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Full Length Research Paper

Effects of root restriction on the ultrastructure of phloem in grape leaves

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In this study, the ultrastructure of phloem and its surrounding parenchyma cells in grape leaves from plants subjected to root restriction or without (control) was systematically investigated for the first time through transmission electron microscopy. The results show that the sieve element (SE) and companion cells (CC) in the main veins of leaves subjected to root restriction were smaller in size than those in the control leaves. The number of plasmodesmata between CC and SE in leaves subjected to root restriction was greater than in the control leaves, but the number of plasmodesmata between phloem parenchyma cells (PP) in leaves subjected to root restriction was less than in the control leaves. Also, the average diameters of SE and CC in the branch veins were smaller in leaves subjected to root restriction than in the control leaves, but their cell walls were thicker and the number of plasmodesmata between PP was less. In the minor veins, the SE and CC were smaller in leaves subjected to root restriction than in the control leaves, and the number of plasmodesmata between CC and SE, SE and PP, and CC and PP were greater. Moreover, less intercellular space among PP cells was observed in minor veins of leaves subjected to root restriction, which was in contrast to the main veins where more intercellular space among PP cells was observed in the leaves subjected to root restriction compared to control leaves. These results therefore demonstrated that changes in the ultrastructure of the phloem of grape leaves are a result of adaptation to the stress of root restriction.

Key words: Root restriction, leaf, phloem, ultrastructure.

INTRODUCTION

Root restriction is a powerful approach to improve the efficiency of crop plants by controlling the shoot size and the partitioning of assimilates between vegetative and reproductive organs (Carmi, 1986). In recent years, root restriction has been developed to manipulate plant vigor by altering the environment of the root systems in grape (Wang et al., 2001; Zhu et al., 2006; Xie et al., 2009), apple (Myers, 1992), mandarin (Yakushiji et al., 1996), peach (Costa et al., 1992; Boland et al., 1994), cherry (Webster et al., 1997) and persimmon (Ogawa et al., 1997). Root restriction was regarded as one type of physical stress for roots of fruit trees. Under root restriction stress, plants showed reduction in vegetative growth and leaf photosynthetic capacity (Kharkina et al., 1999; Goto et al., 2002; Zhu et al., 2006; Shi et al., 2008). These reductions were closely related to the structure of plant organs (Guerfel et al., 2009), but little is known

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Abbreviations: CC, Companion cells; **SE,** sieve element; **PP,** phloem parenchyma cells.

about the response of plant organ structure to root restriction.

The main function of phloem is to transport photosynthetic products from the leaves where they were produced, to other parts of the plant. Frommer and Sonnewald (1995) reported that phloem loading played a key role in photoassimilate partitioning within plants. Root restriction increases partitioning of assimilate to reproductive organs (Wang et al., 2001). Two mechanisms of phloem loading, symplasmic and apoplasmic, have been identified in leaves (Turgeon, 1996; Kempers et al., 1998). Although, the development and ultrastructure of phloem tissue have been studied in a wide variety of plant species (Wang and Huang, 2003; Liesche et al., 2011), effects of root restriction stress on ultrastructural features of the phloem loading zone of leaves have not been studied.

In addition, there is no report describing the phloem loading processes of leaves on plants subjected to root restriction. Understanding the responses of phloem ultrastructure to root restriction is essential for a holistic perception of plant resistance mechanisms to root-restricted condition. Hence, the purpose of this study was to determine the effects of root restriction on phloem ultrastructure and the mechanism of phloem loading in grape leaves.

MATERIALS AND METHODS

The experiment was carried out during the 2008 growing season at the experimental farm at Shanghai Jiaotong University, Shanghai, China (3111N, 12129W). Two groups of 20 uniform 3-year-old grapevines (***Vitis vinifera*×*Vitis labrusca*** cv. Kyoho) were selected. Vines in one group were subjected to root restriction by being planted in 10 L plastic pots in a mixture of sand, loam and perlite (1:1:1). Vines in the second group were planted in a raised bed (50 cm deep) in the same medium and served as the controls. One shoot was trained vertically on each vine and axillary shoots were removed. Each vine was thinned to one shoot with one cluster before bloom, leaving the basal cluster. The number of berries in the cluster was thinned to about 50. The space between each vine was 60 cm.

