AN INVESTIGATION OF GRAFT HEALING WITH SPECTROPHOTOMETRIC METHOD
(ORAL PRESENTATION)

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Abstract

This study was carried out to investigate the graft healing two apple varieties (Amasya, Co-op 40) which were grafted onto Quince A rootstock used Winter Banana interstock. For this purpose total peroxidase activity and total phenolic compounds were determined by spectrophotometric assay in bark samples taken from graft partners. Peroxidase activity was found higher in Co-op 40/Winter Banana/Quince A combination (24.45 ΔA/g.dk; 13.25 ΔA/g.dk;16.54 ΔA/g.dk) graft partner than Amasya/Winter Banana/Quince A combination (9.56ΔA/g.dk; 14.63;15.84). On the contrary total phenolic compounds were lower. Total phenolic compounds higher in graft zones for both combinations Five years after planting, the seedlings Amasya/ Winter Banana/Quince A combination. were observed to have died. This suggested that low peroxidase activity and high total phenolic substance content might be related to graft incompatibility. The findings showed that peroxidase activity and total phenolic compounds could be used as a parameter in early determination of graft incompatibility.

Keywords: Total peroxidase activity, total phenolic compounds, graft incompatibility, apple

Introduction

Successful grafting is influenced by the plant genetics, growth characteristics and physiological and biochemical factors. Differentiation of the callus into vascular tissue (xylem and phloem vessels) is the result of a complex developmental process involving process involving structural and physiological changes, which aim to restore the transport system. During this process, lignin is synthesized in the cells to become part of the transport system (Bidabadi et al. 2017). Graft incompatibility in fruit trees is one of the greatest obstacles in rootstock breeding (Davarynejad et al., 2008). Although an increasing number of studies have tested for graft incompatibility in herbaceous and woody plants, there is limited information available as well as biochemical and the molecular mechanisms involved are not well understood (Pina and Errea, 2008). Analysis of isozymes and peroxidase activity can be used for prediction graft incompatibility. Early and accurate prediction of graft incompatibility is of great importance because incompatible combinations could be avoided while compatible ones could be selected (Gökbayrak et al., 2007; Petkou et al., 2004). Peroxidase activities and isozymes patterns have been reported to parallel hormonally-induced changes in tissue growth and differentiation (Feucht et al., 1983).
Phenolic compounds are a group of secondary metabolites found in higher plants, which play a number of important roles; they are found in the structure of plants and involved in a great number of metabolic pathways. Phenolic compounds are also found in the environment surrounding the plant and in plant–pathogen interactions. Phenolic compounds are important because of their role in lignification, which occurs during the graft fusion process, and their indirect effects on other plant growth regulators (Errea et al. 2001; Mng’omba et al. 2008). In addition, when damage occurs in a plant, the phenolic compounds serve as a chemical barrier or wall in the damaged cells to protect the plant from infection or rotting. When plant tissue is damaged, it produces a chemical response involving either the oxidation of the inner phenolic compounds or the production of mono- or polyphenolic compounds. Phenolic compounds have recently gained importance in the detection of graft incompatibility because of their role in lignification and a variety of biochemical reactions (Prabpree et al. 2018).

Peroxidases have been implicated in a wide range of cellular reactions such as phenolic compound oxidation, indole-3-acetic acid oxidation, lignification and polysaccharide cross-linking (Lee et al., 2001). Also they are known as stress enzymes toward pathogens, salt, metal ions. There are several external symptoms to detect graft incompatibility including graft union uniformity, lack of lignification, yellowing of foliage, decline in vegetative growth and vigor and anatomical abnormalities (Hartmann et al., 1997; Gülen et al., 2005). Appearance of these symptoms could take several years. An early and accurate detection of graft incompatibility is of great importance because it can avoid the use of incompatible graft combinations and help toward the selection of compatible combinations (Gökbayrak 2007). Stress situations can lead to the accumulation of phenolic compounds, which have been implicated in the different mechanisms regardrelated to the scion-stock relationship (Usenik and Stampar 2002).

In this study, combination of Amasya and Co-op 40 apple varieties with Quince A by using Winter Banana interstock was evaluated in terms of total peroxidase activity and total phenolic content and the mechanism of graft fusion was investigated.

**Material and Method**

Plant material of this study are one-year old trees of apple varieties of Amasya and Co-op 40 which were grafted Quince A with used Winter Banana interstock. Saplings were grafted at the beginning of September. Samples were taken before and 12 months after grafting. Barks were removed by using a razor blade 4 cm above and below the graft union and graft zone and these were frozen right away in liquid N2 and stored -80°C until used.

**Total peroxidase activity**

Enzyme extraction was conducted according to Gülen et al. (2002) and Güçlü and Koyuncu (2012). Ground tissues were homogenized by using buffer phosphate extraction solution (0.1 M potassium phosphate (pH 7.5); 30 mM boric acid; 50 mM L-ascorbic acid; 17 mM sodium metabisulfite; 16 mM dithiocarbamic acid; 1 mM EDTA and 4% (w/v) PVP-40, and final pH was adjusted to 7.5. Sixty milliliter of extraction solution was added to 0.6 g samples and homogenized at 10,000 rpm for 30 min at 4°C. Supernatant was used for electrophoresis. Peroxidase activity was determined by spectrophotometric method according to Eryılmaz (2007). The reaction mixture (consisted of 0.25% (v/v) guaiacol in 1 ml 0.1 M sodium phosphate buffer, pH 7.0, containing 0.1% hydrogen
peroxide 100 μl) was added to initiate the reaction and followed spectrophotometrically at 470 nm. The absorbance increase at 470 nm due to the guaiacol oxidation was recorded for 2 minutes.

