

Changes in Endogenous Hormones and Polyamines of Fruit During Growth and Development of Pear Fruits

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Abstract: The longitudinal length and transverse diameter of fruits of two pear cultivars, Zaomi and Whangkeumbae, were measured every 10 days after full blossom (DAFB) in the field. Concentrations of endogenous hormones and polyamines in pulp and seeds during fruit growth and development were also determined with high performance liquid chromatography (HPLC) in this study. The results indicated that: (1) Fruit grew in a parallel trend as “fast-slow-fast” between these two cultivars, whereas both the longitudinal length and transverse diameter of Zaomi pear fruits were longer at the same stage; (2) Zaomi pears contained lower concentrations of indole acetic acid (IAA) and gibberellic acid (GA₃) in pulp than Whangkeumbae, but included higher Zeatin (ZT) levels at the early stage and abscisic acid (ABA) level at the late stage; (3) IAA, ZT concentrations in seeds of Zaomi pears were higher and the peak value of IAA titer appeared earlier, while concentrations of GA₃ in seeds of these two cultivars changed approximately following a unimodal curve and bimodal curve, respectively. Whangkeumbae pears maintained higher level of ABA in seeds at the late stage; (4) The lower spermine (*Spm*), higher putrescine (*Put*) and spermidine (*Spd*) concentrations were measured in the pulp of Zaomi at the early stage. Zaomi pears contained lower concentration of *Put*, slightly higher levels of *Spd* and closer titer of *Spm* in seeds in contrast to those of Whangkeumbae; (5) *Put* and *Spd* in pulp and seeds of Zaomi and Whangkeumbae pears obviously predominated in free total polyamines in the given stage of fruit growth and development. In conclusion, changes in concentration and holding time of endogenous hormones and polyamines found in the pulp and seeds were little different between the two cultivars. Whangkeumbae pears held the longer time both in the early and middle stages of fruit growth and development. However, Zaomi pears developed into maturing stage more rapidly, which resulted in a shorter period during the whole fruit growth and development.

Key words: Pear • Endogenous hormone • Polyamines • Fruit growth and development • Ripening season

INTRODUCTION

Pears are by far one of the most important deciduous tree fruits and are widely grown in China. China which had the widest growing area had the highest yield produced during the past continuous decade in the world. Pear production can promote economic development in the countryside.

Fruit growth and development is one of the foremost life activities for fruit trees and is regulated by complex interactions. These include physiological and environmental factors such as endogenous growth regulators, fruit load of current season, rootstock and

scion cultivar, tree vigor and climate. A proper balance of these factors is required for optimal fruit development. The early research showed that reproductive development was positively correlated with some biochemical substances. These were mainly endogenous hormones and polyamines. The concentrations of them changed continuously after pollination and fertilization [1, 2]. This behavior in part controlled fruit growth, development, maturation and senescence. It also would produce different effects under different climate conditions and among cultivars belonging to different ripening seasons. Besides the five traditional plant hormones the new plant growth-regulating substance, polyamines not only can

facilitate assimilating carbon, transferring and distributing organic compounds produced by photosynthesis but can also interact with certain plant hormones to regulate fruit growth and development [3]. As far as interaction between any given polyamines and endogenous hormones was concerned, much work has been done for polyamines and ethylene during fruit maturity and senescence. It has also covered polyamines and abscisic acid (ABA) in fruit trees under adverse conditions and also for endogenous hormones or polyamines alone during fruit development, but with few combinations [4]. Therefore, two pear cultivars belonging to different ripening seasons were selected to measure concentration of four endogenous hormones, three free polyamines in the pulp and seeds from anthesis to harvest in this experiment. The results were expected to favor the mechanism, with which fruit development could be regulated in the given time and space and optimum guidance could be provided to cultural practices of pears and other fruit crops.

MATERIALS AND METHODS

Plant materials: Each four 5-year-old trees with uniform growing vigor for the early ripening-season pear cultivar Zaomi and the middle one WhangKeumbae grafted on *Pyrus pashia* Buch-Ham rootstocks were selected for experiment in 2006. Trees were spaced 1.5×3.0 m apart in one orchard with the typical paddy soil and subtropical humid monsoon climate located in Pi County, Sichuan province, China. Cultural management such as fertilization and pest control were the same as those used in a commercial orchard (without fruit thinning and bagging practices). Both of them are oriental pear cultivars.

