Reduction of Early Fruit Abscission by Main-branch-girdling in Macadamia Is Related to the Favorable Status of Carbohydrates and Endogenous Hormones

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Abstract. Fruit abscission occurring severely in the early fruit development affects macadamia yield. Developing effective methods to improve fruit retention is a priority for macadamia cultivation and production. Girdling is an important horticultural practice that has been widely used to increase fruit yield. Previous studies have shown that girdling fails to increase macadamia yield despite enhancing the early fruit set, but few have examined the effect of girdling on its related physiological mechanism. The objective of this study was to investigate the effects of main-branch girdling (MBG) on early fruit retention and also on the levels of carbohydrates and endogenous hormones in the leaves, bearing shoots and fruit of macadamia. Herein, MBG was performed at fruit set using a single-blade knife on 9-year-old macadamia trees (*Macadamia integrifolia*). Results showed that MBG significantly reduced young fruit drop, concurrent with significant increases in the contents of starch in both the leaves and the bearing shoots and in glucose, fructose, and sucrose levels in the husk and seed. It was suggested that the availability of carbohydrate for fruit retention was improved by MBG. Additionally, MBG increased indole-3-acetic acid (IAA), gibberellin (GA₃), and zeatin-riboside (ZR, a type of cytokinin) concentrations and decreased abscisic acid (ABA) contents in the husk and the seed, indicating that MBG reduced the early fruit drop by modifying the balance of endogenous hormones. Therefore, a positive interplay between carbohydrates and endogenous hormones induced by MBG was involved in the reduction of early fruit abscission in macadamia.

<3% of the yield of macadamia in the world (Ning et al., 2019).

Like many tropical trees, macadamia is mass flowering. A mature tree in a season can produce more than 10,000 racemes each consisting of 100 to 300 flowers (Ito, 1980). However, more than 90% of the flowers with unsuccessful fertilization are abscised in the first 2 weeks after anthesis (Sakai and Nagao, 1985; Trueman and Turnbull, 1994a). Following this initial drop, more than 80% of the immature fruit are abscised during the 3 to 8 weeks postanthesis (Trueman and Turnbull, 1994b; Wallace et al., 1996). This problem of the excessive fruitlet drop also occurred commonly in macadamia orchards across the production regions in China, such as Guangdong (Xu et al., 1995), Yunnan (Tao et al., 2005), and Guangxi (Zheng et al., 2011). Thus, this phenomenon has posed a major challenge to the development of the macadamia industry.

Studies on cherry (Blanusa et al., 2006), apple (Zhu et al., 2011), litchi (Kuang et al., 2012), and citrus (Talon et al., 1997; Gómez-Cadenas et al., 2000; Mahouachi et al., 2009) confirmed the close relationship between the carbohydrate availability to the developing fruitlets and their likelihood of abscission. The immature fruit abscission in macadamia was presumably caused by a shortage of available carbohydrates for rapid fruit development (McFadyen et al., 2011, 2012a, 2012b). Furthermore, fruit set and fruit development are initiated by the phytohormones signals (Goetz et al., 2007; Picken, 2015). Gibberellins (GAs) and cytokinin (CTK) were considered as the positive regulators in fruit set and development (Iglesias et al., 2007; McAtee et al., 2013), and IAA played a key regulating role in fruit retention (Kuang et al., 2012; Xie et al., 2018). Plant growth regulators, such as benzyladenine (Trueman, 2010), aminoethoxyvinylglycine (McFadyen et al., 2012b), and *N*-(2-Chloro-4-pyridyl)-*N'*-phenylurea (Zeng et al., 2016), were applied to increase fruit retention of macadamia by foliar spray before or after anthesis. The effects of plant growth regulators on the retention of young fruit were ascribed to improved carbohydrate availability (Li et al., 2015; Zeng et al., 2016). Moreover, IAA- and CTK-induced fruit set has been reported by inhibition of ethylene production and downregulation of ethylene biosynthesis and response genes (Martínez et al., 2013; Shinozaki et al., 2015). In fact, ethylene and abscisic acid (ABA) were shown to be involved in fruit abscission induced by carbohydrate starvation stress (Botton et al., 2011; Iglesias et al., 2006; Li et al., 2015).

