Study of the conditions of synthesizing scopolentin in tobacco plants and scopolentin capacity as a means of controlling *Orobanche spp.*

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Abstract

Best (1948) reports that the levels of scopoletin in the tobacco plants increase following TSWV infection. The analysis of experimental results carried out by Wender (1968) led him to the conclusion that the synthesis of scopoletin in the plants is indused by various inhibiting factors of different nature - chemical, physical, biological, climatical etc. Sargents & Skoog (1961) have isolated and identified scopoletin in primary tobacco roots and Whitney (1986) suggested that scopoletin inhibits the process of germination of broomrape seeds. Wender (1968) reaches the conclusion that some factors of different types which inhibit tobacco development cause the production and accrual of scopoletin in tobacco plants.

The aim of our work is to study the effect of different inhibiting factors on the level of scopoletin synthesis in tobacco roots and its potentialities for broomrape control.

The results obtained show that the levels of chlorogenic acid and scopoletin are highest in tobacco roots infected with TSWV, as compared with plants grew under other stress factors.

As a result of the study, it was found that, the roots of tobacco plants infected with *Tomato spotted wilt virus* show the greatest change in the content of chlorogenic acid and scopoletin in comparison with the control group, while for tobacco subject to other stress conditions, even of similar type – viral infection with *TMV*, the content of the polyphenolic complex in plant roots is similar to the one of plants in the control group.

Key words: tobacco; broomrape; polyphenols; chlorogenic acid; scopoletin

INTRODUCTION

Polyphenolic substances are important substances contained in many plants, including tobacco. They are easily oxidized i.e. they are antioxidants. This property makes them one of the indicators showing changes in plants.

The main representatives of polyphenols in tobacco are as follows: chlorogenic acid (3-0-caffeoylquinic acid), its isomers - neochlorogenic acid (5-0-caffeoylquinic acid) and 4-0-caffeoylquinic acid as well as the flavonoid rutin (Kallianos, 1976; Court, 1977; Snook & Chortyk 1982). Tobacco also contains the following in much smaller quantities: scopoletin, caffeic acid and the glycosides scopolin and kaempferol-3-rutinoside (Stotesbury, 1993).

According to Best (1948) scopoletin is formed and accrued in the tissues of tobacco plants infected with TSWV. Sargent & Skood (1961) isolate and identify scopoletin from tobacco roots.

Wender (1968) reaches the conclusion that some factors of different types which inhibit tobacco development cause the production and accrual of scopoletin in tobacco plants. Such are pesticides 2-4 D (Fumitsugu et al., 1982) and maleic hydrazide (Winkler et al., 1969).

Whitney (1986) supposes that scopoletin inhibits the germination of *Orobanche spp.* seeds. Bozukov (1999) reports that plants showing symptoms of infection with TSWV are only mildly attacked by *Orobanche spp.* or not attacked at all.

The purpose of this study is to find the effect of stress factors of different types – chemical, physical, biological, on the quantity of the polyphenolic complex (chlorogenic acid (ChA) and scopoletin in the roots of tobacco plants exposed to the effect of such factors and the effect on the attack by the severe flower root parasite *Orobanche spp.*

MATERIALS AND METHODS

The tests were performed in the experimental field of the Institute of Tobacco and Tobacco Products with large leaf tobacco Burley 21. The plants in the phase of active growth before the formation of generative organs (inflorescence) were subject to the following stress conditions:

1. Treatment with glyphosate (Raundap) - 100 ml/dka;
2. Treatment with maleic hydrazide (MX-30) - 2000 ml/dka;
3. Infection with TSWV (*Tomato spotted wilt virus*);
4. Infection with TMV (*Tobacco mosaic virus*);
5. Disturbed irrigation regime (drought for 20 days).

As a control group we used healthy non-treated plants grown in conditions optimal for their development. Treatment with glyphosate and maleic hydrazide was performed with backpack sprayer; the lower leaves of the plants were sprayed, not the top ones as the traditional practice is. We used plants from the experimental areas that were naturally infected with TSWV and TMV and the drought was performed by stopping irrigation. During the period of the experiment - the month of August in the area of the experimental field no precipitation was reported, which would affect the reported stress factor. The average daily temperature during the drought period (20 days) was 31.7 ^{0}C.