From February to September, vines were maintained in a ventilated plastic house under natural light at the experimental farm. After budburst, 1 L of a complete liquid fertilizer (Hydro Co. Ltd., Israel) containing 32.7 mg NO_3^--N, 22.0 mg NH_4-N, 58.7 mg Urea-N, 120 mg P_2O_5, 120 mg K_2O, 20.0 mg MgO, 0.167 mg B, 0.067 mg Cu, 0.467 mg EDTA-Fe, 2.667mg Mn, 0.027 mg Mo, and 0.167 mg Zn was applied to each vine once a week. Tensiometers were placed at 15 cm depth in the rooting-zone to monitor soil moisture. Vines were watered by drip irrigation to maintain the soil moisture at ≥ -3.0 kPa from replanting to veraison, and ≥ -5.0 kPa from veraison to harvest.

Tissue preparation for structural observation

The method described by Zhang et al. (2004) was used to prepare leaf tissue for structural observation. Pieces (3 mm^3) were cut from the main veins, branch veins and minor veins of fully expanded healthy mature vine leaves (Figure 1). The pieces were rinsed in fresh fixative and placed under a low vacuum to remove the air from the intercellular spaces (Wang and Huang, 2003). Then the pieces were kept in 5% (v/v) glutaraldehyde in 100 mM pre-cooled phosphate buffer (pH 7.0) for 6 h. The penetration of the glutaraldehyde buffer was enhanced by placing the tissue in the fixative under vacuum. After an extensive rinsing with cold phosphate buffer (pH 7.0), the tissue cubes were post-fixed in 1% (w/v) OsO_4 overnight at room temperature. Following another extensive rinse with the same buffer, the samples were dehydrated through a graded ethanol series (30 to 100%) followed by 100% acetone, and then infiltrated for 24 h with Spurr's epoxy resin at room temperature. Polymerization was conducted at 68°C for 8 h. Ultrathin sections (approximately 60 to 90 nm in thickness) were cut by glass knife, and then mounted on 100-mesh copper grids coated with 0.3% Formvar film for the ultrastructural observations, which were conducted using a JEM-100S transmission electron microscope(JEOL Ltd., Tokyo, Japan).

Measurement of plasmodesmal density

The method for measurements of plasmodesmal density was adapted from Kempers et al. (1998) and Zhang et al. (2004). Five series of transverse ultrathin sections were prepared from the Spurr-infiltrated flesh samples, in which each group was cut at a distance of approximately 20 µM from the previous one. From each group, six pieces of ultrathin sections were picked at random and put on the 100 mesh copper grids. Five fields (each consisting of phloem and its surrounding PP) were observed from each ultrathin section. Plasmodesmata were counted at all cell interfaces, that is the interfaces between SE/CC, SE/PP, CC/PP and PP/PP in each selected field. The results of the plasmodesmal counting were given as the number of plasmodesmata per micron of specific cell/cell interface length in the transverse section, which is referred to as plasmodesmal density (Fisher and Oparka, 1996).

Statistical analysis

Five replications in each treatment were used. The comparison of pairs of values was analyzed by t-test and levels of significance were represented by *P < 0.05, **P < 0.01 and NS (not significant). SPSS was used to analyze the data.

RESULTS

Ultrastructural features of the main veins

The sieve elements of the main veins in leaves were bigger than companion cells for both treatments (Figure 2A and D). The sieve element and companion cells of main veins in leaves under root restriction were smaller in size than the control treatment (Figure 2B and C). Companion cells under root restriction in particular, have more mitochondria and vacuoles than control treatment (Figure 2B), and the companion cells under root restriction also contained some paramural bodies at the cell wall adjacent to SE and some big vesicles in their vacuoles (Figure 2B). The number of plasmodesmata between CC and SE under root restriction was more than under the control treatment, but the number of plasmodesmata between PP under root restriction was less than control treatment (Table 1). No plasmodesmata were observed between PP and SE or CC and under

Figure 1. Sampling site of the leaf used for ultrastructure determination.

both treatments, resulting in symplastic isolation between them.

Ultrastructural features of the branch veins

The size of sieve element was larger than the size of companion cells under both treatments (Figure 3A and B). The average diameters of SE and CC in branch veins were smaller under root restriction than control treatment, but cell walls were thicker under root restriction than control treatment (Figure 3C and D). No plasmodesmata between sieve elements and companion cells in branch veins were observed under either treatment, but plasmodesmata between PP were found (Table 1). There were more plasmodesmata between PP in the branch veins under the control treatment than under the root restriction treatment.