Girdling, defined as the removal of a ring of bark around the branches or trunk, is an important technique and has been widely used to improve fruit retention and increase fruit yield (Annabi et al., 2019; Goren et al., 2003; Khadaker et al., 2011). The effects of girdling have been related to the interruption of phloem transport pathway, which resulted in the blocking of the downward transport of photosynthates (Urban and Alphonsout, 2007; Wang et al., 2006), basipetal flow of IAA (Dann et al., 1985), and acropetal flow of CTK (Havelange et al., 2000), thereby increasing carbohydrate

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Macadamia (*Macadamia integrifolia* and *Macadamia tetraphylla*, along with their inter-specific hybrids) is indigenous to the eastern Australia and grown in the tropical and subtropical frost-free regions across the world for their nutritious kernel. The crop flushes mainly in spring and late summer (Stephenson et al., 1986) and blossoms usually during late winter to early spring (Wallace et al., 1996; Wilkie et al., 2009), allowing the fruit to reach maturity $\approx$ 28 weeks postanthesis (Nagao and Sakai, 1988; Trueman et al., 2000). By the end of 2016, China had developed a macadamia industry of $\approx$ 160,000 ha and become the largest player of macadamia production in the world (Ning et al., 2019). Due to the low tree productivity even in the mature orchards, the total production of the nut in shell in China was only $\approx$ 12,301 t in 2016, which accounted for

availability for the developing organ above the girdle (Casanova et al., 2009; Rivas et al., 2006) and modifying the hormonal balance in the canopy (Kong et al., 2012; Shivashankara et al., 2019). However, girdling can cause root starvation due to the decrease in carbohydrate supply to the lower part of the girdle and the gradual depletion of root carbohydrate reserves (Goren et al., 2003; Moscatello et al., 2017), while the transport of water and soluble mineral nutrients through xylem is not directly affected.

In macadamia, girdling is also applied to improve fruit set. It has been reported that branch girdling increased the early fruit set (Trueman and Turnbull, 1994b; Williams, 1980) and that repeat trunk girdling increased yield in two out of four seasons (McFadyen et al., 2013). Cormack and Bate (1976) suggested that girdling delayed and mitigated the onset of carbohydrate depletion by reducing the shoot growth of macadamia. However, the physiological mechanism through which girdling affects fruit set and retention is less well understood. To explore the responses of macadamia to girdling at fruit set and determine how fruit retention are affected by girdling, an MBG with the strip width of 6 mm at the main branch diameter of 6 to 8 cm, differed from the previous reports of branch girdling using a wider girdle to the smaller branch (Trueman and Turnbull, 1994b; Williams, 1980), was performed after fruit set, and the effects of MBG on fruit retention and the contents of carbohydrates and endogenous hormones were examined during the early fruit development of macadamia.

The objectives of this study were 1) to test the efficacy of MBG in mitigating the early fruit drop and 2) to determine how MBG affected the status of carbohydrates and endogenous hormones in the leaves, bearing shoots and fruits, with an attempt to assess the relationship between fruit retention and the levels of carbohydrates and endogenous hormones when MBG was performed on macadamia trees.

Materials and Methods

Plant materials. The experiment was conducted in a mature macadamia orchard located at South Subtropical Crops Research Institute (lat. 21°27' N, long. 110°32' E, 65 m a.s.l.), Zhanjiang, China, from March to June 2019. The climate is subtropical, with a mean annual rainfall of 1600 mm and mean annual temperature of 23 °C. In the orchard with a lateritic soil of medium fertility, trees of macadamia variety 'Nanya-2' (*Macadamia integrifolia*) grafted on 'Hinde' rootstock were grown at 5 × 6 m. Irrigation and fertigation were performed according to local practices and pests were controlled when necessary. Eight 9-year-old trees with similar canopy size and initial fruit set were selected for the experiment, which were performed with eight replicates using individual trees as the experimental block.

