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## Total Protein Content and SDS-PAGE in Pear Scions Grafted on Quince A and Pear Seedling Rootstocks

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**Abstract:** This study was conducted to investigate the effects of various rootstocks on the total soluble protein content of pear scions, and to detect a polypeptide as a marker that would be associated with pear/quince compatibility/incompatibility. Bark samples were collected from 5 year-old pear scions grafted on QA and pear seedling rootstocks (PS). Total soluble protein contents of bark tissues of 4 pear scions, Passa Crassana (PC), Beurre Hardy (BH), Beurre Bosc (BB) and Bartlett (BT), were determined using the Bradford assay. Protein profiles of bark tissues taken from the graft combinations were determined using SDS-PAGE. In general, the scions grafted on QA had a greater total protein content than those on PS rootstock. The highest protein content was determined in BH/QA combination. However, no linear correlation was detected between total protein content and graft compatibility. In SDS-PAGE, the protein profiles of the scions were similar. However, a 63 kDa protein band determined in compatible pear scions (PC and BH) was faintly observed in an intermediate compatible scion (BB), but was not detected in the incompatible scion (BT). Our results indicated that this polypeptide could be associated with pear/quince graft compatibility/incompatibility.

**Key Words:** Graft incompatibility, pear, quince, protein, SDS-PAGE

### Quince A ve Armut Çöğür Anaçlarına Aşılı Armut Çeşitlerinde Toplam Protein İçeriği ve SDS-PAGE

**Özet:** Bu çalışma, armut çeşitlerinin toplam çözünebilir protein içerikleri üzerine farklı anaçların etkisinin araştırılması ve armut/ayva aşılı uyuşma/uyuşmazlığı ile ilgili olabilecek bir markörün belirlenmesi için yapılmıştır. Örnekler, armut çöğür anacı ve QA üzerine aşılı 5 yaşlı armut çeşitlerinden toplanmıştır. Dört armut çeşidinin, Passa Crassana (PC), Beurre Hardy (BH), Beurre Bosc (BB) ve Bartlett (BT), kabuk dokusundaki toplam çözünebilir protein içeriği Bradford Assay yöntemi kullanılarak saptanmıştır. Aşılı kombinasyonlarının kabuk dokusunun protein profilleri ise SDS-PAGE kullanılarak belirlenmiştir. Genelde, QA üzerine aşılı çeşitler, armut çöğür anacı üzerine aşılı olanlardan daha fazla toplam protein içeriğine sahip olmuştur. En yüksek protein içeriği BH/QA kombinasyonunda belirlenmiştir. Fakat, aşılı kombinasyonlarının toplam çözünebilir protein içerikleri arasında doğrusal bir ilişki saptanamamıştır. SDS-PAGE'de ise, dört armut çeşidinin protein profilleri benzer yapı göstermiştir. Ancak, 63 kDa protein bandı uyşur armut çeşitlerinde (PC ve BH) belirlenmiş, orta derecede uyşur çeşitte (BB) silik bir şekilde gözlenmiş, fakat uyşmayan çeşitte (BT) saptanamamıştır. Sonuçlar, bu polipeptidin armut/ayva aşılı uyşmazlığı ile ilgili olabileceğini göstermiştir.

**Anahtar Sözcükler:** Aşılı uyşmazlığı, armut, ayva, protein, SDS-PAGE

### Introduction

The ability of 2 different plants, grafted together, to produce a successful union and to develop satisfactorily into one composite plant is termed compatibility

(Santamour, 1988). The development of a compatible graft is typically comprised of at least 3 independent events: (a) cohesion of the stock and scion, (b) proliferation of callus cells at the graft interface and (c)

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vascular dedifferentiation across the graft interface (Moore, 1983a). Moore and Walker (1981a, 1981b, 1981c) and Moore (1983b) described and compared the anatomical and physiological events taking place in compatible autografts and incompatible heterografts between 2 herbaceous species. Even if the results can not be applied to all genotypes, they clearly demonstrate that the initial phases of graft formation and wound response are similar for compatible and incompatible grafts. Since unsuccessful grafts may still complete the developmental sequence of the events reported by Moore (1983a) grafting is not always successful.

