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Cambial Isoperoxidases Related to Graft Compatibility in Pear-Quince Graft Combinations

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Abstract: This study was initiated to survey the graft compatibility characteristics of some selected quince clones known as "S.Ö." with regard to isoperoxidase markers. Compatible (Beurre Hardy = BH) and incompatible (Bartlett = BT) pear cultivars were grafted on quince A (QA) and 13 S.Ö. quince clones by T budding. Bark tissues were collected from the union (4, 8 and 12 weeks after grafting) and from unbudded rootstocks in addition to current-year shoots of 2 pear scions to investigate 2 anodal isoperoxidase bands. Isoperoxidase profiles of the samples were visualized by native polyacrylamide gel electrophoresis and no marked difference was detected among the isoperoxidase profiles of the samples collected 4, 8 and 12 weeks after budding. Many isozyme bands were observed in common in the 2 scions. However, one anodal peroxidase (A, Rf = 0.88) was detected in BH but not in BT samples. This isoperoxidase was also detected in QA and 5 of the S.Ö. quince clones. Isoperoxidase B (Rf = 0.68) was detected in BH but not in BT or any of the rootstocks. Considering the graft union samples, all the combinations with BH contained bands A and B whereas incompatible graft union tissues (BT/QA) lacked both. Graft union samples involving BT and 5 S.Ö. quince clones (35-160, 54-298, 40-214, 58-316 and 58-315) had both isoperoxidases. The data indicated that these 5 S.Ö. quince clones might form compatible graft unions with BT.

Key Words: Quince (*Cydonia oblanga*), grafting, incompatibility, peroxidase, pear (*Pyrus communis*).

Armut-Ayva Aşı Kombinasyonlarında Aşı Uyuşmasıyla İlgili Cambial İzoperoksidazlar

Özet: Bu çalışma, "S.Ö" olarak adlandırılan bazı seçilmiş ayva klonlarının aşı uyuşma özelliklerinin izoenzim markörleri dikkate alınarak araştırılması üzerine kurulmuştur. Bu çalışmada, uyuşur (Beurre Hardy = BH) ve uyuşmaz (Bartlett = BT) olarak bilinen armut çeşitleri Quince A (QA) ve 13 seçilmiş S.Ö. ayva klonu üzerine T aşı ile aşılanmıştır. İki anodal isoperoksidaz bandının araştırılması için aşılamadan 4, 8 ve 12 hafta sonra aşı noktasından, aşılanmamış anaçlardan ve iki armut çeşidinin yıllık sürgünlerinden kabuk örnekleri toplanmıştır. Örneklerin izoperoksidaz profilleri aktif poliakrilamid jel elektroforezi (PAGE) ile görüntülenmiş ve aşılamadan 4, 8 ve 12 hafta sonra aşı noktasından toplanan örneklerin izoperoksidaz profilleri arasında büyük farklılıklar görülmemiştir. İki armut çeşidinde bazı ortak bantlar görülmüşse de bir anodal peroksidaz, A (Rf = 0.88); BH çeşidinde belirlenmiş ancak BT çeşidinde görülmemiştir. Bu izoperoksidaz, QA ve S.Ö klonlarının beş tanesinde de belirlenmiştir. İzoperoksidaz B (Rf = 0.68), BH çeşidinde belirlenmesine rağmen BT ve hiçbir anaçta görülmemiştir. Aşı noktasından alınan örnekler göz önüne alındığında ise, BH ile oluşturulan tüm aşı kombinasyonları A ve B bantlarını bulundururken uyuşmaz aşı kombinasyonu (BT/QA) her iki banttan da yoksun olmuştur. BT ve beş S.Ö. ayva klonu (35-160, 54-298, 40-214, 58-316 ve 58-315) ile ilgili olan aşı noktası örnekleri her iki izoperoksidaza da sahip olmuştur. Sonuçlar, bu beş S.Ö. ayva klonunun BT ile uyuşur aşı bölgesi oluşturabileceğini göstermiştir.