Main branch girdling and sample collection. Six main branches with similar size and initial fruit set at different positions of the canopy were chosen from each tree at 25 d

after anthesis. Three were girdled at branch diameter of 6 to 8 cm using a single-blade knife. Two separate circular cuts, 6 mm apart, were carefully made by a vernier caliper, and the outer bark and phloem tissues around branch were removed. The remaining three branches were used as the control. For each branch, 60 similar sized racemes with comparable initial fruit set were selected, wherein 30 were used for recording the fruit number on each raceme and 30 for fruit sampling. The fruit sampled at regular intervals was separated into the husk and seed, and immediately frozen and ground to fine powder in liquid nitrogen and stored at -80 °C for analyses. Additionally, two bearing shoots with similar diameter size and raceme number were collected from each branch, and then the mature leaves at the second and third nodes from the top of the bearing shoots and the stems of these bearing shoots were sampled every 10 d until 30 d after treatment when the girdle healed. After washing with pure water and drying in shadow, these leaf and stem samples were heated at 105 °C for 20 min to denature enzymes and then oven-dried for ≈60 h at 60 °C to a stabilized dry weight, followed by grinding into fine powder for later analysis.

Investigation of fruit set. After MBG treatment, the fruit number on each marked raceme was recorded at 10-d intervals until 30 d after treatment, and the average number of fruit per raceme, accumulative fruit drop rate, and relative fruit drop rate were determined. The average number of fruit per raceme was calculated as the mean fruit number of all tagged racemes, the accumulative fruit drop rate was the percentage of the total number of fruit drop from the day of treatment against the initial fruit set. The relative fruit drop rate was the percentage of fruit drop during the period between two investigation dates against the fruit number per raceme on the date of the first investigation of the two.

Measurement of the starch content. An aliquot of 0.2 g of ground powders was homogenized with 10 mL of 80% ethanol and incubated in a water bath at 85 °C for 1 h. Then, the homogenate was centrifuged at 8000 g_n for 15 min and the supernatant was discarded. After repeating the above-mentioned procedures, the precipitate was extracted with 10 mL of 80% (w/v) calcium nitrate in a boiling water bath for 1 h. After filtration, the filtrate was collected and used to determine the content of starch according to the I_2 -KI method (Deng et al., 2008).

Analysis of carbohydrate composition. The extraction and determination of carbohydrates was performed according to the protocol of Zeng et al. (2016). Sample (0.5 g) was homogenized with 5 mL of 80% ethanol and incubated in a water bath at 85 °C for 20 min. Then, the homogenate was centrifuged at 8000 g_n for 20 min at 4 °C, and the precipitate was extracted again with 5 mL 80% ethanol following the aforementioned procedures. The supernatants of the two centrifuges were combined, adjusted to a volume of 10 mL with 80% ethanol, and evaporated at 85 °C to remove ethanol. The condensate was diluted

to 2 mL with distilled water and filtered. The filtrate was used for high-performance liquid chromatography (HPLC) analysis using 75% acetonitrile as the mobile phase, which had been ultrasonically degassed for 2 h before use. A sample volume of 10 μ L, a flow speed of 1.0 mL/min and a column temperature of 35 °C were applied. The used LC-10A HPLC system (Shimadzu Co., Kyoto, Japan) was equipped with an Agilent NH_2 chromatographic column (150 mm × 4.6 mm) and a refractive index detector. The quantification of carbohydrates was performed according to external standard solution calibration. Standard sugars (sucrose, fructose, and glucose) were purchased from Sigma Chemical Co.

Analysis of endogenous hormones. ZR was one of the most abundant cytokinin and occurred at high level in the developing seed, which was involved in promoting apoplastic unloading of assimilates during the early fruit development (Arnau et al., 1999). Here, the extraction, purification, and determination of endogenous levels of ZR, together with IAA, GA_3 , and ABA, were performed as described in a modified method of enzyme-linked immune sorbent assay (ELISA) (He, 1993; Yang et al., 2001). Samples ground powders (0.5 g each) were homogenized in an ice bath with a small amount of polyvinylpyrrolidone and 5 mL phosphate buffer solution (PBS; 50 mmol/L, pH 7.5) containing 80% methanol and 1 mmol/L butylated hydroxytoluene. After centrifuging at 8000 g_n for 20 min at 4 °C, and the supernatant was passed through a C_{18} Sep-Pak cartridge (Waters Corp., Millford, MA), which was prewashed successively with 10 mL 100% and 5 mL 80% methanol. The hormone fractions were dried under N_2 and then dissolved with 2 mL PBS (50 mmol/L, pH 7.5) for analysis by ELISA.

