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Research Article

P-Protein Structure in Functional and Non-Functional Secondary Phloem in *Armeniaca vulgaris* Lam. (*Rosaceae*)

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Abstract: P-proteins (phloem proteins) and their conformational changes in the functional and non-functional sieve elements in secondary phloem of *Armeniaca vulgaris* Lam. (*Rosaceae*) were examined with an electron microscope. Scattered throughout the cell, the fibrillar components of p-protein in active sieve elements were much denser sieve plate surfaces. P-proteins in inactive sieve elements, different from those of active ones, were observed to appear as disordered, short, thread-like fibrils with fern-like crystalline structure.

Key Words: p-protein, *Armeniaca vulgaris* Lam., secondary phloem

Armeniaca vulgaris Lam. (*Rosaceae)*'de Fonksiyonel ve Fonksiyonel Olmayan Sekonder Floemde P-Protein Yapısı

Özet: *Armeniaca vulgaris* Lam. (*Rosaceae*)'ın sekonder floeminde fonksiyonel ve fonksiyonel olmayan elekli boru hücrelerinde p-proteinler (floem proteinleri) ve yapısal değişiklikleri elektron mikroskobunda incelendi. Aktif elekli borularda daha çok uzun fibriller yapıda olan p-proteinleri, bütün hücre boyunca dağılmış olmakla birlikte daha çok elek yüzeylerinde yoğun olarak görüldü. İnaktif elekli boru hücrelerinde ise düzensiz, kısa ipliksi fibriller ve eğrelti benzeri kristalin yapılar şeklinde gözlendi.

Anahtar Sözcükler: p-protein, *Armeniaca vulgaris* Lam., sekonder floem

Introduction

In higher plants, translocation of nutrients takes place inside the phloem. This translocation occurs via the plasma membrane of the sieve tubes, which are composed of a series of connected sieve elements. The mechanism of the longitudinal assimilate movement in sieve tubes has been explained by two different models of phloem loading: 1. Symplastic loading, 2. Apoplastic loading. Symplastic loading has been investigated by Gamalei (1991), Van Bel et al. (1992) and Turgeon (1996). According to them, in symplastic loading, the nutrients are located via continuous plasmodesmatal links. Komor et al. in 1996 claimed that apoplastic loading is the result of the active translocation of assimilate molecules via the plasma membranes of sieve cells or companion cells. The sieve cells differentiate from cambium, firstly experience nuclear degeneration, and then lose their nuclei, and some of their cytoplasmic components and organelles. The remaining cytoplasmic contents are scattered peripherally and the centre of the cell is free for the translocation. On the other hand, in most of the higher plants, dispersive and nondispersive protein bodies appear in both young and mature sieve elements. Several researchers have suggested that p-proteins (phloem proteins) play an active role in the translocation of photosynthetic products via the sieve tubes (Thaine, 1996; Fenson, 1972). Having examined the dispersion of p-proteins in the sieve elements of *Cucurbita maxima* Duch. in 1973, Evert et al. reported that p-proteins are scattered peripherally in both the lumina and sieve pores. Thorsch and Esau in 1988 examined the sieve elements in the primary phloem of *Euphorbia pulcherrima* Willd ex Klotsch with an electron microscope. They found two types of proteinaceous inclusions, both of which are cytoplasmic in origin. While one of them exhibits the

typical form of p-protein composed of tubular units, the other is composed of tubes that have changed into striated fibrils by stretching. The researchers concluded that the second type of inclusion is a dispersing protein body and the first type, in contrast, characterizes the sieve elements in differentiation. Behnke and Richter in 1990 found tubular p-proteins in primary sieve elements of *Rhizophora mangle* L.. Having observed cytoplasmic inclusions in the sieve elements of *Gmelina arborea* Roxb. again in the same year (1990), Vishwakarma and Desphande suggested that those inclusions might be a type of crystalline p-protein which had not been previously reported in the family *Verbenaceae*. In 1990, Wu and Hao investigated the ultrastructure of p-protein in Hevea brasiliensis Mull. Arg. during sieve tube development and after wounding. They found that the conformational changes of p-protein in Hevea brasiliensis Mull. Arg. occurred not only during the differentiation of the sieve elements, but also after their wounding. The researchers concluded that both conformational and morphological changes might occur in p-proteins due to the changes taking place in the cell as regards environmental conditions (Palevitz & Newcomb, 1971). The aim of our study was to investigate the conformational changes of p-proteins in the functional and non-functional sieve elements in the secondary phloem of *Armeniaca vulgaris* Lam.

