Proceedings of the 51st Italian Society of Agricultural Genetics Annual Congress
Riva del Garda, Italy – 23/26 September, 2007
ISBN **978-88-900622-7-8**

Poster Abstract – E.07

DEVELOPING A METHOD FOR SUGAR BEET CHLOROPLAST TRANSFORMATION

DE MARCHIS F.*, WANG Y.**, BELLUCCI M.*, ARCIONI S.*

*) Institute of Plant Genetics – CNR, Research Division of Perugia, Via della Madonna Alta 130, 06132 Perugia (Italy)
**) Institute of Animal Science, CAAS, Yuan Ming Yuan, West Road, Haidian, Beijing, 100094 (China)

sugar beet, plant regeneration, chloroplast, biolistic transformation

Sugar beet (*Beta vulgaris* L.) is an important industrial crop of the temperate zone. Although improvements in various sugar beet traits, such as sugar yield or disease resistance, have been achieved trough conventional breeding, other traits such as herbicide resistance could be introduced in this crop by genetic engineering. Unfortunately, this species is still considered recalcitrant to genetic transformation and a routine method for regeneration of transgenic plants is lacking. Moreover, a lot of concern**s** could arise from the introduction of transgenic sugar beet in the field due to its well documented cross-compatibility with the wild-relative sea beet (*B. vulgaris* ssp. *maritima*). In many crop species chloroplast DNA is not transmitted trough pollen. Thus the development of transplastomic sugar beet**s** plants could avoid the risk of gene flow between the commercial transgenic sugar beet and wild-type plants or relative wild species.

We screened twenty-six *Beta vulgaris L.* varieties from Italian, German, and North American germplasm for optimal tissue culture response. Two regeneration protocols have been used. The first one is based on indirect regeneration from callus obtained from hypocotyls and cotyledons, whereas the second one is based on direct regeneration from leaf petioles. Four varieties have been identified that give high frequency regeneration (ca. 20%) from leaf petioles with the direct regeneration method. To attempt sugar beet chloroplast transformation, four vectors were constructed called pSB1, pSB2, pSB1-*bar* and pSB2-*bar*. Vector pSB1 was constructed cloning the chloroplast sugar beet genes *rrn16*, *trnV* and *rps12/7* in pBlueScript.KS plasmid. pSB2 is the same as pSB1 except for the chloroplast genes cloned in the vector that are *rbcL* and *accD*. Both the *aadA* gene, encoding spectinomycin resistance under control of the chloroplast P*rrn* promoter, and the reporter *gfp* gene regulated by the chloroplast *psbA* promoter, have been inserted in the intergenic spacers of the two vectors. Other than *aadA* and *gfp* genes, plasmid pSB1-*bar* and pSB2-*bar* contain a cassette for expression of the *bar* gene. Preliminary experiments were carried out on leaf petioles using the direct regeneration method to optimize the transformation parameters. Three thousands petiole explants, derived from sterile plants grown *in vitro* have been bombarded with pSB1 or pSB2 DNA and they are currently cultured on regeneration medium containing spectinomycin 50 mg/l.