent E2F *cis* element, shown previously to be functional by chromatin immunoprecipitation, increased its activity. However, both these sites did not appear to be strictly necessary for meristematic expression whereas the presence of a portion of the 5' untranslated region of *AtDRTS2* was found to be essential. This evidence was further confirmed by an experiment of "gain of expression" in which the insertion of the 5' untranslated region of *AtDRTS2* downstream of the *AtDRTS1* promoter, which is active only in vascular tissues, was able to confer a strong additional activity to this promoter also in root meristems. This result suggests the possibility to engineer chimeric promoters to improve gene expression for biotechnological applications. Studies are currently underway to further characterize the functional features of the 5' untranslated region of *AtDRTS2*.

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