Evaluation of production conditions of tomato grafted with different tobacco rootstocks and determining nicotine content and quality of fruit

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Abstract: This study aimed to investigate the effects of grafting tomato on different tobacco rootstocks on quality factors and nicotine content. The commercial variety (Solanum lycopersicum ‘H2274’) (BIOTECH) of the tomato was used as the scion plant, and six different tobacco (Nicotiana tabacum L.) rootstocks were used: Taşova, Tekel, Muş, Samsun, Dişbudak, Hasankeyf cultivars. Cleft grafting method was used in all plants. Yield of non-grafted and grafted plants grown in open-field conditions was calculated, and there was a significant increase in yield in grafted tomatoes compared to non-grafted tomatoes. There was significantly increased lycopene and β-carotene levels (mg kg⁻¹), especially in ‘Tekel’, ‘Taşova’, ‘Samsun’, and ‘Hasankeyf’ tobacco grafts. There was a statistically significant difference between grafted and non-grafted plants according to 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical retention capacities and total phenol (TP) values. Evaluation of quality determinants including pH values, titratable acidity values (citric acid %), soluble solid content (SSC) (°Brix), fruit size ratios, showed that tomatoes grafted with ‘Muş’ tobacco rootstock were of higher quality. There was no significant difference between grafted and non-grafted plants according to nicotine analysis of the tobacco-grafted tomatoes, and due to acceptable ranges of nicotin level on tobacco grafted tomato plants were considered to be suitable for consumption. It could be concluded that grafting practices have significantly positive effects on tomato yield and quality.

Key words: grafting; nicotine; quality; tobacco; tomato; yield

Ovrednotenje pridelovalnih razmer paradižnika cepljenega na različne podlage tobaka in določitev vsebnosti nikotina in kakovosti plodov


Ključne besede: cepljenje; nikotin; kakovost; tobak; paradižnik; pridelek
1 INTRODUCTION

Antioxidants are compounds that protect cells from the damage of unstable molecules known as free radicals. Reactive free radicals, formed in metabolic reactions such as respiration and digestion, contain one or more unpaired electrons and have the potential to cause serious damage to the body (Diplock, 1998). Although the human body has its own antioxidant defense system to prevent damage, environmental factors decrease defensive resistance and render it inadequate in damage prevention. In order to limit this damage, herbal antioxidants that collaborate with the body’s various defense systems are considered effective alternatives. These exogenous, natural antioxidants include compounds such as: vitamins C and E, selenium, β-carotene, lycopene, lutein and other carotenoids, flavonoids, phenolic acid, and terpenes (Hennig & Toborek, 1993; Aruoma, 1994; Burr, 1994). In addition to natural antioxidants, nowadays, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary-butylhydroquinone (TBHQ), and propyl-gallate (PG) are used. However, studies have shown that these synthetic antioxidants have side effects (Kehrer & Digiovanni, 1990). Therefore, there is a growing interest in natural antioxidants in the fields of food chemistry and medicine (Madhavi et al., 1995).

This study was conducted to evaluate the antioxidant capacities, quality, nicotine content, and yield of tobacco-grafted tomato plants compared to non-grafted tomato plants, considering open-area production conditions, under the ecological conditions of the Ankara region.

2 MATERIAL AND METHODS

2.1 SOWING AND GRAFTING

For grafting trials, the H2274 (Biotech) commercial variant of the tomato plant (Lycopersicum esculentum Mill.) was used as the scion, whereas six different commercial tobacco (Nicotiana tabacum L.) variants were used for rootstocks: Tekel (TE), Muş (M), Taşova (T), Samsun (Nicotiana tabacum) (S), Hasankeyf (Nicotiana rustica L.) (H), and Dişbudak (D).

Seeds were germinated in greenhouse conditions at a humidity of 45-55 % and a temperature of 23-25 °C. Cleft grafting was made when tomato seedlings had 3-4 leaves, and tobacco seedlings had 6-7 leaves. The grafted seedlings were grown in a conditioning chamber for fusion in 16/8 day/night period at a humidity of 90-95 % and a temperature of 23-25 °C degrees for 10 day.

2.2 CULTIVATION OF GRAFTED PLANTS IN OPEN-FIELD CONDITIONS AND CALCULATION OF YIELD

Successfully grafted plants of various combinations as well as control group plants consisting of H2274 seedlings were transferred to a pre-cultivated two-acre field. Seedlings were watered with the drip irrigation system with 41 h⁻¹ irrigation capacity according to weather conditions, and appropriate maintenance was carried out. Fruit yield was expressed as the mass of harvested tomato fruit per plant.