When plants that are in the same family but different genera are grafted together, the chances of a successful union become more remote (Hartmann et al., 1997). Although inter-generic grafts are rarely used, they are necessary in the propagation of some plants such as pear. Since quince is the only genus that provides a satisfactory dwarfing rootstock for pear, use of this clonal rootstock is inevitable in intensive pear production. However, some pear varieties, such as Bartlett, do not have good unions on quince. Thus, these pear varieties are grown by double-working, using a body stock, such as Beurre Hardy, which is compatible with both the quince rootstock and the pear scion (Tukey, 1978; Lombard and Westwood, 1987). However, this technique is not desirable in nurseries due to its considerably increasing nursery costs (Baldini et al., 1977; Hartmann et al., 1997).

In this respect, knowing the mechanism of incompatibility would help to establish criteria for selecting appropriate plant materials for grafting. The observation of external symptoms or anatomical abnormalities after grafting were the pre-selection methods used to determine graft incompatibility, the visible symptoms of which would take years to become apparent. Additionally, early anatomical observations may not correlate with long-term graft survival (Andrews and Marques, 1993). Thus the prediction of incompatible graft combinations is a very important area of study for preventing economic losses due to graft incompatibility. In this respect, recent studies have been focused on detecting some isozyme or protein (polypeptide) markers to predict graft incompatibility using effective techniques.

Lachaund (1975) suggested that incompatibility could be avoided, to a certain extent, where similarity of protein composition between the partners would increase

the probability of graft success. The comparison of protein profiles of graft combinations to predict graft incompatibility using SDS-PAGE was studied in *Prunus* species (Huang et al., 1984; Schmid and Feucht, 1985) and in *Vitis vinifera* (Masa, 1986, 1989). Total soluble protein content of compatible and incompatible peach/plum grafts was also studied by Moreno et al. (1994). It was reported in the study that total soluble protein concentration was lower in incompatible grafts.

Although cellular necrosis caused by hydrocyanic acid at the graft interface has been suggested as a main cause of incompatibility between pear and quince (Gur et al., 1968), the molecular bases of graft compatibility/incompatibility are still unclear. This research was initiated to investigate the total soluble protein content and polypeptides profile of 4 pear scions, Passe Crassane (PC), Beurre Hardy (BH), Bartlett (BT) and Beurre Bosc (BB), grafted on QA and PS rootstock using SDS-PAGE. The objectives of this survey were to observe the effects of various rootstocks on the total soluble protein profiles of the scions and to identify polypeptides that would be associated with compatible or incompatible pear/quince combinations.

## Materials and Methods

### Plant materials

Current-year shoots of 5 year-old pear scions (PC, BH, BT and BB) grafted on QA and pear seedling rootstock were collected at the USDA-ARS, National Clonal Germplasm Repository, Corvallis, OR, USA. In addition, some bark samples from QA and PS rootstocks were also taken from below the graft union of the trees. PC/QA and BH/QA graft combinations are considered compatible, while BT/QA and BB/QA are considered incompatible and moderately compatible combinations, respectively (Tukey, 1978; Lombard and Westwood, 1987). Triplicate samples of bark and cambial tissues were scraped from the shoots using a razor blade, ground in liquid N<sub>2</sub> and stored at -80 °C until used.

### Protein extraction

Protein was extracted from bark tissues using the extraction methods described for other woody plants (Arora et al., 1997, 1992) with a few modifications. Ground bark tissues (1.5 g) were homogenized at 4 °C in borate buffer (50 mM sodium tetraborate, 50 mM ascorbic acid, 1% β-mercaptoethanol, 1 mM PMSF, pH

9.0) and insoluble polyvinylpyrrolidone (PVPP) paste made with borate buffer using a 1:5:2 (tissue: buffer: PVPP paste) extraction ratio. Crude extraction was homogenized for 60 s followed by 30 s at 6 speeds with Polytron. Samples were shaken on a gyratory shaker for 15 min at 4 °C followed by centrifugation at 26000 g for 1.5 h at 4 °C. The resulting supernatant was collected and filtered through 0.4 and 0.2 µm filters. Protein content was measured using the Bradford assay method described by Arora and Wisniewski (1994).

