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Poster Abstract – E.03

CHLOROPLAST TRANSFORMATION IN SUGAR BEET BY PARTICLE BOMBARDMENT

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aminoglicoside 3'-adenylyltransferase (aadA), biolistic apparatus, chloroplast DNA transformation, green fluorescent protein, spectinomycin

In the past twenty years, plastids have become attractive targets in the field of plant biotechnology. Plastid transformation is routinely used only in tobacco, thus it is very important for the application of chloroplast engineering to extend the range of species in which this technology can be achieved. Here, we describe the development of a chloroplast transformation system for the sugar beet (*Beta vulgaris* L. ssp. *vulgaris,* Sugar Beet Group) by biolistic bombardment of leaf petioles and the production of sugar beet transplastomic plants. Despite being a dicotyledonous plant and the considerable attention it has received due to its importance as an agricultural crop for sugar production in the Northern Hemisphere, sugar beet is considered to be a recalcitrant species with respect to genetic transformation, displaying poor reproducibility and genotype dependency. Moreover, there are no reports of sugar beet chloroplast DNA transformation at present, making this field of research open to exploration. The production of transplastomic sugar beet lines with maternal inheritance of transgenes could solve problems related to outcrossing between genetic modified varieties and conventional varieties or wild relatives. It is well documented that the sugar beet easily inter-crosses with its wild relative, the sea beet [(*Beta vulgaris* L. *ssp. maritima (L.) Arcang.*)], or with annual weed beets that occur in the field. Although *Beta vulgaris* is a biennial crop that is harvested before its reproductive stage and is not expected to flower in the first year, early flowering can occur through occasional vernalization at low spring temperatures. Therefore, unintentional pollination of sea beets or weed beets is possible. The use of chloroplast genetic engineering could drastically reduce the probability of transgenic pollen dispersal.

To develop a chloroplast transformation system for this species, different methods for plant regeneration were tested for all the varieties analysed. Homoplasmic plastid-transformed plants of breeding line Z025 were obtained. Transformation was achieved using a vector that targets genes to the *rrn16/rps12* intergenic region of the sugar beet plastome, employing the *aadA* gene as a selectable marker against spectinomycin and the *gfp* gene for visual screening of plastid transformants. g*fp* gene transcription and protein expression were shown in transplastomic plants. Detection of GFP in Comassie blue-stained gels suggested high GFP levels. Microscopy revealed GFP fluorescence within the chloroplasts. Our results demonstrate the feasibility of engineering the sugar beet chloroplast genome.