The experiment was carried out early in 2006, in which year the full flower blooming date was March 20 and March 18 for Zaomi and WhangKeumbae, respectively. The corresponding period of fruit growth and development was 100 and 130 days and finished in 257, 210 grams of final fruit. The longitudinal length and transverse diameter of 20 fruits were measured. Fifteen to twenty fruits (3-5 fruits per tree) were sampled at 10 days interval from anthesis to commercial harvest for endogenous hormones and polyamines analysis. At every sampling day about 1.0000 gram pulp and seeds were frozen with liquid nitrogen for 2 to 3 minutes after it was weighed and immediately stored at -70°C until analyzed.

Endogenous hormones and polyamines analysis: The high performance liquid chromatography (HPLC) was used to determine concentrations of four endogenous

hormones, which referred to the methods of Ding *et al.* [5], Chen *et al.* [6]. Also three polyamines, which referred to the methods of Flores and Galston [7], Liu *et al.* [8] and Liu *et al.* [9].

Two repetitions were carried out and statistically analyzed with DPS 6.5 software.

RESULTS

Changes in longitudinal length and transverse diameter of fruit:

Seasonal changes in longitudinal length and transverse diameter of fruits are shown in Fig.1. As for growth of Zaomi pears it could be divided into four stages (in Fig.1-a): (1) Initial 30 DAFB characterized by a little fast growth and longer longitudinal length; (2) From 30 to 50 DAFB characterized by fruit growing relatively slowly; (3) From 50 to 90 DAFB characterized by growing fast again and by faster growth of the transverse diameter; (4) From 90 to 100 DAFB characterized by leveling off to maturity. Based on these four stages and seed development, the period from 0 to 30 DAFB could be approximately looked as the early stage of fruit development, the period from 30 to 70 DAFB as the middle stage and the period from 70 to 100 DAFB as the late stage.

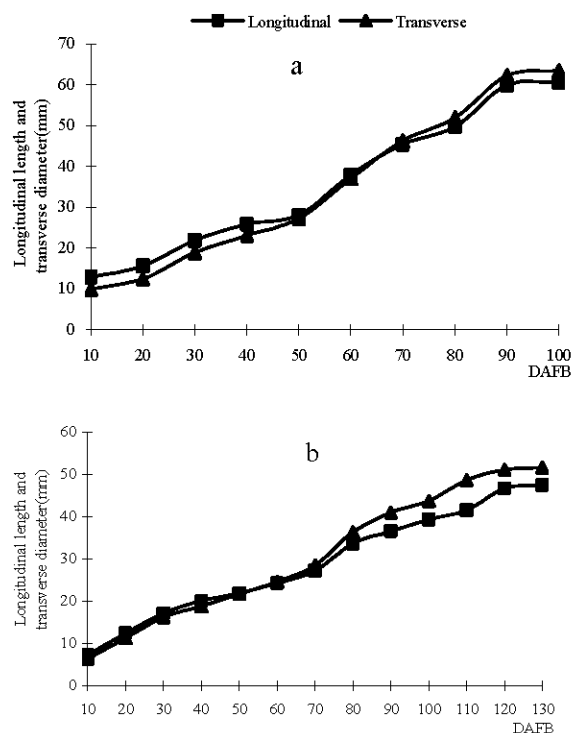


Fig. 1: Changes in longitudinal length and transverse diameter of Zaomi pears (a) and WhangKeumbae (b) pears

