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Anatomical characteristics of young stems and mature leaves of dwarf pear

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ABSTRACT

The anatomical characteristics of young stems and mature leaves of dwarf and standard pears were compared through light microscopy (paraffin sections) and scanning electron microscopy to understand the tissue structure of dwarf pear inherited from 'Le Nain Vert' (*Pyrus communis* L.). Compared with the standard type, the dwarf type showed a larger stem cross-sectional area but a smaller wood/bark ratio. The dwarf type exhibited more cell layers in both collenchyma and cortical parenchyma than the standard type. The dwarf type also had lower vessel diameter and vessel density than the standard type. Longitudinal sections showed that the dwarf type had shorter vertical length of cortical parenchyma cells and thicker cell wall than the standard type. The cells of the cortical parenchyma and pith parenchyma of the dwarf type were deformed and loosely arranged, and numerous cavities were formed in the tissue. Furthermore, the dwarf type had thicker leaves and palisades with more cell layers than the standard type. The results of this study are essential to further elucidate the physical, biochemical and molecular mechanisms of the dwarf tree architecture.

1. Introduction

Dwarfing and closely spaced planting are important evolutions in modern fruit culture and production. Developing suitable germplasms, such as dwarfing rootstocks and dwarf cultivars, is significant for intensive cultivation and effective orchard management (Fideghelli et al., 2003). Many dwarfing apple rootstocks and dwarf cultivars are currently used in production, but these resources are limited in pear cultivation (Elkins et al., 2012). Therefore, breeding of dwarf pear cultivars or dwarfing rootstocks is urgently needed to meet the demand of modern pear production systems.

'Le Nain Vert', a French cultivar of *Pyrus communis*, originated from a chance mutated seedling (Rivalta et al., 2000; Fideghelli et al., 2003) and was released in the 1930s. This cultivar possesses compact crowns, short internodes and dwarf statures. In the 1980s, a group of dwarf pear hybrids was produced from the hybridisation of 'Le Nain Vert' with other European pears at East Malling Research Station, the United Kingdom. We introduced hybrid seeds of the dwarf pear from this institute in 1991. Several seedlings with dwarf characteristic were subsequently raised, one of which was named 'Aihuali'. In 2002, we crossed 'Aihuali' with 'Chili' (*Pyrus bretschneideri*), a main cultivar in China, and obtained a hybrid population separated into dwarf and standard phenotypes (Fig. 1) at a 1:1 ratio ($P > 0.05$). The dwarf characteristic in pear is controlled by a single dominant gene, designated as *PcDw*, and we developed several genetic markers linked to it (Wang et al., 2011). However, this gene remains unknown.

Several hypotheses have been proposed to explain the biological mechanisms, such as anatomical (Olmstead et al., 2006; Trifilà et al., 2007; Tombesi et al., 2011), nutritional (Jones, 1976; Schechter et al., 1991; Fallahi et al., 2001) and hormonal (Kamboj et al., 1999a,b; Richards et al., 1986; Sorce et al., 2002) characteristics, induced by rootstocks that led to the slow growth of scion cultivars in fruits. However, dwarf tree architecture does not represent the dwarfing ability for scions as those dwarfing rootstocks. These characteristics are separately inherited. Thus, their mechanisms are, at least to some degree, different.

Although some theories have been proposed and several indicators based on these theories, such as wood/bark ratio, palisade/spongy ratio, vessel diameter and vessel density (Kurian and Iyer, 1992; Olmstead et al., 2006; Tombesi et al., 2011; Zorić et al., 2012; Hajagos and Végvári, 2013), can be used to distinguish dwarfing rootstocks, the mechanisms of the growth restriction of dwarf fruit trees are complex and poorly perceived. To date, the

