

Comparative analysis of leaf photosynthetic characteristics and fruit sugar content in trees of *Citrus* cultivar ‘Huangguogan’ of different age

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Abstract

Citrus trees of cv. Huangguogan, 8-, 30-, and 280-year-old (8s, 30s, and 280s, respectively), were used to explore the photosynthesis and related enzyme activity. In spring shoot leaves, net photosynthetic rate (P_N) of the 280s was significantly lower than that of the 30s and 8s, but it was opposite in summer shoot leaves. P_N of the summer shoots was higher than that of the spring shoots. P_N was significantly positively correlated with stomatal conductance, intercellular CO_2 concentration, and transpiration rate, but was negatively correlated with maximum photochemical quantum yield of PSII photochemistry. The effect of age on variable fluorescence/minimal fluorescence was similar to that of minimal fluorescence, variable fluorescence, maximal fluorescence yield of the dark-adapted state. Fruits of the 280s had significantly lower sugar components than those of the 8s and 30s. Results showed that fruits of the 8s and 30s actively accumulated photosynthesis products, which was verified by the higher P_N in the spring shoots, lower acidic invertase and neutral invertase activities, and higher sucrose phosphate synthase than that of the 280s.

Additional key words: chlorophyll fluorescence; flavor; sugar component.

Introduction

Citrus is a commercially important genus of the family Rutaceae and is widely cultivated for its fruit worldwide (Biswas *et al.* 2011). Naturally occurring and cultivated hybrids include commercially important fruits, such as the oranges, grapefruit, lemons, some limes, and some tangerines. Hybrid ‘Huangguogan’ (*Citrus reticulata* × *Citrus sinensis*) has more than 300 years of cultivation history in Shimian County of Sichuan Province, China (Xiong *et al.* 2017). In recent years, the planting area of ‘Huangguogan’ has expanded rapidly in southwestern China, but the quality of ‘Huangguogan’ fruits of different ages differs.

The flavor and quality of fruits are mainly determined by sugars, organic acids, ripeness, amino acids, and aromatic compounds (Zhang *et al.* 2012). Starch and sugars are the main end products of photosynthesis, and their contents in plants provide key information related to plant productivity. The partitioning between starch and sugar is regulated in the cell in response to various stimuli inside

and outside the cell (Rosa *et al.* 2009). The physiological mechanisms controlling biomass allocation are important for plant growth performance (Poorter *et al.* 2012). Sugars, especially sucrose, are used by the plant as a carbon source for supporting growth, development, reproduction, and metabolism (Gibson 2005, Basson *et al.* 2010) and as a signal molecule to regulate the expression of genes related to photosynthesis and heterotrophic metabolism (Koch 1996, Roitsch 1999). In most of the higher plants, sugar acts similarly to hormones as it helps transition from the plants' nutritional state to regulate growth and floral transition. Soluble sugars in leaf and root, particularly sucrose and hexoses (glucose and fructose), are highly sensitive to environmental stresses, and act as cellular respiratory substrates and osmotic regulators to maintain cellular homeostasis (Roitsch and González 2004, Gupta and Kaur 2005, Mishra and Dubey 2008, Maestri *et al.* 2010, Ruan *et al.* 2010). However, the plant cannot utilize sucrose directly – sucrose must be first cleaved into hexoses by invertase (EC 3.2.1.26) (Zhang *et al.* 2014) or sucrose synthase (SS, EC 2.4.1.13) (Koch 2004, Roitsch

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Abbreviations: AI – acidic invertase; A/N-INV – alkaline/neutral invertase; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximum photochemical quantum yield of PSII photochemistry; F_v'/F_m' – actual photochemical efficiency; g_s – stomatal conductance; HK – hexokinase; INV – invertase; NPQ – nonphotochemical quenching; P_N – photosynthetic rate; PPP – pentose phosphate pathway; q_p – photochemical quenching coefficient; ROS – reactive oxygen species; SPS – sucrose phosphate synthase; SS – sucrose synthase; Φ_{PSII} – effective quantum yield of PSII.

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and González 2004). Invertases produce glucose, while sucrose synthase produces uridine diphosphate (UDP) glucose; thus, invertases form more hexoses and are thought to have a better ability to regulate gene expression and stimulate specific sugar sensors (Koch 2004).

