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COMPARISON OF DYNAMIC CHANGES IN ENDOGENOUS HORMONE LEVEL, WATER CONTENT AND WATER-SOLUBLE SUGARS IN *CAMELLIA OLEIFERA* OF DIFFERENT AGES

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Abstract

The aim of this study is to investigate and understand the dynamic changes and the possible functions of hormones, water and sugar content during the development of *C. oleifera*. We analyzed and compared WWC, WSS and five endogenous hormones (DHZR, ZR, GA, IAA and ABA) contents between May and August in *C. oleifera* plants at different ages from the same orchard. The results showed that WWC, WSS, DHZR, ZR, GA, IAA and ABA contents varied from 61.64%-74.15%, 21.24mg/g-62.44mg/g, 6.237ng/g-38.342ng/g, 6.215ng/g-16.721ng/g, 5.668ng/g-18.024ng/g, 24.753ng/g-147.507ng/g and 66.541ng/g-156.212ng/g, respectively. WWC showed a decrease-increase-decrease variation pattern, while the WSS contents demonstrated a generally increasing trend with increasing tree age. The levels of ABA in leaves sampled in August was higher than that in May, but the opposite was found for the GA and IAA levels. Tree ages were negatively correlated with ZR and GA concentration at the 1% or 5% significance level. The results of this study broaden our understanding of the interrelationships between phytohormones, WWC, and WSS content in the growth and development of *C. oleifera*.

Key words: *C. oleifera*; Tree age; Water content; Water-soluble sugar content; Endogenous hormone levels.

Introduction

Camellia oleifera Abel originates from China and is an edible oil species with a high antioxdative value. It is also a cash crop in southern China and can help a lot of people in mountain areas to keep them out of poverty. In recent years, *C. oleifera* has been planted in large areas. The transition from vegetative to reproductive growth of *C. oleifera* proceeds in response to environmental factors, which can cause changes in the levels of endogenous hormones, water and soluble sugar content.

C. oleifera, a member of the botanical family Theaceae, is a broad-leaved evergreen shrub that originated in China. It is one of the world's four woody edible-oil trees. Compared to other old species such as palm, olive and coconut, *C. oleifera* has great significance to the security of the grain and oil. It is mostly planted in mountainous regions (Ma *et al*., 2011; Shu, 2013; Zhang *et al*., 2007). It can not only reduce the high demand of edible oil supply, but also can replace the arable land for growing grain (Wu *et al*., 2015). The area devoted to cultivation of *C. oleifera* in China is ~3.67 $\times 10^{10}$ m^2. Therefore, *C. oleifera* is important to the economy of southern China. *C. oleifera* is widely distributed in 18 provinces in southern China such as Guangxi, Yunnan, Fujian, Hunan, and Jiangxi, but it is less common in the northern parts of South East Asia. As a tree species, *C. oleifera* not only has a high economic value, but also plays an important role in biological fire resistance, water and soil conservation, and ecological environmental improvement. Considering the importance of *C. oleifera*, ways to improve and enhance the fruit and oil yield are essential. Therefore, it is very important to to study some of the chemical factors that influence the development of *C. oleifera* such as endogenous hormones, water, and water-soluble sugar content.

Water is essential to plant life and is a very important ecological factor during plant growth and development. Furthermore, water can affect plant secondary metabolism processes and the accumulation of effective components step by step (Gao and Liang, 2004). Sugars are important primary metabolites which act as a main source of energy, precursors for anabolic reactions and in signaling (Hedrich *et al*., 2015). Sugars show an identical pattern throughout maturation (Liu *et al*., 2008). Plant hormones are active at extremely low concentrations, and they interact with specific target tissues to induce specific physiological responses, such as coordinating growth, development, and responces to environmental stimuli (Han *et al*., 2011). The plant hormones GA, IAA, ABA, DHZR, and ZR play significant roles in cell division, cell growth and elongation (Liu *et al*., 2008; Wang *et al*., 2006). These hormones therefore play vital roles in the regulation of plant development. ZR and DHZR are the most active cytokinins, and the mechanism by which they affect the formation and thickening in plants, such as the sweet potato, involves promotion of cell division, suppression of cell death and enhancement of cell expansion (Matsuo, 1983).

