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Comparison of photosynthetic and morphological characteristics, and microstructure of roots and shoots, between columnar apple and standard apple trees of hybrid seedlings

Comparación de características fotosintéticas y morfológicas, y microestructuras de raíces y tallos, entre plantas de manzana columnares y comunes de plántulas híbridas

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Abstract. The photosynthetic and morphological characteristics, and microstructure of roots and shoots, were studied between columnar apple and standard apple trees derived from the crosses of 'Fuji' × 'Telamon' and 'Gala' × 'Telamon'. The results showed that chlorophyll A, chlorophyll B, the content of chlorophyll A+B, and leaf area index were higher in columnar apple trees than in standard apple trees. In a day cycle, the net photosynthetic (Pn) and transpiration rates (Tr) were higher in the columnar apple trees than in standard apple trees. The daily variations of Pn and Tr were of a double peak cure. Light saturation points were higher and light compensation points were lower in columnar than in standard apple trees. There were significant differences in the phenotype between columnar and standard apple trees. Columnar apple trees had fewer branches, they were very compact, and the internode length was shorter than that in the standard apple trees. The highest percentage of short-shoots in columnar apple was 73.5%. The percentage of short-shoots in standard apple trees derived from the crosses of 'Fuji' × 'Telamon' and 'Gala' × 'Telamon' was 38.4% and 41.4%, respectively. At the micro level, these two types of apple also had differences on the microstructure of roots and shoots. The duct diameter of roots and shoots was wider for columnar than standard apple. The number of ducts in roots was also higher in columnar than standard apple. However, there were no significant differences in the number of ducts on shoots of the two apple tree types. Columnar apple trees had higher photosynthetic rates and light energy utilization ratios than standard apple trees, which was beneficial to early fruiting and high yield of columnar apple.

Keywords: Columnar apple; Photosynthetic rate; Morphological characteristics; Microstructure.

Resumen. Se estudiaron las características morfológicas y fotosintéticas, y la microestructura de raíces y tallos, entre los árboles de manzana erguidos y comunes derivados de los cruzamientos 'Fuji' × 'Telamon' y 'Gala' × 'Telamon'. Las clorofilas A y B, el contenido de clorofila A+B, y el índice de área foliar fueron mayores en los árboles de manzana erguidos que en los comunes. En un ciclo diurno, las tasas fotosintéticas (TF) y de transpiración (TR) netas fueron mayores en los árboles de manzana erguidos que en los comunes. Las variaciones diarias de TF y TR tuvieron dos valores máximos. Los puntos de saturación de luz fueron mayores, y los de compensación de luz menores, en los árboles de manzana erguidos que en los comunes. Hubo diferencias significativas en el fenotipo de ambos tipos de árboles de manzana. Los árboles de manzana erguidos tuvieron menos ramas, fueron muy compactos, y la longitud del entrenudo fue más corta comparado a los árboles de manzana comunes. El mayor porcentaje de tallos cortos en los árboles erguidos de manzana fue 73,5%. Los porcentajes de tallos cortos en árboles de manzana común derivados de las cruzas 'Fuji' × 'Telamon' y 'Gala' × 'Telamon' fueron 38,4 y 41,4%, respectivamente. Estos dos tipos de árboles de manzana también presentaron diferencias en la microestructura de raíces y tallos. El diámetro de los conductos de las raíces y los tallos fue mayor para los árboles erguidos que los comunes de manzana. El número de los conductos en las raíces también fue mayor en los árboles erguidos que en los comunes de manzana. Sin embargo, no hubo diferencias significativas en el número de conductos en los tallos de los dos tipos de árboles de manzana. Los árboles erguidos tuvieron mayores tasas fotosintéticas y relaciones de utilización de energía lumínica que los árboles de manzana comunes. Esto fue benéfico para una fructificación temprana y alto rendimiento en los árboles de manzana erguidos.

Palabras clave: Manzana de árboles erguidos; Tasa fotosintética; Características morfológicas; Microestructura.