Among the enzymes involved in sucrose metabolism, SS and invertase (INV, EC 3.2.1.26) are involved in the cleavage of sucrose, while sucrose phosphate synthase (SPS, EC 2.4.1.14) catalyzes the synthesis of sucrose (Guo *et al.* 2002). INVs are important for carbohydrate supply to sink tissues; they play a key role in regulating, amplifying, and integrating different signals, which leads to the transportation of end product of photosynthesis from source to sink (Koch 2004, Roitsch and González 2004). There are two main groups of INVs in plants, namely acidic invertase (AI) and alkaline/neutral invertase (A/N-INV) (Dahro *et al.* 2016). AI maintains tolerance mechanisms to osmotic stress (Wang *et al.* 2000), salinity (Balibrea *et al.* 2003), heat (Li *et al.* 2012), heavy metals (Huang *et al.* 2008, 2011; Xiong *et al.* 2008), and pathogen invasion (Sturm and Tang 1999). Maintaining carbohydrate balance and primary metabolic pathways is the basis for stress resistance in plants (Stobrawa and Lorenc-Plucińska 2007). The close relationship between A/N-INV and reducing sugar content is assumed to regulate and control photosynthesis and carbon metabolism in chloroplasts, respectively (Lou *et al.* 2007, Vargas *et al.* 2008, Lobo *et al.* 2015), and thus supply some energy products to repair injury from abiotic and biotic stress (Vargas *et al.* 2007, Martín *et al.* 2013). In addition, INVs are considered to be closely related to hexokinase (HK), which provides phosphorylates glucose to regulate ROS balance (Foyer and Shigeoka 2011, Xiang *et al.* 2011, Keunen *et al.* 2013). In chloroplasts, hexokinase-derived glucose-6-phosphate participates in the oxidative pentose phosphate pathway (PPP), an important mechanism to control the removal of H₂O₂ (Zeeman *et al.* 2004, Giese *et al.* 2005). Compared with other photosynthetic enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is considered to be inefficient, which may be due to its low saturation, low CO₂ fixation rate, and low affinity for CO₂ (Young *et al.* 2016). Therefore, it is generally believed that photosynthetic enzymes serve important functions in determining fruit quality, especially in terms of sucrose metabolism.

The nature and concentration of sugar, organic acid, cellulose, and secondary metabolites are dynamically changed during fruit development, and largely affect taste characteristics and organoleptic quality (Patel *et al.* 2015, Dong *et al.* 2019). Sugar, which is mainly in the form of sucrose, fructose, and glucose, plays an key role in the quality and flavor, and the differences in sugar content determine the sweetness and color of fruit (Dai *et al.* 2016). Previous research has found that xylose and arabinose were the main sugar component in early fruit development, whereas fructose, glucose, and sucrose showed a significant increase during fruit maturation (Deng *et al.* 2019). The ripening process of citrus fruits is usually accompanied by the accumulation of sugars and the degradation of organic acids (Liu *et al.* 2007). Stomatal closure, lower chlorophyll

(Chl) content and altered Rubisco activity are considered the main limiting factors for P_N , carbohydrate synthesis, and consequent sugar accumulation (Ye *et al.* 2019).

The importance of sucrose metabolism in the development of plants has been studied with regard to the pathway of sugar signaling (Koch 2004). However, functional analysis of the six enzymes in the current study has been limited and studies regarding the effects of different ages on the photosynthetic characteristics of citrus trees are scarce. Therefore, we sought to understand differences in photosynthesis and its end products and related enzyme activities in 'Huangguogan' trees of various ages. We examined quantitative changes in important carbohydrates through measures of photosynthetic activity and Chl fluorescence and related enzymes in leaves of 'Huangguogan' trees of different ages during fruit growth as well as sugar contents in ripe fruits. Here, we present our analysis of the relationship between the net photosynthetic rate (P_N) and other measures of photosynthetic gas-exchange parameters in 'Huangguogan' leaves. This study aims to examine the effects of different tree ages on the sugar accumulation mechanism of citrus and provide a scientific basis for further study on photosynthetic physiology of citrus trees of different age.

Materials and methods

Plant materials: All the field-grown 'Huangguogan' trees were cultivated in the National Agricultural Science and Technology Park of Ya'an, Sichuan Province, China. The canopy size, tree vigor of different ages 'Huangguogan' trees was basically the same. Stands of 8-year-old (8s), 30-year-old (30s), and 280-year-old (280s) 'Huangguogan' trees were maintained under the same conditions (*i.e.*, fertilization, soil management, disease control, and pruning) as described by Liao *et al.* (2016) more than five years. Each group had four replicates, so the total number of the experimental 'Huangguogan' plants was 12 (3 groups × 4 replicates), all the planting spacing was 4 × 5 m.