C. oleifera mainly grows vegetatively during the juvenile state. Vegetative growth dominates in the early fruiting stage (about 4 years). *C. oleifera* not only shows vegetative growth, but also reproductive growth as it enters the fruiting stage. At this time, vegetative growth and reproductive growth are interdependent and influence one another (Pan, 2006; Tan, 2013). Plant reproductive growth is based on the nutrition growth, and the vegetative growth is the premise of the reproductive growth of plant. Flowering is usually associated with changes in hormone and sugar levels. The transition to flowering often proves to be very challenging in individual development research. The transition from vegetative growth to reproductive development proceeds in response to environmental factors. These factors often exert inductive effects through changes in hormone and sugar levels (Liu *et al*., 2008; Wang *et al*., 2008).

C. oleifera depends on vegetative growth in the seedling stage to the treelet stage. Then a variety of cytochromes, tissues and organs began to differentiate step by step, to became the buds, flowers and finally the fruit with the accumulation and adjustment of nutrition and the change of levels and diferent kinds of plant endogenous hormones (Zhuang, 2008). Previous studies on *C. oleifera* mainly focused on variations in hormones and other cellular constituents during development (Zhou *et al*., 2013), the effects of exogenous hormones and growth regulators on *C. oleifera* growth (Gao *et al*., 2012; Li *et al*., 1998; Wen *et al*., 2005), and observations on morphological and anatomical characteristics of flower bud differentiation (Luo, 2012; Wang *et al*., 2011). Nevertheless, water content, soluble sugar content, and the concentrations of endogenous hormones at different ages have not been reported in *C. oleifera*. The objective of this study is to investigate the differences in endogenous hormone levels, soluble sugar content, and water content between the floral bud pre-differentiation stage and the pistil and stamen mature stages in plants of different ages in *C. oleifera* in the same orchard. The information from this study can then be used to clarify the relationship between flower bud formation and hormone, soluble sugar, and water contents in *C. oleifera*.

Material and Methods

Plant materials and growth conditions: Experiments were conducted in Shucheng county, located in the Dabieshan mountains of western Anhui province (31°05'N, 116°31'E) at approximately 220.3 m above sea level. This is on the northern edge of the *C. oleifera* growing area in China, and represents the typical ecotype in the distribution of *C. oleifera*. The study site is in the subtropical zone and has a mild continental monsoon climate, with annual precipitation of ~1100 mm. The maximum, minimum, and annual mean temperatures are 40.5°C, -16.3°C and 15.6°C, respectively, and the average annual sunshine duration is 1969.0 h.

The Shucheng variety (Dabieshan No.1) was used as the experimental material; plants were 2, 4, 6, 10, 14, 50, and 70 years old. Tissue samples were collected from leaves at two different developmental stages in early May and late August, floral bud predifferentiation stage (May 7, 2013), and pistil and stamen mature stage (August 31, 2013).

Twenty-one *C. oleifera* plant samples (approximately 2 g fresh weight each) were frozen in liquid nitrogen and stored at -80°C. These samples were analyzed at China Agricultural University, Beijing, China.

Determination of bud and leaf endogenous hormone contents: Fresh tissue (1.5 g) was used to measure the contents of the endogenous levels of indole-3-acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), dihydro-zeatin riboside (DHZR), and zeatin riboside (ZR). The immunoenzymatic method of analysis, as described by Li and Meng (1996), was used with some modifications. Freeze-dried powdered tissues were stirred and extracted in 10 ml of cold 80% methanol containing butylhydroxytoluene (1 mM) as an antioxidant for 24 h at 4°C in the dark after homogenization. The tissue samples were then centrifuged for 15 min at 10,000 rpm (Avanti 30 centrifuge, Beckman), and the supernatant was transferred to a flask. The residue was washed and extracted with 2 ml of cold methanol for another 12 h, and centrifuged under the same conditions. The supernatants were combined and decolorized using Sep-Pak C-18 cartridges twice so that the dilution curve was parallel to the curve of the standards. After filtration, elutes were vacuum-dried with a rotary evaporator at 37°C to remove the methanol. The residue was re-dissolved in a buffer containing 0.05 mM l Tris, 1 mM $MgCl_2$, 150 mM NaCl, 0.1% gelatin, and 0.1% Tween 20. Hormone contents were determined by ELISA as described by Li and Meng (1996). The hormone level measurements for all 42 *C. oleifera* plant samples were repeated three times, and the standard errors were calculated. The antigens and antibodies were purchased from Chinese Agricultural University.