The mouse monoclonal antigen and antibodies against IAA, GA_3 , ZR, and ABA, and immunoglobulin G-horse radish peroxidase used in ELISA assays were produced by the Phytohormones Research Institute, China Agricultural University, China (He, 1993). The quantification of these endogenous hormones was performed using standard curves, which were all generated at high coefficients of quadratic correlation ($R^2 > 0.998$).

Statistical analysis. Statistical analyses of data were performed using SPSS software (Version 11.5.0; SPSS Inc., Chicago, IL). Differences between treatment and control were analyzed using the procedure of independent-sample t test, and the least significant difference ($P < 0.05$) was applied to compare data.

Results

Effect of MBG on young fruit abscission. Fruit number on the racemes decreased clearly during the early fruit development of macadamia ($P < 0.05$), and MBG treatment at 25 d after anthesis improved the retention of young fruit. The average number of fruit per raceme was 2.27 at 30 d after treatment (55 d after anthesis), which was 1.7-fold greater than the control (Fig. 1A). During the

Fig. 1. Effect of main-branch girdling (MBG) treatment on (A) fruit number per raceme, (B) cumulative fruit drop rate, and (C) relative fruit drop rate in macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$.

20 d after MBG treatment, the accumulative fruit drop rate in the treated trees increased rapidly to 49.9%, which was significantly ($P < 0.05$) lower than 63.3% in the control (Fig. 1B). From 20 to 30 d after treatment, the rate of the accumulative fruit drop in the treatment remained significantly ($P < 0.05$) lower than that in the control, but the increment of the accumulative fruit drop rate in the treated trees was only 4.7% during this period, which was obviously lower than 6.8% in the control. The relative fruit drop rate in the treatment increased to a peak value of

33.2% within the 20 d after treatment, which was lower than that in the control (42.4%), but an insignificant level was found between the treatment and the control. Subsequently, the relative fruit drop rate rapidly reduced to 9.0% from 20 to 30 d after treatment, which was significantly ($P < 0.05$) lower than 19.6% in the control (Fig. 1C). These results suggested that a severe fruit drop of macadamia occurred in the period of early fruit development, and MBG could effectively relieve the early fruit abscission.

Effect of MBG on starch contents in leaves and bearing shoots. The changes of starch in the leaves (Fig. 2A) and bearing shoots (Fig. 2B) of MBG treatment showed a similar pattern to those in the control, decreasing initially and increasing later. However, the starch contents in the leaves did not change obviously until 30 d after treatment when the level of starch in the treatment were significantly ($P < 0.05$) higher than that in the control. In the case of bearing shoots, the minimum in the treated bearing shoots occurred at day 10 after treatment, which was 10 d earlier than that in the control. Compared with the control, MBG treatment significantly ($P < 0.05$) reduced the starch content at 10 d after treatment, but increased it during 20 to 30 d after treatment (Fig. 2B). The results indicated that MBG promoted the accumulation of carbon nutrition in the leaves and the bearing shoots.

Effect of MBG on carbohydrates in fruit tissues. During the early fruit development, the contents of glucose in both the husk and the seed decreased (Fig. 3A and D), whereas those of fructose increased steadily (Fig. 3B and E). Compared with the control, MBG significantly ($P < 0.05$) increased glucose content in the seed at 30 d after treatment, and also in the husk at day 10 and day 30. Similarly, fructose level in the husk was also significantly ($P < 0.05$) increased by MBG at day 20 after treatment, as well as that in the seed at 10 to 30 d after treatment. In addition, sucrose contents in the husk and seed displayed an increasing trend in the treatment, but an opposite change was found in the control (Fig. 3C and F). Further, the contents of sucrose in both the husk and the seed were significantly

($P < 0.05$) enhanced by MBG within 20 to 30 d after treatment. The results suggested that MBG produced a large impact on the sugar composition in both the husk and the seed.