Gas exchange and Chl fluorescence measurements: Net photosynthetic rate (P_N), transpiration rate (E), intercellular CO₂ concentration (C_i), stomatal conductance (g_s), and the Chl fluorescence were monitored by portable photosynthesis measuring instrument (LI-6400, Licor, Lincoln, NE, USA). The photosynthetic active radiation and CO₂ concentration were 1,200 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$ and 400 $\mu\text{mol}\text{mol}^{-1}$, respectively. g_s and C_i were determined at a saturated light intensity of 1,000 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$, and 70% relative humidity. On the periphery and middle of the tree canopy (about 1.8 m above the ground), the 4th leaves from top to bottom on the spring and summer shoots of the current year were selected randomly for each replicate during the fruit-expanding period (10 September 2018). The following Chl fluorescence parameters were measured using a LI-6400 infrared gas analyzer (Licor, Lincoln, NE, USA), *i.e.*, minimal fluorescence yield of the dark-adapted state (F_0), maximal fluorescence yield of the dark-adapted state (F_m), minimal fluorescence yield of the light-adapted state (F_0'),

maximal fluorescence yield of the light-adapted state (F_m'), steady-state fluorescence yield (F_s), and electron transport rate (ETR). The maximal quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$, effective quantum yield, $\Phi_{PSII} = (F_m' - F_s)/F_m'$, photochemical quenching coefficient, $q_p = (F_m' - F_s)/(F_m' - F_0)$, and nonphotochemical quenching, $NPQ = (F_m - F_m')/F_m'$ were calculated according to the methods of Xie *et al.* (2018).

Enzyme extraction and activity analysis: In total, 24 samples of the 4th to 6th leaf from top to bottom of current-year spring and summer shoots were selected randomly from each replicate at 9:00 h, immediately frozen in liquid nitrogen, and stored at -80°C until analysis. Photosynthetic metabolizing enzymes were prepared from frozen tissues as described by Zhang *et al.* (2011b). AI, NI, SPS, SS, Rubisco, and HK activities were assayed using the Plant AI, NI, SPS, SS, Rubisco, and HK ELISA test kits (Shanghai BOYE Biology Science and Technology Co. Ltd., Shanghai, China) according to the manufacturer's instructions.

Sugar composition: In total, 24 fruit samples were randomly selected from each replicate at 9:00 h when fruits were ripe (25 March 2016), immediately frozen in liquid nitrogen, and stored at -80°C until analysis. Glucose, fructose, and sucrose contents were analyzed using high performance liquid chromatography (LC-1260, Agilent Technologies, Sacramento, CA, USA) according to the methods of Zhang *et al.* (2012). Samples were isolated with Innoval NH_2 column (4.6×250 mm, $5 \mu\text{m}$, Agela Technologies, Tianjin, China) at room temperature. The HPLC experiment was completed using a mobile phase [acetonitrile:water = 80:20, v/v], 20- μL sample volume, flow rate of 1 mL min^{-1} , column temperature of 30°C , and detection temperature of 40°C .

Statistical analysis: All experiments were performed in triplicate ($n = 3$). The data were analyzed using Duncan's multiple range test in the XLSTAT program (version 2010)

at the $P < 0.05$ level of significance.

Results

Photosynthesis and Chl fluorescence: The P_N , g_s , C_i , and E of leaves taken from the spring shoots of the 280s was significantly lower than those from the 30s and 8s, respectively (Fig. 1), but the opposite trend was observed in the summer shoots. There was no significant difference in P_N , g_s , C_i , and E between 30s and 8s both in spring and summer shoots.

Regarding the indicators of Chl fluorescence, F_0 , F_m , and F_v in the leaves of the 280s from both spring and summer shoots were significantly higher than those of the 30s and 8s (Fig. 2A–C). There were no significant differences between leaves of different ages of ‘Huangguogan’ trees in terms of F_v/F_m and F_v/F_m' (Fig. 2D,F). The effect of age on F_v/F_0 was similar to trends seen in F_0 , F_v , and F_m individually; specifically, F_v/F_0 was significantly higher in the leaves of the 280s than those of other ages (Fig. 2E).

The relationships between P_N and g_s , C_i , E , and F_v/F_m are demonstrated in Fig. 3. P_N was positively and significantly correlated with g_s ($R^2 = 0.8839$), C_i ($R^2 = 0.4249$), and E ($R^2 = 0.9414$), but negatively correlated with F_v/F_m ($R^2 = 0.0677$). Our results indicate that g_s , C_i , E , and F_v/F_m accounted for 88.4, 42.5, 94.1, and 6.8%, respectively, of the change in P_N .

Photosynthesis-related enzyme activities: The activities of AI, neutral invertase, SS, SPS, Rubisco, and HK in leaves were all significantly related to the tree age (Fig. 4). The AI, NI, and HK activities of leaves from the spring shoots were higher than those from the summer shoots, but SPS and Rubisco showed the opposite trend. Interestingly, the SS activity of leaves from the spring shoots was higher than that of leaves from the summer shoots in the 280s, but this trend was reversed in the 30s and 8s.