Measurement of water-soluble sugar (WSS) content: The soluble sugar content was measured according to the method described by Plummer (1987); 0.5 g leaf samples were homogenized by grinding with quartz sand. The leaf homogenate volume was adjusted to 50 mL, incubated for 1 h at room temperature, and then filtered. A 0.5-mL sample of the extract was used for the measurement. The experiment was repeated three times.

Measurement of water content: The fully-expanded third leaf from the top per replicate was weighed and recorded as fresh weight (W_f). The sample was dried in the oven at 105°C for 30 min and then at 80°C until the weight became constant. The sample was weighed and recored as the dry weight (W_d).

Water content was calculated using the following formula:

Wet base water content (WWC): $(W_f - W_d)/W_f \times 100\%$, where W_f = fresh weight and W_d = dry weight.

Analytical methods: Data from this study were presented as the means ± SE for at least three independent experiments. Analysis of variance (ANOVA) and Duncan’s test at $P = 0.05$ or $P = 0.01$ were used to compare the variations of endogenous hormone levels and soluble sugar parameters in samples of different ages and different sample time treatments using SPSS 17.0 software.

Results

Changes in leaf wet base water content (WWC): The water contents in leaves of different ages sampled at two different times of the year are presented (Fig. 1). In the two stages, the WWC among the different age groups ranged from 65.67%-74.15% (May) and 61.64%-73.02% (August). The water contents in May and August showed a decrease-increase-decrease pattern of variation. The maximum WWC in *C. oleifera* was in the May sample from the 2-year-old plants, while the minimum WWC was in the August sample from the 70-year-old plants. Additionally, the WWC in the May sample from the 6-year-old plants was low (67.94%), and the samples taken in August from the 4-year-old plants were much lower as 63.35%. The WWC in leaves sampled in May were significantly higher than in the August samples. This can be explained because the tissues were younger and fresher, and therefore the water content was higher. There were significant differences in water content among the plants of different ages ($p>0.01$).

Changes in leaf water-soluble sugar (WSS) content: The variation in WSS content in leaves from trees of different ages sampled in May and in August is presented (Fig. 2). The WSS content in *C. oleifera* leaves showed an increasing trend for the two sampling times. In both May and in August, the maximum leaf WSS contents were 52.93 $\mathrm{mg \cdot g^{-1}}$ and 62.44 $\mathrm{mg \cdot g^{-1}}$, respectively in the 70-year-old plants. In the May samples, the minimum WSS was 21.24 $\mathrm{mg \cdot g^{-1}}$ in the 6-year-old plants, whereas the minimum WSS in August was 26.67 $\mathrm{mg \cdot g^{-1}}$ in the 2-year-old plants. The WSS contents for all *C. oleifera* plants was significantly higher in August than it was in May ($p<0.01$). The WSS contents of the 2- and 70-year-old plants were significantly different than in the plants of other ages. There were also significant differences in the WSS contents among the different aged plants ($p<0.01$). In summary, the WSS contents of juvenile trees were the lowest, followed by adult trees, with that of senescent trees being the highest over the life cycle of *C. oleifera*.

Changes in five endogenous hormone levels in leaves: The DHZR, ZR, GA, IAA, and ABA contents in 1-year-old leaves sampled from plants of different ages were determined. The results show that the contents of five endogenous hormones vary with plant age and sampling date.

DHZR contents of *C. oleifera* in the two sampling periods exhibited a low-high-low trend with respect to increasing tree age. Leaf DHZR content in May increased sharply in *C. oleifera* plants from 2- to 4-years of age (>3-fold), after which it declined with increasing age, reaching its lowest level in the oldest trees. The samples taken in August showed an approximately 2-fold increase in DHZR content going from the 2- to 10-year-old plants, and then dropped in older trees, reaching the same level as in the May sample for the 70-year-old trees (Fig. 3).