Effect of MBG on the endogenous hormones in fruit tissues. The levels of IAA, ZR, and ABA in the husk gradually decreased during the early fruit development, and MBG treatment increased the IAA content but decreased the ABA level significantly ($P < 0.05$) at day 30 after treatment, whereas the content of ZR was not influenced by MBG (Fig. 4A, B, and D). The change in GA₃ content in the husk showed a pattern of decreasing initially and increasing later in both the control and the treatment (Fig. 4C). However, the level of GA₃ in the treatment was significantly ($P < 0.05$) higher than that in the control in the period from 20 to 30 d after treatment.

Unlike the husk, the change patterns of IAA, GA₃, and ABA in the seed were similar in the control and the treatment, decreasing initially and increasing later (Fig. 5A, C, and D). However, MBG treatment significantly enhanced GA₃ content ($P < 0.05$) at day 10 and day 30 after treatment and decreased ABA level at 30 d after treatment. Although IAA content in the treatment was generally augmented relative to that of the control, no significant difference was found after MBG treatment. The content of ZR in the seed exhibited an increasing trend in both the treatment and the control, and the ZR level in the treatment was significantly ($P < 0.05$) higher than that in the control at 30 d after treatment (Fig. 5B).

Compared with the control, MBG generally enhanced the ratios of (IAA+GA₃+ZR)/ABA in both the husk and the seed, and the increase was significant ($P < 0.05$) at 30 d after treatment in the husk and at day 10 and day 30 in the seed (Fig. 6). These results suggested that MBG exerted a great effect on the composition and proportion of the endogenous hormones in the early fruit.

Discussion

The immature fruits of macadamia shed severely during the early fruit development. In

Fig. 2. Effect of main-branch girdling (MBG) treatment on the starch contents in (A) leaves and (B) bearing shoots of macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$.

Fig. 3. Effect of main-branch girdling (MBG) treatment on the contents and composition of carbohydrates in the (A–C) husk and (D–F) seed of macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$.

the current study, fruit set from 25 d after anthesis was investigated and the wave of fruitlet drop occurring in the period of 25–45 d after anthesis was confirmed, which resulted in an accumulative fruit drop rate higher than 70%. The results were consistent with previously published reports (Sakai and Nagao, 1985; Tao et al., 2005; Trueman and Turnbull, 1994b; Xu et al., 1995; Zeng et al., 2016).

Girdling is a technique known to increase fruit set in many crops including apple (Hoying and Robinson, 1992), kiwifruit (Boyd and Barnett, 2011), avocado (Trochoulis and O'Neill, 1976), and citrus (Rivas et al., 2006). In the current study, MBG with a 6-mm girdle at fruit

set (25 d after anthesis) retained 69% more fruit per raceme in the following 30 d. This was similar to the effect for girdled macadamia branches using girdle widths of 10 mm at 7 d postanthesis (Trueman and Turnbull, 1994b) and 30 mm (Williams, 1980) in autumn, where the early fruit set increased. Unlike the study in citrus that branch girdling performed at anthesis delayed the natural fruitlet drop (Mahouachi et al., 2009), the MBG did not change the process of fruit drop in macadamia which agreed with the report by Trueman and Turnbull (1994b). Thus, the results indicated that MBG effectively mitigated the early fruit abscission in macadamia.

The amounts of available carbohydrates for fruit growth, especially those of soluble sugars, have been documented to regulate fruit abscission (Botton et al., 2011; Iglesias et al., 2003; Yang et al., 2011). In macadamia, fruit set was also considered to be controlled by available carbohydrates in the tree through a competition mechanism (McFadyen et al., 2011, 2012a, 2013). However, carbohydrate resources within a tree could be manipulated by branch or trunk girdling to minimize competition for resources between fruit and rapidly growing shoots. It has been demonstrated that girdling promoted carbohydrate accumulation above the girdle portion of the tree by preventing the basipetal movement

Fig. 4. Effect of main-branch girdling (MBG) treatment on the contents of endogenous (A) indole-3-acetic acid (IAA), (B) zeatin-riboside (ZR), (C) gibberellin (GA₃), and (D) abscisic acid (ABA) in the husk of macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$.