2.3 DETERMINING PIGMENT CONTENT

Lycopene and carotene extractions were performed in accordance with the low volume hexane extraction method protocol (Fish et al., 2002). In this study, 0.05 %
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(w/v) butylated hydroxytoluene (BHT) in acetone, 95 % ethanol, and hexane were used.

Previously pureed non-grafted and grafted tomatoes in various combinations were weighed as 0.5 g, and afterwards, 0.05 % (M/V) (BHT) in acetone, and 95 % ethanol and hexane were added and vortexed. Distilled water was added to the samples which were shaken in the 180-rpm shaker. Supernatant of the samples brought to room temperature and spectrophotometer (HITACHI U-1800) readings were measured at 453 nm for β-carotene and 503 nm for lycopene. The results were presented as mg kg⁻¹.

2.4 DETERMINING TOTAL PHENOLIC CONTENT

The total content of phenolic compounds was determined using the Folin-Ciocalteu method (Slinkard & Singleton, 1977). The regularly harvested grafted and non-grafted tomatoes, were dried for 48 hours at 60 °C in oven and pulverized to a powder form. 10 % etanolwas added to 0.05 g of powder material which was incubated at 4 °C for overnight. After incubation, the samples were centrifuged at 5000 rpm for 5 minutes, and the supernatant was filtered through a 0.45 µm filter. Folin reagent, dH₂O and 10 % sodium carbonate were added to the samples and incubated for 30 minutes in 40 °C water bath. The samples were measured with spectrophotometer at 765 nm, and the results were expressed as standard gallic acid equivalents (GAE).

2.5 DPPH FREE RADICAL SCAVENGING ACTIVITY

Spectrophotometric evaluation of electron retention capacity and stable DPPH free radical scavenging activity of the tomato samples was performed in accordance with the protocol specified by Sharma and Bhat (2009). The powders obtained from previously prepared grafted and non-grafted tomato samples were mixed with 1 ml methanol and centrifuged at 5000 rpm. Samples prepared in different dilutions were mixed with 200 mM methanolic DPPH and left to incubate in dark for 30 minutes.

A 50 % inhibition concentration (IC50) was calculated using the concentration-dependent inhibition percentage (I % = (Ablank-Asample / A blank) × 100) curve, and these values were compared with the IC50 of standard antioxidants.

2.6 NICOTINE ANALYSIS

Pureed tomato samples were homogenized with distilled water in a glass homogenizer (Yasinok et al., 2009). After homogenization process nicotine extraction with toluene was performed. Diphenylamine was used as the internal control during the extraction. Nicotine analysis of the extracted samples was carried out with phosphorus detector gas chromatography using appropriate columns and standards at Anadolu University Plant, Drug and Scientific Research Center (EEskisehir, Turkey).

2.7 QUALITY ASSESSMENT

2.7.1 Fruit height and diameter

The diameters and lengths of the products of the grafted and non-grafted tomato plants were measured using fruit calipers and yielded average results.

2.7.2 pH and titratable acidity

pH was analyzed potentiometrically using the INOLAB brand WTW series pH meter. A homogenous mixture of freshly collected and pureed grafted and non-grafted tomatoes was prepared, and pH was measured. For the acidity analysis, the homogeneous samples were mixed with distilled water and titrated to pH 8.1 using 0.1 M NaOH. The acidity of tomatoes was calculated as citric acid according to the following formula:

acidity % (in citric acid) = S × 0.0064 × F × 100 / sample amount (ml or g)
S = Consumption, amount of 0.1 N NaOH spent (ml)
F = Factor of sodium hydroxide solution (F = 1 if the solution has a normality of 0.1) (Flores et al., 2010).

2.7.3 Soluble solids content (SSC)

Soluble solids content was determined using FUJI handheld refractometer. A sufficient amount of fluid from tomato samples were placed on the prism of the refractometer and the brix (amount of substance dissolved in 100 g) readings were taken.

2.8 STATISTICAL ANALYSIS

All data was expressed as mean ± standard error of the means (SEM), and derived from at least four replicates. IBM SPSS Statistics 25 package program was used for statistical evaluation. Descriptive statistics are expressed as mean and standard deviation for continuous data and
frequency and percentage for discrete data. In this regard, in the comparison of mean values of continuous variables between two groups, Independent Sample t-test was used for parametric tests and Mann Whitney U test for non-parametric tests. In the comparison of continuous variables when there were more than two groups, One-way ANOVA was used for parametric tests and Kruskal Wallis test for non-parametric tests. Results of the analysis of tested hypotheses was compared with a $p$ value of 0.05 in which values less than 0.05 were considered statistically significant.