The levels of ZR with respect to tree age exhibited different patterns in the May and August samples. In May, the ZR content initially rose from 14.149 $\mathrm{ng \cdot g^{-1}}$ in the 2-year-old plants to 16.721 $\mathrm{ng \cdot g^{-1}}$ in the 4-year-olds, remained relatively constant in the 6- and 10-year old trees, and then decreased to 6.215 $\mathrm{ng \cdot g^{-1}}$ in the 70-year-old trees. In the August samples, the ZR content exhibited a slight low-high-low trend with increasing tree age to 50 years, then decreased sharply in the 70-year-old trees. The ZR content in the 2-, 4-, 6- and 10-year-old samples taken in May was higher than those taken in August, but the opposite was found for the 14-, 50-, and 70-year-old trees (Fig. 3).

For GA content, there were no significant differences for the trees at any age between the samples taken in May compared to those taken in August. For the plants between 2- and 4-years of age, a remarkable decrease in the GA level was observed, and this time period coincided with the transition from the seedling stage to the sapling stage. After that, the GA levels first increased in the 6- and 10-year-old trees and then decreased in the older trees(Fig. 3).

The concentration of leaf IAA in the samples taken in May and in August showed nearly identical profiles for the trees from 2- to 70-years of age. The IAA levels displayed a high-low-high-low trend with increasing tree age for both sampling times, with the overall concentrations higher in May than in August. In summary, IAA contents showed statistically significant differences with increasing tree age (Fig. 3).

For all *C. oleifera* plants between 4- and 70-years old, the ABA content in May was lower than it was in August. In the 2-year-old plants, the ABA content were the same in May and in August. For the samples taken in May, the ABA content in trees from 4 to 14 years of age decreased from 93.615 $\mathrm{ng \cdot g^{-1}}$ to 66.541 $\mathrm{ng \cdot g^{-1}}$, and then rose from 66.541 $\mathrm{ng \cdot g^{-1}}$ to 91.297 $\mathrm{ng \cdot g^{-1}}$ in trees from 14- to 70-years of age. In August, ABA content reached its peak (150.498 $\mathrm{ng \cdot g^{-1}}$) in leaves of 4-year-old trees of *C. oleifera*, at which time the trees entered the fast vegetative growth stage. The *C. oleifera* trees aged 6, 10, and 14 years were in full fruit stage, whereas the 50- and 70-year-old trees were in the senescence stage (Fig. 3). There were statistically significant differences in the ABA levels in trees of different ages between the samples taken in May and those taken in August.

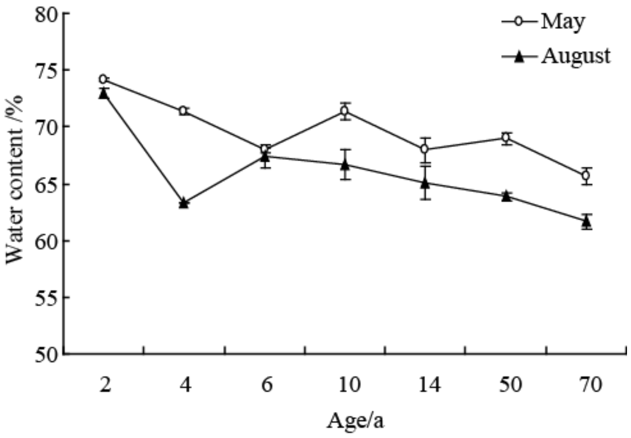

Fig. 1. Comparison of leaf water content in *C. oleifera* trees of different ages (2-, 4-, 6-, 10-, 14-, 50-, and 70-years of age) in May and August.

