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PHYTOSTEROLS AND POLYPHENOLS IN RECIPROCALLY GRAFTED TOBACCO–TOMATO PLANTS

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Key Word Index—*Nicotiana tabacum* L.; *Lycopersicon esculentum* Mill.; Solanaceae; 3-β-hydroxysterols; polyphenols; reciprocal grafting.

Abstract—Intact and reciprocally grafted tobacco and tomato plants were studied to examine their roles as scions and stocks in the formation, composition, and total amount of 3-β-hydroxysterol and polyphenols. Intact tobacco plants have a higher phytosterol content than do tomato plants. Tobacco leaves from the Tob/Tom grafted plants contained much less phytosterol than leaves from intact tobacco plants. The distribution of four major sterols, however, did not vary significantly. The concentration of total polyphenols in intact tobacco and tomato was about the same, but tobacco was high in chlorogenic acid, and tomato was high in rutin. Tobacco leaves from Tob/Tom grafted plants showed only a slight decrease in total polyphenol concentration compared with intact tobacco. Tomato roots appeared to contribute to the increased total polyphenol per plant in either intact tomato or in Tob/Tom grafted plants, despite the fact that no polyphenolic compounds were detected in the tomato root itself. Grafting may provide a limited technique for reducing the phytosterol concentration of tobacco leaves.

INTRODUCTION

RECIPROCAL grafting techniques involving tobacco (*Nicotiana tabacum* L) and tomato (*Lycopersicon esculentum* Mill.) plants have frequently been used for physiological and biochemical investigations.[1–3] Those investigations generally centered on tobacco alkaloids, particularly on the loci of alkaloid formation.

In the current study, grafting techniques are used to examine two other major groups of organic components of tobacco plants, namely, phytosterols and polyphenols. Recent studies reveal that tobacco quality and usability depend on many leaf characteristics, including phytosterols and polyphenols.[4] In a recent examination of more than 100 tobacco varieties differing in genetic background and cultural practices, a three-fold variation in phytosterols and a five-fold variation in polyphenols was observed.[5] Available information on physiology and biochemistry of these compounds suggests that it is feasible to regulate their levels in tobacco.[6]

The purpose of the present experiments is to examine the levels and composition of phytosterols and polyphenols in tobacco leaf from graft combinations of tobacco shoot onto

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tomato root, and in other plant parts from reciprocal grafts. Results of these studies may provide information on the specific roles of tobacco shoot and root in the formation of these compounds, and for determining whether grafting tobacco onto tomato may induce desirable changes in leaf tobacco.

RESULTS AND DISCUSSION

In tobacco shoots, the concentration of phytosterols was almost double that in tomato shoots. Similarly, the concentration of phytosterols in tobacco roots also was almost double that of tomato root (Table 1). In the tobacco shoot, most of the sterols were in the leaf, and only a small amount was in the stem.

Table 1. Total and individual phytosterols in intact and reciprocally grafted tobacco and tomato plants

Plant materials	Total phytosterols Conc. (mg/gm*)	Total amount (mg/plant)	% of total sterol fraction† Cholesterol	Campesterol	Stigmasterol	Sitosterol
Tom. shoot from intact plant	0·125	34·4	15·76	6·09	40·52	37·62
Tom. root from intact plant	0·060	4·1	9·91	9·12	55·62	25·35
Tom. shoot on tom./tob. graft	0·176	45·8	23·13	8·92	33·52	34·42
Tom. root from tob./tom. graft	0·090	4·0	8·92	6·77	58·81	25·49
Tob. shoots from intact plant	0·235	32·9	11·40	17·40	51·89	19·31
Tob. root from intact plant	0·137	8·2	8·96	27·27	47·60	16·17
Tob. shoot on tob./tom. graft	0·159	28·6	11·68	16·92	60·64	10·76
Tob. root from tom./tob. graft	0·036	1·8	6·28	23·91	45·04	24·77

* Fr. wt basis.
† Determined as acetate derivatives.

The composition of phytosterols in tobacco and tomato was very similar. Stigmasterol was the principal sterol, sitosterol was next, and campesterol and cholesterol made up the rest. In tomato, a brassicasterol-like compound was also found in small amount, but it was not measured because of lack of reference material. The same compound, however, was barely detectable in tobacco plants.

Tobacco shoots from Tob./Tom. grafted plants contained a lower concentration (mg/g fresh material) of phytosterols in comparison with that in intact tobacco plants. Similar decreases also occurred in tobacco root from Tom./Tob. grafted plants. In reciprocal grafts, the concentration of phytosterols increased in tomato shoots from Tom./Tob. grafts and in tomato root from Tob./Tom. grafts, in comparison with those in respective intact plants. The relative distribution of the four sterols were slightly different in intact and grafted plants.