#### Sample Preparation and SDS-PAGE

Proteins were precipitated according to the method described by Lim et al. (1999) by adding trichloroacetic acid (TCA, 10%) to 1.3 ml of sample from the supernatant. Samples were centrifuged at 16 000 g for 30 min at 4 °C, after 30 min incubation at 4 °C. Protein pellets were washed 3 times with cold acetone by centrifugation at 16 000 g for 30 min at 4 °C. After the first acetone wash, the pellets were broken physically using a sealed pipette tip. Dried pellets were resuspended in SDS-PAGE sample buffer (65 mM Tris-HCl, 10% (v/v) glycerol, 2% (w/v) SDS, pH 6.8 and 5% β-mercaptoethanol with Bromphenol blue). Discontinuous SDS-PAGE was performed with a PROTEAN II electrophoresis unit (Bio-Rad) using 4% stacking gel and 12.5% separating gel. An equal amount of total protein (30 µg) was used for each sample and gels were stained with Coomassie Brilliant blue G-250.

Total soluble protein contents of the 4 scions (PC, BH, BT and BB) grafted on QA and PS rootstocks were analyzed with 3 replications, and the results were statistically analyzed using the Tukey test.

## Results

### Total Soluble Protein Contents of the Graft Combinations

Total soluble protein content of the scions showed some differences depending on the rootstocks (Table 1). Comparing total protein content of pear scions grafted on QA and PS rootstock, the scions grafted on QA had greater total protein content than the combinations with PS rootstock. The mean total protein content of QA was 2.60 mg g<sup>-1</sup>, whereas it was 1.60 mg g<sup>-1</sup> in PS. However, there was no difference among the compatible (BH/QA, PC/QA), moderately compatible (BB/QA) and incompatible graft combinations, except for BH/QA (3.72 mg g<sup>-1</sup>).

Regarding the total protein content of the scions, and ignoring the rootstock effect, only the BH scion had a markedly high protein content (2.70 mg g<sup>-1</sup>). The moderately compatible scion BS had almost the same total protein content as the PC and BT scions. Consequently, no linear correlation was detected among the graft combinations.

### Total Protein Profiles of the Graft Combinations in SDS-PAGE

SDS-PAGE analysis was replicated at least 3 times with similar results and data from the representative analysis are presented here.

In general, total protein profiles were similar in the bark tissue of the 4 pear scions grafted on QA and PS rootstocks (Figure 1). Moreover, certain pear scions grafted on either rootstock had almost the same polypeptides, with a few minor differences. Regarding the protein profiles of pear scions grafted on QA, they

Table 1. Bark total protein content (mg g<sup>-1</sup>) of the pear scions grafted on Quince A (QA) and pear seedling (PS) rootstocks.

Scion	Rootstock		Means <sup>a</sup> (Scion)
	QA	PS	
Passa Crassane	2.22	1.62	1.92 b
Bartlett	2.32	1.54	1.93 b
Beurre Bosc	2.14	1.58	1.86 b
Beurre Hardy	3.72	1.67	2.70 a
Means <sup>b</sup> (Rootstock)	2.60 a	1.60 b	D 1% (Rootstock): 0.021 D 1% (Scion): 0.037

<sup>a</sup> Values within columns followed by same letter are not significantly different at P < 0.01 according to the Tukey test.

<sup>b</sup> Values within rows followed by same letter are not significantly different at P < 0.01 according to the Tukey test.

showed almost the same polypeptides, as those of the QA rootstock (itself) had polypeptides (Figure 2). The only significant difference, a 63 kDa protein band, was determined between compatible (PC and BH) and incompatible (BT) scions. PC, BH and BB had a 63 kDa protein band, whereas BT did not. This 63 kDa protein band was also detected in the QA rootstock itself. Consequently, this protein band is common in compatible and moderately compatible pear scions and QA rootstock.