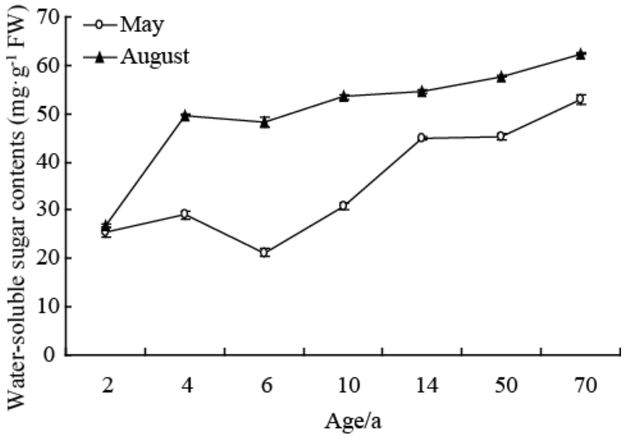

Fig. 2. Comparison of water-soluble sugar content in leaves of *C. oleifera* trees of different ages (2-, 4-, 6-, 10-, 14-, 50-, and 70-years of age) in May and August.

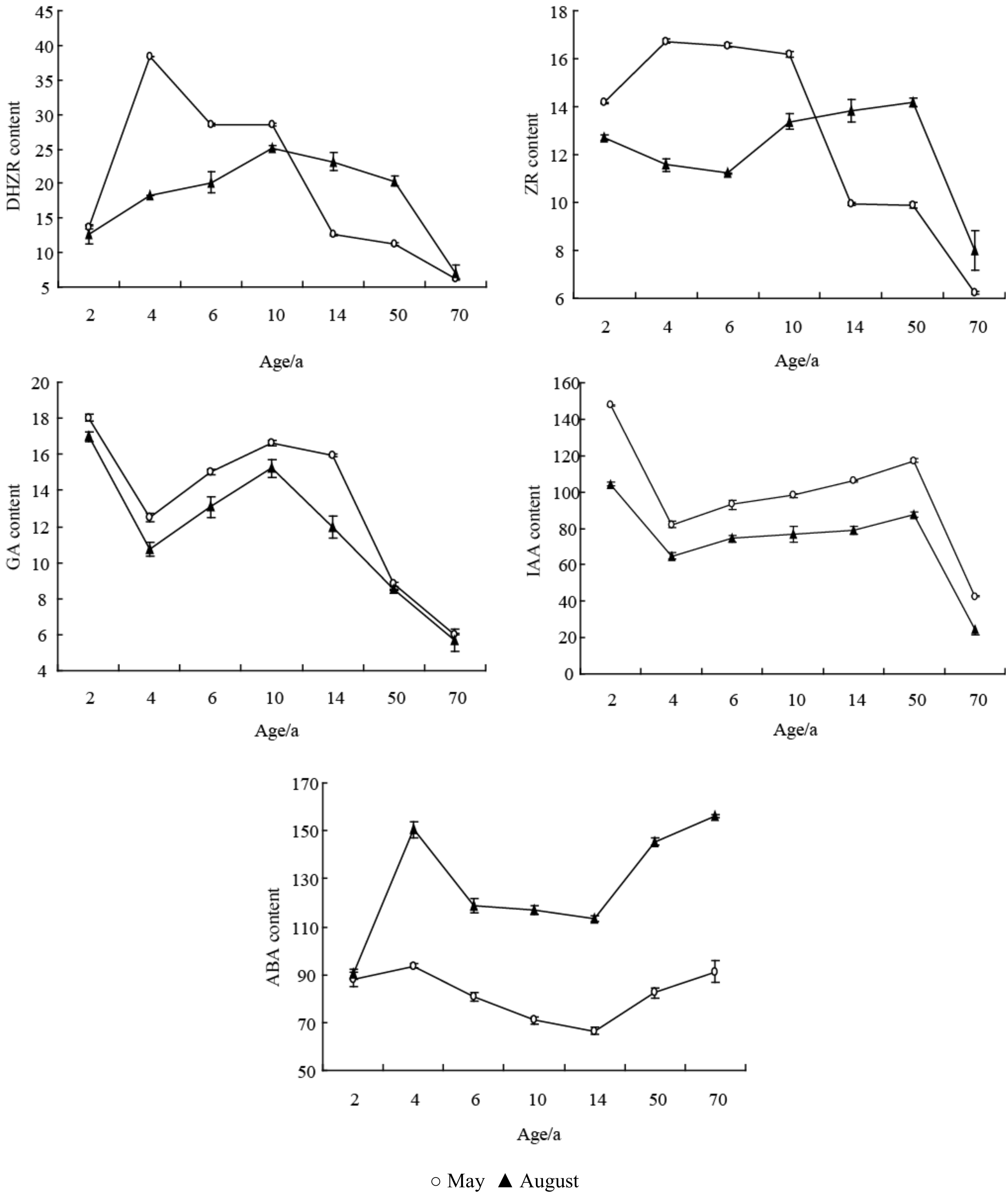

Fig. 3. Comparison of the five phytohormones DHZR, ZR, GA, IAA, and ABA contents (ng·g^{-1} FW) in leaves of *C. oleifera* trees of different ages in May and August.

Correlations between contents of endogenous hormones, water, and WSS: The changing patterns of endogenous hormone levels, WWC, and WSS contents in *C. oleifera* trees at different ages are shown (Table 1). A highly significant negative correlation was observed between WWC and WSS content in leaves (r=-0.90**). The correlation analysis between WWC and WSS content and the concentration of endogenous hormones in leaves indicated that there was a significant positive correlation between WWC and the concentrations of GA and IAA (r=0.86** and r=0.82*, respectively). There was a significant negative correlation between WSS content and the concentrations of ZR and GA (r=-0.73* and r=-0.82*, respectively). These correlations imply that ZR, GA, and IAA play key roles in WWC and WSS content.

Table 1. Correlation coefficients between concentrations of endogenous hormones, water content, water-soluble sugar content, and tree age.

Index	WWC	WSS	DHZR	ZR	GA	IAA	ABA
WSS	-0.90**						
DHZR	0.19	-0.39					
ZR	0.65	-0.73*	0.85**				
GA	0.86**	-0.82*	0.45	0.78*			
IAA	0.82*	-0.65	0.18	0.65	0.73*		
ABA	-0.67	0.52	-0.13	-0.49	-0.87**	-0.7	
Tree age	-0.71*	0.83*	-0.7	-0.88**	-0.90**	-0.59	0.62

1) DHZR, dihydro-zeatin riboside; ZR, zeatin riboside; GA, gibberellins; IAA, indole-3-acetic acid; ABA, abscisic acid

2) WWC, wet base water content; WWS, water-soluble sugar

3) * and ** Significance at 0.05 or 0.01 probability level, respectively