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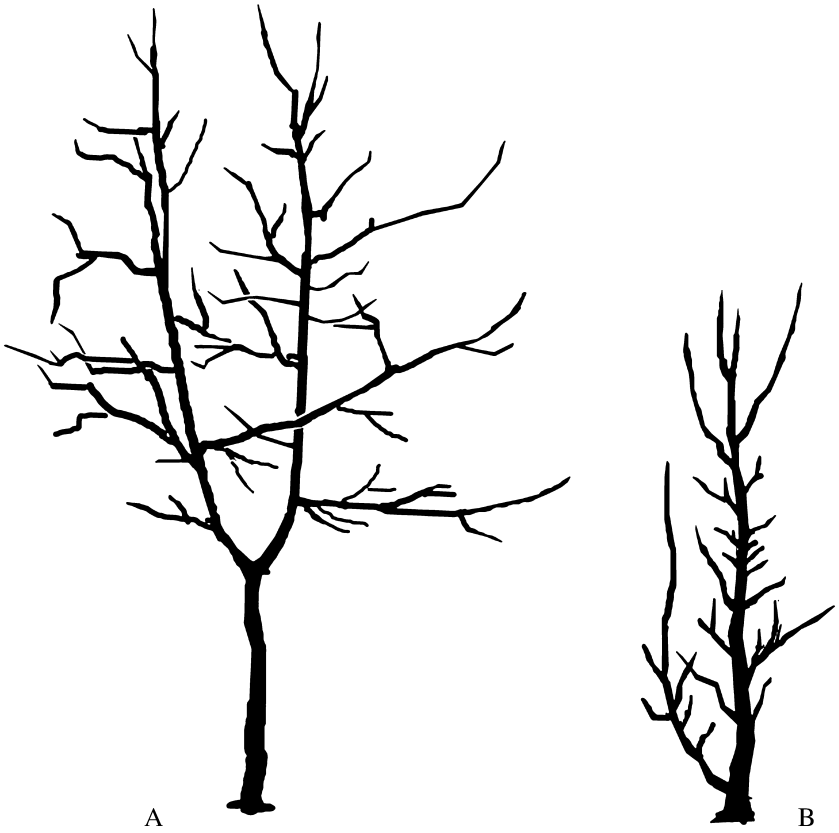

Fig. 1. Silhouettes derived from in situ photographs to display the different pear tree architectures. (A) Standard type and (B) Dwarf type.

mechanism of the dwarf tree architecture from 'Le Nain Vert' pear has not been explained. In this study, the anatomical characteristics of the young stems and mature leaves of the dwarf- and standard-type pears separated from 'Aihuali' × 'Chili' were compared to further analyse the biological mechanisms underlying the tree architecture.

2. Materials and methods

2.1. Plant materials

A hybrid pear population was created in 2002 by crossing 'Aihuali', a seedling from the European pear variety 'Le Nain Vert' (*Pyrus communis* L.), with 'Chili' (*P. bretschneideri* Rehd.), a main Chinese cultivar in pear production. This population was planted in the field at the fruit experimental station of Qingdao Agricultural University (Laiyang, Shandong). Three dwarf hybrids and three standard hybrids were randomly selected from the population for anatomical comparison. The phenotypes were easily evaluated because of the clear distinction between the tree forms.

2.2. Observation by light microscopy

Segments and mature leaves of the vigorously growing primary shoots (the fourth internode and the fourth or fifth leaf from the base) were collected from the dwarf- and standard-type hybrid plants (five segments and five leaves were sampled from each tree). Each segment was cut into a fragment of 0.5 cm long, whilst each leaf was cut into a square approximately 0.5 mm × 0.5 mm from the middle part of the leaf, with the midrib as the central axis. These samples were immersed in formalin–aceto–alcohol solution (63:30:5:2, ethanol:distilled water:acetic acid:formalin) for 24 h. Then, the samples were dehydrated in a graded series of ethanol concentrations, permeated with wax, embedded in wax and then mended as described by Purvis et al. (1964). The samples were sliced into 12 μm-thick sections using a rotary microtome (Lecia Rm 212RT; Wetzlar, Germany) and pasted on glass slides. The wax was eliminated in xylol and xylol:ethanol (1:1) for 7 min, and rehydration was performed in a graded series of ethanol concentrations (100% → 95% → 85% → 70%) for 1 min each. The sections were stained with safranin (3%) for 24 h and gently washed in increasing ethanol concentrations (70% → 85% → 95%) for 5 s each. The sections were transferred into fast green dye solution (1%) for 3 s, dipped twice into absolute ethanol for 5 s each and then transferred into xylol:ethanol (1:1) and xylol for 5 min. The slides were mounted with synthetic resin and observed and photographed under a light microscope (Nikon ECLIPSE 80i; Tokyo, Japan). By this method, lignified cells were stained with safranin dye, whereas the other cells were stained with fast green dye.

2.3. Observation by scanning electron microscopy

The sample preparation for scanning electron microscopy was the same as that for light microscopy. Segments were prepared using a rotary microtome (Lecia Rm 212RT; Wetzlar, Germany) and gently dipped in xylol for 4 h at 45 °C to remove the wax. Xylol was replaced every hour for this step. The samples were treated with xylol:ethanol (1:1) and absolute ethanol for 1 h and then transferred into tert-butyl alcohol. Finally, the segments were dried using a freeze dryer (JFD-320; Tokyo, Japan) and then metallised with gold using an ion sputtering apparatus (JFC-1600; Tokyo, Japan). Observation and micrography were performed under a scanning electron microscope (JEOL-7500F; Tokyo, Japan).