Study on changes in concentrations of four endogenous hormones in different fruit positions during citrus fruit growth and development

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Abstract: Changes in concentrations of indol-3-acetic acid (IAA), abscisic acid (ABA), gibberellin ($GA_{1/3}$) and ribosylzeatin (ZR) were investigated in different fruit positions (rind, pulp and seed) during fruit growth and development of parthenocarpic 'Guoqing 1' satsuma mandarin and self-pollinated 'Huanong Bendizao' tangerine. The results were showed that concentrations of IAA, $GA_{1/3}$ and ZR in the rind of 'Guoqing 1' were relatively high during young fruit growth, and then IAA in the rind and pulp was decreased progressively, whereas significant peaks were observed for ABA in the pulp and $GA_{1/3}$, ZR in the rind and pulp during fruit enlargement, and ABA concentrations were obviously increased in the rind and pulp during fruit maturation. In 'Huanong Bendizao', notable peaks were found for IAA in the seed, rind and pulp and seed ABA at the stage of fruit enlargement, $GA_{1/3}$ and ZR concentrations in the seed were relatively high and significantly increased during fruitlet growth and early fruit enlargement, whereas the levels of four endogenous hormones in the rind and pulp were relatively low and changed a little as time passed. Relationship between fruit growth and development and dynamics of endogenous IAA, ABA, $GA_{1/3}$ and ZR concentrations in different fruit parts was discussed herein.

Key words: Citrus (*Citrus reticulata* Blanco); fruit; endogenous hormone

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Endogenous hormones accumulation in fruit plays important roles in fruit growth and development and the processes of some other physiological metabolism. Generally, the growth of seedless fruits depends upon the growth hormone in the ovary (fruitlet), while fertilization can promote the increase of the growth hormones content, which can accelerate fruit growth and development. The change rules in endogenous hormones concentrations in fruit or ovary (fruitlet) during fruit development have been reported so much (Kondo *et al.*, 2001; Kojima *et al.*, 1994, 1996, 1999; Pozo, 2001; Zhang *et al.*, 1994; Chen *et al.*, 2002; Xiao *et al.*, 2004), whereas the changes in endogenous hormones in different fruit parts from different types of fruits have seldom been reported. Thus, in this study, parthenocarpy and self-pollinated citrus were taken as experimental materials, changes in

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concentrations of four endogenous hormones such as IAA, ABA, $GA_{1/3}$ and ZR in the structural parts of fruit were comparatively determined and analysed during the growing days, with the aim of probing into the relationship between the two different fruits growth and development and changes in concentrations of endogenous hormones.

1 Materials and methods

The experiment was conducted on 10-year-old trees of 'Guoqing 1' satsuma mandarin and self-pollinated 'Huanong Bendizao' tangerine grafted on trifoliate orange (*Poncirus trifoliata* Raf.) growing at the experimental orchard of Huazhong Agricultural University (three blocks of five trees for each cultivar). The trees were received the same orchard management program. Fruits were collected from 50 days after full bloom (DAFB) to final harvest, and were sampled every half a month. 2—5 fruits were collected from each tree at each harvest. Fruits were representative of average size of fruits on each tree. After being sampled, the fruits and the component parts of fruit (rind, pulp and seed) were immediately frozen in liquid nitrogen and stored at -40 ℃ for analysis.

Endogenous phytohormones (IAA, ABA, $GA_{1/3}$ and ZRs) in the rind, pulp and seed of citrus fruit were measured by enzyme linked immunosorbent assay (ELISA) according to the method of Wu (1988). The ELISA reagent were supplied by Nanjing Agricultural University.

2 Results and analysis

2.1 Changes in IAA concentrations in fruit during fruit growth and development

IAA concentrations were relatively high at 50 days after full bloom (DAFB) in the rind from 'Guoqing 1', and increased to peak at 65 DAFB, thereafter decreased rapidly and came to 2.1 nmol/g at 95 DAFB, then changed a little as the season progress. Pulp IAA concentration was 5.5 nmol/g at 50 DAFB, and then gradually decreased (Fig. 1: A).

