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Changes in Activity of PG, PE, Cx and LOX in Pulp during Fruit Growth and Development of Two Different Ripening-Season Pear Cultivars

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Abstract: The activities of polygalacturonase (PG), pectinesterase (PE) and cellulase (Cx) and lipoxygenase (LOX) in pulp of two pear cultivars, Zaomi and Whangkeumbae, were detected every 10 days from anthesis to fruit maturation in this study. The results indicated that: (1) PG activity increased gradually along with fruit development of the two pear cultivars. It peaked at 50 Days after Full Blossom (DAFB) for Zaomi pear and peaked at 30, 90 and 130 DAFB for Whangkeumbae pear. Zaomi pear fruits contained higher activity of PG at the middle stage but had no statistically significant differences with Whangkeumbae pear fruits; (2) The activity of PE kept at high level at early stage and decreased gradually along with fruit development, but rose in slightly range before maturation for the two cultivars. It peaked at 10, 70 and 100DAFB for Zaomi pear and peaked at 10, 80 and 130DAFB for Whangkeumbae pear which contained higher activity at early stage than Zaomi pear and had lower level of activity at middle and late stage; (3) The activity of Cx was a little low at early stage and increased along with fruit development up to maturation following with slightly decrease for the two cultivars. It rose dramatically from 30 to 70DAFB and from 80 to 90DAFB for Zaomi pear fruits, whereas from 20 to 30DAFB, from 50 to 70DAFB and from 80 to 120DAFB for Whangkeumbae pear; (4) The two pear cultivars contained a little low level of LOX activity at initial stage and it increased equably along with fruit development. It rose remarkably from 20 to 40DAFB and from 50 to 90DAFB for Zaomi pear, but increased in slow rate for Whangkeumbae pear during the whole fruit development. In conclusion, activities of the five enzymes changed in a little similar trend during fruit growth and development for these two pear cultivars, but the peaks of activity appeared in different time.

Key words: Pear . PG . PE . Cx . LOX . fruit growth and development . ripening-season

INTRODUCTION

Fruit growth and development is a highly coordinated, genetically programmed and an irreversible phenomenon involving a series of physiological, biochemical and organoleptic changes that lead to the development of a soft and edible ripe fruit with desirable quality attributes. It is one of the foremost life activities for pear fruit trees and is regulated by complex interactions. A many of survey in field indicated that date of anthesis and its holding time differed in few days, about 2 to 5 days, among the different ripening-season pear cultivars in the same habitat. However, the whole days of fruit growth and development were so remarkably different. It could be up to 75 days between the earliest and latest pear cultivar. The early researches showed that reproductive development was positively correlated with some biochemical substances which in part controlled the processes of fruit growth, development, maturation and senescence. At the same time the enzymes of cell wall could regulate its degradation, which would result in fruit softening and maturating [1]. The three most important enzymes of cell wall, polygalacturonase (PG), pectinesterase (PE) and cellulase (Cx), all of which positively accelerated cell wall softening and degradation, could directly influence fruit growth and development and the date of fruit maturating [2-4]. And another kind of enzyme, lipoxygenase (LOX) plays crucial role in fruit maturating and softening through accelerating cell wall softening and degradation, could directly influence fruit growth and development and the date of fruit maturating [2-4]. And another kind of enzyme, lipoxygenase (LOX) plays crucial role in fruit maturating and softening through accelerating peroxidization reaction catalysed by itself. The products of peroxidization might destroy cell membrane, deactivation of enzymes of protein and might be as the substances synthesized into jasmonic acid (JA) and abscisic acid (ABA). They could also join in the

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processes of biological synthesis from 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene [5, 6]. Most of researches for these enzymes focused mainly on their physiological effects on fruit softening during storage [7-9]. The activity of these enzymes was determined on few researches during fruit growth and development. Especially, the relationship between these enzymes and different maturating dates of pear cultivars has not been reported in the past researches. Therefore, the early and middle ripening-season pear cultivars, Zaomi and Whangkeumbae, were selected to measuring the activity of PG, PE, Cx and LOX in pulp from anthesis to harvest in this experiment. The results were expected to elucidate, to some extent, that different ripening-season pear cultivars had obvious different maturating dates.

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, both of which were grafted on *Pyrus pashia* Buch-Ham rootstocks, were selected for experiment. All the trees were spaced 1.5×3.0 m apart in the same 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 contrzol 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 pear, respectively. The corresponding period of fruit growth and development was 100 and 130 days and finished in 257, 210 grams of final fruit. Fifteen to twenty fruits (three to five fruits per tree) were sampled for each time from anthesis to commercial harvest at 10 days interval. And about 2.000 gram pulp for each cultivar was stored at –70°C after it was weighed. Each sample was treated in triple repetitions.

Measurement of enzymes activity: The activity of PG, PE and Cx were measured referring to the methods of Zeng [10] and LOX activity was measured referring to the methods of Chen [11].

All the data were statistically analyzed with SPSS 11.5 software.

RESULTS

Changes in activity of PG, PE and Cx in pulp during fruit growth and development: Seasonal changes in acitivity of PG, PE and Cx in pulp of Zaomi and WhangKeumbae pears are shown in Fig. 1.

