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Short Communication

Anomalous accumulation of selenium by genetically modified potato, stable to Colorado beetle

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ABSTRACT

Although ordinary and genetically modified potatoes stable to Colorado beetles (CPB), *Leptinotarsa decemlineata* Say, are known to possess very small differences in chemical composition, nothing is known about selenium (Se) accumulation by these plants. Using a fluorimetric method of analysis, we have demonstrated extremely high Se accumulation in leaves of CPB-resistant potatoes (more than 1 mg kg^{-1} dry weight) and moderate accumulation levels of Se in tubers (1.39 times more than in ordinary plants). Leaves of genetically modified potatoes are shown to possess a decreased concentration of ascorbic acid (1.5 times less than controls) and slightly elevated levels of nitrates. The possibility of Se participation in the protection of genetically modified potatoes against CPB is discussed.

1. Introduction

Potatoes are the fourth largest crop grown in the world, exceeded only by wheat, rice and corn. The major limiting factor in growing potatoes in many areas of the world, including Russia, is the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (CPB), which is resistant to most classes of chemical pesticide (Georghiou and Lagunes-Tejada, 1991; Metcalf and Metcalf, 1993). According to the United States Department of Agriculture, potato producers in the USA spend $20–40 million each year on CPB pest control (Glenda, 2008).

A prospective solution to this problem is to use a protein (Cry 3A protein) toxic to CPB, produced by a naturally occurring soil bacterium, *Bacillus thuringiensis* (Bt) (Shelton et al., 2002). The mechanism of Cry 3A protein on CPB does not differ from that of other Bt Cry proteins, known to possess high selectivity for different pests. The primary action of Cry toxins is to lyse midgut epithelial cells in the target insect by forming pores in the apical microvilli membrane of the cells leading to severe septicemia and insect death (de Maagd et al., 2001; Bravo et al., 2005).

The high selectivity of Bt Cry proteins makes them harmless to human beings, animals or beneficial insects that help to control other crop pests. Nevertheless, the use of Cry proteins in commercial spray applications has been limited as a result of their relatively high cost, rapid environmental inactivation and poor crop coverage (Benedict and Altmab, 2001).

To date, Bt Cry genes have been inserted into numerous plant species, such as maize, cotton, potato, tomato, rice, eggplant and canola, to produce their own Cry proteins (Shelton et al., 2002; De Maagd, 2004; Federici, 2002). The benefits of using such Bt-plants include increased crop yields, reduced pesticide use, less environmental damage and reduced labor. CPB-resistant potatoes were first developed and sold in the USA in 1994 under the NewLeaf trademark to control CPB (Perlak et al., 1993). Successful utilization of CPB-resistant potatoes was achieved in Mexico and South America. European countries were reluctant to embrace the technology, fearing unknown health and environmental consequences. Although there is no commercial production of Bt-potatoes in Russia, the Russian Center of Bioengineering has adapted the technology developed by Monsanto (St Louis, MO), one

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Table 1
Concentration of Se, vitamin C and nitrates in potato leaves.

Cultivar	Se, μg kg^{-1} dry weight		Vitamin C, mg kg^{-1} fresh weight		Nitrates, mg kg^{-1} dry weight	
	Control	CPB-resistant potatoes	Control	CPB-resistant potatoes	Control	CPB-resistant potatoes
Lugovskoy	221 ± 15	1150 ± 71	820 ± 50	570 ± 50	148 ± 12	173 ± 14
Nevsky	196 ± 14	3127 ± 108	1060 ± 80	500 ± 40	158 ± 14	188 ± 16
Elyzabeth	230 ± 18	1860 ± 50	920 ± 70	740 ± 50	145 ± 15	165 ± 17
M ± SD	216 ± 16	2046 ± 721	930 ± 98	603 ± 101	150 ± 5.6	176 ± 9.5
CV, %	6.7	58.8	10.6	16.7	3.7	5.4

Significance of differences between CPB-resistant plants and control ones $P < 0.001$.

of the world's biggest biotechnology companies, for three varieties of potatoes commonly grown in Russia.

A general property of Bt-plants is an elevated level of lignin. This peculiarity is poorly expressed in CPB-resistant potatoes, where not only lignin, but also starch, carbon and nitrogen concentrations are close to those of non-modified plants (Flores et al., 2005). The lack of information on the selenium (Se) content in Bt-crops has prompted our investigation of the Se status in CPB-resistant potatoes, as this element is implicated in the protection mechanism of plants against insect attack (Hanson et al., 2003; Poschenrieder et al., 2006).