Anahtar Sözcükler: Ayva (*Cydonia oblanga*), aşı, uyuşmazlık, peroksidaz, armut (*Pyrus communis*).

Introduction

Many of the problems in understanding 'graft incompatibility' have arisen from our failure to define the term precisely and to have that definition accepted by all investigators. Mosse (1962) has written that the only certain criterion of incompatibility is the characteristic

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interruption in cambial and vascular continuity that leads to the spectacular smooth breaks at the point of union, and further that at the point of union no normal vascular tissue develops. The gap thus formed is filled by proliferating ray tissue that does not lignify normally (Santamour, 1988a). Whether or not the above quotes are correct, the failure to reconstitute a structurally sound and physiologically functional continuity of vascular tissue (both xylem and phloem) has to be the cornerstone of the incompatibility in woody plants. The earliest methods used to detect graft incompatibility relied on external symptoms such as graft union malformations, yellowing of foliage, decline in vegetative growth and vigor, and marked differences in growth rate of scion and rootstock (Hartmann et al., 1997), or anatomical abnormalities after grafting. This requires waiting until the symptoms are visible, which may take years. Additionally, early anatomical observations may not always correlate with long-term graft survival (Andrews and Marguez, 1993).

Santamour (1982) demonstrated a significant relationship between cambial peroxidase isoenzyme banding patterns and sectional taxonomic classification in Acer (maple). The orginal intent of the maple study was to investigate potential variation among interspecific and intraspecific enzyme banding patterns that could be related to graft compatibility. The theory behind this approach was outlined by Santamour (1980): (1) lignification is essential for a strong and permanent graft union, (2) peroxidase isoenzymes mediate the polymerization of cinnamic alcohols to lignin and also the bonding of lignin to carbohydrates, and (3) the greater the similarity of isoperoxidase bands between stock and scion, the greater the chances of long-term graft compatibility.

Quince is a common rootstock for pear (*Pyrus communis* L.) because of size control, which makes high-density orchards possible. However, quince is graft-incompatible with some of the major pear cultivars such as Bartlett (Tukey, 1978; Hartmann et al., 1997). Thus, there is a need to develop new selections of quince that are compatible with major pear cultivars. Growth characteristics of 96 quince clones known as S.Ö., selected by Ankara University, were determined during 1974-1980 (Çelik, 1982). In addition, their graft compatibilities were investigated using biochemical analysis (cyanogenic glycoside content). Based on these studies some of these clones and types were selected as potentially compatible for the Bartlett pear cultivar. However, these potential clones or types need to be screened, considering the recent evidence that certain isoperoxidases are involved in graft compatibility between pear and quince (Gulen et al., 2002).

This research was initiated to survey the peroxidase isozyme profiles of some selected S.Ö. quince clones and to compare their peroxidase profiles to quince A (QA) rootstock and 2 pear cultivars, Bartlett (BT) and Beurre Hardy (BH), which are known to be incompatible and compatible graft partners with QA, respectively. Additionally, samples from the graft unions between various quince rootstocks and 2 pear scions were analyzed for peroxidase isozymes to identify isozymes associated with compatible graft combinations. The objective of this survey was to screen 13 selected quince clones, as mentioned above, for the presence or absence of the isozyme bands that may be associated with compatibility or incompatibility, and which were identified in our previous work (Gulen et al., 2002).

Materials and Methods

Plant Material

BH and BT, compatible and incompatible pear cultivars on QA (Tukey 1978) respectively, were grafted onto 1-year-old QA rootstock. These pear cultivars were also grafted onto 13 S.Ö. quince clones selected as potential compatible clones (Çelik, 1982, 1988) by T budding. Triplicate samples (3 different plants) of bark and cambial tissues were scraped from unbudded rootstocks (1-year-old) and scions (current-year growth of ~10-year-old trees of BT and BH) using a razor blade; care was taken to completely exclude xylem tissues. Samples were also collected from the graft unions of budded plants at 4, 8 and 12 weeks after grafting. Samples were right away frozen in liquid N_2 and stored at –80 °C until used.