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INTRODUCTION

Columnar apple has many notable features, such as short internodes, few branches, leaf density, compact structure, and other notable features that help to maximize light interception; thus, columnar apple is the ideal plant type for high-density planting (Kesley et al., 1992). From the 1990s columnar apple was introduced into China. Since then, research began on breeding, molecular markers and associated physiological traits on this apple type (Zhang et al, 1995; Dai et al., 1998; Wang et al., 2002; Dai et al., 2003; Gao et al., 2004; Tian et al., 2005; Zhang et al., 2007). However, there are no reports to date on the morphology, photosynthetic characteristics and microstructure of roots and shoots of columnar apple. In this paper, photosynthesis characteristics, and morphology and microstructure of stems and roots of columnar apple are reported. They are compared with the general apple type (standard apple) (1) to find reasons why higher yields are obtained with columnar apple, and (2) to provide a theoretical basis for the close and dwarf planting of columnar apple.

MATERIALS AND METHODS

Materials. Materials were collected from the Fengshi gardening field at Laixi county, Qingdao city: 3-year-old seedlings of columnar apple and standard apple from two hybrids. The first hybrid was the 'Fuji' × 'Telamon', and the other was 'Gala' × 'Telamon'. They were obtained with the help of our task force at Qingdao Agricultural University.

Experimental methods. *Field observations and determination of morphological indicators.* Research was carried out at the Laboratory of fruit breeding at Qingdao Agricultural University and Fengshi gardening field, in Laixi County, from March to June 2010. Ten plants were selected each from columnar apple and standard apple to measure internode length; plant high crude ratio; ratio of long, thick branches, and the short-, medium- and long-branch numbers.

Leaf area index. Plant crown projection diameter was determined for columnar and standard apple trees. Area of single leaves was measured with a leaf area meter, and thereafter, a mean leaf area was obtained. Leaf area index was calculated according to the formula: mean leaf area × the number of leaves / crown projection area.

Determining the content of Chlorophyll. Half a gram of fresh leaves was ground, and chlorophyll was extracted with 80% acetone, little quartz and $CaCO_3$. Absorbance was detected using a UV-2100 spectrophotometer. Thereafter, chlorophyll-type A and B contents were calculated following Sun et al. (2010).

Determination of photosynthetic parameters. Three plants each of columnar and standard apple trees with the same growth potential were selected to determine photosynthetic parameters. Determinations were made with a CIRAS-1 portable photosynthesis analyzer. Measured indexes included: Net photosynthetic rate (Pn, μmol/m^2/s); Transpiration rate (Tr, mmol/m^2/s); Stomatal conductance (Gs, mmol/m^2/s); Intercellular CO_2 concentration (Ci, μmol/mol), etc. Determinations of Light response curves were determined using the following light intensities: 0, 50, 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1200, 1400 and 1600 μmol/m^2/s. Thereafter, light compensation and light saturation points were obtained through linear regression of the straight portion of the light response curve.

Root and stem microscopic observation. Selected three sections of stems and roots each from columnar apple and standard apple were placed in FAA, and taken back to the laboratory into a constant temperature refrigerator. Thereafter, samples were cut into small pieces about 0.5 cm.

A fifth of a gram of agarose was placed into a flask. Twenty milliliters of distilled water were then added and dissolved in a microwave oven to make up a 0.1% agarose solution. The above tissue fragments were embedded with agarose solution immediately and frozen. These frozen tissues were sliced with a HM525 microtome. Thickness of slices was about 16μm. They were stained with milk powder oil and 0.1% aniline blue dye. Microstructure of roots and stems were observed using a Nikon E80i microscope, and vessel diameter was measured. Ten to fifteen observation fields were taken per sample. All samples were replicated three times.

Statistical methods. SPSS 13.0 and Excel 2007 were used for data analysis.

RESULTS

Morphological comparison between columnar and standard apple. Figure 1 shows that branches of standard apple trees are slender while those of columnar apple are short and strong. Internode length was significantly lower in columnar than standard apple trees (Table 1). The number of mature branches and those with spurs were significantly different between columnar and standard apple trees. The highest ratio of spur and mature branches can get to 73.5% in columnar apple, and about 40% in standard apple. The percentages of slender branches in standard apple were 39.1% and 39.3% in both groups. These ratios in both groups of columnar apple were 19.3% for group 1 and 15.7% for group 2; differences between these groups were significant ($p<0.05$). However, differences were not significant ($p>0.05$) for medium branches.