Sugar components: In the current study, we measured fructose, glucose, and sucrose contents of ‘Huangguogan’

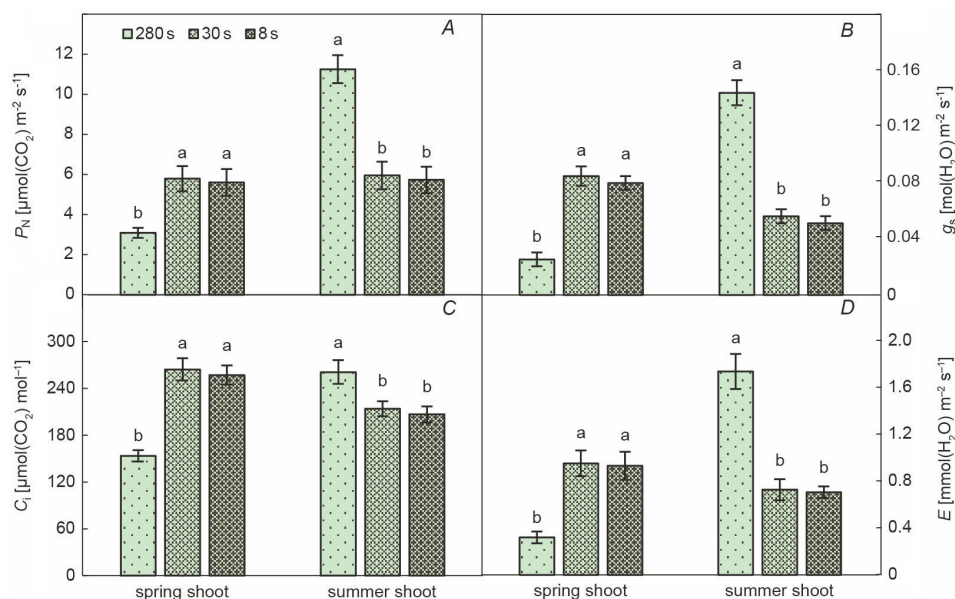


Fig. 1. Changes in the net photosynthetic rate, P_N (A), stomatal conductance, g_s (B), intercellular CO_2 concentration, C_i (C), and transpiration rate, E (D) in the leaves of ‘Huangguogan’ trees of different ages. Values in each column with different letters are significantly different at $P < 0.05$. Data are provided as mean \pm SD of three independent measurements.