Native Polyacrylamide Gel Electrophoresis of Acidic Isoperoxidases

Bark and cambial samples (collected as described above) were extracted according to the procedure described by Gulen et al. (2002). Ground tissues were homogenized in extraction buffer (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 readjusted to 7.5 with NaOH). Homogenates were centrifuged at 16 000 g for 30 min at 4 °C, and the supernatant was used for electrophoresis.

Discontinuous polyacrylamide gel electrophoresis (PAGE) was performed with a PROTEAN II electrophoresis unit (Bio-Rad) according to the system described by Davis (1964) for anodal isoperoxidases. Five percent stacking gel and 10% separating gel were prepared for both systems. An equal volume of the sample (20 µl) was loaded for each sample. Electrophoresis was performed at 20 mA until the samples entered the separating gel (about 30 min), and at 40 mA for 3 h, thereafter. Gels were stained for peroxidase using the method described by Wendel and Weeden (1989). Gels were then rinsed with distilled water, fixed and stored in 10% glycerol. The relative distance (Rf value) of the bands on the gel was calculated as described by Manganaris and Alston (1992) using Rf = 1.0, the distance to the fastest band (or the finish point of the running), and Rf = 0.0, the starting point of the running.

Results

All 3 extraction samples yielded identical profiles. The data from a single representative experiment are presented here.

Native PAGE profiles of isoperoxidases of nonbudded scion and rootstock revealed predominantly anodal isoperoxidases, which are presented in Figure 1. Analysis of profiles revealed an isoperoxidase band (band A; Rf = 0.88) that was present in BH (a compatible pear scion), absent from BT (an incompatible pear scion), and present in QA and 5 of the S.Ö. quince clones. Another isoperoxidase band (band B; Rf = 0.68) was observed in the BH scion, but not in BT or in any of the rootstocks. Consequently, band A was the common band in the BH scion and most of the rootstocks, band B was unique to BH, and BT lacked both bands A and B.

The isoperoxidase patterns of the graft union samples taken 4, 8 and 12 weeks after budding were identical, with some minor exceptions. The only difference among the sampling periods was the intensity of some bands, particularly bands A and B. Both bands were present with darker intensities in the samples taken 4 weeks after budding than in the other samples. Therefore, a