In 'Huanong Bendizao', IAA concentrations remained constant during 65—125 DAFB in the seed, whereas dramatically rose to peak of 10.1 nmol/g at 140 DAFB. Rind IAA were relatively low during 50—110 DAFB, and began to rise at 125 DAFB and reached peak of 5.0 nmol/g at 140 DAFB, followed by a decrease, except for an obvious increase at final harvest time. Whereas pulp IAA changed slightly during 50—80 DAFB, and presented peaks of 6.1 and 4.4 nmol/g at 110 DAFB and 140 DAFB, respectively (Fig. 1: B).

In addition, it was shown that IAA concentrations in fruit from 'Guoqing 1' were relatively high at the stage of fruitlet growth (50—80 DAFB), IAA concentrations were significantly higher in the rind than in the pulp, while the peaks of IAA concentration in fruit from 'Huanong Bendizao' were presented at the stage of fruit enlargement (eg. at 140DAFB), and seed IAA concentration was the highest among them.

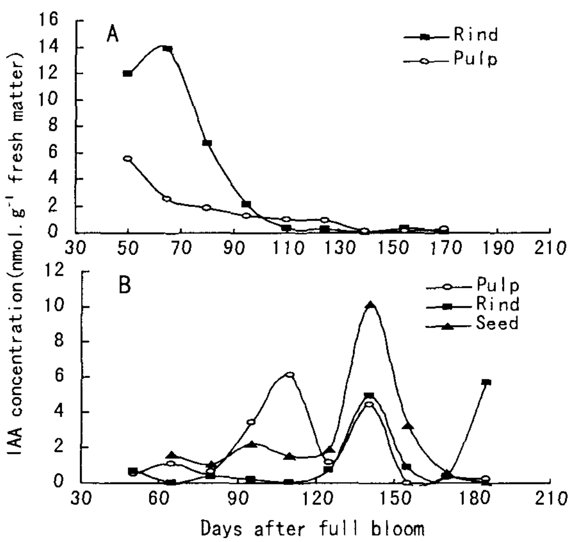

Fig. 1 Changes of IAA concentrations in different fruit positions (rind, pulp and seed) during citrus fruit growth and development of 'Guoqing 1'(A) and 'Huanong Bendizao'(B)

2.2 Changes in ABA concentrations in fruit during fruit growth and development

As shown in figure 2-A, ABA concentrations in the rind and pulp from 'Guoqing 1' were 0.7 and 0.4 nmol/g at 50 DAFB, respectively, and then both decreased. Rind ABA concentrations were around 0.4 nmol/g during 65—140 DAFB, but gradually increased to 0.8 nmol/g from 155 DAFB to final harvest. Whereas pulp ABA concentration decreased to 0.2 nmol/g at 95 DAFB, followed by an increase to 0.7 nmol/g, although a decrease at 155 DAFB was observed.

ABA concentrations in the seed from 'Huanong Bendizao' remained relatively steady during 65—110 DAFB, and began to rise at 125 DAFB and came to peak of 3.1 nmol/g at 140 DAFB, thereafter presented decreasing trend. ABA in the rind and pulp remained relatively low during 50 — 140 DAFB, while progressively increased to 2.7 nmol/g from 155DAFB to final harvest (Fig. 2:B).

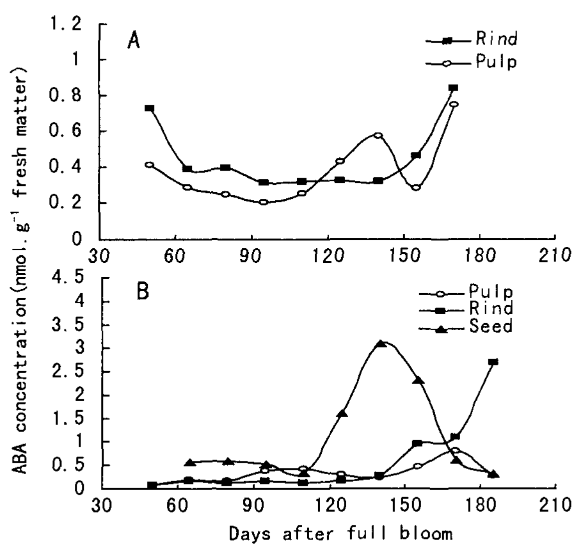

Fig. 2　Changes of ABA concentrations in different fruit positions (rind, pulp and seed) during citrus fruit growth and development of 'Guoqing 1' (A) and 'Huanong Bendizao' (B)