Solanum tuberosum L. belongs to a large group of agricultural crops which accumulate very low concentrations of Se (less than 100 μg kg^{-1} dry weight). Not being an essential element in plants (Kabata-Pendias and Pendias, 2001), Se participates in antioxidant defense, deactivating free radicals and hydrogen peroxide, known to be produced as a result of biotic and abiotic stress, plant defoliation (Ślesak et al., 2008), UV-radiation, drought, etc. (Germ et al., 2005, 2007; Seppänen et al., 2003). As Se is a synergist of vitamin C, and also participates in nitrate metabolism, it was important to characterize all three components of potato leaves in CPB-resistant plants.

2. Materials and methods

2.1. Potato samples

Three cultivars of CPB-potatoes, Lugovskoy, Nevsky and Elizabeth (10 plants in each group), and the same cultivars of control plants ($n = 10$) were planted in the experimental field of Russian Bioengeneering Centre, Moscow region (lat 55°45′N, long 37°37′E) on Dernovo-Podzol heavy loamy soil with humus content of 2.5–3.2%, 175 mg kg^{-1} P, 10.18 mg kg^{-1} NO_3, 244 μg kg^{-1} Se, and 0.2 mg kg^{-1} Cd. Soil pH was equal to 6.5.

Leaves of individual plants were collected in July, dried at room temperature to constant weight, and homogenized. After harvesting at the beginning of September, the skin and inner tissue of three tubers from each plant were separated, cut into thin slices, dried at room temperature to constant weight, and homogenized.

2.2. Determination of Se, vitamin C and nitrate

Acid digestion fluorimetry was used for determining the Se concentration in leaves, skin and inner part of tubers (Alfthan, 1984). The detection limit was 0.45 ng Se. Calibration range was 0.02–1.5 μg. Accuracy and precision of the method were verified by analyzing a certified sample of lyophilized cabbage (Institute of Nutrition, Moscow), which gave a value of 150 ± 5 μg kg^{-1} (11 series, 14 observations), the certified value being 150 ± 3 μg kg^{-1}. Vitamin C content in fresh potato leaves was determined using a visual titration method with sodium 2,6-dichlorophenol indophenolate (AOAC, 1980). Nitrate concentrations were measured in water extracts of dried leaves using nitrate-tester Morion 0K-2 (Russia).

2.3. Statistical analysis

Statistical analysis was performed by Student's *t*-test.

3. Results

The biochemical characteristics of the potato leaves are presented in Table 1. Compared to control plants, the leaves of CPB-resistant potatoes accumulate 9.5 times more Se, and 1.17 times more nitrates. On the contrary, the amount of vitamin C is 1.5 times lower than in the control. Differences between cultivars are greater in genetically modified plants. Characteristic concentration ranges in leaves of CPB-resistant plants are: 1150–3127 μg Se kg^{-1}, 500–740 mg ascorbic acid kg^{-1} and 165–188 mg nitrates kg^{-1}.

Se accumulation by tubers of control and CPB-resistant potatoes is shown in Fig. 1. Bt-modification results in a 1.39 times increase of Se concentration in the inner part of tubers, with only a 1.07 times increase in the skin. In contrast to control plants, all three CPB-resistant potato varieties demonstrate lower Se concentration in the skin than in the inner tissue of tubers.

4. Discussion

The huge increase of Se concentration in potato plant leaves as a result of Bt-modification corresponds to the increase in the biological accumulation coefficient from 0.89 to 8.4. It is significant that with such a strong accumulation of Se, transport of the element from leaves to tubers is relatively low, so that the Se leaf:tuber ratio in control plants is equal to 2.16, and in CPB-resistant potatoes, 14.7. This latter value is 5 times lower than leaf:tuber Cry3A protein ratio. Indeed, according to Mendelsohn et al. (2003), the main quantity of

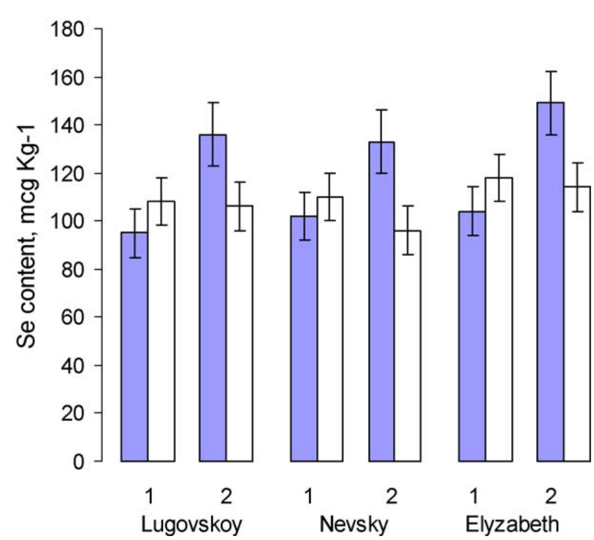

Fig. 1. Se accumulation by potato tubers: (1) control and (2) CPB-resistant potatoes. Dark columns, inner tissue; white columns, tuber skin.