We found the number of Orobanche spp. on tobacco plants after which we plucked them; then we washed, cut and dried their roots in a drying cabinet and ground them - 10 days after treatment, in case of the typical symptoms of phytotoxicity due to glyphosate and maleic hydrazide, 20 days after stopping irrigation and selection of plants infected with the above viruses.

The following was used for extraction and isolation of polyphenols from the analyzed samples: the method of fractionation with solid phase extraction with cartridge C_{18} (Court, 1991). The resulting fraction containing the polyphenolic substances was filtered through a membrane filter of 0.45 µm and was delivered for separation in the method of high performance liquid chromatography (HPLC) (in Snook & Chortyk, 1982). The repeatability of the method was good with permanent standard deviation of 2 - 5 % for chlorogenic acid and about 10 % for scopoletin. The peaks of chlorogenic acid and scopoletin were identified against the peaks of pure substances (reference substances). We calculated the content of chlorogenic acid and scopoletin using the area of peaks of the relevant pure substances.

The analyses were performed with five repetitions. The average values were used for calculation of the content of chlorogenic acid and scopoletin.

RESULTS AND DISCUSSION

The analysis of the polyphenolic complex in roots of tobacco of the Burley type under different stress conditions shows (Fig.1 and Fig.2) that the basic components were chlorogenic acid (peak 1) and scopoletin (peak 2). In insignificant quantities, there are peaks of other polyphenol substances such as isomers of chlorogenic acid, kaempferol-3-rutinoside, etc.

The roots of tobacco plants infected with *Tomato spotted wilt virus show* (Fig. 1) greatest change in the content of chlorogenic acid and scopoletin in comparison with the control group (Fig. 2).

The data in Table 1 show that in plants infected with *TSWV* the content of chlorogenic acid is increased 2 times and the content of scopoletin 3 times. For tobacco subject to other stress factors, even of similar type – viral infection (*TMV*), the content of polyphenolic complex in plant roots is similar to the one of the control group and sometimes even lower. E. g. there is no significant difference in the content of chlorogenic acid (ChA) with plants treated with glyphosate and such grown under disordered irrigation regime against the content in the control group. The last factor (drought) shows the same values for scopoletin quantity.