Ultrastructural features of the minor veins

In the minor veins, the sieve elements were bigger than companion cells under both root restriction and control treatments (Figure 4A and B), and the sieve elements and companion cells in minor veins were smaller under root restriction than under the control treatment (Figure 4A and B).

The companion cells under control treatment contained a dense cytoplasm with abundant mitochondria, ER and vesicles but CC in minor veins contained small vacuolar structures with fewer mitochondria and endoplasmic reticula under root restriction (Figure 4A and B). The number of plasmodesmata between CC and SE, SE and

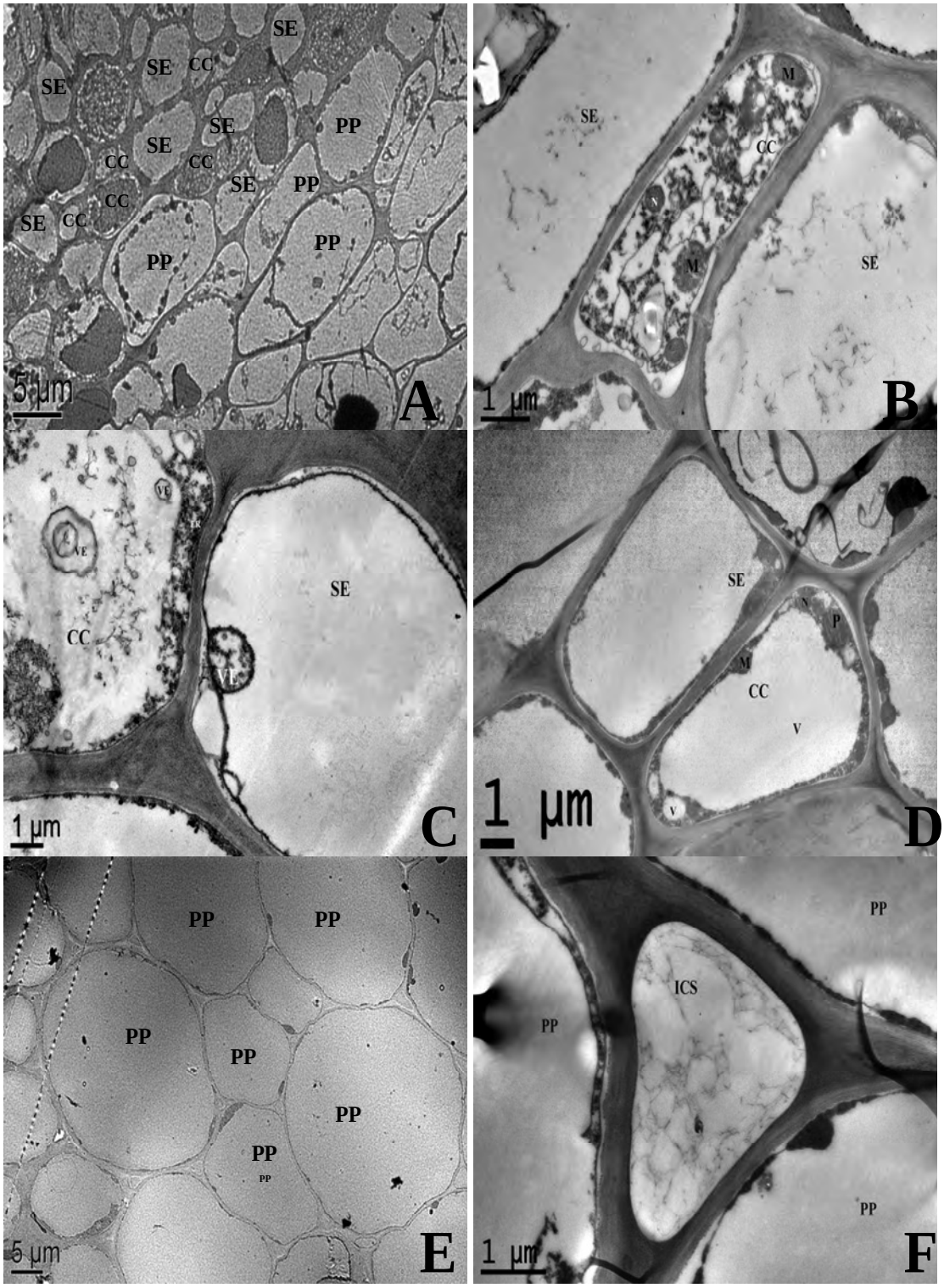

Figure 2. Ultrastructure of transverse sections of the main vein phloem in leaves subjected or not subjected to root restriction. (A) Main vein in leaf subjected to root restriction, (B) Sieve element-companion complex in leaf subjected to root restriction; (C) and (D) Sieve element-companion complex in control leaf; (E) Phloem parenchyma cells in leaf subjected to root restriction; (F) Phloem parenchyma cells in control leaf. Abbreviations: AI, aphakia; CC, companion cell; CW, cell wall; ER, endoplasmic reticulum; ICS, intercellular space; M, mitochondrion; MB, multivesicular body; N, nucleus; NCW, nacreous cell wall; NS, nacreous-walled sieve element; NU, nucleolus; P, plastid; PL, plasmolysis; PP, phloem parenchyma cell; S, starch grain; SE, sieve element; TI, tonoplast invagination; V, vacuole; VE, vesicles.

Table 1. The plasmodesmal density (number of plasmodesmata μm^{-1}) between different cell types in mature grape leaves from plants subjected to root restriction (RR) or not subjected to root restriction (control).

Parameter	Treatment	Plasmodesmal density (No. μm^{-1})			
		SE/CC	SE/PP	CC/PP	PP/PP
Main vein	Control	0.4	NO	NO	0.6
	RR	0.9	NO	NO	0.2
	Difference[z]	*	NS	NS	*
Branch vein	Control	NO	NO	NO	0.7
	RR	NO	NO	NO	0.3
	Difference	NS	NS	NS	*
Minor vein	Control	1.2	0.6	0.8	NO
	RR	1.8	0.8	1.2	NO
	Difference	*	NS	*	NS

[z]Differences determined by t-test, where *P < 0.05, NS = not significant; NO, not observed.

PP, CC and PP under root restriction was greater than under the control treatment, but plasmodesmata between PP were not observed under either treatment (Table 1).

Ultrastructural features of the phloem parenchyma

