Screening of *Solanum tuberosum* cultivars for the "*ac2*" genetic modification

R. Pribylova[1], J. Ptacek[2], I. Pavlik[1], M. Bartos[1]

[1]Veterinary Research Institute, Brno, Czech Republic
[2]Potato Research Institute Ltd., Havlickuv Brod, Czech Republic

ABSTRACT: In 2001, genetically modified potatoes carrying the *ac2* gene were developed. This gene was originally found in amaranth seeds (*Amaranthus caudatus*) and is expressed into the fungicidal peptide Ac-AMP2. The purpose of the present study was to test 105 potato cultivars (*Solanum tuberosum*) registered in the Czech Republic for the presence of the above mentioned genetic modification "*ac2*" with the use of a previously published method (Pribylova et al., 2006). The method was based on the simultaneous detection of the *ac2* gene from amaranth seeds as well as the *StTS1* gene from potatoes as an internal amplification control. The results showed none of tested potatoes cultivars were positive for the genetic modification "*ac2*". These results confirmed the currently valid legislative in the Czech Republic and the European Union, where the use of genetically modified potatoes carrying the gene for the fungicidal peptide from amaranth is not allowed, was respected.

Keywords: GMO; transgenic potato; food safety; *Amaranthus caudatus*; antimicrobial peptide

Abbreviations: Ac-AMP2 = *Amaranthus caudatus* antimicrobial protein 2; Bt = *Bacillus thuringiensis*; EC = European Commission; GM = genetically modified; GMO = genetically modified organism; PCR = polymerase chain reaction; *StTS1* = *Solanum tuberosum* putative trehalose synthase gene

Biotech crops achieved several milestones in 2006: the annual area of biotech crops exceeded 100 million hectares for the first time and the number of farmers growing biotech crops exceeded 10 million. The accumulated area from 1996 to 2006 increased 60-fold, making it the fastest adopted crop technology in recent history (Clive, 2006).

In 2006, the 22 countries growing biotech crops comprised eleven developing countries and eleven industrial countries. In Europe, Spain continued to be the lead country (60 000 hectares in 2006). In the other five European countries (France, Czech Republic, Portugal, Germany, and Slovak Republic) the Bt maize area increased over 5-fold and growth in these five countries is expected to continue in 2007 (Clive, 2006).

The principle of the "*ac2*" genetic modification was a transformation of potato plants (*Solanum tuberosum*) with the *ac2* gene from seeds of *Amaranthus caudatus* (Liapkova et al., 2001). The ac2 gene encodes the Ac-AMP2 antimicrobial peptide (De Bolle et al., 1993, 1996). Ac-AMP2 peptide belongs to a group of chitin-binding proteins (Broekaert et al. 1992), which are able to bind to chitin in fungal cell wall, causing a consequent change in its polarity and resulting in the inhibition of fungal growth (Selitrennikoff, 2001). The unique properties of Ac-AMP2 suggest it may play a role in seed defense against invasion by fungal organisms (Broekaert et al., 1992; Cammue et al., 1994), and thus transgenic potatoes have been produced to obtain a similar attribute.

Supported by the Ministry of Agriculture of the Czech Republic (Grant No. MZE 0002716201) and by the Ministry of Education, Youth and Sports of the Czech Republic (Grants No. 331/2006 and MSM 6010980701).

According to Regulation (EC) No 1829/2003 on Genetically Modified Food and Feed, a Community Register of GM Food and Feed containing GM products, which may be legally used in the market of the European Union, was made. At present, twenty one modifications of GM maize, rape, soy, cotton, bacterial and yeast biomass are listed on this register (Anonymous, 2006a). Concerning pending authorisations, a list of Genetically Modified Food and Feed Applications (Under Regulation (EC) No 1829/2003) also exists. Take potatoes into consideration only GM amylopectin potato event EH-92-527-1 occurs on the list nowadays (Anonymous, 2007).

Despite the prohibition of the above-mentioned modification of potatoes in the European Union, it is convenient to have possibilities for its revelation in the case of its illegal import. The method for GM "*ac2*" detection was published previously in our laboratory (Pribylova et al., 2006). At present, 167 cultivars of *Solanum tuberosum* are registered in the Czech Republic (Anonymous, 2006b). The aim of the present study was to test 105 cultivars of *S. tuberosum* from the market and from the Potato Research Institute in the Czech Republic.