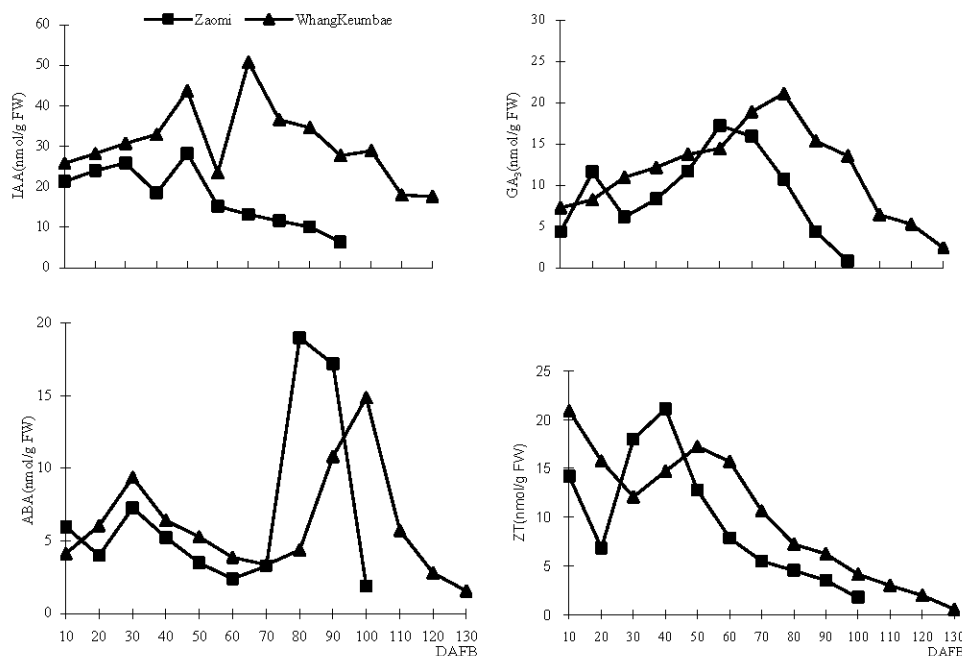


Fig. 2: Changes in endogenous hormones in the pulp during fruit growth and development of Zaomi and WhangKeumbae

Similar changes existed in WhangKeumbae pear fruits but included a longer period in every stage (in Fig.1-b). Also, considering the seed development conditions, the period from 0 to 50 DAFB could be approximately looked as the early stage, the period from 50 to 100 DAFB as the middle stage and the period from 100 to 130 DAFB as the late stage.

Changes in endogenous hormones found in the pulp during fruit growth and development: Seasonal changes in endogenous concentration of IAA, GA₃, ABA and ZT in pulp of Zaomi and WhangKeumbae pears are shown in Fig.2.

Relatively high concentrations of IAA in pulp of these two cultivars were measured at the initial days (about 7 to 15 DAFB). Then it followed with gradual increase up to a peak at 30 and 50 DAFB for Zaomi and WhangKeumbae pears, respectively and up to another peak at 50 and 70 DAFB. During the whole period of fruit development, IAA levels in pulp of Zaomi were lower than that of WhangKeumbae, of which the first and second peak value was 168.79% and 179.84% of that of Zaomi. And the corresponding time of peak was late for 20 days.

As far as concentrations of GA₃ found in the pulp was concerned, it changed in a bimodal curve peaking at 20 and 60 DAFB for Zaomi. For WhangKeumbae, it

increased constantly from initial level to a maximum at 80 DAFB. This maximum value was 122.44% of that of Zaomi pears at 60 DAFB. It was obvious that GA₃ level in pulp of these two cultivars rapidly increased from the middle stage, but decreased to an extremely low level at harvest.

Similar changing trend in ABA concentrations existed in pulp between the two cultivars, which maintained a constant low level before the sharp increase except the value at 30 DAFB. However, concentrations of ABA in pulp of Zaomi, WhangKeumbae pears increased rapidly from 70 and 80 DAFB and peaked at 80 and 100 DAFB, respectively. It followed with a continuous decline to a very low level at harvest. The peak value of Zaomi was 127.78% of that of WhangKeumbae, which held a higher level for a longer time.

The concentrations of ZT in the pulp of Zaomi pears were a little high at initial days and peaked at 40 DAFB. It reached to a maximum at 10 DAFB for WhangKeumbae pears, which was as many as 147.27% of that of Zaomi at 10 DAFB and gradually decreased to harvest with low level as Zaomi did.

Changes in endogenous hormones found in seeds during fruit growth and development: Changes in endogenous concentrations of IAA, GA₃, ABA and ZT in seeds during fruit growth and development are shown in Fig.3.