Materials and Methods

Samples were taken from one-year-old stems of *A. vulgaris* growing in Ankara University's Botanical Garden in spring and autumn. Sampling dates for functional and non-functional secondary phloem were based on light microscope observations. After being excised, each piece of stem was immediately immersed in 5% glutaraldehyde in a 0.1 M phosphate buffer at pH 7.2. The materials were fixed at room temparature under vacuum for 2 hours and washed for 3 hours in the buffer. They were then post fixed with buffered 1% osmium tetraoxide for 3 hours. The tissue was dehydrated in a graded ethanol series and embedded in epon 812. Sections were cut with glass knives and stained with resorcin blue for light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate. Ultrastructural observations were made using a Jeol CX II transmission electron microscope at 80 Kv.

Results

The first visible indicator of sieve cell differentiation is the thickening of cell walls. Afterwards, the nucleus loses its normal shape, the nuclear membrane exhibits a wavy appearance and the content of the nucleus becomes granular (Fig. 1A). Rough ER mostly manifests a peripheral dispersion. It is possible to observe mitochondria, ribosomes, plastids and large vacuoles during differentiation. In this study, dividing mitochondria was observed as well during differentiation (Fig. 1B).

It was noticed that nucleus and ribosomes had disappeared in the mature sieve elements of *Armeniaca vulgaris* Just after the beginning of differentiation, the cell wall manifested much more thickening than that of adjacent parenchymatous cells. Cell wall thickening exhibited an irregular outline on the inner surface. With the increase in thickening, the inner surface of the cell wall became wavy in appearance and the cell lumina shrank gradually (Fisher, 1990). In the thickened walls, the structure was mostly lamellated. Peripherally situated mitochondria and plastids were the only organelles in the mature sieve tubes. It was rather difficult to distinguish between mitochondria and plastids as they were similar in size. At the beginning of the active period of sieve tubes, fibrillar components of p-proteins were observed to be irregularly dispersed in the whole cell lumina (Fig. 1C). At this stage, some spindle shaped crystallines were noticed as well among the p-proteins of fibrillar type (Fig. 1D). Then, p-proteins, especially those in the sieve plates, transformed into hair-like fibrillar structures (in May samples) (Fig. 1E). The long fibrils passing through the sieve pores looked like a bunch of fibers stretched on both sides. Small tubular or channel like pieces were also observed among the fibrils, especially in the sieve pore entrances of the active sieve elements (Fig. 1F). The pores in the active sieve elements of *Armeniaca vulgaris* appear to be plugged by fibrillar p-protein substances (Knoubblauch & Van Bel, 1998). Much different situations were observed in the sections taken from the samples of the inactive month, December. It was seen that callose accumulation had begun in sieve plates during the resting season. From the beginning of autumn onwards, an increase was reported in the number of plastids. Those plastids, round or oval in shape, are S-