Comparison of content of Chlorophyll and Leaf area index. Leaf contents of Chlorophyll A, Chlorophyll B and Chlorophyll A+B were significantly higher in columnar than standard apple trees (Table 2). Leaf area indexes were also higher in columnar than standard apple trees. Within group 1, for example, leaf area indexes were 12.9 times higher in columnar than standard apple trees (Table 2).

Fig. 1. Morphological comparison of columnar apple with standard apple. A – C – E: Standard apple; B – D – F: Columnar apple.
Fig. 1. Comparación morfológica entre árboles de manzana erguidos y comunes. A – C – E: Comunes; B – D – F: Columnares.

Table 1. Comparison of internodes and shoots between columnar and standard apple trees.
Tabla 1. Comparación de entrenudos y tallos entre los árboles de manzana erguidos *versus* comunes.

Combination	Type	Internode length (cm)	Number of long-shoots	Percent of long-shoots (%)	Number of middle-shoots	Percent of middle-shoots (%)	Number of short-shoots	Percent of short-shoots (%)
Group 1	Columnar	1.38±0.16B	8.9±2.8c	19.3	5.5±1.8b	11.9	31.8±8.0B	68.8
	Standard	2.18±0.36A	16.7±4.9a	39.3	8.2±1.8ab	19.3	17.6±7.5C	41.4
Group 2	Columnar	1.05±0.12B	10.4±2.8bc	15.7	7.2±5.2ab	10.8	48.8±14.4A	73.5
	Standard	2.03±1.06A	16.7±4.3a	39.1	9.6±3.7a	22.5	16.4±6.4C	38.4

Values are means ± SE. Different capital letters (A, B and C) within each column represent significant differences at p=0.01. Different lowercase letters (a, b and c) within each column represent significant difference at p=0.05.
Los valores son promedios ± EE. Letras mayúsculas diferentes (A, B y C) dentro de cada columna representan diferencias significativas a p=0,01. Letras minúsculas diferentes (a, b y c) dentro de cada columna representan diferencias significativas a p=0,05.

Table 2. Comparison of chlorophyll content between columnar and Standard apple trees.
Tabla 2. Comparación del contenido de clorofila entre los árboles de manzana erguidos versus comunes.

Combination	Type	Chlorophyll A content (mg/g)	Chlorophyll B content (mg/g)	Chlorophyll (A+B) content (mg/g)	Leaf area index
Group 1	Columnar	1.878±0.164A	0.771±0.077A	2.688±0.243A	5.82±0.98A
	Standard	1.561±0.150B	0.493±0.033B	2.157±0.115B	0.45±0.14C
Group 2	Columnar	1.291±0.159BC	0.471±0.036B	1.891±0.215BC	2.64±0.93B
	Standard	0.877±0.133D	0.302±0.060C	1.251±0.213D	0.61±0.30C

Different capital letters within each column indicate significant differences at p=0.01.
Letras mayúsculas diferentes en la misma columna indican diferencias significativas a p=0,01.

Leaves were sparser in common than columnar apple trees. Leaf area indexes were usually less than 1.0 in standard apple tree. However, columnar apple trees usually had shorter branches, a lower crown projection and higher leaf area index. This suggests that columnar apple trees can efficiently use light energy and produce higher yields.

Daily variations of leaf photosynthetic rates in columnar and standard apple. During the growing season, columnar and standard apple trees showed the same pattern of variation of photosynthetic rates (Fig. 2). Photosynthetic rates were significantly higher in columnar than standard apple trees (Fig. 2).

Plant transpiration rates were related to net photosynthetic rates (Fig. 3). The higher the photosynthetic rate, the higher the plant transpiration rate. Columnar apple trees showed a similar daily transpiration rate pattern than standard apple trees. However, diurnal variations of transpiration rates were significantly higher in columnar than standard apple tree during the growing season (Fig. 3).

Comparison of the leaf light compensation and saturation points in columnar and standard apple trees. Columnar apple trees showed higher light compensation and lower light saturation points than standard apple trees (Table 3). This indicates that columnar apple trees can resist lower light intensities than standard apple trees.

Comparison of root and stem microstructures between columnar and standard apple trees.