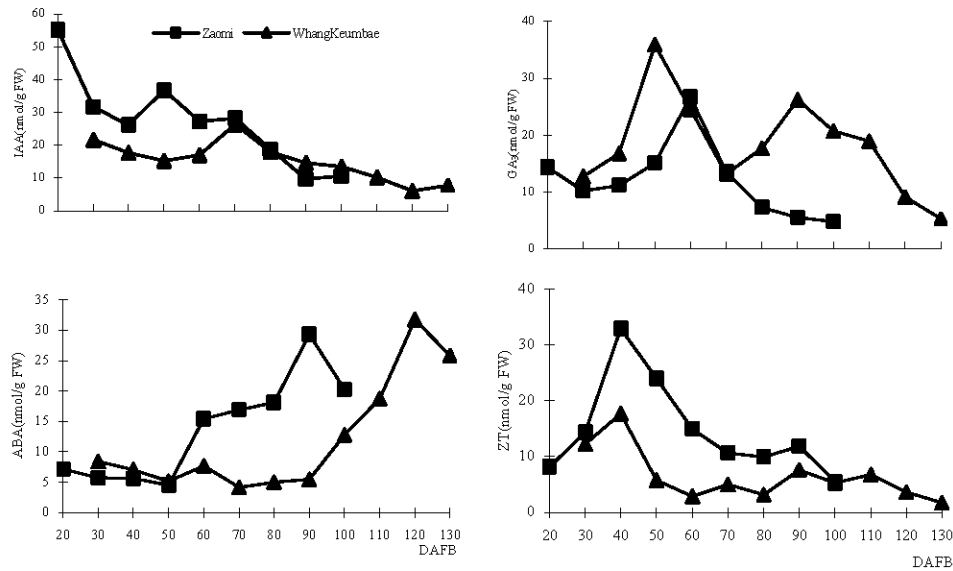


Fig. 3: Changes in endogenous hormones in seeds during fruit growth and development of Zaomi and WhangKeumbae

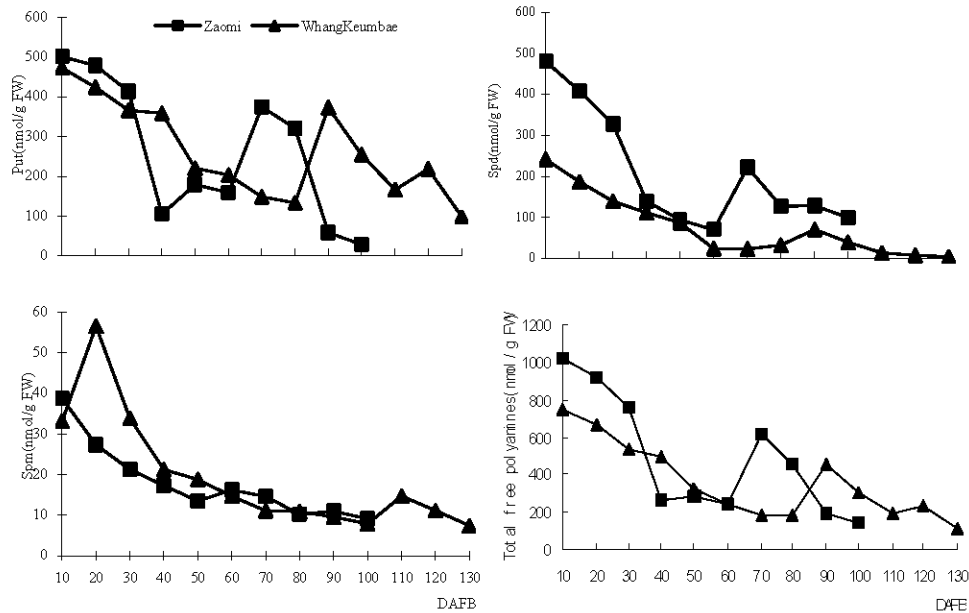


Fig. 4: Changes in endogenous polyamines in the pulp during fruit growth and development of Zaomi and WhangKeumbae

Zaomi pears contained a very high level of IAA at the initial days, peaked at 20 DAFB and peaked again at 50 DAFB. Then it followed with a constant decline to a slight rise before harvest. WhangKeumbae pears developed with a relatively parallel concentration curve of IAA but peaked at 70 DAFB, with 47.68% of the maximum value of Zaomi pears.