There were many more intercellular spaces among PP cells in the main veins of grape leaves from control plants, compared to those found in leaves from plants subjected to root restriction (Figure 2E and F). Severe plasmolysis appeared in PP cells in branch veins in the leaves from plants subjected to root restriction (Figure 3E and F). In contrast to the main veins, more intercellular spaces among PP cells in the minor veins occurred in leaves from plants subjected to root restriction than in controls (Figure 4C to E).

DISCUSSION

Since structure is often a meaningful guide to function, the ultrastructure of phloem in leaves is expected to yield clues to the mechanisms of phloem loading in grape vine under root restriction. Reduced photosynthesis as a result of root restriction is a well recognized phenomenon (Goto et al., 2002). Shi et al. (2008) found that the limitation to leaf photosynthesis by root restriction was mimicked by water stress. As the root restriction stress progressed, the capacity of CO_2 assimilation in the Calvin cycle was also affected (Shi et al., 2008). In our previous study, during full bloom and veraison, when vines were well watered, water potential in plants subjected to root restriction decreased rapidly during a sunny day, reaching the critical value prior to the following noon or midday, while water potential of control plants remained stable (Wang et al., 2001). Therefore, water stress occurred most days because of the reduced available water resulting from root-zone restriction. Marur et al. (1996) also reported that cotton leaves under water stress had 55% less soluble sugars, lower starch content, and reduced leaf export rates of assimilated carbon. This suggested that water stress decreased both source and sinks activities due to reduced carbon fixation and assimilate export.

The phloem of mature leaves had two overlapping functions: Loading photoassimilate from the mesophyll and exporting this material out of the lamina. In this study, CC in minor veins in leaves from control plants had a dense cytoplasm, abundant mitochondria, endoplasmic reticula, multivesicular bodies, vesicles and plastids; while in leaves from plants subjected to root restriction the CC in minor veins had cytoplasm containing numerous but small vacuolar structures with few mitochondria and endoplasmic reticula. These ultrastructural responses of phloem under root restriction were caused by biochemical and biophysical adjustments. We also found that root restriction induced damage to the chloroplasts, including thylakoid swelling and disruption of the chloroplast envelope.