The endogenous hormones that were significantly positively correlated with the ZR concentration were DHZR and GA (*r*=0.85** and *r*=0.78*, respectively). There was a significant positive correlation between GA content and the concentrations of IAA (r=0.73*), whereas a highly significant negative correlation was observed between GA and ABA content (r=-0.87**).These correlations indicate that leaf endogenous hormones interact with one another.

We also found that tree age was positively correlated with leaf WSS content (*r*=0.83*). There was a highly significant and negative correlation between tree age and the concentrations of ZR (*r*=-0.88**), and GA (*r*=-0.90**). Tree age was also significantly and negatively correlated with leaf WWC (*r*=-0.71*).

Discussion

Camellia oleifera is a perennial woody plant, and it exhibits a large variation in WWC, WSS content, and the concentrations of five endogenous phytohormones during the developmental stages defined by trees of seven different ages (2-, 4-, 6-, 10-, 14-, 50-, and 70-years of age). The transition from vegetative growth to reproductive growth and development proceeds in response to environmental factors. These factors often bring into play inductive effects by evoking changes in endogenous phytohormone and sugar levels (Liu *et al*., 2008). This is a critical step in plant reproductive development, because if plant endogenous hormone levels, water content, and sugar contents do not change, seed abortion and fruit abscission can occur. Therefore, a better understanding of the changes during plant development is essential to the establishment of strategies for yield improvement in economically important fruit trees (Liu *et al*., 2008; Zhou *et al*., 2000). Because plant endogenous hormones are known to play a significant role in plant growth and development, a number of studies have been conducted on the relationship between hormone contents and formation of leaves and flower buds (Liu *et al*., 2008), although no consistent results have been published for the growth stages of *C. oleifera* at different ages.