MATERIAL AND METHODS

Biological material – potato cultivars

(1) *Solanum tuberosum* cultivars (Table 1) originated from two institutions: The potatoes numbered

Table 1. The list of tested cultivars of *Solanum tuberosum*

No.	Cultivar	No.	Cultivar	No.	Cultivar	No.	Cultivar
1	Accent	27	Felsina	53	Lady Christl	79	Raja
2	Adela	28	Filea	54	Lady Rosetta	80	Red Scarlett
3	Adora	29	Folva	55	Laura	81	Remarka
4	Agria	30	Fresco	56	Lenka	82	Rosara
5	Albina	31	Granola	57	Liseta	83	Rosella
6	Amylex	32	Impala	58	Livera	84	Samantana
7	Anosta	33	Innovator	59	Magda	85	Sante
8	Apolena	34	Javor	60	Marabel	86	Satina
9	Arnika	35	Karin	61	Marena	87	Saturna
10	Asterix	36	Karlena	62	Markies	88	Secura
11	Berber	37	Karmela	63	Merkur	89	Sibu
12	Bettina	38	Katka	64	Milva	90	Signal
13	Bionta	39	Kerkovske rohlicky	65	Minerva	91	Solara
14	Bolesta	40	Klera	66	Miriam	92	Symfonia
15	Borka	41	Kobra	67	Molli	93	Tabor
16	Boubin	42	Kordoba	68	Monalisa	94	Tara
17	Camilla	43	Korela	69	Mondial	95	Tegal
18	Cicero	44	Kornelie	70	Morene	96	Tomensa
19	Cinja	45	Korneta	71	Nicola	97	Ukama
20	Colette	46	Koruna	72	Ornella	98	Vaneda
21	Dali	47	Krasa	73	Pacov	99	Vera
22	Desirée	48	Kreta	74	Panda	100	Veronika
23	Delikat	49	Krista	75	Producent	101	Aneta
24	Disco	50	Krumlov	76	Provento	102	Kariera
25	Donald	51	Krystala	77	Provita	103	Ditta
26	Fambo	52	Kuras	78	Quarta	104	Red Anna
						105	Nela

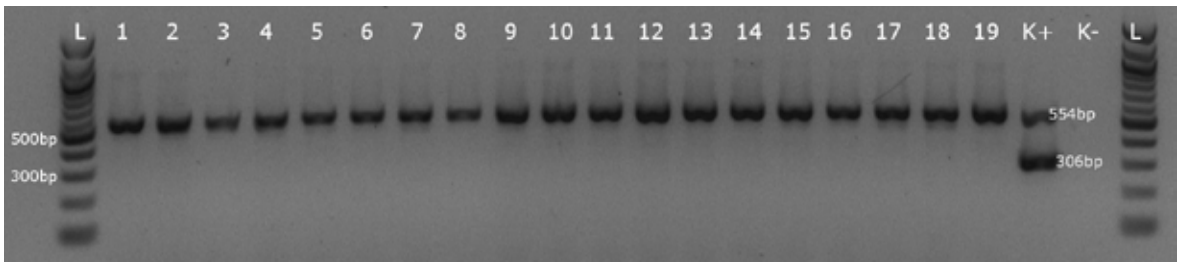

Figure 1. Detection of the "*ac2*" genetic modification in *Solanum tuberosum* – cultivars No. 1 to 19

From the left: 100 bp ladder (lane L), tested cultivars of *Solanum tuberosum* (lane 1–19), positive PCR control (lane K+), negative PCR control (lane K–) and 100 bp ladder (lane L)

from 1 to 100 originated from Potato Research Institute Ltd., Havlickuv Brod, Czech Republic. DNA was isolated from potato plants cultivated in *in vitro* conditions using the GenElute™ Plant Genomic DNA Kit (Sigma, USA).

(2) The potatoes numbered from 101 to 105 were obtained from Ing. Roman Rozsypal (Research Institute of Crop Production, Prague, Czech Republic), as well as potato no. 13, 19, 28, 32, 47, 53, 60 and 83. DNA was isolated from sprouted tubers using the DNeasy Plant Kit (QIAGEN, Germany) as described previously (Pribylova et al., 2006).

Polymerase chain reaction

The method for the detection of genetically modified potatoes carrying the *ac2* gene for the fungicidal peptide from *A. caudatus* was previously described by Pribylova et al. (2006). Briefly: the *ac2* gene from amaranth and simultaneously the endogenous gene from potato (*StTS1* gene) were amplified in one reaction test tube. All PCR detections were performed in microtubes with a volume of 20 µl. The PCR reaction mixture contained one unit of HOTStar *Taq* DNA polymerase (QIAGEN, Germany), 10 pmol of each primer and 2 µl of isolated DNA. A mixture containing the plasmid with the cloned *ac2* gene and the plasmid with the cloned *StTS1* gene was used as a positive control.