**Total phenolic compounds**

Small sections of bark (4 cm above and 4 cm below the graft union, 1 × 2 cm.) were removed using a razor blade and immediately frozen in liquid nitrogen. The polyphenol analyses were performed according to the procedure of Usenik et al. (2006). The samples were extracted using acetone-water (80:20, v/v) containing Triton X-100 (0.4%) for 10 d at 4 °C according to the procedure of Treutter and Feucht (1988). In a mortar, 10 mg of dry plant material was homogenized with 2 mL of the extraction solution. After the extraction, the solvents were removed in vacuo at 40 °C and the residue dissolved in 2 mL of methanol. The samples were clarified using centrifugation at 6000 × g for 15 min and then filtered through a 0.45 μm membrane filter. Folin-Ciocalteu method was used to determine the total phenolic content, and readings were calculated as catechin equivalent in 690 nm.

**Results and Discussion**

Total peroxidase activity of graft partner’s were shown in Table 1. As seen as Table 1 Peroxidase activity was found higher in Co-op 40/Winter Banana/Quince A combination (24.45ΔA/g.dk; 13.25ΔA/g.dk; 16.54ΔA/g.dk) graft partner than Amasya/Winter Banana/Quince A combination (9.56ΔA/g.dk; 14.63; 15.84). Table 1. Total peroxidase activities of graft partners(ΔA/g.dk).

<table>
<thead>
<tr>
<th>Graft partners</th>
<th>Total peroxidase activity (ΔA/g.dk)</th>
</tr>
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<tbody>
<tr>
<td>Co-op 40/Winter Banana/Quince A</td>
<td></td>
</tr>
<tr>
<td>Co-op 40</td>
<td>24.45</td>
</tr>
<tr>
<td>Winter Banana</td>
<td>13.25</td>
</tr>
<tr>
<td>Quince A</td>
<td>16.24</td>
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<tr>
<td>Amasya/Winter Banana/Quince A</td>
<td></td>
</tr>
<tr>
<td>Amasya</td>
<td>9.56</td>
</tr>
<tr>
<td>Winter Banana</td>
<td>14.63</td>
</tr>
<tr>
<td>Quince A</td>
<td>15.84b</td>
</tr>
</tbody>
</table>

Despite the using of same rootstock and interstock in both combination because of different cultivars first combination had higher peroxidase activity. This showed us that different varieties grafting on the same rootstocks could produce different results. Because of peroxide roles in lignification, there has been increasing attention into graft incompatibility in recent years. Some research works were previously conducted on graft incompatibility in fruit trees in terms of peroxidase isoenzyme activity. The relationship between high peroxidase activity and graft compatibility was previously reported by many research works (Donaldson, 2001; Fernandez-Garcia et al., 2004; Feucht et al., 1983). There is an augmentation in both rootstocks and scion peroxidase activity after the grafting. Peroxidase is reported as a stress enzyme by previous researchers (Has-Schön et al., 2005; Lee et al., 2001; Rajeswari et al., 2008). When phenolic compounds content of the graft zone were evaluated in two different combinations, it was determined that the graft zone in Amasya / Winter Banana / Quince A combination had higher phenolic content in the bark samples. (Table 2).
Table 2. Total phenolic compounds of graft zone

<table>
<thead>
<tr>
<th>Graft partners</th>
<th>Total phenolic compounds (mg/g)</th>
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</thead>
<tbody>
<tr>
<td><strong>Graft zone bark</strong></td>
<td></td>
</tr>
<tr>
<td>Co-op 40/Winter Banana</td>
<td>27.70</td>
</tr>
<tr>
<td>Winter Banana/Quince A</td>
<td>22.54</td>
</tr>
<tr>
<td><strong>Amasya/Winter Banana/Quince A</strong></td>
<td></td>
</tr>
<tr>
<td>Amasya/Winter Banana</td>
<td>31.33</td>
</tr>
<tr>
<td>Winter Banana/Quince A</td>
<td>25.54</td>
</tr>
</tbody>
</table>

When damage occurs in a plant, the phenolic compounds serve as a chemical barrier or wall in the damaged cells to protect the plant from infection or rotting. When plant tissue is damaged, it produces a chemical response involving either the oxidation of the inner phenolic compounds or the production of mono- or polyphenolic compounds. Healthy cells adjacent to the damaged cells start the repair process and become active in the accumulation of key enzymes, simple phenolic compounds (chlorogenic acid) and polymeric compounds (lignin) (Errea 1998). Phenolic compounds have recently gained importance in the detection of graft incompatibility because of their role in lignification and a variety of biochemical reactions (Prabpree et al. 2018).

A combination of Amasya / Winter Banana / Quince A containing a high total amount of phenolic compounds with low peroxidase activity suggests that this combination may be a problem in the fusion of the graft fusion. As a result, graft incompatibility is a complicated anatomical and physiological process, and extensive studies to unravel its mechanism still continue. Methods developed for the early detection of possible incompatible combinations will prevent financial loss and delays. Moreover, a specified technique will allow early rootstock selection.

References