Water is an important ecological factor for plant life. Under different water conditions, plant physiological and biochemical processes can be affected to different degrees. Furthermore, water conditions can affect the secondary metabolism process in plants and the accumulation of effective components (Gao & Liang, 2004). Thus, *C. oleifera* trees of different ages and growth stages have different WWC in leaves, and there are substantial differences in water requirements to meet the needs of growth and development. In previous study, leaf WWC measurements showed that leaf WWC increased with advancing tree age in *C. oleifera*, and there were no significant differences in the leaf WWC among the different ages (Wen, 2013). The results of the present study indicated that variation in leaf WWC in trees of different ages in May was consistent with the variation in August, and showed a decrease-increase-decrease pattern. Moreover, the WWC of juvenile trees was significantly higher than it was in the senescent trees. The physiological roles of WWC on the development of leaves in *C. oleifera* needs to be investigated further.

Sugars are essential primary metabolites, acting as energy equivalents, precursors for anabolic reactions and as signals (Hedrich *et al*., 2015). Sugars showed an identical pattern throughout maturation in *Castanea mollissima* (Liu *et al*., 2008). It has been reported that sugar levels are the highest in tobacco (*N. tabacum*) leaves that were beginning to senesce, as compared with younger and older leaves on the same plant (Masclaux *et al*., 2000). Thus, the increasing sugar could be seen as a sign of senescence during plant growth and development. Our results showed a similar pattern for WSS contents in the samples taken in May and August. In both growth stages, the WSS contents demonstrated a generally increasing trend with increasing tree age, which coincided with the progression from juvenile stage to adult stage to senescence; in other words, from the vegetative to the reproductive phase. WSS contents of *C. oleifera* in May were significantly lower than in August (P<0.01), which coincided with floral bud differentiation and as the leaves became more mature. These results are consistent with the findings of Masclaux *et al*. (2000) and Yoshida (2003).

DHZR is one type of cytokinin that plays an important role in cell division, suppression, and expansion. The contents of DHZR in seed potato were found to be higher when the physiological ages of seed potato were older. When the physiological age of the seed potatoes was >30 days, the DHZR levels in the buds declined sharply (Woolley & Wareing, 1972; Xiao & Guo, 2007). DHZR concentrations in large tubers were significantly higher than in middle-sized or small tubers at the early thickening period. The DHZR concentrations in the top or inner parts of the tubers were significantly higher than in the ends or outer parts (Matsuo & Mitsuzono, 1988; Wang *et al*., 2005). These results all show that when the tissues and organs are younger, the relative DHZR content is higher. However, the concentration of DHZR in *C. oleifera* has never been reported. In this study, the concentration of DHZR was higher in May than it was in August in trees from 2- to 10-years of age, and the opposite was found in trees that were 14-, 50-, and 70-years old. *C. oleifera* trees that are 4-, 10-, and 70-years of age are typical representatives of the juvenile, full fruit, and senescence stages, respectively. Our results indicate that cell division was rapid and extensive in the juvenile and full fruit stages in *C. oleifera*, and that it was much slower in senescence stage trees.

The cytokinin ZR is a plant growth regulator that plays a central role in cell division and shoot formation (Dewitte *et al*., 1999; Friml, 2003; Li *et al*., 2010). The results of the present study demonstrated that the ZR content in May was higher than it was in August for the 2-, 4-, 6-, and 10-year-old trees, whereas the opposite was observed for the trees from 14- to 70-years of age. Therefore, the higher levels of ZR in juvenile *C. oleifera* in May and the higher levels of ZR in adult trees in August indicate that the high ZR concentration in young trees affected processes such as cell division and vegetative bud and leaf formation in the floral bud pre-differentiation stage. The high ZR concentration in adult *C. oleifera* not only affected cell division, leaf and reproductive bud formation in the pistil and stamen mature stage, but also was considered to be a component of the floral stimulus. These results are similar to the findings of Liu *et al*. (2008) and Bernier *et al*. (1993).

