STRESS MARKERS IN CHLORSULPHURON TOLERANT TRANSGENIC TOBACCO PLANTS

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Summary. Transgenic tobacco plants, carrying *als*3R gene, and expressing tolerance to chlorsulfuron were treated with Glean©. The *als*3R gene was introduced by *Agrobacterium*-mediated transformation in the cultivar Nevrokop 1146. Tolerant plants avoid the effect of the herbicide, expressing a changed acetolactatsynthase, encoded by the introduced *als*3R gene. The following stress markers: peroxidase activity, quantity of proline, hydrogen peroxide and products of lipid peroxidation were studied in transgenic and control (sensitive) plants. Sensitive tobacco plants expressed physiological changes typical for the oxidative stress reaction. The markers studied showed lower values in herbicide-tolerant transgenic plants.

Key words: chlorsulphuron (Glean©), herbicide, tobacco, transgenic plants, stress markers

Abbreviations: ALS – acetolactatsynthase, MDA – malondialdehyde, FW – fresh weight, cv – cultivar.

INTRODUCTION

A large number of herbicides are still used in agriculture, despite of the numerous attempts for restricting their application. In this respect, breeding of herbicide-tolerant and resistant crops is a necessity in agriculture. Such lines and cultivars can be

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obtained by selection of induced mutants or introduction of foreign genes for resistance to certain herbicides into plants.

Chlorsulfuron (Glean©) is a sulfonylurea herbicide characterized by very low application rates, excellent crop selectivity and low mammalian toxicity (Beyer et al., 1988). Selectivity is based on the ability of plants to metabolize the herbicide molecules to non-phytotoxic products (Sweetser et al., 1982). Sulfonylureas inhibit the activity of acetolactatsynthase (ALS), which catalyzes the first step in the biosynthesis of the aromatic amino acids valine, leucine and isoleucine (Chaleff and Mauvais, 1984). The introduced *als*3R gene into tobacco plants encodes a modified ALS that is not affected by the herbicide, thus making the plant tolerant.

Treatment of plants with herbicides induces physiological changes and leads to stress reactions. These changes are not always accompanied by visible damages. The physiological responses to different stress factors including herbicides are similar. The changes in pigment content (Yordanov, 1992), proline quantity (Reddy and Veerajnegulu, 1991), and the products of lipid peroxidation (Kramer et al., 1991; Sergiev et al., 2000) are considered as possible stress-induced markers.

The aim of this study was to investigate the effect of chlorsulfuron on proline quantity, guaiacol-peroxidase activity, hydrogen peroxide content and the products of lipid peroxidation in tolerant and sensitive tobacco plants.

MATERIALS AND METHODS

Plant material

The transgenic tobacco line tolerant to chlorsulfuron (Glean©) was engineered in the AgroBioInstitute, Sofia by introducing the *als*3R gene in the cultivar Nevrokop 1146 via *Agrobacterium*-mediated transformation (Valkov et al., 1998).

Sensitive (control) plants from tobacco cv. Nevrokop 1146 and a tolerant line, were used throughout the experiments. Plants were grown in pots in a growth chamber (light was supplied by cool-white fluorescence tubes at 60 μmol m^{-2} s^{-1} PFD and temperature 20-25°C) until they formed 6^{th} – 8^{th} leaf. Half of the tolerant and sensitive plants were treated with 0.33 mg Glean© per plant. Samples were taken from 3^{rd} and 4^{th} leaves on 1^{st}, 3^{rd}, 10^{th} and 15^{th} day after herbicide treatment.

Proline was determined following the method of Bates et al. (1973).

Activity of guaiacol peroxidase (EC 1.11.1.7) was measured spectrophotometrically by monitoring the formation of tetraguaiacol, according to the method of Hart et al. (1971).

Protein was estimated according to the method of Bradford (1976).

Malondialdehyde was determined according to Dhindsa et al. (1981), including TCA (trichloroacetic acid)/TBA (thiobarbituric acid) addition and a heat/cool cycle and calculated using its extinction coefficient 155 $mM^{-1}.cm^{-1}$.