Fig. 5. Effect of main-branch girdling (MBG) treatment on the contents of endogenous (A) indole-3-acetic acid (IAA), (B) zeatin-riboside (ZR), (C) gibberellin (GA₃), and (D) abscisic acid (ABA) in the seed of macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$.

of photosynthetic assimilates through the phloem (Annabi et al., 2019; Quentin et al., 2013; Vemmos et al., 2012). After MBG treatment, the change of starch level in the treated leaves kept pace with that in the control, only at day 30 after treatment MBG led to a significantly larger accumulation of starch compared with the control. However, starch content in the treated bearing shoots was initially decreased, indicating that early on the leaves might not have been photosynthesizing sufficiently to provide resources which would have come from reserves from other parts of the tree, and as a result of the increased usage from local resources. Subsequently, the content of starch was increased by MBG treatment resulting in more carbon nutrition accumulating in bearing shoots. Hence, MBG increased the content of carbohydrates in both the leaves and the bearing shoots and thus improved the retention of early fruits. Such a response is similar to that reported in citrus

(Mataa et al., 1998) and pistachio (Vemmos et al., 2012).

Developing fruit was considered as the strongest sink for carbohydrates. The overall results on ungirdled branches showed that the total amount of glucose, fructose, and sucrose in the husk and the seed decreased, which might not support the increasing demand of energy source for the growing fruit. After MBG treatment, the levels of these three soluble sugars in both the husk and the seed were significantly increased. Therefore, MBG promoted fruit retention by increasing the carbohydrate level of fruit, suggesting that the availability of carbohydrates in the fruit might be a limiting factor in determining the capability of fruit retention in macadamia tree. Sucrose is the major end-product of photosynthesis, rapidly hydrolyzed to glucose and fructose by invertase activity. A large body of evidence confirmed that glucose and sucrose

functioned as both nutrients and signals to regulate fruit development (Botton et al., 2011; Iglesias et al., 2007; Liu et al., 2013; Ruan et al., 2010). In the present study, the contents of glucose and sucrose in the early fruit were significantly increased by MBG, indicating that the improved sugar nutrition in the fruitlets after MBG satisfied fruit growth. Furthermore, the fructose contents in the husk and seed increased steadily in both the treatments, supporting the view that the growing fruit accumulated fructose when sucrose was transported and broken down (Berüter and Feusi, 1997).

Besides carbon nutrition, the endogenous hormones in fruit were involved in regulating fruit abscission (Bangerth, 2000; Gillaspay et al., 1993). As is well known, IAA is essential for fruit retention (Else et al., 2004; Kuang et al., 2012), ethylene and ABA have been implicated in the abscission of young

Fig. 6. Changes in the ratio of (IAA+GA₃+ZR)/ABA in the (A) husk and (B) seed after main-branch girdling (MBG) treatment in macadamia. Data are the means $\pm$ SE of eight replicates. Different letters at each individual time point indicated significant differences between treatment and control (CK) by Student *t* test at $P < 0.05$. IAA = indole-3-acetic acid; GA₃ = gibberellin; ZR = zeatin-riboside; ABA = abscisic acid.

fruit (Blanusa et al., 2006; Eccher et al., 2013; Talon et al., 1997). Xie et al. (2018) reported that IAA was able to inhibit citrus fruitlet abscission by repressing ethylene biosynthesis as a result of the decreased sensitivity of abscission zone to ethylene. In this study, the IAA content in macadamia husk increased remarkably after MBG, accompanied by a significant increase in sugar level. Botton et al. (2011) suggested that the increased sugar in apple served as signal to induce reactive oxygen species burst and cell senescence, resulting in a reduced IAA export and thus increased sensitivity of abscission layers to ethylene. The results of the current study did not support their findings, however.

