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Poster Abstract – A.28

COPY NUMBER AND TRANSCRIPTION LEVEL IN TRANSFORMANTS OF A WILD POTATO SPECIES (*SOLANUM CARDIOPHYLLUM)*

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In order to assay the effect of regeneration events on both transgene copy number and expression level, three different genotypes of *S. cardiophyllum* were used in genetic transformation experiments. In particular we used a wild type clone (CPH1C 2n=2x=24) and two regenerated derivatives, N11B (2n=2x=24) and C6B (2n=4x=48). *S. tuberosum cv.* Désirée (2n=4x=48) was used as control. *Agrobacterium*-mediated transformation was done according to either Millam (*In* : "Transgenic Crops of the World – Essential Protocols" pp 257-270, Ed. I. Curtis, Kluwer Academic) 2004) or Karp *et al.* (Plant Cell Tiss Organ Cult 3:363-373 1984) procedure by using a *p35S::GUS-INT* vector harboring the transgene *uidA* under a double 35S promoter. DNA and RNA were extracted from transformed plants by DNeasy Mini kit and RNeasy Mini kit, respectively. *UidA* copy number and mRNA relative abundance was assayed by Real Time qPCR.

Transgene copy number stably integrated in transformant genomes varied significantly between genotypes. On average, *S. cardiophyllum* transformants integrated more copies of *uidA* transgene than *S. tuberosum* (respectively 5.5 copies *vs.* 1.7 copies). The origin of explants affected the transgene copy number (Fig. 1). In fact, transfromants coming from the wild type CPH1C showed a significantly higher transgene copy number compared to transformats coming from N11B and C6B. Also, significant differences were observed in copy number between transformants coming from diploid and tetraploid derivatives, suggesting the transgene integration being affected by the ploidy level (Fig. 1). Analysis of larger samples of transformants will confirm this hypothesis.

UidA transcript showed significant variations between species. Again, *S. cardiophyllum* transformants showed higher level of the *uidA* transcript compared to *S. tuberosum* ones. In fact, *S. tuberosum* transformants performed an average relative quatification of 30.6 compared to *S. cardiophyllum* transformants with a relative abundance of the *uidA* transcript of 147.7. Within *S. cardiophyllum* transfromants, a strong effect of the origin of explants was observed on the *uidA* transcript abundance. Transformants coming from the wild type CPH1C showed a significantly higher transcript RQ when compared to transformants coming from N11B and C6B. Finally, transcript level was higher in regenerants deriving from 4X C6B than in those produced from 2X N11B. This work suggests that regeneration events may compromise the transgene integration through *Agrobacterium*-mediated transformation and limit the transcription of the transgene in further transformants. Our results also suggest that the ploidy level of explants may affect both transgene copy number and transcription level. Additional studies will investigate molecular

mechanisms elicited by somaclonal variations and affecting transgene expression in transformants coming from regenerated tissues.