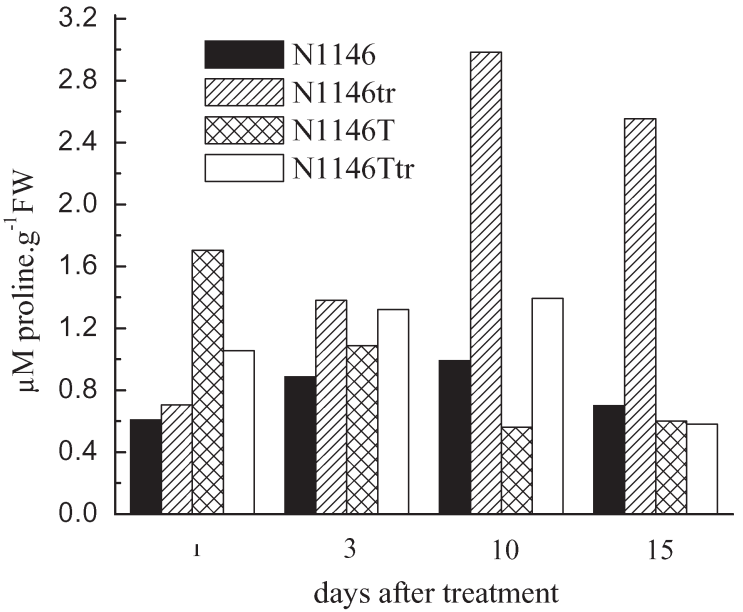
The endogenous content of hydrogen peroxide was determined specrophotometrically and calculated using a standart curve (Heath and Packer, 1968).
Statistical analyses. Results were evaluated statistically using LSD (P≤0.05).

RESULTS

Proline

Proline levels in the sensitive plants were increased already one day after the herbicide treatment. On the 15th day after treatment proline content reached 201.1 % compared to the control (sensitive) untreated plants (Fig. 1). An increase in proline content in the treated tolerant plants was observed. At the end of the experiment all tolerant plants (treated and untreated) had lower levels of proline (82-84%) as compared to the sensitive plants.

Fig. 1. Changes in proline content in tolerant and sensitive tobacco plants after chlorsulfuron treatment. LSD (P≤0.05=0.497).

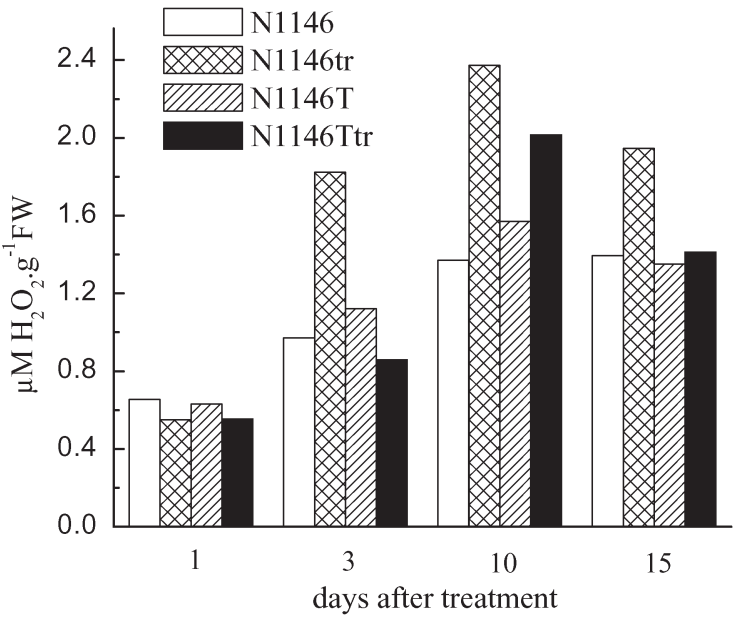


N1146 – untreated sensitive plants of cv. Nevrokop 1146
N1146tr - sensitive plants of cv. Nevrokop 1146 treated with chlorsulfuron
N1146T – untreated tolerant tobacco plants of cv. Nevrokop 1146
N1146Ttr – tolerant tobacco plants of cv. Nevrokop 1146 treated with chlorsulfuron

