

Expression of sweetpotato-like cry genes to control weevils,
Abstract presented at the International Association of Plant Biotechnology (IAPB) congress in June 2010

Milagros Ormachea, Katterinne Prentice, Jose Carlos Tovar, Marc Ghislain[†]
Applied Biotechnology Laboratory, International Potato Center, P.O. Box 1558, Lima 12,
Peru

Weevil active proteins were identified previously with efficacy against the two African weevil species, *Cylas puncticollis* and *C. brunneus*, responsible of huge production loss of the sweetpotato in sub Saharan Africa. Transformation of sweetpotato with their respective genes (*cry7Aa1* and *cry3Ca1*) yielded a total of 17 events with either *cry* gene. In order to identify events that are high expressers of the Cry protein, we tested transcriptional activity through real-time PCR based on SYBR Green I methodology and DAS-ELISA detection of the expressed protein using rabbit polyclonal antibodies. Five and nine transformed events bearing the *cry7Aa1* and *cry3Ca1* genes, respectively, were analyzed for transgene expression in the leaf, taking as reference the event with the lowest expression (LE). Two *cry7Aa1* events showed statistically significant differences from the LE, with expression levels of 10.19 and 1.78 higher than the LE. Likewise, two *cry3Ca1* events showed statistically significant differences from the LE, with expression levels of 54.65 and 2.78 times higher than the LE. These results were confirmed by DAS-ELISA analysis for protein expression in the leaves. The two *cry7Aa1* events had protein expression levels of 0.03 and 0.009 ug/gr of fresh weight, and the two *cry3Ca1* events had protein expression levels of 0.04 and 0.03 ug/gr of fresh weight. All other events had very low protein expression levels. Because the promoters used to guide gene expression were taken from the genes of the two major proteins in the tuberous root, it is expected that gene expression in the tuberous roots will be higher. This will allow us to localize the expression, a strategy used for insect resistance management.

[†]Present address: International Potato Center Sub-Saharan Africa Regional Office P.O. Box 25171, Nairobi 00603, Kenya