GA is known to have positive effects in promoting stem growth by stimulating both cell division and cell elongation (Swarup *et al*., 2002). The present study showed a similar pattern (decrease-increase-decrease) of GA content for the two growth stages. The concentration of GA was lower in August than in May for trees from 2- to 70-years of age. A low GA content was observed when *C. oleifera* was going to flower. Al-Khassawneh *et al*. (2006) and Hartmann *et al*. (2010) also reported that exogenous GA promoted flowering in the black iris (*I. nigricans* Dinsm.), which may be attributed to the growth-promotion effect of GA in stimulating and accelerating cell division, and increasing cell elongation and enlargement.

IAA is the predominant auxin in most plants, and higher levels are present in young and growing tissues (Bartel, 1997). It is interesting to note that, in our present study, the patterns of change seen in leaf IAA contents were similar between May and August. IAA contents in the two growth stages showed a decrease-increase-decrease pattern in *C. oleifera* trees from 2- to 70-years old. In 4- to 10-year-old trees, a sharp increase in bud IAA level was observed that coincided with the change from vegetative growth to reproductive growth. A slow increase in leaf IAA was observed at the same time the trees changed from vegetative growth to reproductive growth in trees from 4- to 50-years of age. The levels of IAA in May were significantly higher than in August in trees of all ages. Further experiments are needed to determine the exact roles of IAA in *C. oleifera*. A significant increase in endogenous IAA content during bud outgrowth has also been reported in other plant species (Gocal *et al*., 1991).

ABA has been previously reported to be involved in regulating the transition from the vegetative to the reproductive phase (Finkelstein *et al*., 2002), and higher ABA levels are characteristic of maturity. In the present study, the ABA content in *C. oleifera* trees in May and in August showed a increase-decrease-increase pattern in trees from 2- to 70-years of age. The levels of ABA in May were significantly lower than in August. The results of leaf ABA content are resembled with earlier studies (Galoch, 1985; Valdés *et al*., 2004) where the authors postulated that ABA affects plant senescence and is involved in maturation. These authors reported that the mature phase was characterized by high ABA levels, whereas juvenile plants showed lower levels of ABA. To the contrary, our results for *C. oleifera* indicate that higher levels of ABA are characteristic of the juvenile state, whereas mature and senescencing trees are characterized by lower ABA levels.

Differences in tree age can influence water content, soluble sugar content, and endogenous hormone levels in leaves in some woody plants, which can aid in plant development and the transition from vegetative to reproductive growth. Our results indicated that the contents of water and soluble sugar in leaves directly affects the concentrations of endogenous hormones, and were significantly correlated with age in *C. oleifera* trees. Statistical analysis did not show any significant correlations between tree age in *C. oleifera* and changes in the concentration of DHZR, IAA, and ABA. Characterization of ABA response mutants in Arabidopsis has revealed alterations in sugar and starch levels (Brocard-Gifford *et al*., 2003; Meinke *et al*., 1994). In *Chenopodium rubrum*, cytokinin (including DHZR and ZR) has been shown to induce the expression of both a hexose transporter and a cell wall invertase, suggesting that cytokinins may participate in regulating sugar transport (Ehness and Roitsch, 1997).

Conclusions

We investigated changes in hormone levels, water content, and WSS content in *C. oleifera* trees from 2- to 70-years of age. Our results showed different patterns variation in the WWC, WSS content, and endogenous hormone levels for DHZR, ZR, GA, IAA, and ABA in the trees of different ages sampled in May and in August. The contents of water and WSS showed opposite change patterns. WWC had a decrease-increase-decrease pattern variation, while the WSS contents demonstrated a generally increasing trend with increasing tree age. Both WWC and WSS content were significantly correlated with age in *C. oleifera*. There was a significant positive correlation between leaf WWC and the levels of GA and IAA in leaves, and significant negative correlations between leaf WSS content and leaf ZR and GA levels. We found significant and negative correlations between tree age and the concentrations of leaf ZR and GA. The data presented in this paper indicate that quantitative changes in endogenous hormone levels and WCC and WSS content may correspond to different growth and development mechanisms (e.g. sprouting, branching leaves, flowering, and fruiting) in *C. oleifera*.

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