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Peptide selection and antibody generation for the prospective immunorecognition of Cry1Ab16 protein of transgenic maize

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ABSTRACT

The introduction of genes isolated from different *Bacillus thuringiensis* strains to express Cry-type toxins in transgenic crops is a common strategy to confer insect resistance traits. This work intended to extensively *in silico* analyse Cry1A(b)16 protein for the identification of peptide markers for the biorecognition of transgenic crops. By combining two different strategies based on several bioinformatic tools for linear epitope prediction, a set of seven peptides was successfully selected as potential Cry1A(b)16 immunogens. For the prediction of conformational epitopes, Cry1A(b)16 models were built on the basis of three independent templates of homologue proteins of Cry1A(a) and Cry1A(c) using an integrated approach. PcH_736-746 and PcH_876-886 peptides were selected as the best candidates, being synthesised and used for the production of polyclonal antibodies. To the best of our knowledge, this is the first attempt of selecting and defining linear peptides as immunogenic markers of Cry1A(b)-type toxins in transgenic maize.

1. Introduction

Maize (*Zea mays* L.) plays a determinant role, both in human and animal nutrition, representing more than 36.5% of the total area of cereal production in 2014 (FAOSTAT, http://faostat3.fao.org/home/E). However, this crop is a common target of insects, nematodes and mites that propagate very rapidly, leading to low production rates of maize and, consequently, to great economic losses. To solve this problem, several biotechnological solutions have been adopted. One frequent strategy to confer insect resistance concerns the introduction of genes isolated from different *Bacillus thuringiensis* strains, which is a group of gram-positive bacteria that produces parasporal crystals (composed of polypeptide protoxins) during sporulation (Guo et al., 2012). In insects, the protoxins are activated by gut proteases to generate Cry toxins that are highly specific to their target species. Cry proteins are considered as innocuous to humans, animals and plants, and are completely biodegradable, being faced as viable alternatives for the control of insect pests in agriculture (Bravo, Gill, & Soberón, 2007). These crystalline (Cry) inclusions are classified as delta-endotoxins, encompassing a high number of toxins that share structural/functional similarities and display high specificity towards particular groups of insects (Ibrahim, Griko, Junker, & Bulla, 2010). So far, genes expressing Cry1, Cry2 and/or Cry3 class-type toxins have been isolated from *B. thuringiensis* strains and used for the generation of different genetically modified (GM) crops (Dehury et al., 2013; ISAAA, http://www.isaaa.org). In the specific case of maize, genetic constructions expressing Cry1 and Cry2 toxins are known to have specific insecticidal activity against insects from Lepidoptera order (e.g. mosquitoes, moths), while Cry3 proteins have toxic activity against insects from Coleoptera order (e.g. beetles) (Ibrahim et al., 2010; ISAAA, http://www.isaaa.org).

Maize is the second most cultivated biotech plant, thus contributing for about 30% of the global biotechnological area of 179.7 million hectares in 2015 (James, 2015). Among the GM plants, maize is the crop with the highest number of GM events (142), although only 29 have valid authorisation for food and feed, as well as for importing and processing, inside the European Union

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Table 1
List of web server programs used for the prediction assessment.

Name	Function	URL	References
BLAST	Basic Local Alignment Search Tool	http://blast.ncbi.nlm.nih.gov/Blast	–
BioEdit v.7.2.5	Sequence alignment editor	http://www.mbio.ncsu.edu/BioEdit/bioedit.html	–
DiscoTope 2.0	B-cell discontinuous epitope prediction	http://www.cbs.dtu.dk/services/DiscoTope/	Kringelum et al. (2012)
ElliPro	Prediction of linear and discontinuous B-cell epitopes	http://tools.iedb.org/ellipro/	Ponomarenko et al. (2008)
ExPASy Peptide Cutter	Enzymatic cleavage sites	http://web.expasy.org/peptide_cutter/	–
IEDB	B-cell linear epitope prediction	http://tools.iedb.org/bcell/	–
Chou & Fasman Beta-Turn Prediction	Beta-turn prediction	–	Chou and Fasman (1978)
Emini Surface Accessibility Prediction	Accessibility prediction	–	Emini et al. (1985)
Karplus & Schulz Flexibility Prediction	Flexibility prediction	–	Karplus and Schulz (1985)
Kolaskar & Tongaonkar Antigenicity	Antigenicity	–	Kolaskar and Tongaonkar (1990)
Parker Hydrophilicity Prediction	Hydrophobicity prediction	–	Parker et al. (1986)
Bepipred Linear Epitope Prediction	Linear epitope prediction	–	Larsen et al. (2006)
NCBI	DNA and protein sequences	http://www.ncbi.nlm.nih.gov	–
PEP-FOLD3	Peptide structure prediction	http://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py?form=HCA#forms::PEP-FOLD3	Shen et al. (2014)
POPI 2.0	T-cell epitope prediction	http://e045.life.nctu.edu.tw/POPI/	Tung and Ho (2007)
RCSB PDB	(Research Collaboratory for Structural Bioinformatics-Protein Data Bank. Information of 3D Structure	http://www.rcsb.org/pdb/home/home.do	–
SWISS-MODEL	SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information	http://swissmodel.expasy.org/interactive	Biasini et al. (2014)
UniProt	Protein primary/secondary structures	http://www.uniprot.org/	–