2.3 Changes in $GA_{1/3}$ concentrations in fruit during fruit growth and development

$GA_{1/3}$ concentrations were relatively high in the rind from 'Guoqing 1' during 50 — 65 DAFB, and obviously decreased at 80 DAFB, thereafter remained at relatively low levels up to final harvest, although an obvious increase was detected at 95 DAFB. Pulp $GA_{1/3}$ concentrations changed slightly and their values were relatively low throughout the growing season, with the exception of that the two small peaks were found at 80 DAFB and 140 DAFB, respectively (Fig. 3:A).

$GA_{1/3}$ in the seed from 'Huanong Bendizao' were relatively high and changed little during 65—95 DAFB, while peaks were present at 110 DAFB, thereafter progressively decreased up to 140 DAFB, followed by a slight increase. Whereas $GA_{1/3}$ concentrations were relatively low and remained relatively stable in the rind and pulp throughout fruit development (Fig. 3:B).

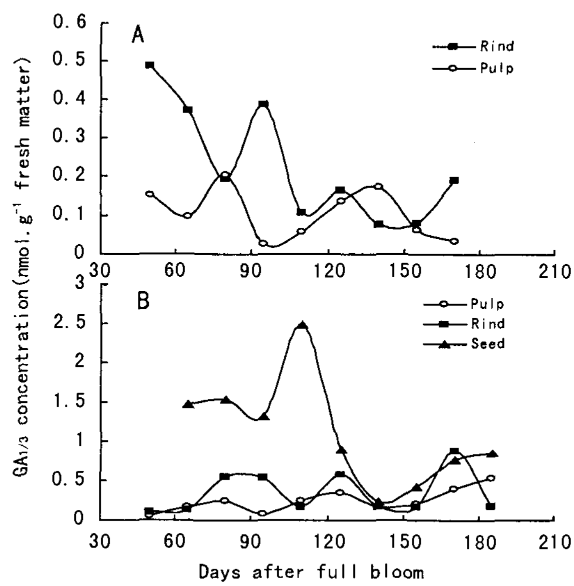

Fig. 3　Changes of $GA_{1/3}$ concentrations in different fruit positions (rind, pulp and seed) during citrus fruit growth and development of 'Guoqing 1' (A) and 'Huanong Bendizao' (B)

2.4 Changes in ZR concentrations in fruit during fruit growth and development

There were not obvious change rules in ZR concentrations in the rind and pulp. For example, rind ZR presented peaks at 65 DAFB and 155 DAFB, respectively. Increases were also detected for pulp ZR at 95 DAFB and 155 DAFB, respectively. Furthermore, the concentrations and fluctuations of ZR were smaller in the pulp than in the rind.

ZR concentrations in the seed from 'Huanong Bendizao' were relatively high, for instance, seed ZR was 0.7nmol/g at 65 DAFB, and increased to peak of 1.1 nmol/g at 80 DAFB, thereafter gradually decreased to 0.02nmol/g at 125 DAFB and then kept relatively constant. While ZR in the rind and pulp were relatively low and changed slightly during the growing season. In addition, ZR concentrations were much higher in the seed than in the rind and pulp.