Although, the responses of phloem structure to root restricted stress is not yet studied, many studies have been conducted on the response of phloem structure to other environmental stresses, such as water stress (Schurr et al., 2000) and light stress (Wang and Huang, 2003). Root restriction could induce water and nutrient stress, so under root restriction the comprehensive stresses may result in the changes of phloem ultrastructure in leaf. In this study, results showed that there were more plasmodesmata between CC/SE, SE/PP and CC/PP in minor veins in leaves from plants subjected to root restriction than those from control plants. The

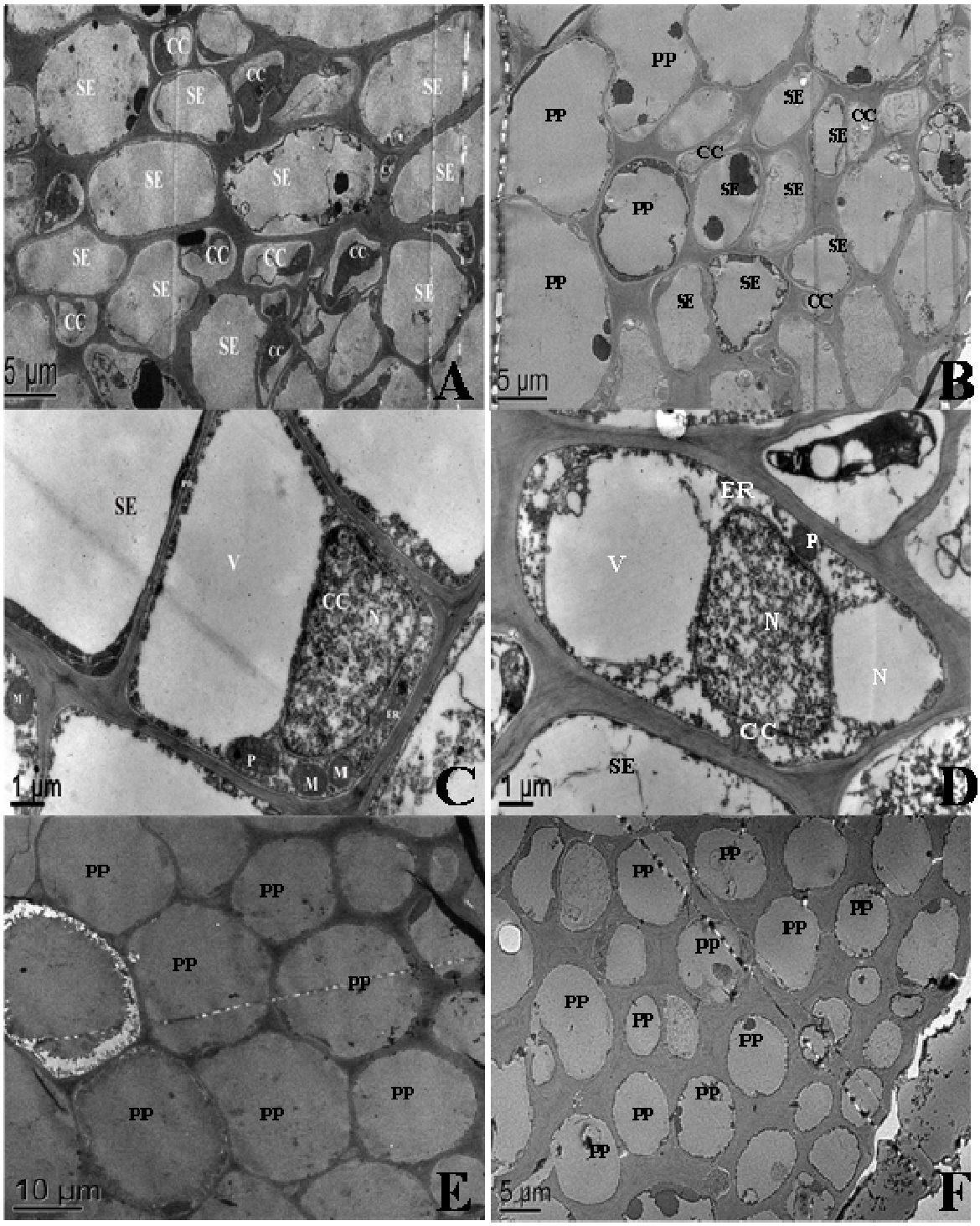

Figure 3. Ultrastructure of transverse sections of the branch vein phloem of leaves subjected or not subjected to root restriction. (A) Branch vein in leaf subjected to root restriction; (B) branch vein in control leaf; (C) sieve element-companion complex in leaf subjected to root restriction; (D) sieve element-companion complex in control leaf; (E) phloem parenchyma cells in leaf subjected to root restriction; (F) phloem parenchyma cells in control leaf treatment.