As for concentration of GA₃, Zaomi pears always contained similar or lower levels than WhangKeumbae

pears at the same phase. GA₃ levels in seeds of these two cultivars started to increase rapidly from 40 DAFB. They reached a maximum at 60 DAFB for Zaomi, at 50 DAFB for WhangKeumbae following with another peak at 90 DAFB.

Zaomi pear seeds contained a low level of ABA concentrations during the early stage and increased sharply between 50 to 60 DAFB, following with a slight rise during the next 20 days. Finally it peaked at 90 DAFB.

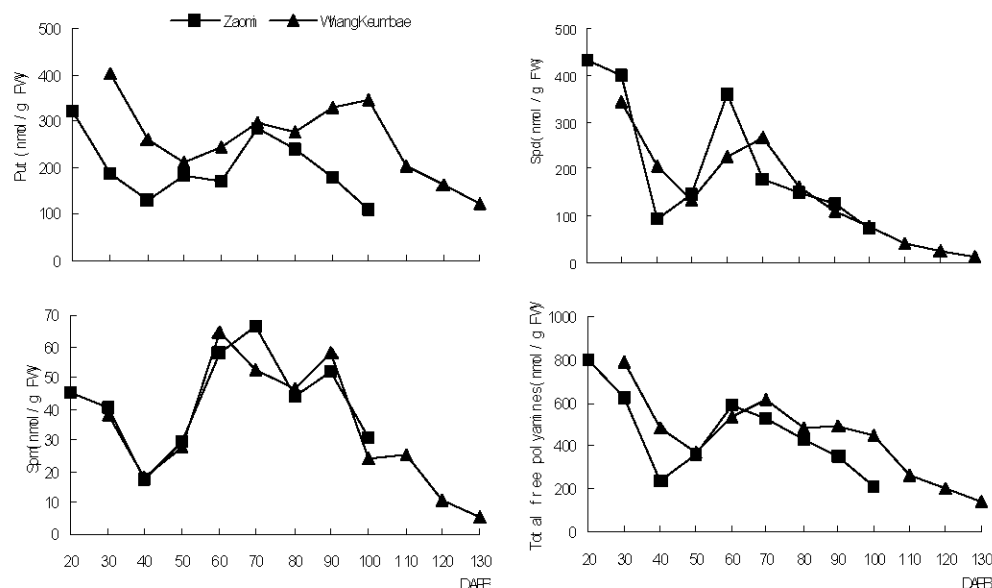


Fig. 5: Changes in endogenous polyamines in seeds during fruit growth and development of Zaomi and WhangKeumbae

As for WhangKeumbae pears, it changed slightly in ABA concentration with a continuous low level before 90 DAFB. After this it increased abundantly and peaked at 10 days before harvest with higher value than that of Zaomi pears.

The low levels of ZT concentration in seeds of the two cultivars were measured at initial stage. They then increased rapidly and both peaked at 40 DAFB. However, Zaomi pears contained higher level at the same stage of fruit development.

Changes in endogenous polyamines found in the pulp during fruit growth and development: Seasonal changes in endogenous concentration of *Put*, *Spd*, *Spm*, and total free polyamines in the pulp are shown in Fig.4.

The initial concentration of *Put* was in maximum at 10 DAFB for these two cultivars and then decreased gradually. It shifted to increase rapidly from 60 DAFB for Zaomi and 80 DAFB for WhangKeumbae and reached to another peak at 70 and 90 DAFB, respectively. At harvest WhangKeumbae pears contained higher titer than Zaomi pears, but both kept in a low level.

The relatively high concentration of *Spd* in pulp was measured at the initial stage and gradually decreased to a low level at harvest despite some rise during the middle stage. However, Zaomi pears contained higher level at the same stage. It was 198.64% of that of WhangKeumbae pears at 10 DAFB and was similar to *Put* level in pulp at the corresponding stage in early season. It was lower

during the middle and late stages. Also, *Spd* titer of WhangKeumbae pears was only 50-60 percent of *Put* concentration at the same developing stage.