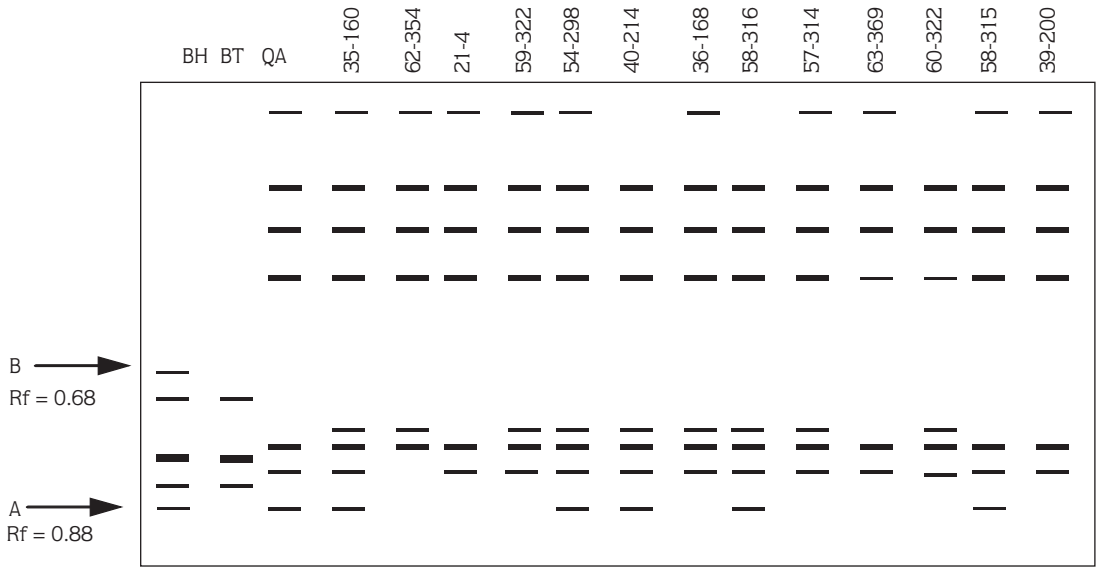

Figure 1. Native polyacrylamide gel electrophoresis of peroxidase isozymes of 2 pear cultivars, QA (quince A) rootstock and 13 S.Ö. quince clones (35-160, 62-354, 21-4, 59-322, 54-298, 40-214, 36-168, 58-316, 57-314, 63-369, 60-322, 58-315 and 39-200). Analysis of profiles revealed an isoperoxidase band (A; Rf = 0.88) that was present in BH (Beurre Hardy, a compatible pear scion), absent from BT (Bartlett, an incompatible pear scion), and present in QA and 5 of the S.Ö. quince clones. Another isoperoxidase band (B; Rf = 0.68) was observed in BH, but not in BT or in any of the rootstocks.

representative profile (of samples taken 4 weeks after budding) is presented in Figure 2. The data indicated identical isozyme profiles across all graft unions involving a BH scion and quince rootstocks. Therefore, the data are not presented here. However, differences were observed among graft unions involving BT and quince rootstocks. Thus, only data for the BH/QA graft union are presented here; along with all the graft combinations with BT (Figure 2). In general, the isoperoxidase bands observed in the graft union samples, particularly in the zone where bands A and B were present, were darker than those in unbudded graft partners (compare Figures 1 and 2).

The graft union phenotype shown in Figure 2 is the sum of both scion and rootstock banding patterns as shown in Figure 1, with a few exceptions. Bands A and B were noted as the main differences. Data from Figures 1 and 2 were summarized and are presented in Table 1 for the sake of clarity. The data presented in Table 1 indicated that isoperoxidases A and B were not present in some graft union samples even though they were present in nonbudded individual graft partners. Conversely, these isoperoxidases were present in some graft union samples in spite of their absence from one or both graft partners. For example, all graft union samples involving BH and quince rootstocks contained both bands A and B, whereas BT/QA union samples contained neither band A nor B, even though nonbudded QA samples contained band A.

Discussion

The specific role of peroxidase in graft compatibility/incompatibility is not clearly understood. However, Buchloh (1960) stated that a strongly lignified graft union was essential for graft compatibility. Harkin and Obst (1973) also reported that peroxidase was the only enzyme involved in the polymerization of p-coumaryl alcohols to lignin, and this provided the basis of much research into the relation between cambial isoperoxidases and graft compatibility. In addition, Santamour (1988b) suggested that dissimilarities in isoperoxidase composition between stock and scion could result in abnormal lignification and also a lack of vascular connections at the graft union, resulting in an incompatible combination. Based on this hypothesis, it is well established that different isoperoxidases are involved in the production of structurally different lignins that may have different bonding (with cell wall carbohydrates) characteristics (Santamour, 1988c).