Root microstructure. Diameter and number of xylem vessels were larger in columnar than standard apple trees (Fig. 4). Phloem and parenchyma cells, however, showed no significant differences between columnar and standard apple trees (Fig. 4).

Fig.2. Daily variations of leaf photosynthetic rates in columnar and common apple trees.
Fig. 2. Variaciones diarias de las tasas fotosintéticas foliares entre los árboles de manzana erguidos versus comunes.

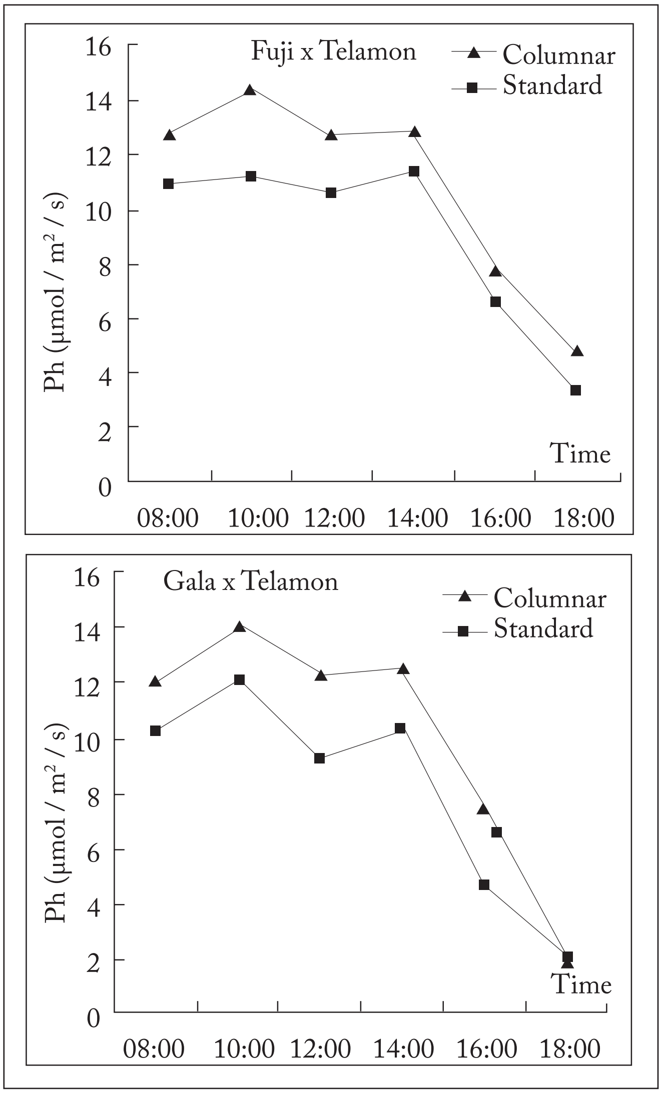

Fig.3. Daily variations of leaf transpiration rates in columnar and standard apple trees.
Fig. 3. Variaciones diarias de las tasas de transpiración foliares entre los árboles de manzana erguidos versus comunes.