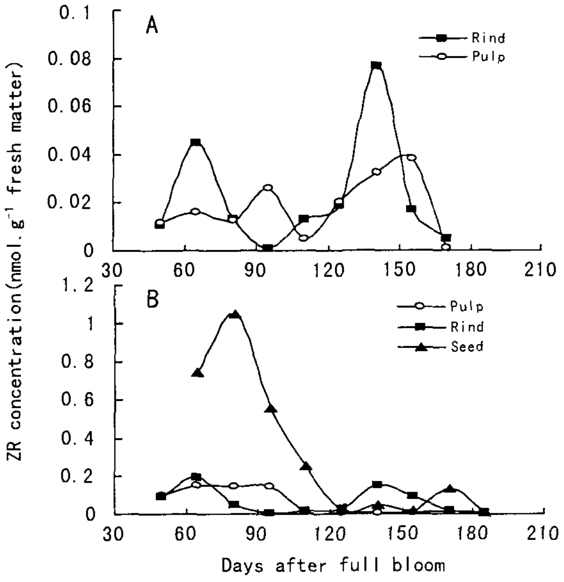

Fig. 4 Changes of ZR concentrations in different fruit positions (rind, pulp and seed) during citrus fruit growth and development of 'Guoqing 1' (A) and 'Huanong Bendizao' (B)

3 Discussion

The fruit growth curves of 'Guoqing 1' and 'Huanong Bendizao' were similar and could be divided into three stages: stage of fruitlet growth (before 80 DAFB), stage of fruit enlargement (from 80 DAFB to 140 DAFB) and stage of fruit maturation (from 140 DAFB to final harvest) (Xiao *et al.*, 2004a, 2004b). The present research shows that IAA, $GA_{1/3}$ and ZR in the rind from parthenocarpic 'Guoqing 1' were relatively high or presented peaks during 50-65 DAFB (Fig. 1, 2, 3), which is the same physiological process that terminates fruitlet growth and initiates fruit extension, which indicated that growth hormones accumulation in the rind play important roles on fruit set and fruit growth and enlargement during this period, in accordance with the result on seedless persimmon by Kojima (1999) and on parthenocarpic 'Kamei' satsuma mandarin by Xiao *et al.* (2005). In this experiment, it's manifested that ABA and $GA_{1/3}$ in the pulp and ZR in the rind and pulp presented obvious increases during fruit enlargement (Fig. 2, 3, 4), which is in harmony with fruit extension and growth at this time. It's reported that ABA related to substance conversion in fruit, sugar accumulation and initiation of fruit maturation (Chen *et al.*, 2002), So, it was speculated that the conspicuous increase of ABA concentrations in the pulp at the stage of fruit enlargement maybe relate to above mentioned mechanism. Whereas ABA in the rind and pulp obviously increased during fruit maturation (Fig. 2), which was indicated that fruit maturation and senescence initiated with ABA increase at this time, in agreement with previous research on other species (Kojima *et al.*, 1994, 1996, 1999; Chen *et al.*, 2002).

It was revealed that there were relationships between IAA and GA and ovary (fruitlet) growth and enlargement (Kojima *et al.*, 1994; Pozo, 2001; Xiao *et al.*, 2004). It was shown that IAA in the seed, rind and pulp and ABA in the seed from self-pollinated 'Huanong Bendizao' presented significant peaks during fruit enlargement (Fig. 1, 2), which manifested that IAA was in relation to fruit growth and development, and ABA maybe play important roles in modulating substance conversion in fruit, such as starch and sugar were accumulated etc. Thus, it was speculated that it was very important to keep balance between IAA and ABA, which maybe modulate fruit growth and development. In the present work, it was found that $GA_{1/3}$ and ZR in the seed from 'Huanong Bendizao' were at relatively high levels during 65-110 DAFB (from the stage of fruitlet growth to the early stage of fruit enlargement), whereas IAA and ABA in the seed were relatively low (Fig. 1, 2, 3, 4), which showed that fruit growth mainly relate to $GA_{1/3}$ and ZR at the early stage, thereafter fruit enlargement and growth and carbohydrate accumulation were mainly correlated with IAA and ABA at the latter stage. The results were demonstrated that peaks of seed $GA_{1/3}$ and ZR concentrations at the early stage and seed IAA and ABA concentrations during fruit enlargement were obvious higher than those in the rind and pulp, endogenous hormones levels in the rind and pulp were relatively low and changed slightly as the time progress (Fig. 1,2,3, 4), which was not only shown that seed produce many different hormones with the seed growth and development, which can maintain a certain metabolism intensity in fruit and keep carbohydrate to effuse progressively into fruit from source parts, but also was indicated that the differences of endogenous hormones in the two types of citrus fruits should relate to different fruit types (seed and seedless) and different courses of fruits growth and development.