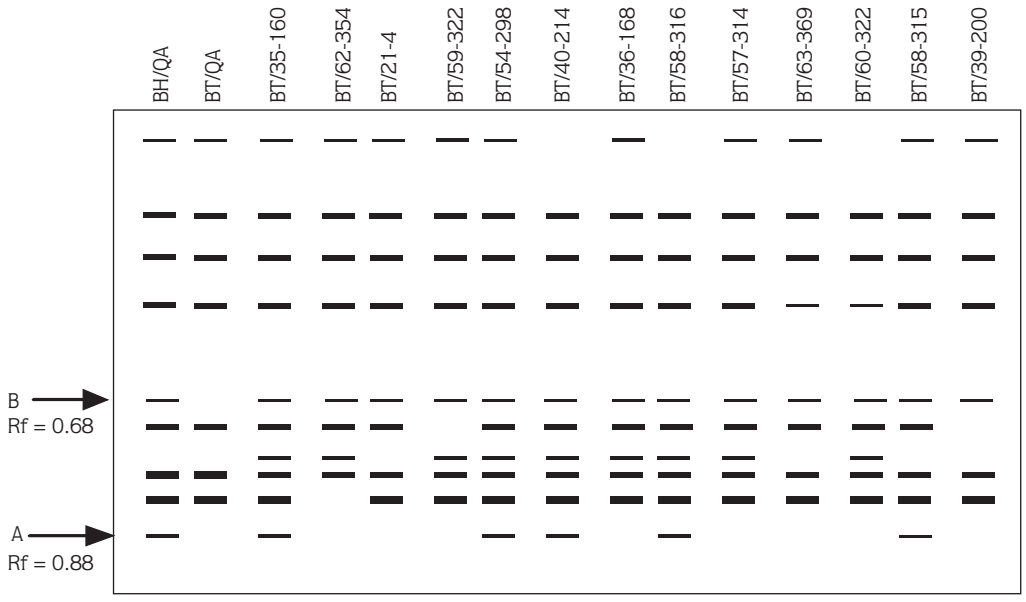

Figure 2. Native polyacrylamide gel electrophoresis of peroxidase isozymes of graft union samples (4 weeks after grafting) of all the graft combinations representing BT/QA (Bartlett/quince A, an incompatible combination), BT/S.Ö. quince clones (35-160, 62-354, 21-4, 59-322, 54-298, 40-214, 36-168, 58-316, 57-314, 63-369, 60-322, 58-315 and 39-200) and BH/QA (Beurre Hardy/quince A, a compatible combination). The isoperoxidase bands, A (Rf = 0.88) and B (Rf = 0.68), are common in BH/QA and 5 of the BT/S.Ö. quince clones (35-160, 54-298, 40-214, 58-316 and 58-315).

Table 1. Native PAGE profiles of isoperoxidases A and B (as shown in Figures 1 and 2) for 2 pear cultivars (Bartlett = BT and Beurre Hardy = BH), quince A (QA), and 13 S.Ö. quince clones (35-160, 62-354, 21-4, 59-322, 54-298, 40-214, 36-168, 58-316, 57-314, 63-369, 60-322, 58-315 and 39-200).

Cultivar/clone	Unbudded	Graft union w/BH	Graft union w/BT
Cultivars			
BH	A, B	ND	ND
BT	__z	ND	ND
Rootstocks			
QA	A	A, B	__
35-160	A	A, B	A, B
62-354	__	A, B	B
21-4	__	A, B	B
59-322	__	A, B	B
54-298	A	A, B	A, B
40-214	A	A, B	A, B
36-168	__	A, B	B
58-316	A	A, B	A, B
57-314	__	A, B	B
63-369	__	A, B	B
60-322	__	A, B	B
58-315	A	A, B	A, B
39-200	__	A, B	B

[z] Neither A nor B detected; ND = not done.

Some research findings regarding the relationship between graft compatibility and cambial peroxidase isozyme pattern in various genera suggest that the matching of isoperoxidase bands between scion and rootstock could be used as an index for graft propagation (Santamour et al., 1986; Santamour, 1988a, 1988b, 1988c, 1989). In a recent report, Gulen et al. (2002) also demonstrated a significant relationship between cambial peroxidase isoenzyme banding pattern and graft compatibility in pear cultivars and various selected Turkish quince clones. Two anodal isoperoxidase bands, A (Rf = 0.88) and B (Rf = 0.68), were detected in pear/quince combinations as isozyme markers in early quince rootstock selection considering their graft compatibility characteristics.