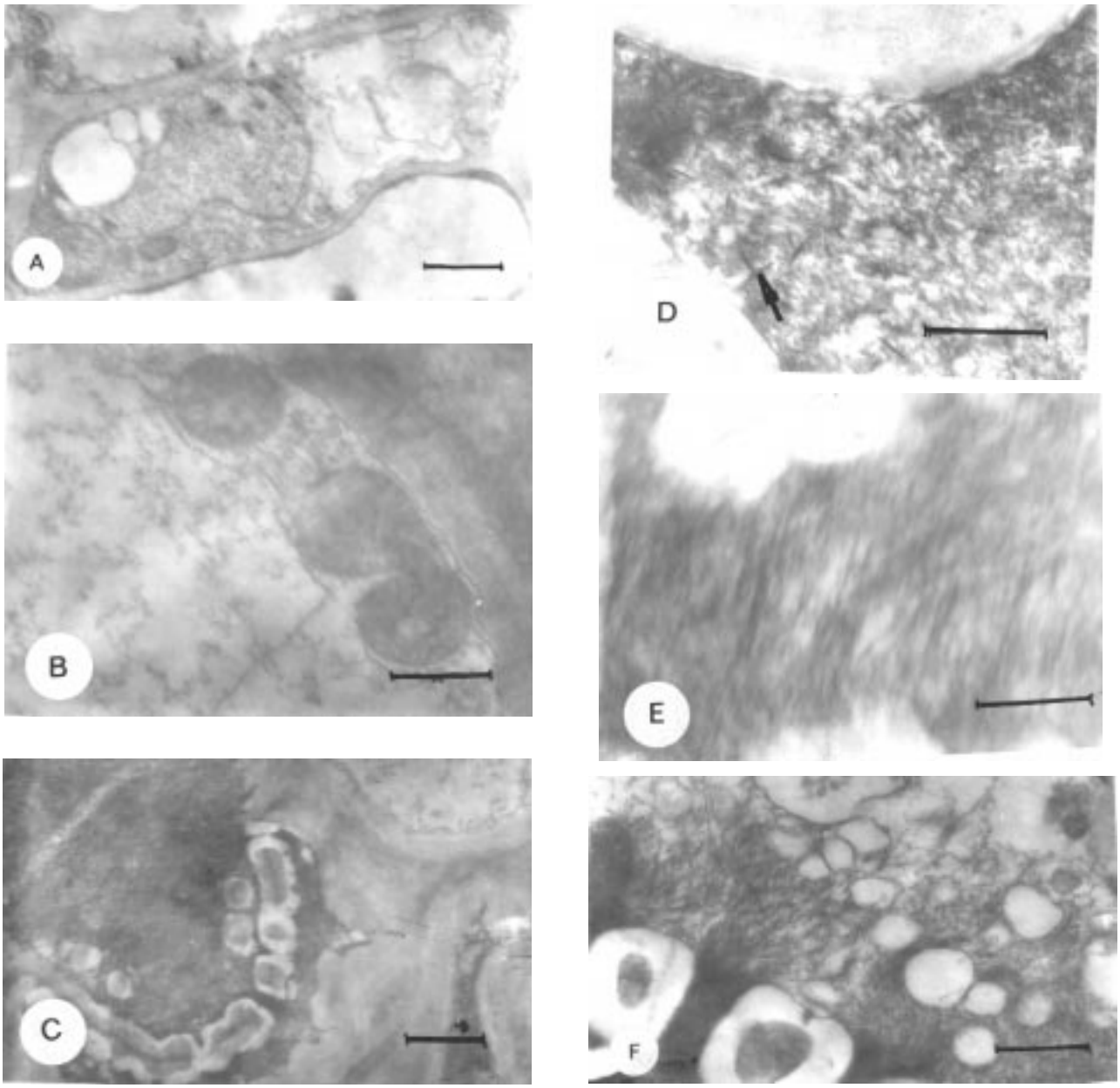

Figure 1. A) Cell components of differentiating sieve elements. Bar=2 mm. B) Dividing mitochondrium in differentiating cell. Bar=1 mm. C) Fibrillar components of p-protein in active sieve elements. Bar=2 mm. D) Spindle shaped crytallin structure in active sieve elements (arrow). Bar=1 mm. E) Long hair-like structure of p-protein. Bar=5 mm. F) Small pieces of tubule like structure near the sieve pores (arrow) Bar=1 mm.

type plastids generally including one starch grain. Callose accumulation occurred gradually around the plastids as well (Fig. 2A). Then, with the increasing amount of callose, the pores became plugged. The fibrillar components of p-proteins encountered in active sieve elements transformed into electron dense aggregates (Fig. 2B). Most of the plasmodesmatal links in the inactive sieve elements between the adjacent cells were noticed to have disappeared. At the beginning of the inactive period, the cell wall reached its maximum thickness, and parallel to this, the cell lumina shrank to a considerable extent. As the callose increases in the cell lumina, mostly near the sieve plate, plastids or starch grains released from the plastid membrane appear to be embedded in callose. The

structures of some p-proteins in the inactive sieve tubes transformed from long regular fibrillar forms to irregular thread fibrils (Fig. 2C). As far as was observed, in some inactive sieve elements, p-proteins were fern-like crystalline structures (Fig. 2D). This sort of p-protein structure has never been reported before.

Discussion

This study shows that conformational changes occurred in the p-proteins of metabolically functional and non-functional sieve elements. It was reported previously by other researchers that structural changes had occurred in p-protein in some plant species. However, it was noted that these changes occurred among differentiating sieve elements and among mature sieve elements (Thorsch & Esau, 1988; Wu & Hao, 1990; Cronshaw & Esau, 1967). In the plants they have examined, researchers have found that p-proteins in mature sieve elements appear in striated fibrillar form. Our findings also support those of researchers, as almost 85% of the total p-proteins in the mature sieve elements of *Armeniaca vulgaris* were observed to be composed of striated fibrils. On the other hand, it was noticed that a small amount of p-proteins in the mature sieve elements transformed into small tubular or channel-like structures. However, no evidence was found regarding the presence of organized, microtubular structured p-proteins in *Armeniaca vulgaris* by Thorsch