Peroxidase activity

Peroxidase activity increased in both treated tolerant and sensitive plants (Fig. 2). Peroxidase activity measured three days after treatment was increased by 87.6% as compared to the untreated sensitive plants. Enhanced peroxidase activity was observed in the tolerant plants 10 days after treatment (47.5 %), but it was lower compared to the activity in the treated sensitive plants (72.99 %).

Fig. 2. The effect of chlorsulfuron on peroxidase activity in tolerant and sensitive tobacco plants. LSD (P≤0.05=0.422).

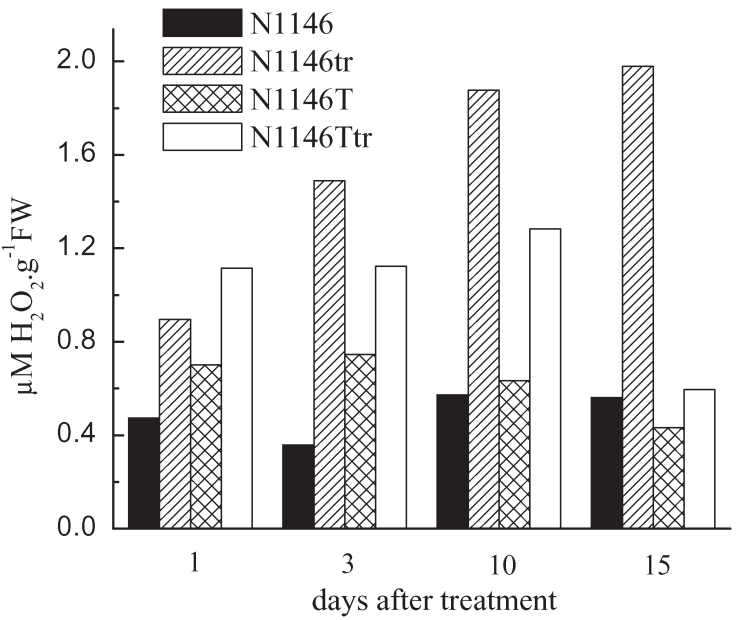


N1146 – untreated sensitive plants of cv. Nevrokop 1146
N1146tr - sensitive plants of cv. Nevrokop 1146 treated with chlorsulfuron
N1146T – untreated tolerant tobacco plants of cv. Nevrokop 1146
N1146Ttr – tolerant tobacco plants of cv. Nevrokop 1146 treated with chlorsulfuron

Hydrogen peroxide

Higher content of hydrogen peroxide was found in both tolerant and sensitive treated plants. The increase was above 200 % compared to the untreated transgenic and control plants (Fig. 3). The content of hydrogen peroxide decreased in the treated tolerant plants reaching the normal level by day 15 after treatment. In the sensitive treated plants it remained high till the end of the experiment.

Fig. 3. The effect of chlorsulfuron on hydrogen peroxide content. LSD (P≤0.05=0.32).



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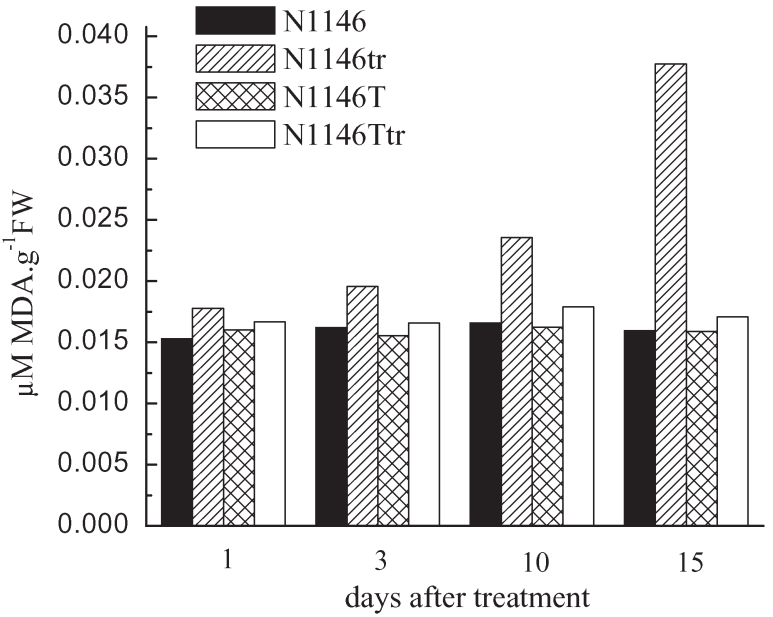
Malondialdehyde