Since anodal isoperoxidases reportedly regulate lignification, which may be related to graft compatibility (Walter and Gordon, 1975; Gaspar et al., 1982; Walter, 1992), only anodal isoperoxidases were considered in this study. Many bands were observed, but only 2 consistent bands, referred to as bands A (Rf = 0.88) and B (Rf = 0.68) by Gulen et al. (2002) in pear/quince (intergeneric) graft combinations were easily identifiable (Figures 1 and 2). Results from the present study support the conclusion reached by Santamour et al. (1986) and Gulen et al. (2002). Although only 1 compatible or incompatible graft combination was used in this study, our data suggest that a specific isoperoxidase (band A; Rf = 0.88) may be associated with the compatibility of *Pyrus communis*/*Cydonia oblanga*. This was the only band observed in both QA and BH (a compatible scion) but absent from BT (an incompatible scion) (Figure 1). In addition, none of the BT isoperoxidases matched the isozymes in QA.

Our data also indicated an interesting point in which incompatible graft union samples lacked bands A and B even though they were present in one or both graft partners. Conversely, compatible graft union tissues contained isozyme bands A and B, one or both of which were absent from either or both graft partners. For example, the BT/QA graft union (an incompatible combination) lacked isozymes A and B, despite the

presence of isozyme A in QA. These observations together with the presence of isoperoxidases A and B in graft union samples of compatible combinations (BH/QA) suggest that the activity of A and B may be associated with graft compatibility between pear and quince, which was also stated in our previous study (Gulen et al., 2002). Regarding this hypothesis and the data presented in Table 1 and Figure 2 it can be argued that 5 of the 13 S.Ö. quince clones and types (35-160, 54-298, 40-214, 58-316 and 58-315) used in this study might form compatible graft unions with BT. Previous research (Çelik, 1982, 1988) supports this notion. Çelik (1982, 1988) selected S.Ö. 40, 35 and 58 clones as the most compatible for Bartlett pear depending on their growth and development characteristics in addition to their prunasin contents and β-glycosidase activities. The appearance or disappearance of isoperoxidases (as discussed above) may be due to altered gene expression. These changes may be triggered by a signal produced by the contact between the 2 graft partners. In a recent study (Pirovana et al., 2000), 60 putative genes that were expressed differentially in compatible and incompatible combinations were identified using an in vitro model system comprised of cell cultures from compatible and incompatible pear/quince combinations.

Deloire and Hebant (1982) reported increased peroxidase activity in incompatible grafts compared to autografts. We were not able to detect any significant increase in peroxidase activity in the pear/quince combinations studied, which is consistent with our previous work (Gulen et al., 2002). On the other hand, Copes (1978) reported much darker staining of certain isoperoxidases from tissues of compatible unions or from nonunion areas. In our study, graft union samples of both compatible (BH/QA) and incompatible (BT/QA) combinations showed darker staining, particularly in the zone where bands A and B were present, than in nonunion samples (compare Figures 1 and 2). This result is consistent with the study that described different staining intensities between graft union and nonunion samples (Gulen et al., 2002). This observation suggests that peroxidase activity may be increased by wounding, which was also stated by Macheix et al. (1986). Moreover, the presence of additional isoperoxidase bands in compatible (BH/QA) graft union samples may be correlated to their role in lignin biosynthesis.

In conclusion, only 2 major and variable anodal peroxidase bands (A and B) were found in the plants studied. Because of their stability at various periods of grafting, these isoperoxidases were considered to be probably involved in lignin formation and lignin-carbohydrate bonding and, therefore, in grafting success. Consequently, these isoperoxidase markers, bands A (Rf = 0.88) and B (Rf = 0.68), could be used in pear/quince graft combinations. Results from the isoperoxidase analysis coupled with previous research involving S.Ö. quince clones suggest that 5 of the clones studied, 35-160, 54-298, 40-214, 58-316 and 58-315, are potential compatible clones with the BT pear cultivar.

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