CHLOROPLASTIDIC TRANSIENT EXPRESSION OF THE GREEN FLUORESCENT PROTEIN IN IMMATURE EMBRYOS OF *Zea mays* L.

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Introduction. The maize is of the most significant cereal grain for México from a nutritional, economic, and social perspective(1). The genetic improvement, channeled to the increase of the nutritional quality and greater tolerance to biotic and abiotic stress, may increase the quality and productivity of your crop with a reduction in the costs; however, in some sectors of the society exist a legitimate concern about the potential partners risks, including the possible environmental impact of the transgenes flow to wild species(2). An alternative for the contention of transgenes is the generation of transplastomic plants, due to the maternal inheritance of the plastids in most higher plants(3).

The objective was the subcloning of *mgfp5* and *hph* into a vector for the chloroplasts transformation of maize and demonstrate the transient expression of the GFP produced in immature embryos of *Z. mays* L.

Methods. The chloroplastidic transgenes were designed *in silico* on based to sequences of the NCBI, were synthesized de *novo* and subcloned into the pTLPC135kb vector in the *Aat*II site, using conventional recombinant DNA techniques. The immature embryos of 1-2 mm were bombarded through biolistic technique by nanoparticles flow with helium at low pressure(4). Transient expression was observed after 16 h by epifluorescence microscopy with 30 s of exposure to 5X.

Results. pZmcpDNAGFP was constructed according to the design shown in the Figure 1. In the bombarded embryos (Figure 2) are observe marked differences; the untransformed embryo presents uniformity in your luminance, unlike to the other embryos which having areas with different fluorescence signals. The transformed embryo with pZmcpDNAGFP shows foci of fluorescence clearly delimited, and this it can be interpreted as an evidence of transformation and suggesting the transient expression of GFP in proplastids; the transformed embryo with pCAMBIA1302 exhibits broader zones of fluorescence, expected of the transient expression of GFP in the cytoplasm of every cell transformed.

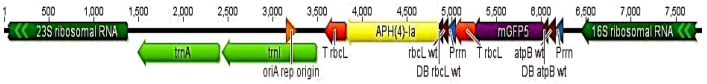

Figure 1. Location of transgenes and chloroplastidic genes in pZmcpDNAGFP. It shows a fragment of the construction (7,661 bp), with the genes required for the selection, expression and homologous recombination.

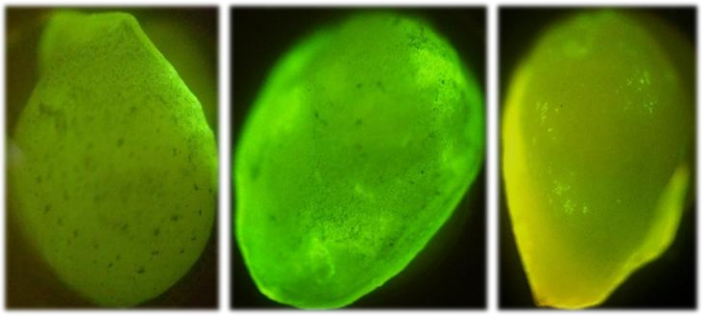

Figure 2. Transient expression of *mgfp5*. On the left is shown an embryo without bombard, on the center one bombarded with pCAMBIA1302, and on the right other bombarded with pZmcpDNAGFP.

Conclusions. pZmcpDNAGFP containing the transgenes *mgfp5* and *hph*; the transient expression assays of GFP in maize immature embryos indicate that it is likely to be present in the proplastids of the bombarded cells and that at least the *mgfp5* gene is functional.

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