**Discussion**

In the limited number of studies on the prediction of graft incompatibility using total protein analysis it was reported that the total protein content in scion bark is related to the incompatibility level of the graft combination (Schmid and Feucht, 1985). In the present study, the highest total protein content (3.72 mg g<sup>-1</sup>) was detected in BH/QA, which is a compatible combination. Since BH is the most effective pear scion used as an interstock for incompatible scions like BT, graft combinations of BH may have the highest protein content. However, this result is inconsistent with another fully compatible combination, PC/QA. On the other hand,

regarding the pear seedling rootstock, all the combinations had markedly low protein contents. In this respect, our data are not parallel to the study by Moreno et al. (1994) reporting the low total protein content in the scion of incompatible combinations of peach/plum grafts, 3 months after grafting. Weak and nonstandard physiological activity in these graft combinations due to weak scion growth and insufficient vascular connection may cause a reduction in protein content in the early stage of grafting.

In general, our data indicated that the protein content increased in the scion on QA as a result of stress. Quince is in the same family as pear, but is part of a different genus. This leads to graft combination stress even if they are compatible. However, the results indicated no clear mechanistic relationship between pear/quince graft compatibility/incompatibility and total protein content of 5-year-old pear scions. Thus, total protein measurements in the early stage of grafting may give a better idea of the composition of and relationship between compatible and incompatible combinations.

Regarding the gel photos (Figures 1 and 2), a 63 kDa protein band was observed in compatible (PC/QA and

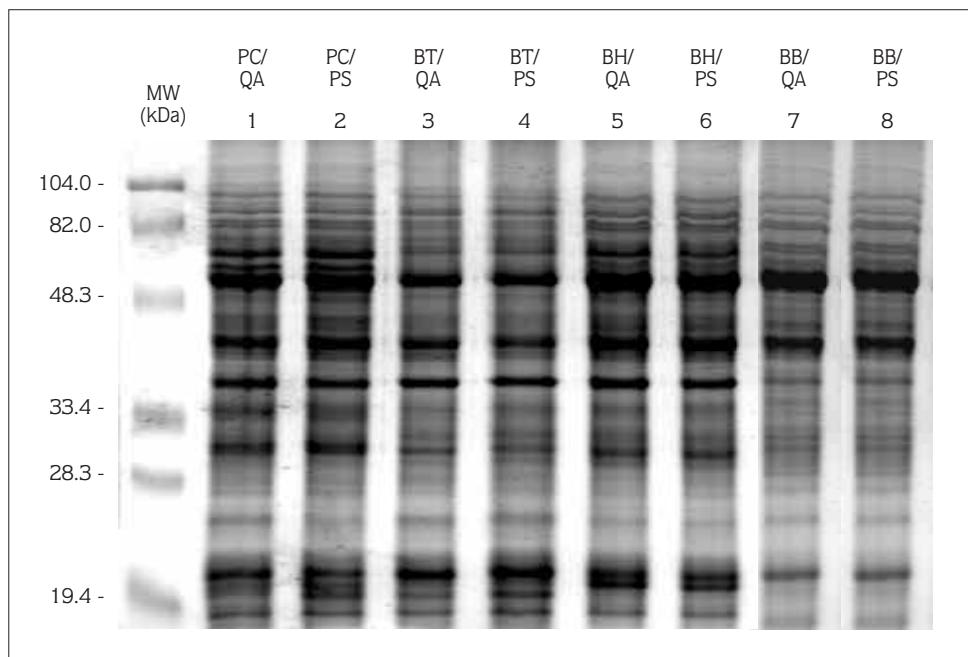


Figure 1. SDS-PAGE profile of bark proteins extracted from 4 pear scions, Passa Crassane (PS), Bartlett (BT), Beurre Hardy (BH) and Beurre Bosc (BB), grafted on QA and pear seedling (PS) rootstocks. In the left lane molecular masses (MW) of protein standards are indicated. An equal amount of total protein (30 µg) was used for each sample.