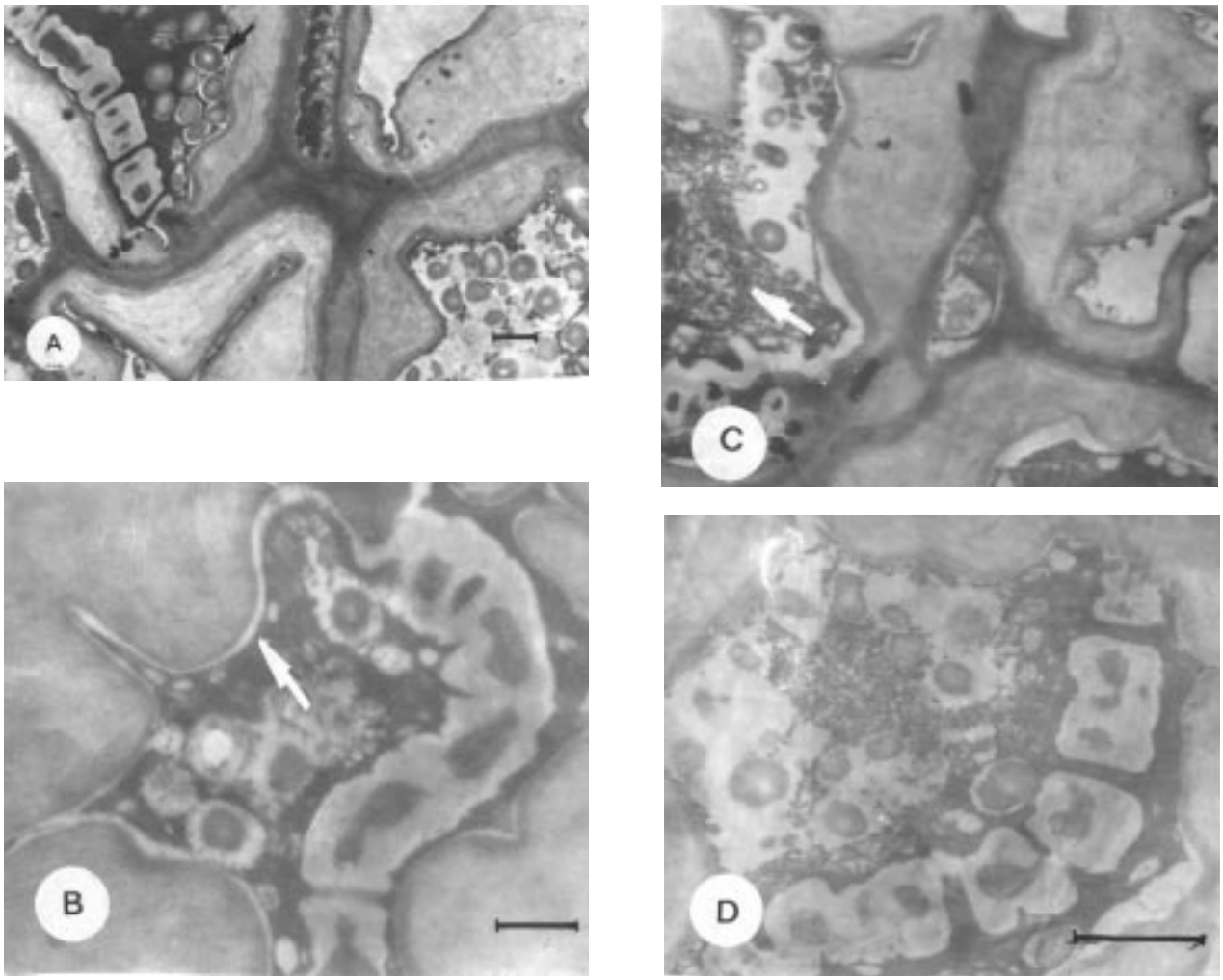

Figure 2. A) Callose accumulation are seen around the plastids (arrow) in inactive sieve elements Bar= 3 mm. B) Electron dense aggregates of p-protein (arrow) Bar=1 mm. C) Disordered thread fibrils of p-protein (arrow) Bar=3 mm. D) Fern-like structure of p-protein in inactive sieve elements Bar=3 mm.

and Esau (1988), who observed these p-proteins in the mature sieve cells in the primary phloem of Poinsettia. S-type plastids have been found in the sieve elements of most plants (Evert & Desphande, 1971; Behnke, 1991). The sieve elements of *Armeniaca vulgaris* contain only S-type plastids, and they probably play a role in callose synthesis due to the previously observed callose accumulation around them. When the sieve elements in the secondary phloem of *Armeniaca vulgaris* were inactivated, it was noticed that their p-proteins underwent some conformational changes. Some researchers have shown the changes in p-proteins during sieve element differentiation (Evert et al., 1973; Thorsch & Esau, 1988). Wu and Hao (1990) reported that wounding experiments led to some conformational changes in p-proteins, pointing out that thread-like proteins transformed into tubular forms in the wounded tissues. In conclusion, our findings indicated that in functional and non-functional sieve elements p-proteins undergo conformational changes, and fibrillar structures are transformed into fern-like structures. These findings support the view that p-protein structures are sensitive to metabolic conditions and have functional significance. It can also be assumed that tubular or channel-like structures in active sieve elements might well have roles in the translocation of substances.

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