Zaomi pears contained one parallel concentration curve of *Spm* with WhangKeumbae pears, which underwent an abrupt increase alone at 20 DAFB and a slight rise at 100 DAFB. For these two cultivars, concentrations of *Spm* were lower than that of *Put* and *Spd* at the same stage of fruit development.

The concentration of total free polyamines changed in a similar trend with *Put* and *Spd*. For Zaomi pears, the free polyamines level in pulp at the early, middle and late stage was 55.13%, 28.68%, 16.19% of the total free polyamines during the whole period of fruit development, respectively and for WhangKeumbae pears, being in contrast to 59.39%, 29.03%, 11.58% of that. It was obvious that seasonal trend of concentration in total free polyamines mirrored the concentrations of *Put* and *Spd* at the given stage of fruit development.

Changes in endogenous polyamines found in seeds during fruit growth and development: Changes in endogenous concentrations of *Put*, *Spd*, *Spm*, and the total free polyamines in seeds during fruit growth and development are shown in Fig.5.

Initial concentrations of *Put* of these two cultivars were the highest and then decreased gradually, shifted to increase from 40 DAFB for Zaomi pears and 50 DAFB for WhangKeumbae pears. Both of them got to another peak

at a lower value at 70 and 100 DAFB, respectively. During the whole period of fruit development Zaomi pears maintained an approximate or lower level at the corresponding stage than WhangKeumbae pears.

The highest *Spd* levels of these two cultivars was measured at initial days. The following decline lasted up to 40 and 50 DAFB for Zaomi pears and WhangKeumbae pears, respectively. After then they shifted to increase rapidly to another peak at 60 and 70 DAFB. Also, *Spd* concentration in seeds was not obviously different between the two cultivars, but was a little higher than that in pulp at the same stage for each cultivar.

As for *Spm*, both of the two cultivars contained much lower level comparing with *Put* and *Spd* at initial days. However, it converted to increase sharply from 40 DAFB, peaked at 70 for Zaomi pears and 60 DAFB for WhangKeumbae pears. Then it decreased gradually until harvest with a relatively high level for Zaomi pears but very low level for WhangKeumbae pears.

Zaomi pears developed seasonally in a parallel curve of concentration for total free polyamines with WhangKeumbae pears, but included lower values. It contained the free polyamines level at the early, middle and late stage with 34.44%, 41.36% and 24.20% of the total free polyamines during the whole period of fruit development, respectively, in contrast to 34.07%, 53.39% and 12.54% for WhangKeumbae pears. It was obvious that *Put* and *Spd* concentration predominated in the total free polyamines at the given stage.

DISCUSSION

Endogenous hormones and fruit development of pears:

The previous studies showed that cell division of fruit worked extremely actively at the early stage of fruit development. During this time most of terminal buds in spring shoots were sprouting and canopy was expanding. And then a good many nutritional materials stored in last winter or synthesized from photosynthesis this year were transferred to satisfy it. Thus, the powerful competition for organic nutrition, which was directly related to high concentration of endogenous hormones such as IAA, GA₃ and ZT in fruit, was favor of fruit setting and early development [10, 11]. In this study, for the middle ripening-season pear cultivar WhangKeumbae, it contained higher concentrations of IAA and ZT in the pulp than the early ripening-season pear cultivar Zaomi at initial stage. Also, it held longer period with high concentrations of these two endogenous hormones and GA₃ (shown in Fig. 2). It has been suggested that IAA

and ZT could accelerate division of cell nucleus and cytoplasm [12]. Based on this result, WhangKeumbae pears might develop with much more active and longer-term cell division than Zaomi pears did. This speculation coincided with the results in apple [13]. It could also be used to elucidate, to some extent, WhangKeumbae had the higher fruit setting efficiency than Zaomi besides genetic makeup. On the other hand, Zaomi pears contained higher concentrations of IAA and ZT in seeds. It might be good for more organic substances to be transferred into seeds so that Zaomi pears developed more rapidly into next phase than WhangKeumbae pears.