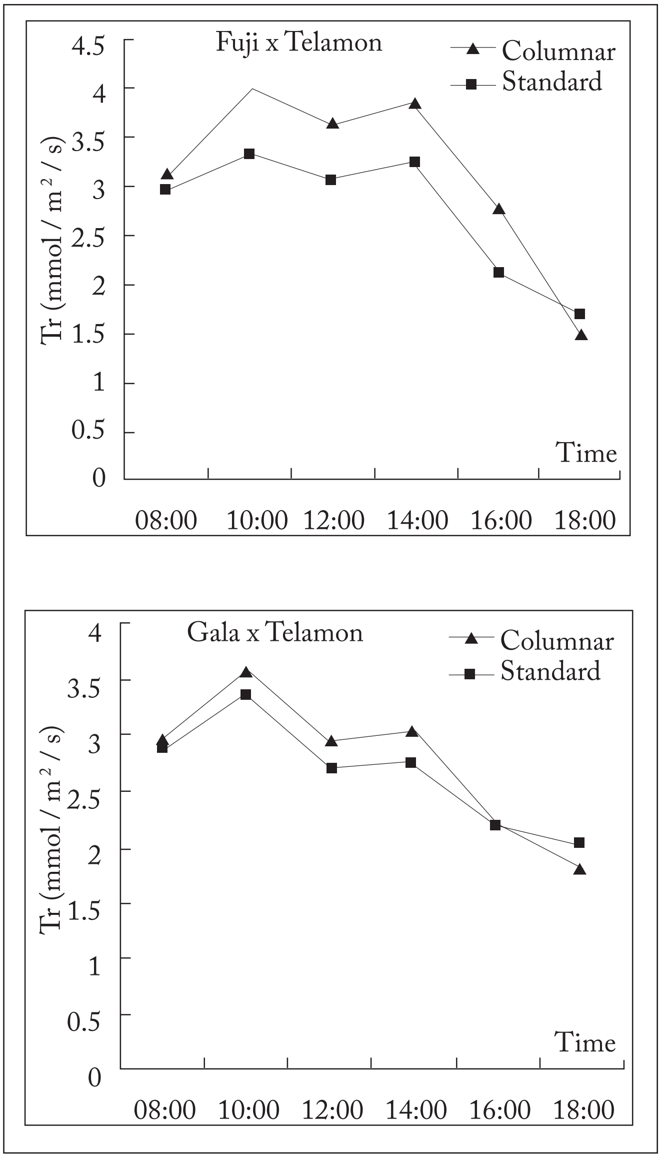

Fig. 4. Comparison of microstructure of roots between columnar and standard apple trees. A, B 'Fuji'×'Telamon' (A: Columnar apple; B: Standard apple); C, D 'Gala'×'Telamon' (C: Columnar apple; D: Standard apple).
Fig. 4. Comparación de la microestructura de raíces entre los árboles de manzana erguidos versus comunes.
A, B 'Fuji'×'Telamon' (A: Manzana columnar; B: Manzana común); C, D 'Gala'×'Telamon' (C: Manzana columnar; D: Manzana común).

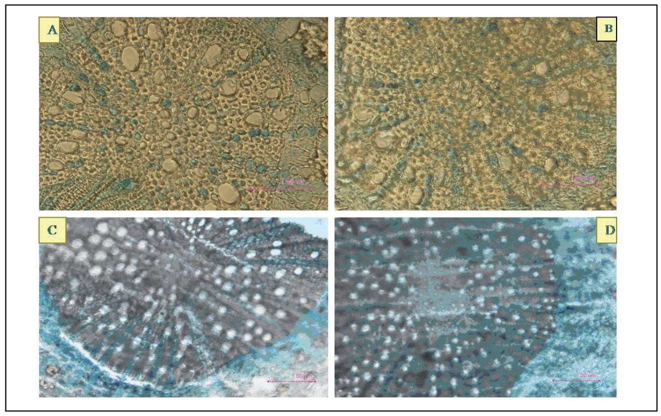

Fig.5. Comparison of microstructure of shoots between columnar apple and standard apple trees. A, B 'Fuji'×'Telamon' (A: Columnar apple; B: Standard apple); C, D 'Gala'×'Telamon' (C: Columnar apple; D: Standard apple).
Fig. 5. Comparación de la microestructura de tallos entre los árboles de manzana erguidos versus comunes. A, B 'Fuji'×'Telamon' (A: Manzana columnar; B: Manzana común); C, D 'Gala'×'Telamon' (C: Manzana columnar; D: Manzana común).

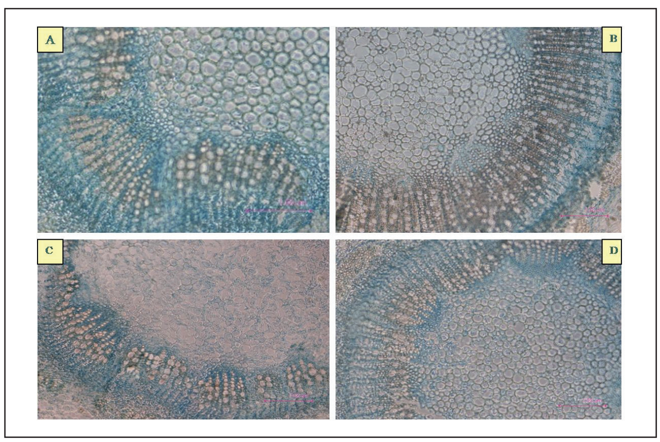

Table 3. Comparison of the compensation and light saturation points in columnar and standard apple trees.
Tabla 3. Comparación de los puntos de compensación y saturación de luz entre los árboles de manzana erguidos versus comunes.