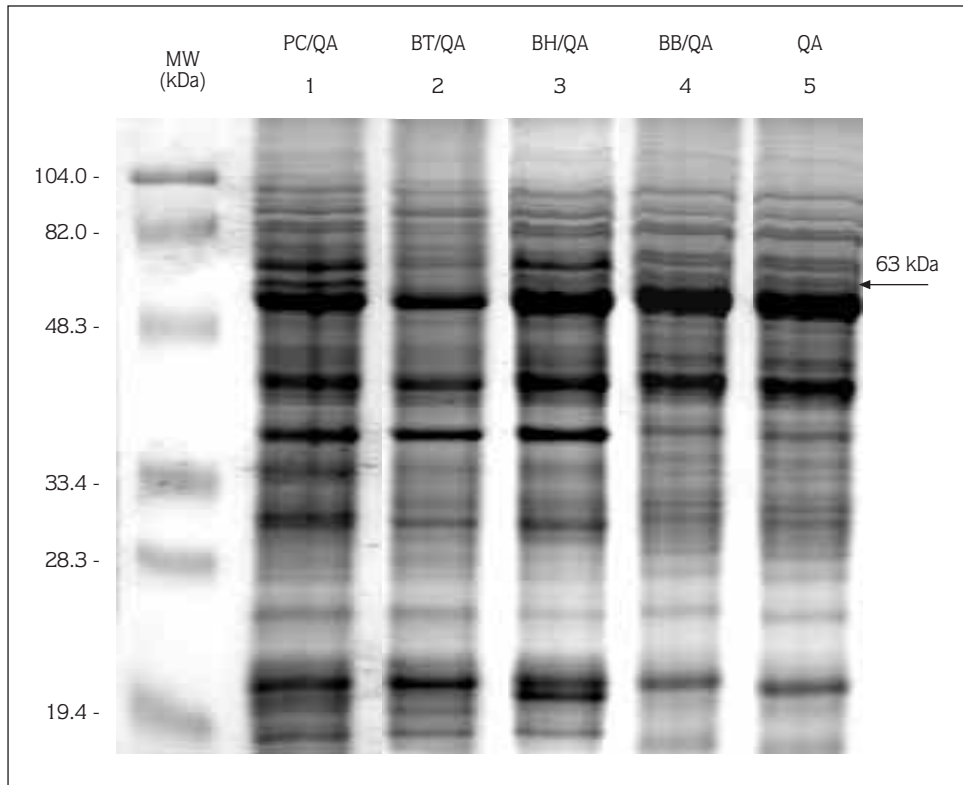


Figure 2. SDS-PAGE profile of bark proteins extracted from 4 pear scions, Passa Crassane (PS), Bartlett (BT), Beurre Hardy (BH) and Beurre Bosc (BB), grafted on QA rootstock. The bark protein profile of QA rootstock (itself) is indicated to the right (lane 5). Molecular masses (MW) of protein standards are indicated to the left. An equal amount of total protein (30 µg) was used for each sample.

BH/QA) and moderately compatible (BB/QA) graft combinations, but not in the incompatible one (BT/QA). It was also detected in the QA rootstock itself. The band intensity of the 63 kDa polypeptide changed depending on the graft combinations. The 63 kDa polypeptide observed in PC/QA was darker than that in the other combinations, which means it could be related to the compatibility levels of the combinations.

So far, very little work has been done on the molecular basis of the rootstock-scion relation. However, it was reported that certain genes are turned on and off, and/or that genetic information may be transmitted between the graft partners of the combination (Ohta, 1991). Even if some characters of the scion are changed by grafting (fruit quality, yield, ripening, growth rate, juvenility, etc.), these are epigenetic (non-genetic) changes. Processes related to direct growth, development and plant structure are referred to as epigenetic because change does not occur in the basic genotype of the plant

during the process, like grafting (Hartmann et al., 1997; Howell, 1998). Furthermore with adhesion of the graft partners and growing as a single plant after grafting the scion becomes autotrophic in later years (Moreno et al., 1994). This physiological evidence may explain why a certain pear scion has almost the same protein profiles, even though it is grafted on various rootstocks. In addition, the plants used in the present study were 5 years old. Thus, they may be mostly autotrophic.

Regarding the 63 kDa protein band, our data indicated that this was detected in the compatible (PC, BH) and moderately compatible (BB) scions grafted on both rootstocks (QA, pear seedling), in addition to the QA rootstock itself. These preliminary results suggest that the expression of the polypeptide may be unique to compatible pear scions, even if it is also expressed in QA. Moreover, it may be a general response in successful combinations.

In conclusion, our data indicated that electrophoretic detection of compatibility/incompatibility using SDS-PAGE protein analysis could be used in pear/quince graft combinations. Hence, a 63 kDa protein band would have a detectable role in pear/quince graft compatibility/incompatibility. More detailed studies are needed to support or disprove this conclusion in a definitive manner.

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