3 RESULTS

3.1 FRUIT YIELD

A statistically significant difference was observed between grafted and non-grafted tomato groups according to total yield means. There was a significant increase in the yield of grafted tomatoes, but there was no significant difference between the grafted groups. According to the data on fruit yield, the lowest yield of fruit mass per plant was observed in the tomatoes grafted to 'Hasankeyf' tobacco rootstock, and the highest value was observed in tomatoes grafted to 'Samsun' tobacco rootstock (Figure 1).

Fruit yield of the harvested tomatoes in the years 2014, 2015, and 2016 was compared according to tobacco types and no significant difference was observed between the types.

3.2 PIGMENT CONTENT

According to lycopene and β-carotene carotenoids, which have strong antioxidant effects, there was no significant difference in 'Muş' and 'Dışbudak' grafted tomatoes compared to the nongrafted control group, while there was a significant difference in the 'Taşova', 'Tekel', 'Samsun', and 'Hasankeyf' grafted groups. When grafts were evaluated among themselves, there were significant differences between 'Tekel', 'Muş' and 'Dışbudak'; between 'Tekel' and 'Muş'; and between 'Muş', 'Samsun', and 'Hasankeyf' tobacco-grafted tomatoes (Figure 2). While there were partial differences when all grafts were evaluated in total, no single graft type showed statistical significance.

Tomatoes of the 'Hasankeyf' grafted plants had the highest lycopene content, whereas tomatoe of the 'Muş' grafted plants had the lowest amount. Similarly, 'Hasankeyf' grafted tomatoes also had the highest β-carotene content, whereas, 'Muş' grafted tomatoes had the lowest amount.

3.3 TOTAL PHENOLIC CONTENT (TPC)

Except for the 'Muş' and 'Taşova' graft types, tobacco-grafted tomatoes showed significant differences in total phenolic compounds compared to the control group. When grafts were compared among themselves, significant differences were found between 'Taşova' and 'Tekel', 'Dışbudak', and 'Hasankeyf'; between 'Tekel' and 'Muş', 'Samsun', 'Dışbudak', and 'Hasankeyf'; between 'Muş', and 'Dışbudak' and 'Hasankeyf'; and between 'Samsun' and 'Dışbudak' and 'Hasankeyf' (Figure 3). The highest total phenol content was found in tomatoes grafted on 'Muş' tobacco.

3.4 DPPH FREE RADICAL SCAVENGING ACTIVITY (FRSA)

Radical scavenging activity of tomato extracts were
determined with decreased absorbance of the reduction of DPPH radicals. The comparison between tobacco-grafted tomatoes and non-grafted tomatoes showed significantly increased activity in tomatoes grafted with ‘Dişbudak’ and ‘Hasankeyf’ tobacco. There was no significant difference in tomatoes grafted with ‘Taşova’ tobacco and non-grafted tomatoes (Figure 4). Tomatoes grafted with ‘Samsun’ tobacco were determined to have the best radical scavenging activity.

According to the total evaluation of methods to determine antioxidant properties, there was a significant increase in tomatoes grafted with ‘Samsun’ tobacco.

3.5 NICOTINE ANALYSIS

There was no significant difference between tomatoes grafted with various tobacco variants and non-grafted tomatoes according to nicotine content. Considering the harvest time, there was no significant difference in nicotine content between newly emerged tomatoes from seedlings and tomatoes collected at the end of the harvest. There was no significant difference in nicotine content among the tobacco types of grafted tomatoes (Figure 5).

3.6 QUALITY PARAMETERS

3.6.1 Physical quality parameters

There were statistically significant differences between the groups. ‘Taşova’, ‘Murş’ and ‘Samsun’ tobacco grafted plants showed significant difference compared to
According to the results, tomatoes grafted with ‘Dişbudak’ tobacco had the highest concentration of water-soluble dry matter. There were significant difference in ‘Tekel’ and ‘Muş’ grafts in terms of pH and titratable acidity, the important taste components and parameters that help prevent deterioration.

4 DISCUSSION

H2274 variant of tomato plants were successfully grafted with ‘Taşova,’ ‘Tekel,’ ‘Muş,’ ‘Samsun,’ ‘Dişbudak,’ and ‘Hasankeyf’ tobacco rootstocks by using cleft grafting method. Previous grafting different types of tomatoes with tobacco conducted in our institution resulted in good survival (Yasinok et al., 2009). Additionally, Khah et

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**Figure 4:** IC50 of free radical scavenging activity in fruits. Standart error of the means were derived from four biological replicates. The different letters emphasize the statistical difference (p < 0.05).