GA₃ was one of the important active substances to promote cell enlargement of fruit [14]. Combining it with IAA and ZT in pulp, one would presume that WhangKeumbae pears might maintain more active cell division at early stage of fruit development. And so both higher concentration of GA₃ and longer holding time were required to satisfy cell enlargement and fruit development at the following middle and late phases. As for ABA, the peaking time of concentration in the pulp and seeds of Zaomi pears was earlier than that of WhangKeumbae pears (shown in Figs. 2 and 3). This might result in shifting to maturity more early. Most views were coincident with it of triggering and hastening fruits to mature although controversial standpoints about ABA existed in fruit [15-17].

In general, it has been certain that fruit development is not linearly graphed as a function of any given endogenous hormone because hormones do not act alone, nor do they have a single function in fruit. They locate in different micro-spaces in fruit and combine differently to contribute to fruit growth and development [18]. Thus, the balance of concentration among all the endogenous hormones in fruit appears to promote its development more properly. And an understanding of receptors, effective working location and concentration and different configurations of endogenous hormones in fruit would enhance general knowledge of the working mechanism of fruit growth and development.

Endogenous polyamines and fruit development of pears:

Most research done on polyamines in fruit during fruit development emphasized the effects on pollination, fertilization and fruit setting [19, 20]. In this study, initial concentrations of *Put*, *Spd*, *Spm* and total free polyamines in the pulp and seeds were a little high for both Zaomi pears and WhangKeumbae pears (shown in Figs. 4 and 5). It might be induced by the vigorous activities of cell division in fruit at the early stage [21]. The relationship of

higher concentration of endogenous polyamines and shorter period of fruit development for Zaomi pears was speculated but still remains far away clear.

As one of the most important endogenous polyamines for tissue development of plant, *Put* reached its second peak of concentration both in the pulp and seeds of the two experimental cultivars at the late stage. But the middle ripening-season cultivar WhangKeumbae occurred later for 30 to 40 days and matured late for 30 days than the early cultivar Zaomi. This result was in part different from that of Toumadjet and Richardson [22] and was relatively in accordance with that of Omar [23] on pear, Liu *et al.* [24] on peach. The controversial conclusions might be induced by the different fruit cultivars, among which ethylene released in different ways to trigger fruit maturation and by the different growing conditions. The past research indicated that high level of *Put* concentration played an active role in triggering biosynthesis of ethylene, which reversely inhibited biosynthesis of *Put* after been triggered [25, 26]. On the other hand, the conjugated polyamines might be as important for cell division and enlargement of fruit as the free polyamines measured in this study [27, 28]. So analysis on them is expected to be carried out in detail.

Up to now, it has not been so clear for polyamines on functioning in fruit growth and development. However, some biochemical approaches such as cloning, locating and expressing for genes of enzymes related to biosynthesis of polyamines applied widely in other crops could be used to perfect this working mechanism. And the fruit growth and development could be modified to some extent in field practices by this theory.

Endogenous hormones and polyamines of fruit and different ripening seasons of pears: Development period of pear fruits produced in south region of China is rich in high temperature, strong light, heat and water. Thus, pears grow relatively rapidly, but had different development periods among different ripening-season cultivars. The field practices showed that the difference of just 2 to 5 days in blooming date would finally cause the difference of 75 days in development period of pear fruits in maximum. In this experiment, Zaomi pears contained higher levels of *Put*, *Spd* and total free polyamines, apparel titer of IAA and lower concentration of ZT in the pulp than WhangKeumbae pears. They bloomed early for only one day, but matured early for 30 days. The past research indicated that the concentration, location and activity of polyamines in fruit, even in the whole tree body were widely influenced by endogenous hormones such as

cytokinins, auxins and gibberellins [29]. They markedly took effects on the activity of polyamine synthases and their precursors and were inversely reacted by polyamines as well. Both the endogenous hormones and polyamines are the main ingredients resulting in different periods of fruit development. In conclusion, an understanding of the proper balance of them in the pulp and seeds could be helpful to gain deeper insight into fruit growth and development of pears, as well as other fruit crops.

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