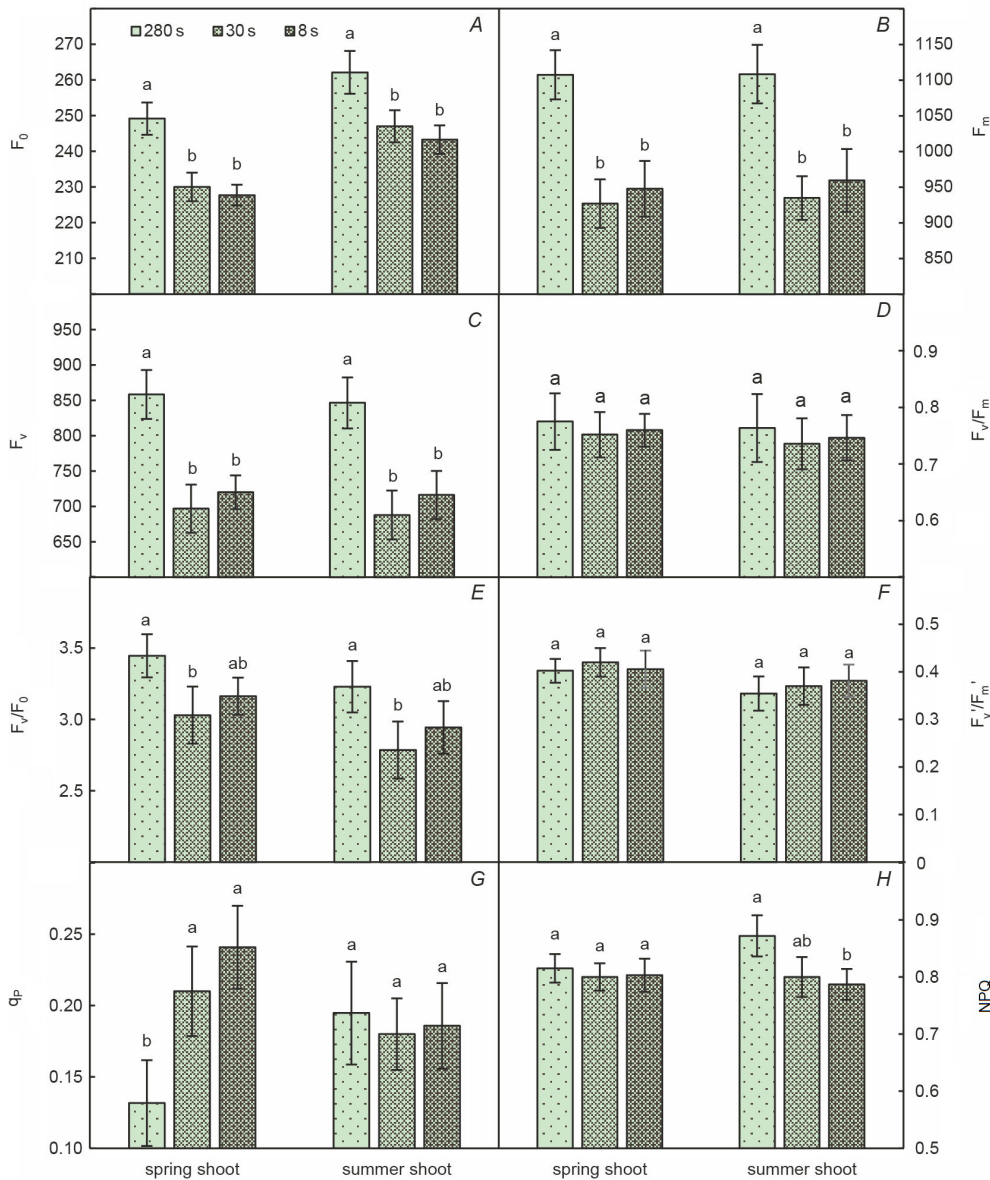


Fig. 2. Chlorophyll fluorescence characteristics of the leaves of 'Huangguogan' trees of different ages. (A) F_0 , minimum chlorophyll fluorescence yield obtained from dark-adapted leaves. (B) F_m , maximum chlorophyll fluorescence yield obtained from dark-adapted leaves. (C) F_v , variable fluorescence of the dark-adapted samples. (D) F_v/F_m , maximal photochemical efficiency of PSII in the dark. (E) F_v/F_0 , potential activity of PSII. (F) F_v'/F_m' , photochemical efficiency of PSII in light. (G) q_p , photochemical quenching. (H) NPQ, nonphotochemical quenching. Different letters above the bars indicate significant differences at the 5% probability level.

fruits when fruit was ripe. Sucrose was the predominant sugar in ripe 'Huangguogan' fruits from the 8s and 30s, but not the 280s. Compared to fruits from the 30s and 8s, fructose, glucose, and sucrose contents were all significantly lower in fruits from the 280s. There was no significant difference between the fruits from the 30s and 8s (Fig. 5A–C). Diametrically, the sucrose/hexose ratio of 280s was lower than that of 30s and 8s, but had no significant effect on sucrose/hexose ratio (Fig. 5D).

Discussion

In the current study, there were major differences in

'Huangguogan' fruit quality with the increasing tree age, especially with regard to sugar contents. Several physiological characteristics and processes may affect P_N , i.e., light, temperature, water quality, soil, and abiotic stresses (Marsic *et al.* 2018, Ohnishi *et al.* 2019, Jahan *et al.* 2020), and these factors can interact to limit photosynthesis and to increase photooxidative damage in sensitive plants (Paudel *et al.* 2016). The accumulation of photosynthetic products is the result of g_s , C_i , E , and chlorophyll fluorescence (Song *et al.* 2011). P_N is the most representative photosynthetic characteristic of plants, and its value directly reflects photosynthetic capacity (Sicher and Bunce 2001). C_i is an important indicator of stomatal activity. In photosynthesis,