increased number of plasmodesmata in the leaves of plants subjected to root restriction meant that they had more potential of efficient symplastic loading than control leaves. In this study, the results showed that root restriction changed the ultrastructure of leaf phloem, although how these changes affected the transport of

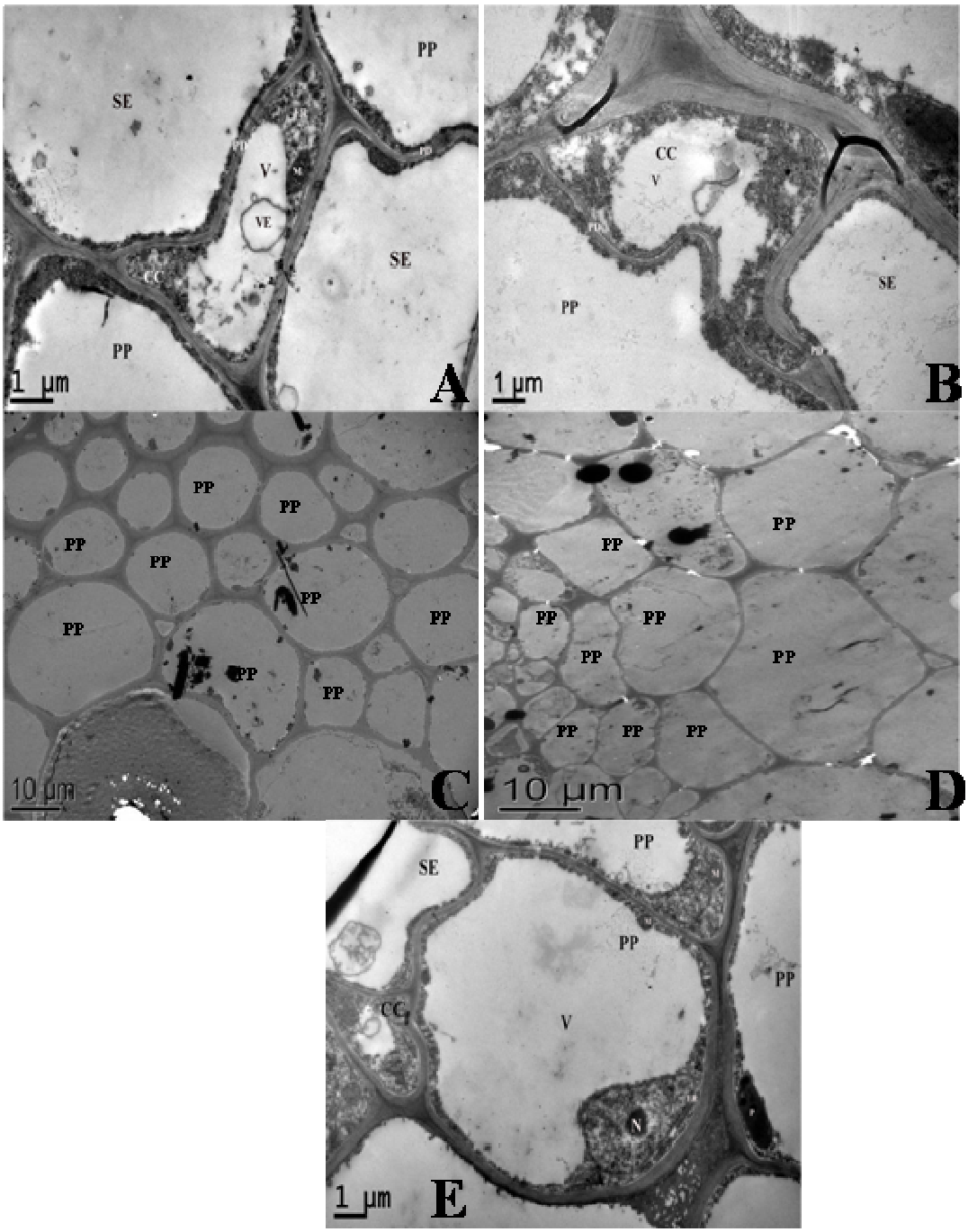

Figure 4. Ultrastructure of transverse sections of the minor vein phloem of leaves subjected or not subjected to root restriction. (A) Sieve element-companion complex in leaf subjected to root restriction; (B) sieve element-companion complex in control leaf; (C) phloem parenchyma cells in leaf subjected to root restriction; (D) and (E) phloem parenchyma cells in control leaf.

photosynthate need more research.

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