Each of the 105 potato cultivars was examined twice by the PCR method and amplification products were visualised on 1.5% agarose gel.

RESULTS AND DISCUSSION

The amplification product of the *ac2* gene with 306 bp in length was not detected in any of the tested cultivars of *S. tuberosum* (Figure 1). The only band detected in each cultivar was a length of 554 bp (Figure 1), which corresponded to the amplification product of the endogenous *Solanum tuberosum* putative trehalose synthase (*StTS1*) gene.

The results showed that GM "*ac2*" was not detected in any of the tested potato cultivars. Due to the fact that the investigated modification is not allowed either in the European Union or in the Czech Republic, the obtained results confirmed that the currently valid legislative has been respected.

Acknowledgements

The authors want to thank Ing. Roman Rozsypal, CSc. (Research Institute of Crop Production, Prague, Czech Republic) for potatoes species mentioned in this work and Catherine Murdoch (Aberdeen University, UK) for the critical grammatical reading of the paper.

REFERENCES

Anonymous (2006a): European Commission, Directorate-General Health and Consumer Protection, Food and Feed Safety. Regulation (EC) No 1829/2006 on Genetically Modified Food and Feed: Genetically Modified Food and Feed Authorised (last update 30-07-2007). http://ec.europa.eu/food/dyna/gm_register/index_en.cfm

Anonymous (2006b): Central Institute for Supervising and Testing in Agriculture: National list of varieties (listed in the State Variety Book by July 1st, 2006) – Potatoes and root crops (in Czech). http://www.ukzuz.cz/index_ooz.php?id=registrace&id0=seznam

Anonymous (2007): European Food Safety Authority. Applications under Regulation (EC) 1829/2003 on

Genetically Modified Food and Feed: GM Food and Feed Applications (last updated: March 2007). http://www.efsa.europa.eu/en/science/gmo/gm_ff_applications.html

Broekaert W.F., Marien W., Terras F.R., De Bolle M.F.C., Proost P., Van Damme J., Dillen L., Claeys M., Rees S.B., Vanderleyden J. (1992): Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine glycine-rich domain of chitin-binding proteins. Biochemistry, 31, 4308–4314.

Cammue B.P.A., De Bolle M.F.C., Schoofs H.M.E., Terras F.R.G., Thevissen K., Osborn R.W., Rees S.B., Broekaert W.F. (1994): Gene-encoded antimicrobial peptides from plants. In: Boman H., Marsh J., Goode J.A. (eds.): Antimicrobial Peptides. (Ciba Foundation Symposium No. 186). John Wiley & Sons Canada Ltd. 91–106.

Clive J. (2006): International Service for the Acquisition of Agri-biotech Applications (ISAAA). ISAAA Brief 35-2006: Global Status of Commercialized Biotech/GM Crops. http://www.isaaa.org/resources/publications/briefs/35/executivesummary/default.html

De Bolle M.F.C., David K.M.M., Rees S.B., Vanderleyden J., Cammue B.P.A., Broekaert W.F. (1993): Cloning and characterization of a cDNA encoding an antimicrobial chitin-binding protein from amaranth, *Amaranthus caudatus***.** Plant Molecular Biology, 22, 1187–1190.

De Bolle M.F.C., Osborn R.W., Goderis I.J., Noe L., Acland D., Hart C.A., Torrekens S., Van Leuven F., Broekaert W.F. (1996): Antimicrobial peptides from *Mirabilis jalapa* and *Amaranthus caudatus***:** expression, processing, localization and biological activity in transgenic tobacco. Plant Molecular Biology, 31, 993–1008.

Liapkova N.S., Loskotova N.A., Maisurian A.N., Mazin V.V., Korableva N.P., Platonova T.A., Ladyzhenskaia E.P., Evsiunina A.S. (2001): Isolation of genetically modified potato plant containing the gene of defensive peptide from *Amaranthus* (in Russian). Applied Biochemistry and Microbiology, 37, 349–354.

Pribylova R., Pavlik I., Rozsypalova Z., Bartos M. (2006): A PCR based method for the detection of genetically modified potatoes by the gene *ac2* from *Amaranthus caudatus.* European Food Research and Technology, 223, 139–142.

Selitrennikoff C.P. (2001): Antifungal proteins. Applied and Environmental Microbiology, 67, 2883–2894.

Received: 2007–03–27
Accepted after corrections: 2007–10–10

Corresponding Author:

Mgr. Radka Pribylova, Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel. +420 533 331 611, fax 420 541 211 229, e-mail: pribylova@vri.cz