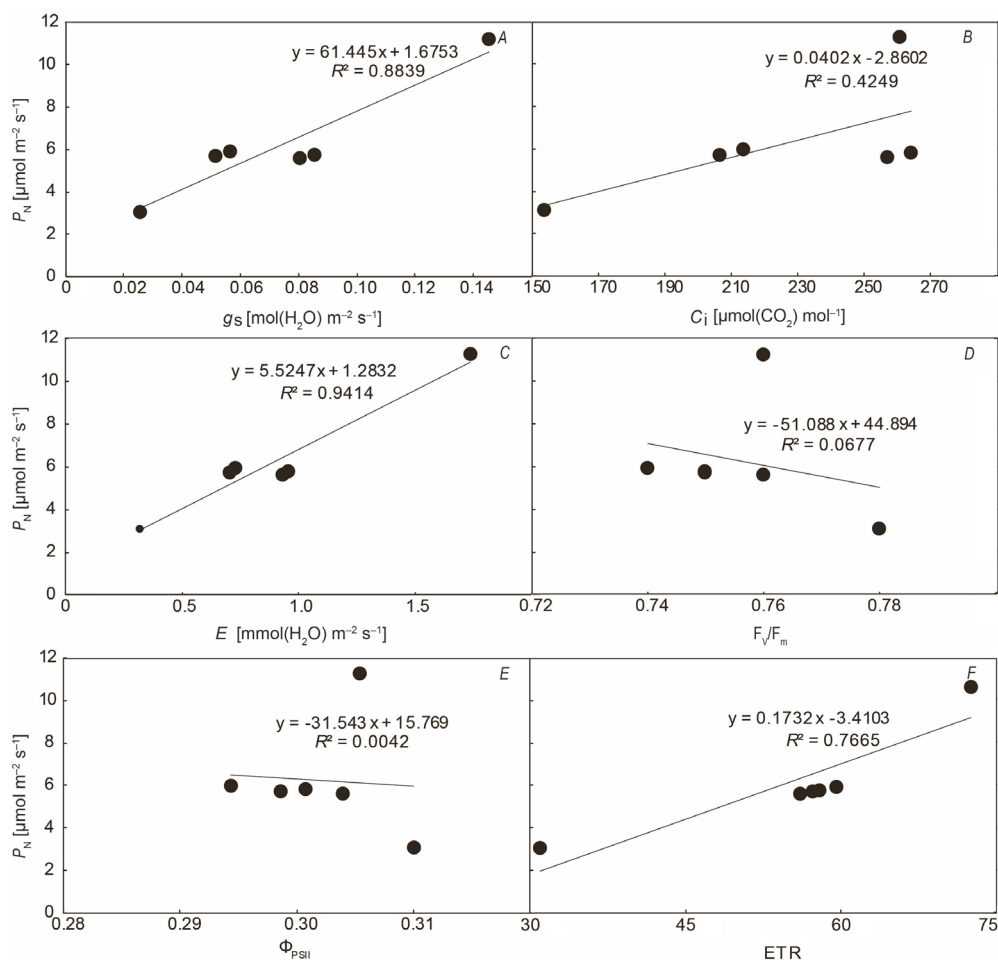


Fig. 3. Linear correlation between net photosynthetic rate (P_N) and stomatal conductance, g_s (A); intercellular CO_2 concentration, C_i (B); and transpiration rate, E (C); maximal photochemical efficiency of PSII in the dark, F_v/F_m (D); effective quantum yield of PSII, Φ_{PSII} (E); electron transport rate, ETR (F).

a decrease in E caused by a decrease in g_s leads to a significant decrease in transpiration, which can slow down the absorption and transportation of water and nutrients (Gao *et al.* 2016). In the present study, the P_N , g_s , C_i , and E values of leaves from the summer and spring shoots of ‘Huangguogan’ trees of different ages showed the same patterns of variation. The P_N of leaves from the 280s were significantly lower than those of the 30s and 8s in the spring shoots, indicating that stomatal closure and altered Rubisco activity are probably the main limiting factor for P_N . Lower P_N led to less sugar accumulation with increasing tree age (Figs. 1, 4). This phenomenon is consistent with previous research results (Yousuf *et al.* 2017, Ye *et al.* 2019). However, the trend was reversed in the summer shoots (Fig. 1), and there were no significant differences in leaves from the spring and summer shoots taken from the 30s and 8s. Among ripe fruits, the fructose, glucose, and sucrose contents in the 280s were the lowest, and these values were significantly different from those of the 30s and 8s (Fig. 5). These results indicated that leaves of the spring shoots might be the main organ for the accumulation of photosynthetic products in ‘Huangguogan’.

Chl fluorescence reflects the changes in thylakoid membrane function and organization, as well as the inhibition of photosynthesis and oxygen evolution through interactions with PSII components (Hussain and Reigosa 2011). F_0 reflects fluorescence when the reaction centers of PSII are all open, and an increase in F_0 indicates initial phase of injury to PSII (Kitajima and Butler 1975). F_0 was significantly higher in the 280s than that in the other two age groups. It is possible that increased F_0 values were due to the disassociation of LHCII and the PSII reaction center in swollen thylakoids (Tsai *et al.* 2019). F_v/F_m represents the conversion efficiency of primary light energy (Guidi *et al.* 2007) and the potential maximum photosynthetic capacity (Zhou *et al.* 2016). The effect of different tree ages on F_v/F_0 was similar to that on F_0 , F_v , and F_m . F_v/F_0 was significantly higher in the 280s than that in the other two groups (Fig. 2E). In contrast, previous studies have found that there is a strong correlation between F_v/F_m and F_v/F_0 (Sharma *et al.* 2014). The result of this study indicated that F_v/F_0 was more sensitive to the increasing age of citrus plant than F_v/F_m . There were no significant differences between the leaves of the 30s and 8s in any of the Chl fluorescence