**Figure 5:** The distribution of nicotine content in H2274 tomatoes and tobacco grafted tomatoes. Standard error of the means was derived from three biological replicates. There was no statistical difference in the control group of the grafted tomatoes, within the grafted groups or the early and the late harvest.
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al. (2006) showed that grafting tomato plants with compatible rootstocks had positive effects on performance and that grafted plants in greenhouse were sturdier than non-grafted plants. Our study revealed a significant difference in yield between grafted and non-grafted plant groups. The results showed that tomatoes were grafted to compatible rootstocks. Grafting was also found to significantly increase fruit weight per plant.

The protective effects of fruits and vegetables against various diseases are believed to stem from the antioxidant compounds they contain including carotenoids, phenolic acids, and flavonoids (Abuajah et al., 2015; Kaur & Kapoor, 2001). Since the methods used to determine the amount of antioxidant activity are performed under different oxidation conditions and vary in substrate, probe, and reaction conditions to measure different oxidation products, more accurate results are obtained by implementing and comparing multiple methods (Frankel & Meyer, 2000).

It is known that antioxidant food products have the ability to prevent bitterness and taste deterioration due to oxidation. In addition to these characteristics, due to their role in preventing several diseases caused by stress or aging, antioxidants have begun to gain importance and have been studied in experimental, clinical and epidemiological research (Zavala et al., 2004). Therefore, it is crucial to measure the changes in antioxidant content of fruits and vegetables.

The natural composition of tomatoes includes antioxidant compounds such as tocopherol, ascorbic acid, lycopene and β-carotene flavonoids, and phenolic acids (Meyer et al., 2000; Maslarova, 2001; Heinonen, 2002). The antioxidative activity of tomatoes results from the synergistic effect of several phytochemicals (Heinonen, 2001; Maslarova, 2001; Heineken, 2002).

Several studies focused on the optimal conditions for maximum biosynthesis of lycopene and β-carotene and had varying results (Dumas et al., 2003). In the current study, lycopene values were between 84.5 mg kg⁻¹ and 112.6 mg kg⁻¹ and the highest values were obtained in tomatoes grafted with Hasankeyf tobacco. Similar to our results, Frusciante et al. (2007) reported that lycopene contents in fresh tomatoes vary between 18.6 and 146.2 mg kg⁻¹ according to data gathered from different resources. Based on the results of the current study, we conclude that 'Taşova', 'Tekel', 'Samsun', and 'Hasankeyf' tobacco grafted tomatoes can be preferred for lycopene and β-carotene pigments with antioxidant characteristics.

Increased DPPH free radical scavenging activity was observed in 'Tekel', 'Muş', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco-grafted tomatoes. The presence of phenolic compounds in 'Tekel', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco tomatoes is important in terms of their role in scavenging radicals. The fact that these tomatoes have a strong antioxidant and anti-radical activity shows that their use in healthcare could be beneficial. There were statistically significant differences in 'Tekel', 'Samsun', 'Dişbudak', and 'Hasankeyf' tobacco grafted tomatoes according to total phenolic content. These results are valuable in determining their role in radical scavenging activity.

In tobacco-tomato grafting, nicotine content was evaluated to examine whether or not yielded tomatoes are suitable or healthy for consumption. Yasinok et al. (2009) found increased nicotine content in grafted fruit. After grafting, a very low level of nicotine was detected in

### Table 1: Physical quality of grafted and non-grafted tomatoes

<table>
<thead>
<tr>
<th>Traits</th>
<th>H2274</th>
<th>'TAŞOVA'</th>
<th>'TEKEL'</th>
<th>'MUŞ'</th>
<th>'SAMSUN'</th>
<th>'DİŞBUDAK'</th>
<th>'HASANKE'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit diameter (cm)</td>
<td>6.18±0.18a</td>
<td>5.22±0.53b</td>
<td>5.8±0.22b</td>
<td>5.04±0.38b</td>
<td>7.3±0.24c</td>
<td>6.52±0.30d</td>
<td>5.08±0.37b</td>
</tr>
<tr>
<td>Fruit Height (cm)</td>
<td>5.44±0.27c</td>
<td>4.12±0.40b</td>
<td>5.62±0.15b</td>
<td>3.92±0.35c</td>
<td>6.22±0.19d</td>
<td>5.4±0.36c</td>
<td>5.66±0.54c</td>
</tr>
<tr>
<td>Fruit Mass (g)</td>
<td>125.44±13.78a</td>
<td>71.98±13.86b</td>
<td>138.24±8.56b</td>
<td>66.64±6.86b</td>
<td>175.79±15.17c</td>
<td>129.53±8.23a</td>
<td>91.74±14.81b</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the means (SEM). The different letters in the same row emphasize the statistical differences (p < 0.05).