References:

Chen FH, Cai HN, Feng ZS, *et al*. 2002 . Changes in hormones levels in fruit during grape fruit development[J]. *J Plant Physiol Mol Biol*, **28**(5): 391－395

Kojima K, Shiozaki K, Koshita Y, *et al*. 1999. Changes of endogenous level of ABA, IAA and GA-like substances in fruitlets of parthenocarpic persimmon[J]. *J Japan Soc Hort Sci*, **68**(2): 242－247

Kojima K, Yamade Y, Yamamoto M. 1994. Distribution of ABA and IAA within a developing Valencia orange fruit and its parts[J]. *J Japan Soc Hort Sci*, **63**(2): 335－339

Kojima K, Yamamoto M, Goto A, *et al*. 1996. Changes of ABA, IAA and GAs content in reproductive organs of satsuma mandarin[J]. *J Japan Soc Hort Sci*, **65**: 237－243

Kondo S, Fukuda K. 2001. Changes of jasmonates in grape berries and their possible roles in fruit development[J]. *Sci Hort*, **91**: 275－288

Pozo L V. 2001. Endogenous hormonal status in citrus flowers and fruitlets: relationship with postbloom fruit drop[J]. *Sci Hort*, **91**: 251－260

Wu SR, Chen WF, Zhou X. 1988. Enzyme linked immunosorbent assay for endogenous plant hormones[J]. *Plant Physiol Commun*, (5): 53－57

Xiao JX, Peng SA, Zhang HY. 2004a. Studies on dynamics of calcium and potassium during fruit development of parthenocarpic and self-pollinated citrus varieties[J]. *Acta Hort Sin*, **31**(1): 7－10

Xiao JX, Peng SA, He HP. 2005. Changes in concentrations of IAA, ZR and GA3 during fruit development of parthenocarpic and self-pollinated citrus cultivars[J]. *J Wuhan Bot Res*, **23**(3): 272－275

Xiao JX, Peng SA, He HP. 2004b. Seasonal changes of total calcium, soluble Ca^{2+} concentrations and Ca^{2+}-ATPase activity of citrus fruit[J]. *J Fruit Sci*, **21**(6): 530－534

Xiao JX, Peng SA. 2004. Studies on dynamics of calcium, boron nutrients and IAA, $GA_{1/3}$ in ovary(fruitlet) around citrus flowering[J]. *J Fruit Sci*, **21**(2): 132－135

Zhang SN, Chen KS, Ye QF, *et al*. 1994. Changes of endogenous IAA, ABA and ZT in pollinated, non-pollinated and parthenocarpic ovary (fruitlet) of citrus[J]. *Acta Hort Sin*, **21**(2): 117－123

柑橘果实生长发育过程中果实不同部位的 4 种内源激素含量变化的研究

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摘 要：以单性结实的国庆 1 号温州蜜柑和自花结实的华农本地早橘为材料，研究了果实生长发育过程中果实不同部位的吲哚乙酸(IAA)、脱落酸(ABA)、赤霉素($GA_{1/3}$)和玉米素核苷(ZR)含量的变化。结果表明：(1)国庆 1 号果皮 IAA、$GA_{1/3}$和 ZR 含量在幼果阶段均相对较高，随后果皮和果肉 IAA 含量均趋下降，而在果实膨大期内果肉 ABA 和果皮、果肉 $GA_{1/3}$、ZR 含量均出现上升峰值，果实成熟采收时果皮和果肉 ABA 含量均明显回升。(2)华农本地早种子、果皮和果肉 IAA 及其种子 ABA 含量均在果实膨大期内出现明显峰值，在幼果阶段至果实膨大初期内种子 $GA_{1/3}$和 ZR 含量均居较高并出现明显上升，对应的果皮、果肉 4 种内源激素水平均相对较低且变幅小。还就两结实类型柑橘果实生长发育与其内源 IAA、ABA、$GA_{1/3}$和 ZR 含量动态的关系进行了讨论。

关键词：柑橘；果实；内源激素