TABLE 2. TOTAL AND INDIVIDUAL PHENOLIC COMPOUNDS IN INTACT AND RECIPROCALLY GRAFTED TOBACCO AND TOMATO PLANTS

Plant materials	Total phenolic compounds		Individual polyphenolic compound (mg/g)				
	Conc. (mg/g*)	Total amount (mg/plant)	Chlorogenic acid	Scopolin	Rutin	Scopoletin	Quinic acid
Tom. shoot from intact plant	1·398	384·5	0·380	—	0·456	—	0·562
Tom. root from intact plant	—†	—	—	—	—	—	—
Tom. shoot on tom./tob. graft	2·042	138·9	0·635	0·004	0·510	—	0·893
Tom. root from tob./tom. graft	—	—	—	—	—	—	—
Tob. shoot from intact plant	1·369	191·7	1·013	0·005	0·083	0·002	0·266
Tob. root from intact plant	0·443	26·6	0·233	0·009	—	0·001	0·200
Tob. shoot from tob./tom. graft	1·239	223·0	0·846	0·006	0·052	0·001	0·334
Tob. root from tom./tob. graft	0·241	11·8	0·166	0·008	—	0·001	0·066

* Fr. wt basis.
† Not detected.

Total polyphenol concentration was almost equal in tobacco and tomato shoots from intact plants, but the composition was quite different (Table 2). The chlorogenic acid content of tobacco was three times higher than that of tomato. On the other hand, the rutin and quinic acid contents in tomato shoots were five times and two times more, respectively, than those in tobacco. Because there was more plant material from tomato shoots than from tobacco, the total amount of polyphenol in tomato was double that in tobacco. No polyphenols were detected in tomato root, and scopoletin and scopolin were absent in both tomato shoots and roots.

Tobacco shoots from Tob./Tom. grafted plants contained lower concentrations of chlorogenic acid and rutin, but a higher level of quinic acid, than those in intact tobacco plants. The net result was a slight increase in total polyphenol content. In tomato shoots from Tom./Tob. grafted plants, there was a general increase in concentration of all polyphenolic compounds, but a decrease in total amount per plant in comparison with the intact plant. The total plant material produced from a grafted plant composed of a tobacco root with tomato shoots was much less than from intact ones.

These results indicated that tomato roots, although they contained no detectable amounts of polyphenols, played an important role in the formation and thus the total amount per plant of polyphenols, regardless of whether the shoots were tomato or tobacco.

CONCLUSIONS

Results from reciprocal grafts between tobacco and tomato plants have demonstrated that there were considerable differences in the distribution and total amount of phytosterols and polyphenols in shoots and root of both plants. One can postulate several possible reasons for such differences, including changes in the formation of precursors, and the

physiological effects of a foreign shoot or root, resulting in interferences with biosynthetic processes.

Total phytosterol concentration of tobacco shoot produced on grafted tomato root, as shown in this study, was reduced 30% from that of intact plants. However, the concentration of polyphenols in tobacco from the same grafts did not decrease significantly. These results indicate that such a grafting technique would only modify phytosterols. Such a limited change may also be brought about through genetic manipulation. It is known that phytosterol content in leaf tobacco is independent of alkaloid level, plant yield, or nitrogen fertilization.[7]

EXPERIMENTAL

A common variety of Maryland tobacco, *Nicotiana tabacum* L. cv. Catterton and of tomato, *Lycopersicon esculentum* Mill., cv. Rutgers, were used in these experiments. Plants of these varieties were used as intact individuals and also after tongued inarch grafting of each scion on the other stock. These samples were grown under greenhouse conditions, in 7 in. soil pots. At maturity (when leaves began yellowing) five plants of comparable size were selected from each treatment, and composite fresh samples were taken for chemical analysis. The treatments included (a) intact tobacco plants, (b) intact tomato plants, (c) tobacco scion grafted onto tomato stock (Tob./Tom.) and (d) tomato scion grafted onto tobacco stock (Tom./Tob.). Shoot and root parts for chemical analyses were separated at the time of harvest. Isolation and determination of total sterols were carried out as described previously.[8] Briefly, they followed the semi-micro gravimetric method of Stedman and Rusaniwskyj.[9] Individual sterols were determined by GLC. Sterols were released from a sterol–digitonin complex by heating with pyridine and then extracting with Et_2O. Free sterols were converted into acetate derivatives with pyridine and acetic anhydride (1:1). The steryl acetate mixtures were dissolved in tetrahydrofuran, then analyzed with a F and M 400 series gas chromatograph (flame ionization detector). The instrument was equipped with a 1·2 m × 6-mm glass column containing 3·8% UCW 98, on GC 80–100 S* (Applied Science Laboratory, Inc.). The column was maintained at 240°, the detector at 250° and flash heater at 300°. The carrier gas was He at 60 ml/min. Cholestane was used as an internal standard. Identification of steryl acetates was made by comparison of their retention times with those of authentic compounds. Determinations of polyphenols generally followed a paper chromatography procedure.[10] A total of four solvent systems and three spray reagents were used. Known amounts of authentic standards were used in each system for semi-quantitative evaluation of phenolic compounds in the samples. Tobacco tissue was extracted 3 × with hot 70% EtOH. The combined extracts were conc. under reduced pressure and chromatographed using tert-AmOH–H_2O (5:1) on Whatman* no. 1 paper, prebuffered at pH 6·75 with phosphate, or EtOAc–pyridine–H_2O (2:1:2).[11] The chromatograms were first examined under UV light, with and without ammonia vapor; they were then sprayed with Hoepfner reagent.[12] To confirm the identification of rutin and scopolin, H_2O was used as solvent.[13] For best separation of quinic acid, BuOH–HOAc–H_2O (4:1:2)[14] was used. The chromatograms were sprayed with Cartwright and Roberts' reagent.[15]

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