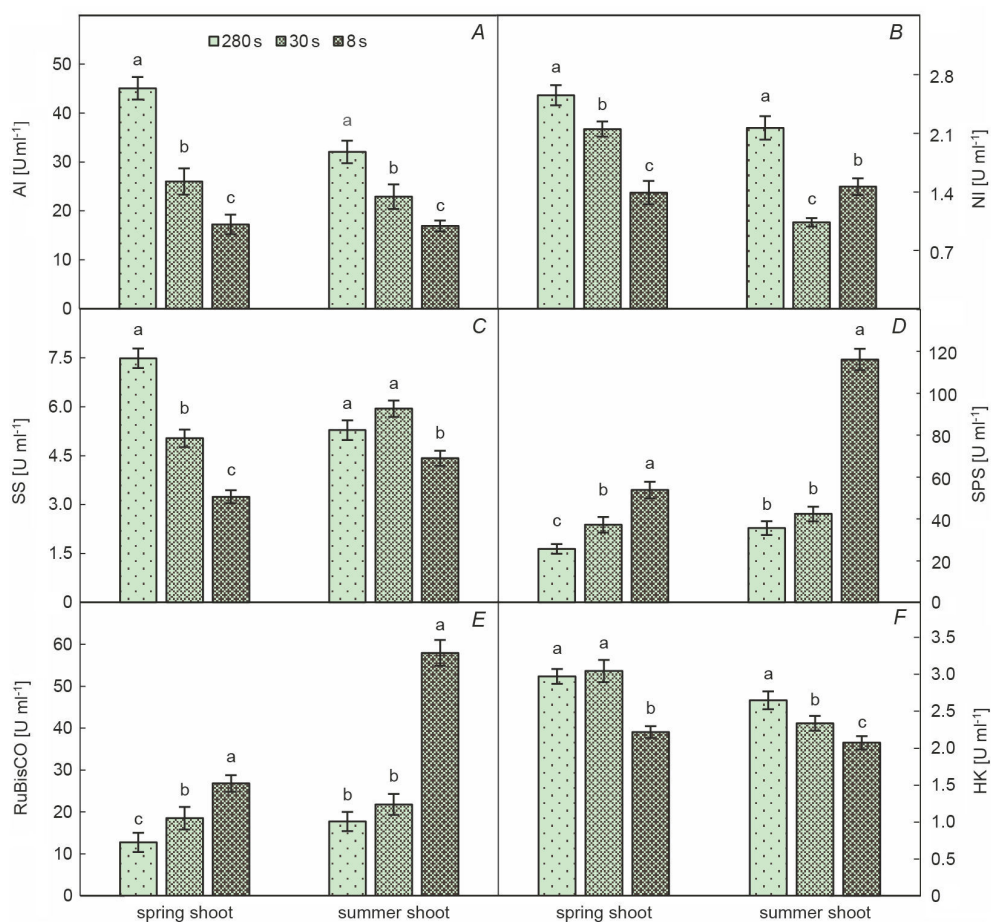


Fig. 4. Photosynthesis-related enzyme activities in spring and summer shoots of ‘Huangguogan’ trees of different ages. (A) acidic invertase, AI. (B) neutral invertase, NI. (C) sucrose synthetase, SS. (D) sucrose phosphate synthase, SPS. (E) ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco. (F) hexokinase, HK. Different letters above the bars indicate significant differences at the 5% probability level.

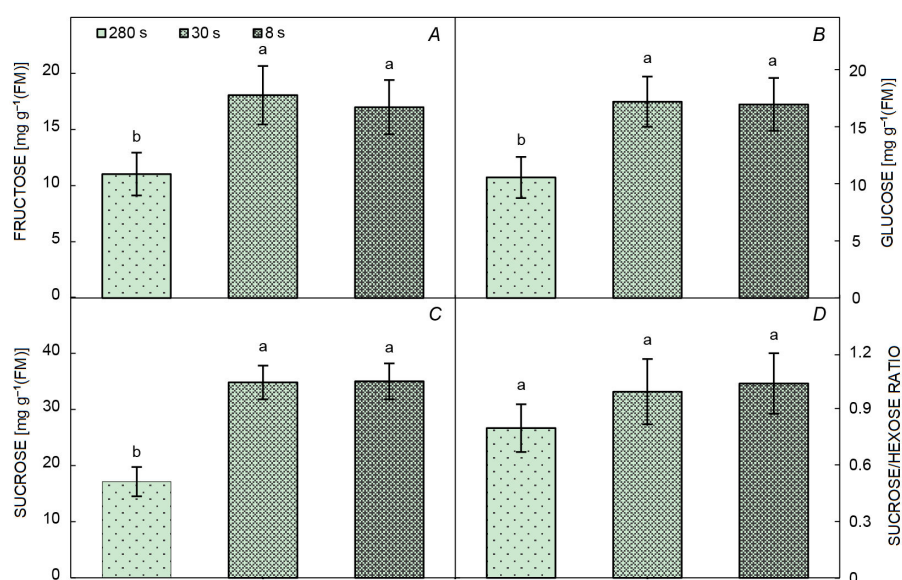


Fig. 5. Fructose (A), glucose (B), and sucrose (C) contents and sucrose/hexose ratio (D) in the fruits of ‘Huangguogan’ of different ages. Different letters above the bars indicate significant differences at the 5% probability level.

parameters in the spring and summer shoots (Fig. 2). This suggests that the photochemical activity of leaves was inhibited to a certain extent by older tree age. However, 'Huangguogan' has relatively high photochemical activity compared to that of other trees, which may have caused the photosynthetic carbon assimilation rate maintained at a relatively high level, especially in the summer shoots. Our analysis of indices related to photosynthesis showed that the leaves of the 8s and 30s exhibited higher q_p and F_v'/F_m' , but lower F_0 , F_v/F_m , and NPQ compared to those of the 280s.

