Congreso Nacional de Biotecnología y Bioingeniería
VII Simposio Internacional de Producción de Alcoholes y Levaduras

*Atc*DNAPHYB MUTANT AS SCORABLE PHENOTYPIC MARKER IN RICE AND TOMATO

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Key word: *Phytochrome, selectable marker, dark*

Introduction. The Public Intellectual Property Resource of Agriculture (PIPRA) provides resources and a collaborative framework to achieve the most effective utilization of IP to advance innovation in agriculture, both for humanitarian purposes and for regional or specialized commercial markets. Fluorescent markers are widely used in molecular biology as labels for nucleic acid probes, antibodies, and receptors as diagnostic indicators and as research tools. The Green Fluorescent Protein (GFP) has recently gained widespread utility as a selectable marker and a fluorescent indicator of cellular events. However, GFP has a narrow spectral range over which it fluoresces, which limits its usefulness as a marker. In addition, licensing the GFP marker or its derivatives for selectable marker applications is cost prohibitory, even for research and non-commercial purposes. Phytochromes are plant pigment proteins that absorb light, initiating physiological responses in higher plants that govern light-sensitive processes such as seed germination, growth, and flowering. There are previous observations, which indicate that plants over-expressing a mutant phytochrome confer a visually scorable phenotype in *Arabidopsis thaliana (At)* transgenic plants (1). This technology is available for licensing from the University of California. Therefore the overall aim was to evaluate a plant-derived mutant phytochrome in transgenic plants of rice and tomato for applications as a phenotypic plant selectable marker.

Methodology. Tomato and rice plants transformed with the CaMV35S-AtcDNAPhyBmutant were obtained by standard *A. tumefaciens* methods by the Ralph M. Parson´s transformation facility (UCDavis). Transgenic and wild-type plants of tomato were grown under light or dark conditions on 0.5X MS salt and 0.6 % tissue culture agar. For both treatments (light and dark), seeds were kept in the dark at 4°C for 2 days then exposed to white light for 2 h to stimulate seed germination before each treatment. For dark treatment plates were kept at 23°C in complete darkness (wrapped with aluminum foil) and for light treatment plates were grown at 23°C under normal conditions of growth (16 h light/8 h dark). For rice, plates were kept at 28°C in complete darkness (wrapped with aluminum foil) during 6 days.

Results and discussion. Tomato and rice plants that overxpress the AtcDNAPHYB mutant exhibit phenotypic difference when compared to wild-type. *AtcDNAPHYB* tomatoes show a clear de-etiolated response (Fig.1). In the dark, transgenic seedlings do not exhibit elongated, hypocotyls. They grow de-etiolated as if light-grown.

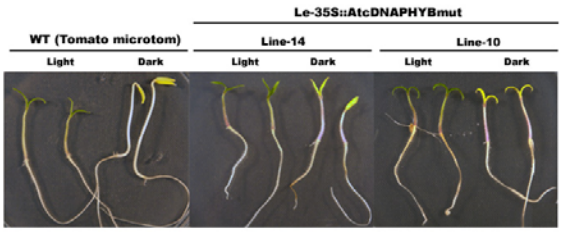

Fig. 1. Phenotype of 10 day-old tomato transgenic plants expressing a phytochromeB mutant.

In figure 2, rice transgenic plants expressing the AtcDNAPHYB mutant show a clear reduction in the size of the coleoptiles compared with wild-type plants.

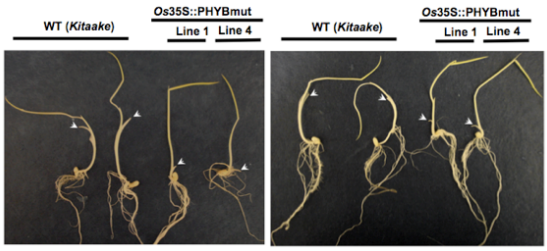

Fig. 2. Phenotype of 6 day-old rice transgenic plants expressing a phytochromeB mutant grown in dark conditions. Arrows indicate the tips of coleoptiles.

Conclusions. We observe phenotypic differences in both transgenic tomato and rice expressing the phytochromeB mutant from *A. thaliana*. PhytochromeB mutant could be a simple and effective scorable marker to screen transgenic plants in both dicots and monocots.

Acknowledgment. This work was supported by a UCMEXUS postdoctoral fellowship and a grant from the Rockefeller Foundation. Thanks to Dr. Clark Lagarias for providing the phytochrome binary vector.

References.

1.- Su, YS, Lagarias JC. (2007). Light-independent phytochrome signaling mediated by dominant GAF domain tyrosine mutants of Arabidopsis phytochromes in transgenic plants. *Plant Cell*. 19(7): 2124-39