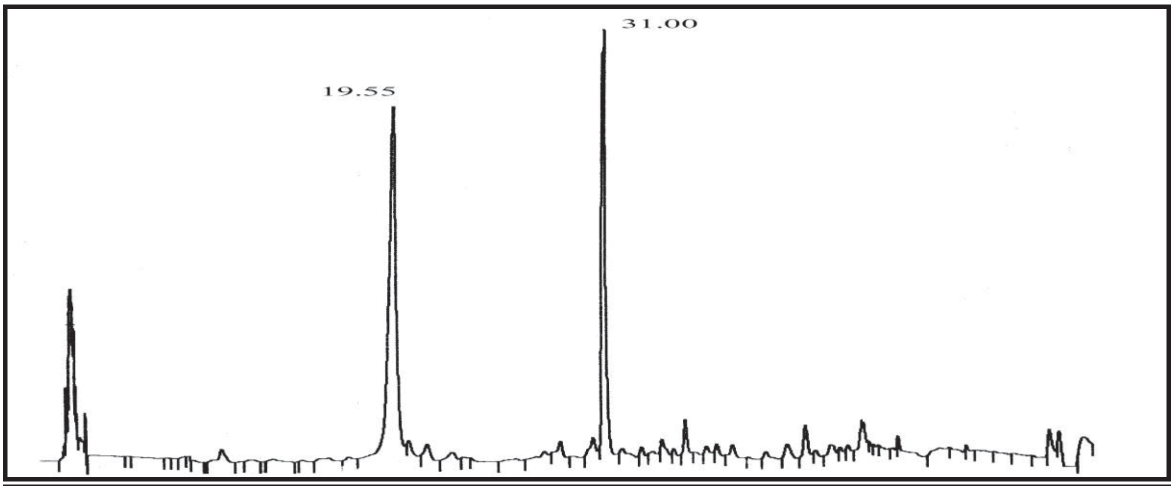

Figure 1. Tobacco plants infected with *Tomato spotted wilt virus*

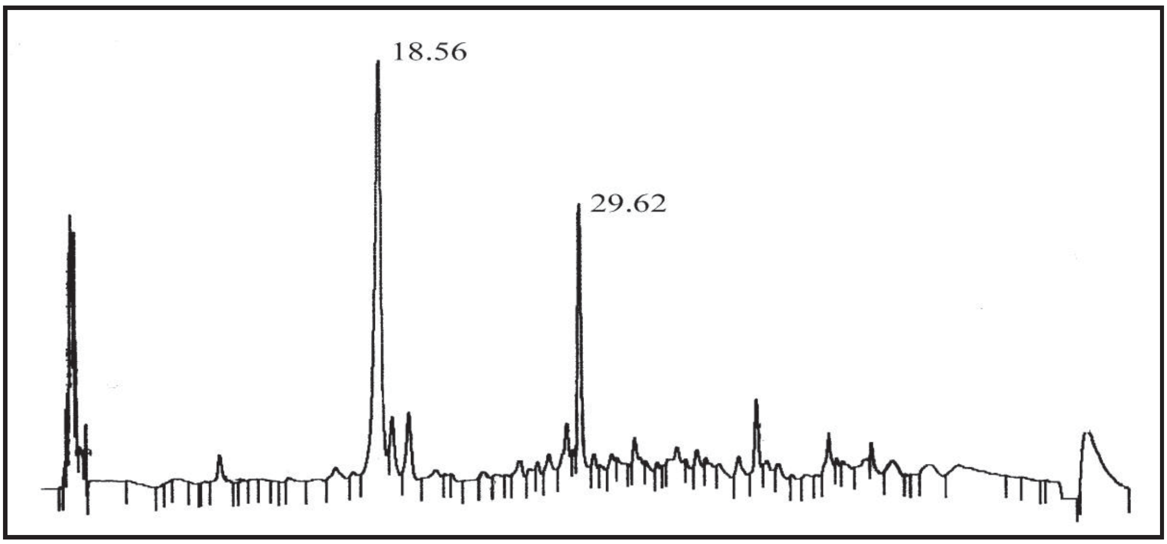

Figure 2. Tobacco plants in control group

Table 1. Polyphenolic complex from roots of Burley tobacco subject to stress conditions

№	Stress factor	Chlorogenic acid (%)	Scopoletin (%)
1.	Glyphosate	0.014	0.0030
2.	Maleic hydrazide	0.026	0.0048
3.	Tomato spotted wilt virus	0.046	0.0090
4.	Tobacco mosaic virus	0.016	0.0046
5.	Drought	0.009	0.0023
6.	Control	0.023	0.0030

The results from chemical analysis of roots of tobacco plants subject to different stress conditions are confirmed from data of *Orobanche spp.* attacking tobacco (Table 2).

The smallest number of parasitic plants is with tobacco infected with Tomato spotted wilt virus

With tobacco treated with glyphosate and maleic hydrazide their number is insignificantly lower in

Table 2. Attack of *Orobanche spp.* against tobacco subject to stress conditions

№	Stress factor	Number of broomrapes per plant
1.	Glyphosate	5
2.	Maleic hydrazide	6
3.	Tomato spotted wilt virus	2
4.	Tobacco mosaic virus	8
5.	Drought	8
6.	Control	8

comparison with the number for the control group while tobacco plants infected with Tobacco mosaic virus and the ones cultivated under disordered irrigation regime (drought) are infected to the same degree as the control group.

CONCLUSIONS

The roots of tobacco plants infected with Tomato spotted wilt virus show the greatest change in the content of chlorogenic acid and scopoletin in comparison with the control group.

For tobacco subject to other stress conditions, even of similar type – viral infection with TMV, the content of the polyphenolic complex in plant roots is similar to the one of plants in the control group.

The number of *Orobanche spp.* with tobacco infected with Tomato spotted wilt virus is the lowest.

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