The products of lipid peroxidation (malondialdehyde content) in the treated sensitive plants increased by 137 % between the 1st and the 15th day after treatment (Fig. 4). The content of malondialdehide remained almost unchanged during the whole experimental period in both treated and untreated tolerant plants.

Fig. 4. Levels of products of lipid peroxidation (MDA) in tolerant and sensitive tobacco plants after chlorsulfuron treatment. LSD (P≤0.05=0.004).



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Discussion

In order to establish whether oxidative stress is involved in the response of the sensitive and tolerant tobacco plants to treatment with chlorsulfuron, we measured the production of proline, guaiacol peroxidase activity, contents of hydrogen peroxide and MDA.

We found that the level of free proline was considerably increased after herbicide treatment in sensitive tobacco plants compared to the untreated control plants, which could be due to enhanced breakdown of proteins. Lower levels of proline was estimated in both treated and untreated transgenic plants. Accumulation of proline in response to various stresses has been reported in a number of plant species under water and salt stress conditions (Hsiao, 1973; Waldren and Teare, 1974); in cold-treated plants (Tantau and Dorffling, 1991), as part of plant defense reaction against abiotic and biotic stresses (Reddy and Veerajnegulu, 1991). The high concentration of proline corresponds to its osmotic role but other functions, including radical detoxification and regulation of cellular redox status have also been suggested (Hare and Cress, 1997).

Plant cells posses highly efficient defence systems for elimination of the harmful effect of oxidative stress (Sergiev et al., 2000). Guaiacol peroxidase is one of the enzymes with antioxidative functions. According to Asada (1992) the physiological function of peroxidase is defensive and the enzyme plays a role in cell wall lignification and tannin production. Our results showed that the activity of guaiacol peroxidase was higher in the treated sensitive tobacco plants compared to both treated and untreated tolerant plants. The results on peroxidase activity correlated with the enhanced levels of hydrogen peroxide observed in the treated sensitive and tolerant plants. The elevated peroxidase activity, however, was not able to decrease hydrogen peroxide content in the treated sensitive plants. On the other hand, the elevated peroxidase activity in the transgenic plants lead to low levels of hydrogen peroxide which might be attributed to the expression of the introduced *als*3R gene encoding resistance to Glean©.

Plant cell death involves membrane destruction. Metabolites such as malondialdehyde serve as indicatiors for membrane status. MDA is a product of peroxidation of polyunsaturated fatty acids and is a widely used stress indicator of plant cell membrane damage (Lam et al., 1999). The amount of MDA in the sensitive plants increased gradually in response to herbicide treatment. In both treated and untreated tolerant plants MDA remained unchanged, indicating the absence of damages in cell membrane as well as in the whole transgenic plants. This result might be due to the tolerance of the tobacco line studied to the Glean® herbicide.

In conclusion, the estimated low levels of proline, peroxidase activity, MDA content and normalized levels of hydrogen peroxide in the tolerant transgenic plants indicate that they are able to overcome successfully the negative effects of herbicide

treatment that could probably be related to the expression of the introduced *als*3R gene. Further investigations on chloroplast antioxidants will elucidate the biochemical aspects of the tolerance of transgenic tobacco plants to chlorsulfuron.

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