Group	Type	Light compensation point ($mol/m^2/s$)	Light saturation point ($mol/m^2/s$)
Group 1	Columnar	1668	202
	Standard	1396	284
Group 2	Columnar	1600	215
	Standard	1192	279

Table 4. Comparison of root and shoot ducts between columnar and standard apples.
Tabla 4. Comparación de los conductos de raíces y tallos entre los árboles de manzana erguidos *versus* comunes.

Combination	Type	Number of root ducts	Diameter of root ducts (μm)	Number of shoot ducts	Diameter of shoot ducts (μm)
Group 1	Columnar	95.0±3.39a	31.26±4.413a	182.4±6.91a	17.08±3.308a
	Standard	86.2±2.39b	23.50±3.453b	180.0±6.75a	13.96±2.467b
Group 2	Columnar	96.0±5.87a	34.84±5.763a	188.6±4.62a	16.69±2.462a
	Standard	89.4±9.61b	19.91±3.251b	186.4±5.61a	12.84±1.835b

Values are means ± 1 SE. Different letters within each column represent significant differences at p=0.05.
Los valores son promedios ± 1 EE. Letras diferentes en cada columna representan diferencias significativas a p=0,05.

Stem microstructure. Diameter of xylem vessels were slightly larger in columnar than standard apple (Fig. 5).

Comparison of root and stem xylem vessels. Number and diameter of root xylem vessels were greater in columnar than standard apple trees (Table 4). However, there were no differences between types of apple trees (Table 4). Also, there were no significant differences for stem vessels between columnar and standard apple trees (Table 4).

The number of stem xylem ducts was greater than that of root xylem ducts (Table 4). However, the reverse was true between root and stem duct diameter (Table 4).

DISCUSSION

Relationship between external morphology and photosynthesis. Morphological features of branches and leaves were closely related to photosynthesis. Du et al. (2010) showed that flooding affected ultrastructure adversely, which resulted in a decreasing photosynthetic capacity of leaves.

Columnar character is one important agronomic trait different from the spur character for dwarf-close-planting. Many favorable traits were found in columnar apple trees: earlier flowering and fruit, and higher yields. Columnar apple trees had more spur branches and higher leaf area indexes. Leaf area indexes of columnar apple trees from 'Fuji'×'Telamon' reached 5.82 leaf square meters/soil square meter. This value was 12.9 times that of standard apple trees. In the offspring of 'Gala'×'Telamon' this difference also reached 4.3 times. The highest leaf area index of standard apple trees was 0.61; this indicates that columnar apple trees can quickly form a certain leaf screen. Columnar apple trees also had significantly larger and darker green leaves (Fig. 1). Other traits have also been found greater in columnar than standard apple. For example, the leaf and palisade tissue thickness have been found to have the following order: columnar > spur-type > common type (Wang et al., 2005). The stomata densities were also higher in columnar than in standard apple types (Liang et al., 2009, 2010). These specific traits of columnar apple contributed to its efficient use of energy to produce photosynthetic products, earlier fruiting and higher yields. In this study, the highest proportion of short branches in columnar apple reached 73.5%, while values in standard apple trees reached only 38.4% and 41.4%. This might be the basis that columnar apple trees have higher yields than standard apple trees (Xue et al., 2010).

Relationship between microscopic root and stem structure and photosynthesis. In the microscopic structure, root ducts were different from those of stems. Vessel diameters in roots and stems of columnar apple were higher than those of standard apple in both groups. This difference was more obvious in roots than in stems.

In this study, results showed that transpiration and net photosynthetic rates were higher in columnar than standard apple trees. This suggests that columnar apple might transport more mineral elements and water to the shoot. This would contribute to explain why vessel diameters are greater in roots than shoots. Phloem sieves were similar in columnar and standard apple trees. It is very likely that translocation of photoassimilates downward is similar in columnar and standard apple. Results of this study appear to explain the earlier flowering and maturity in columnar than standard apple tree.

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