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Poster Abstract – E.22

HIGH EXPRESSION OF THE VACCINIA VIRUS A27L PROTEIN IN TRANSGENIC AND TRANSPLASTOMIC PLANTS

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Orthopoxviruses (OPV) have recently received increased attention, due to the fear of bioterrorism and to the occurrence of zoonotic OPV outbreaks, highlighting the needs for the development of safer vaccines against smallpox and related viruses. To avoid the use of a live virus, the production of subunit protein-based vaccines is an attractive approach. Transgenic plants are ideal means to produce subunit vaccines being them safe, and less expensive to produce and distribute. We are therefore investigating the possibility to produce the immunogenic A27L protein of vaccinia virus (VACV) in tobacco (*cv*. Petit Havana). Transgenic and transplastomic tobacco plants were produced, respectively, by *Agrobacterium*-mediated transformation of the nuclear genome and by biolistic transformation of the plastome. Western blot analysis showed the presence of one band of the expected A27L protein size (15 kDa) in the transplastomic and transgenic lines analysed. Two additional bands at ~30 and 40 kDa were displayed only in the transplastomic samples; these bands were thought to be dimers and trimers, a characteristic property of the VACV protein. Indeed, such results were confirmed by electrophoresis of proteins collected on a gradient, which mostly showed the accumulation of the multimeric forms, important for the biological activity of the protein. ELISA analysis demonstrated that the integration of the *A27L* gene into the chloroplast genome resulted in the accumulation of ~1.6 g/kg fresh weight (equivalent to 18% of TSP). This amount of expression is at least 500-fold higher than the nuclear lines. In fact, values for the transgenic plants analyzed ranged from 1.4 to 3.6 mg/kg fresh weight (0.01-0.04 % TSP). The level of protein accumulation did not decline during leaf development in mature transplastomic plants, suggesting that the protein is stable in transgenic tobacco chloroplasts. Preliminary results indicated that the chloroplast-made A27L protein is recognized by antiserum produced against zoonotic orthopoxviruses. The results obtained herein demonstrate that plastid transformation is a useful for the expression of OPV subunit vaccines. Chloroplast transformation has the additional advantages to co-express multiple antigens in operons and to impose transgene containment through maternal inheritance.