As a stress hormone, ABA levels in the fruit rise rapidly in response to carbon starvation (Iglesias et al., 2006; Li et al., 2015). Moreover, the increased ABA may serve as a sensor of the intensity of the carbohydrate shortage and function as a regulatory signal during fruit abscission induced by carbon starvation (Gómez-Cadenas et al., 2000). After MBG treatment, the ABA levels in the husk and the seed were significantly lowered, similar to the results previously reported for litchi (Zhou et al., 1999). Therefore, MBG decreased fruit drop by reducing the ABA level of macadamia fruit, suggesting that ABA signal could be induced by the carbohydrate shortage and that ABA-sugar crosstalk might involved in the process of fruit abscission, which was in agreement with the results reported by Botton et al. (2011) and Zhu et al. (2011). Rivas et al. (2011) showed that girdling, eliciting a higher ABA content in young than in mature leaves, affected differentially the ABA level of citrus leaves. In the fruitlets, peduncle girdling of grape clusters decreased ABA level but increased the physiologically inactive ABA-glucose ester (Böttcher et al., 2018). Soluble sugars above the girdle, especially glucose, might have favored the synthesis of ABA-glucose ester after girdling (Kong et al., 2012; Sauter et al., 2002). The inhibition of fruitlet abscission was ascribed to the depressed ABA biosynthesis and promoted ABA degradation caused by IAA (Xie et al., 2018). Could a decreased ABA in the young fruit of macadamia after MBG be related to the decreased biosynthesis and the raised transportation of IAA to leaves, as well as to the increased ability of degrading ABA and forming ABA conjugates? It will be of interest to further study the physiological response of ABA in macadamia fruit to girdling.

Commonly, ZR and GA₃ are the most detected endohormones that play an essential role in regulating fruit set and development (Obroucheva, 2014; Qiu et al., 1998; Tyagi et al., 2020). Zhou et al. (2018) reported that girdling increased the contents of GA₃ and ZR in the litchi panicles, which were beneficial to lessen fruit drop. The ZR and GAs levels increased in grape fruitlets after girdling vines (Tyagi et al., 2020). Here, MBG increased remarkably the ZR content in the seed of macadamia, suggesting that a strong sink activity in the fruit was created by MBG via increasing the CTK level of seed to cause

diversion of assimilates to the fruit. Mahouachi et al. (2009) stated that the retention of citrus fruitlet by branch girdling was coincided with previous increases in the GAs concentrations. In this study, MBG significantly increased the GA₃ levels in husk and seed, indicating that the positive effect of MBG on increasing fruit retention was linked to the concentrations of GA₃. Thus, a relationship in the developing fruitlets of macadamia was established between the decreased abscission and the increased levels of GA₃, ZR, and IAA, which is in agreement with the results reported by Qiu et al. (1998), Talon et al. (1997), Mahouachi et al. (2009), and Xie et al. (2018).

Fruit set and development are complex developmental processes that rely on the coordination of different phytohormones (McAttee et al., 2013). Baktir et al. (2004) showed that the relative balance between GA₃-like compound and ABA concentrations of tissues may act as a key regulator of alternate bearing in olive. In the current study, the increase in IAA, GA₃, and ZR contents and the decreased ABA level resulted in the increased ratio of (IAA+GA₃+ZR)/ABA in the fruit, which could account for the higher fruit retention efficacy of girdling, as reported in litchi (Qiu et al., 1998; Zhou et al., 1999) and citrus (Iglesias et al., 2007; Mahouachi et al., 2009). Therefore, MBG appeared to suppress fruit abscission by modifying the balance of endogenous hormones, although there is a need to perform detailed study on the temporal and spatial role of individual hormones within the fruit after girdling.

In summary, MBG effectively increased fruit retention by increasing the accumulation of carbohydrates in both the leaves and the bearing shoots and by improving the carbohydrate availability in the fruit. Moreover, MBG modified the balance of endogenous hormones and increased the ratio of (IAA+GA₃+ZR)/ABA in the fruit chiefly by decreasing the ABA level and increasing the IAA, GA₃, and ZR levels, which was positive for fruit retention. Therefore, a link in the developing fruit of macadamia was established among the reduction of early abscission rate, the lower level of ABA and the higher concentrations of carbohydrates and growth-promoting hormones induced by MBG.

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