Based on these results, when compared to 280s, the 8s and 30s were more effective in utilizing chlorophyll and activating q_p , but less effective in utilizing NPQ, as suggested in previous studies (Allahverdiyeva *et al.* 2005, Aldea *et al.* 2006, Murchie and Lawson 2013). The q_p reflects the share of the absorption of light energy by PSII antenna pigment molecules for photochemical electron transfer. NPQ reflects the proportion of energy absorbed by the PSII reaction center that cannot be used for photosynthetic electron transport, but is dissipated in the form of heat energy (Zhang *et al.* 2011a). We found that q_p was the lowest in the leaves of spring shoots of the 280s, and NPQ slightly increased in these leaves. NPQ can dissipate excess light energy in the form of heat energy, thereby protecting photosynthetic organs from light damage (Chen *et al.* 1995).

As reported by other researchers, sugars, acidity, and aroma are all closely related to fruit quality; overall fruit quality is determined by the total sugar content and composition (Keutgen and Pawelzik 2008). Sucrose is the main component of sweetness, and is transported through sieve elements, and then directly enters into the sink organs through the plasmodesmata or apoplastic space (Zhang *et al.* 2011b, Pressman *et al.* 2012). Sucrose and its main components, glucose and fructose, are some of the most important sugars for the process of carbon assimilation, during which these sugars are transported into sink tissues, such as fruits, shoots, and other tissues (Itai and Tanahashi 2008). In the present study, the fructose and glucose contents of fruits from the 30s and 8s were nearly equal and were the primary sugars in the ripe fruits of these trees. The fructose and glucose contents of the 30s and 8s were also clearly different from those in fruits from the 280s. The differences in fructose and glucose contents, and the sucrose/hexose ratio were associated with changes in AI, NI, SPS, SS, Rubisco, and HK activities. As reported previously, these results suggest that A/N-INV plays an important role in sucrose metabolism (Dahro *et al.* 2016). In addition, NI may increase a fruit sink strength by increasing hexose concentrations (Zhang *et al.* 2012). In plant tissues, SS activity is related to the direction of reversible sucrose cleavage, it has a high demand for carbohydrate biosynthesis and respiratory pathways as well as the synthesis of cellulose and callose (Koch 2004). SS plays a key role in the process of sucrose breaking into UDP-glucose and the possible conservation of energy through activation of the hexose moiety by UDP (Verma *et al.* 2011). In many species, SS plays a very important part in regulating sink strength (Carlson *et al.* 2002). In

the current study, fruits of 8- and 30-year-old trees actively accumulated the end products of photosynthesis, which was verified by the higher P_N of leaves in the spring shoots, more stable Chl fluorescence imaging, lower AI and NI activities, and higher SPS activity relative to the fruits of 280-year-old trees.

The present results show that P_N was positively and significantly correlated with g_s , C_i , and E , but was negatively correlated with F_v/F_m (Fig. 3). This indicated that, with increasing tree age, stomatal limitation became increasingly important limiting factor for CO_2 assimilation. This result coincides with those of Hou *et al.* (2014). The linear relationship between P_N and Φ_{PSII} was negative, and the linear relationship between P_N and ETR was positive (Fig. 3E,F). Although the Φ_{PSII} was negatively correlated with P_N , there was no significant difference in the Φ_{PSII} at different tree ages. All the results proved that stomatal closure and altered Rubisco activity were probably the main limiting factor for P_N with the increasing of Huangguogan plant age.

Conclusions: This study investigated the effects of tree age on photosynthesis, chlorophyll fluorescence, photosynthesis-related enzymes, and fruit sugar content in *Citrus* 'Huangguogan'. With increasing tree age, there was some inhibition of photosynthesis related to the decrease in P_N caused by changes in g_s , C_i , E , and chlorophyll fluorescence. In addition, this study revealed that the photochemical activity of leaves was inhibited to a certain extent by the old age. However, the relatively high photochemical activity of 'Huangguogan' may cause the photosynthetic carbon assimilation rate to be maintained at a relatively high level, especially in the summer shoots. Finally, stomatal limitation was a limiting factor for carbon assimilation in the older trees. The differences observed in fructose and glucose contents and the sucrose/hexose ratio were accompanied by dramatic differences in AI, NI, SPS, SS, Rubisco, and HK activities. Our results provide some preliminary data for elucidating the physiological and biochemical effects of age on citrus. Therefore, the molecular mechanisms by which tree age affects photosynthesis in citrus should be investigated in the future.

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