Development of Alfalfa (Medicago Sativa L.) Transgenic Plants Expressing a Bacillus Thuringiensis Endotoxin and Their Evaluation Against Alfalfa Caterpillar (Colias Lesbia)

F. Ardila
Instituto de Genética, Argentina

M. C. Gómez
Instituto de Genética, Argentina

M. J. Diéguez
Instituto de Genética, Argentina

E. M. Pagano
Instituto de Genética, Argentina

M. Turica
Instituto de Microbiología y Zoología Agrícola, Argentina

The XX International Grassland Congress took place in Ireland and the UK in June-July 2005. The main congress took place in Dublin from 26 June to 1 July and was followed by post congress satellite workshops in Aberystwyth, Belfast, Cork, Glasgow and Oxford. The meeting was hosted by the Irish Grassland Association and the British Grassland Society.

Proceedings Editor: D. A. McGilloway

Publisher: Wageningen Academic Publishers, The Netherlands

Presenter Information

F. Ardila, M. C. Gómez, M. J. Diéguez, E. M. Pagano, M. Turica, R. Lecuona, V. Arolfo, D. Basigalup, C. Vázquez Rovere, E. Hopp, P. Franzone, and R. D. Rios

Development of alfalfa (*Medicago sativa* L.) transgenic plants expressing a *Bacillus thuringiensis* endotoxin and their evaluation against alfalfa caterpillar (*Colias lesbia*)

F. Ardila[1], M.C. Gómez[1], M.J. Diéguez[1], E.M. Pagano[1], M. Turica[2], R. Lecuona[2], V. Arolfo[3], D. Basigalup[3], C. Vázquez Rovere[4], E. Hopp[4], P. Franzone[1] and R.D. Rios[1]

[1]Instituto de Genética "Ewald A. Favret", [2]Instituto de Microbiología y Zoología Agrícola, [3]EEA INTA Manfredi, Ruta 9 Km 636 (5988), Manfredi, Argentina, [4]nstituto de Biotecnología. 1,2 and 4: CICVyA, INTA, Castelar, cc 25 (1712), Argentina. Email: fardila@cnia.inta.gov.ar

Keywords: alfalfa, *Bacillus thuringiensis*, *Colias lesbia*

Introduction Alfalfa (*Medicago sativa* L.) is the most important forage crop in Argentina, with ca. 6 million cultivated hectares. The production of this crop is limited by the alfalfa caterpillar (*Colias lesbia*) which causes a loss equivalent to at least 10% of the biomass per year. No natural tolerance against this lepidoptera was found in alfalfa germplasm, hampering the development of tolerant cultivars by conventional breeding. This pest is usually controlled by using chemical insecticides but this has adverse effects on beneficial insects and the environment. Alternatively, low doses of commercial Bt insecticides (40 to 70 g/ha) also proved to efficiently limit the pest. This observation leads to us consider that the development of alfalfa transgenic plants expressing a suitable member of the *B. thuringiensis cry* gene family could be a useful tool for overcoming this alfalfa yield constraint. The aim of this work was to produce alfalfa transgenic plants expressing a Bt protein and to assess its biological activity against *C. lesbia* under laboratory conditions.

Materials and methods Petioles from alfalfa semi-dormant clones with high *in vitro* regeneration capacity (Moltrasio *et al*., 2004) were submitted to a customized *A. tumefaciens* based transformation protocol. The *npt* II gene, under Nos promotor and Ocs terminator control, was employed as selectable marker. As a source of δ-entomotoxin, an unmodified 2,086 bp, 5´-terminal fragment of the *cry*IA(b) gene from *B. thuringiensis* var. *kurstakii* HD-1 (Geiser *et al*., 1986), with high range of lepidoptera specificity, and controlled by 2 X 35S promoter, AMV enhancer and T7 terminator, was used. Kanamycin was employed as selection agent through all the *in vitro* culture steps. The regenerated plants were analyzed by PCR to evaluate the presence of the transgenes. Expression of the *cry* gene at mRNA level was evaluated by RT-PCR. The presence of the CryIA(b) protein in crude protein extracts from transgenic alfalfa leaves was determined by DAS-ELISA (adgia). A bioassay to test the entomocidal capacity of alfalfa transgenic plants was set up. For that purpose, first instar larvae of *C. lesbia,* obtained from field gathered eggs, were challenged by feeding them with transgenic ELISA positive or control plant leaves.

Results Fifty-one independent alfalfa transgenic plants were obtained and established in the greenhouse. All the plants proved to be PCR positive for both genes. Similarly, transcriptional activity from *cry*IA(b) gene was demonstrated in all the evaluated plants. Duplicated ELISA analyses showed that some plants expressed the Cry protein clearly above the control values. Some of the ELISA positive plants showed an insecticidal activity strongly enough to limit the development of alfalfa caterpillar larvae under laboratory conditions.

Conclusions The results of the present study provides proof of concept of the feasibility of limiting the development of alfalfa caterpillar through the employment of alfalfa transgenic plants expressing a truncated CryIA(b) protein. It is noteworthy to mention that the plants involved in this project were established in the greenhouse 8 years before the evaluations were performed. This underlines the remarkable structural and expression stabilities of these materials. The biological strategy described here paves the way to develop, in the short term, an effective tool for the field control of alfalfa caterpillar.

References

Geiser, M., S. Schweitzer & C. Grimm (1986). The hypervariable region in the genes coding for entomopathogenic crystal proteins of *Bacillus thuringiensis*: nucleotide sequence of the *kurhd*1 gene of subsp. kurstaki HD1, Gene 48 (1), 109-118.

Moltrasio R., C.G. Robredo, M.C. Gómez, A.H. Díaz Paleo, D.G. Díaz, R.D. Rios & P.M. Franzone (2004). Alfalfa (*Medicago sativa* L.) somatic embryogenesis: genetic control and introduction of favourable alleles into elite Argentinean germplasm". Plant, Cell, Tissue and Organ Culture 77 (2): 119-124.