### Table 2: Chemical quality element values of grafted and non-grafted tomatoes

<table>
<thead>
<tr>
<th>Traits</th>
<th>H2274</th>
<th>'TAŞOVA'</th>
<th>'TEKEL'</th>
<th>'MUŞ'</th>
<th>'SAMSUN'</th>
<th>'DİŞBUDAK'</th>
<th>'HASANKEYF'</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSC(°Bx)</td>
<td>4.57±0.22ac</td>
<td>4.47±0.17ac</td>
<td>4.67±0.14a</td>
<td>5.02±0.03b</td>
<td>5.27±0.33bc</td>
<td>5.35±0.24a</td>
<td>5.00±0.11bc</td>
</tr>
<tr>
<td>pH</td>
<td>4.56±0.03b</td>
<td>4.61±0.01bc</td>
<td>4.59±0.01b</td>
<td>4.61±0.01b</td>
<td>4.59±0.03bc</td>
<td>4.53±0.02bc</td>
<td>4.50±0.04bc</td>
</tr>
<tr>
<td>TA(citric acid) %</td>
<td>0.42±0.01cd</td>
<td>0.37±0.01bhc</td>
<td>0.39±0.01b</td>
<td>0.36±0.01b</td>
<td>0.42±0.01bhc</td>
<td>0.43±0.01cd</td>
<td>0.38±0.01d</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard error of the means (SEM). The different letters in the same row emphasize the statistical differences (p < 0.05).
tomato fruits. Dawson (1942) reported a high quantity of alkaloid accumulation in leaves of tomato plants grown on tobacco rootstocks. Andersson et al. (2003) reported that 30–40 % of orally consumed nicotine reaches the systemic circulation, a person could be exposed to almost 21.3 µg of nicotine in his/her diet and only 6.4–8.5 µg nicotine would enter the systemic circulation. Yasinok et al. (2009). In our study nicotin content of non grafted tomatoes obtained 1.10 µg/7 kg fm in early harvested plants and 0.67 µg/7 kg fm at late harvested tomatoes, also in nicotin content of grafted plants, there was no significant difference in nicotine content between non-grafted tomatoes and tobacco-grafted tomatoes. This shows that rootstock and scion selection is important, and indicates that tobacco-tomato graft is reliable and can be used.

According to the analysis of physical parameters, ‘Taşova’, ‘Muş’, and ‘Samsun’ tobaccos yielded larger fruit. This suggests that tomato plants with physically smaller fruit could be improved by being grafted with these tobacco variants.

Low titratable acidity is an indicator of better fruit quality and taste (Özenç et al., 2017). In this context, a significant decrease was observed in tomatoes grafted with ‘Tekel’ and ‘Muş’ tobaccos.

The content of soluble solids content is an important factor in identifying ripeness in fruits and may change depending on fruit type, ripeness phase, and storage conditions (Özbay & Ateş, 2015). High amount of dry-substance is suggested to be associated with longer-lasting fruit (Özenç et al., 2017).

It is reported that the soluble solids content in tomatoes vary between 2.9 % and 7 % (Bargefurd & Harker, 1998; Salk et al., 2008; Ünlü & Padem, 2009; Danneh et al., 2015). Similarly, our results showed a range between 4.48 and 5.35 °Bx. Additionally, the best results were observed in tomatoes grafted with ‘Muş’ and ‘Dişbudak’ tobaccos. This suggests that durability could be achieved by grafting ‘Muş’ and ‘Dişbudak’ tobacco to tomatoes that are not as durable. The analyses showed that ‘Muş’ tobacco grafting enhances physical and chemical quality of tomatoes.

As tomato mostly consists of water, short spoilage time, especially in ripe products, causes commercial problems. Antioxidant compounds have positive effects on deterioration and human health, and tomatoes are a rich source of these compounds. This demonstrates the importance of innovations improving antioxidant characteristics in tomatoes.

According to the results of our study, ‘Samsun’ tobacco was the most compatible and applicable graft for tomato plants in terms of size, yield, and antioxidant capacity. Tomatoes grafted with tobacco can be used as a rich antioxidant source compared to non-grafted tomatoes. Because of the low nicotine content, grafted tomato plants were considered to be safe and suitable for consumption.

The grafting method described in this study may guide vegetable growers, as it increases tomato yield, performance, and quality, also allowing them to make more profit.

5 ACKNOWLEDGEMENT

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6 REFERENCES


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