(EU) in 2016 (GMO Compass, 2016; James, 2015). MON810 and Bt11 transgenic events have been widely cultivated since they present resistance to a specific insect named European corn borer, which is responsible for inducing severe damage in European maize plantations. Both transgenic maize lines were modified to express the Cry1A(b) toxin that is one of the subclasses of Cry1 proteins (ISAAA, http://www.isaaa.org; Kumar et al., 2013). Like other Cry proteins, Cry1A(b) corresponds to the final product of a protoxin of approximately 130 kDa and 1155 amino acids that, after being submitted to different mechanisms occurring at transcriptional, post-transcriptional and post-translational levels, presents a molecular mass of 65 kDa (Ibrahim et al., 2010). The available data regarding this protein suggest that it is composed of 3 domains (I, II and III), also common to other Cry structures (Dehury et al., 2013; Kashyap, 2012), in which the domains II and III are believed to be involved in receptor binding and insecticidal activity (Ibrahim et al., 2010).

In spite of all the extensive safety assessments of GM crops about the potential effects of Cry toxins in human health, GM maize crops like Bt11 or MON810 have been widely cultivated for about 20 years. Presently, the consumption of GM plants is still a matter of concern since it continues dividing consumers' opinions. For an informed consumer choice, the mandatory labelling of processed foods containing or produced of GM crops is in force in many countries including EU (Regulation (EC) No. 1829/2003), continuously requiring for simple, fast and highly sensitive solutions to control the presence of genetically modified organisms (GMO) in foods. To answer this challenge, the interest on biosensor devices has been growing in the field of GMO analysis because of their potential for automation and micro-fabrication of simple and portable detection systems (e.g. visual or electrochemical) (Manzanares-Palenzuela et al., 2016; Plácido et al., 2016). So far, the immunorecognition of proteins expressed by GM plants has only been proposed using enzyme-linked immunosorbent assays (ELISA), but it has not been reported for biosensing devices.

To date, bioinformatics have been pointed out as excellent predicting tools to assess the immunoreactivity of peptides, while reducing the experimental work involved in epitope mapping. The main objective of this study concerned the selection of a set of marker peptides that could function as potential immunogens for the production of novel antibodies for Cry1A(b)16 biorecognition. For this purpose, an extensive *in silico* analysis was performed on Cry1A(b)16 protein, in order to select the best peptide candidates for antibody generation. On the basis of this study, the best peptides were synthesised, purified and used for animal inoculation with subsequent production of functional polyclonal antibodies.

2. Materials and Methods

2.1. Selection of Cry1 sequences and 3D structures

The correspondent protein sequence of Cry1A(b)16 was retrieved from the NCBI database (Table 1) with accession No. AAK55546.1. This protein is a delta-endotoxin produced by *B. thuringiensis* strain AC11 serotype H14, being encoded by a silent *cry* gene with NCBI accession No. AF375608.1. Cry1A(b)16 is the final product of a protoxin with a molecular mass of 130 kDa and an entire primary sequence of protein of 1155 amino acids.

In the absence of an experimental conformational structure of Cry1A(b)16, three homologue proteins, namely one model from Cry1A(a) and two models from Cry1A(c) with RCSB PDB ID: 1CIY, 4ARY and 4W8J, respectively, were selected based on their sequence identity and conformational similarity (Fig. S1, Supplementary material). In order to obtain pdb files of Cry1A(b)16 for the analysis of discontinuous B-cell epitopes, the SWISS-MODEL platform was used to generate three models from the experimental predetermined Cry1A(a) and Cry1A(c) structures.