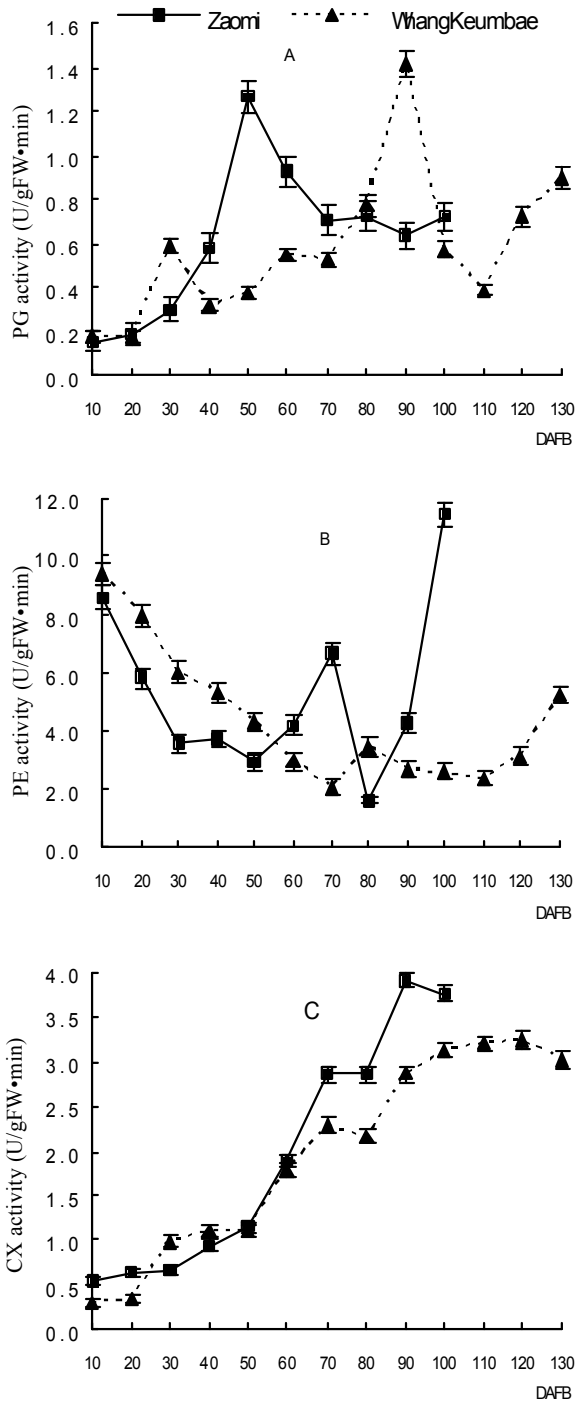

Fig. 1: Changes in PG, PE and Cx activity in pulp during fruit growth and development of Zaomi and WhangKeumbae pear

As showed in Fig. 1A, the activity of PG in pulp of these two pear cultivars changed in different curves and generally increased gradually along with fruit growth

and development. For Zaomi pear, it rose slightly from 10 to 30 DAFB with small increment of 0.0377 U/gFW·min and increased rapidly from 20 to 50 DAFB and reached to a maximum of 1.2640 U/gFW·min at 50 DAFB. After then it decreased slowly, but did not change too much from 70 to 100 DAFB and kept at a relatively high level at harvest. For Whangkeumbae pear, it contained a little low and approximate PG activity in pulp with Zaomi pear at initial days of fruit development. The PG activity almost did not increase during the 20 DAFB, but peaked at 30, 90 and 130 DAFB, respectively, with corresponding values being 0.5885 U/gFW·min, 1.4141 U/gFW·min and 0.8957 U/gFW·min. The second peak value (at 90 DAFB) was approximate with the maximum (at 50 DAFB) of Zaomi pear. An obvious increment appeared 20 days before maturation and finished in 0.8957 U/gFW·min which was not significantly different from that of Zaomi pear. As a whole, the PG activity of Zaomi pear was higher than that of Whangkeumbae pear at the middle stage of fruit growth and development, but was a little similar at the late stage.

As showed in Fig. 1B, PE activity in pulp changed in similar trend for both the two pear cultivars. It kept at a little high level at initial days and reduced gradually along with fruit development, but returned to increase rapidly before maturation and finished in a high level. For Zaomi pear, it contained different peaks of PE activity in pulp at 10, 70 and 100 DAFB, respectively, with corresponding values being 8.6180 U/gFW·min, 6.6635 U/gFW·min and 11.4416 U/gFW·min. At the same time the PE activity reduced dramatically from 10 to 30 DAFB and increased about 10 times during the last 20 days of fruit growth and development. For Whangkeumbae pear, it contained a similar changing trend of PE activity in pulp with Zaomi pear, but the increasing range was much slighter. It reached to three different peaks at 10, 80 and 130 DAFB with corresponding values being 9.4026 U/gFW·min, 3.4463 U/gFW·min and 5.2368 U/gFW·min. However, PE activity reduced gradually since 10 DAFB and got to the trough at 70 DAFB. As the same as that of Zaomi pear it started to return at 20 days before maturation, but was significantly lower at harvest ($p<0.01$). As a whole, PE in pulp of Whangkeumbae pear was higher than that of Zaomi pear at the early stage and was lower at middle and late stages.

As showed in Fig. 1C, a little low level of Cx activity was measured in pulp of the two pear cultivars at early stage and it increased gradually along with fruit development, but decreased slightly before maturation. For Zaomi pear it had rapid increment of Cx activity for two times, from 30 to 70 DAFB, with corresponding value being from 0.6633 U/gFW·min to 2.8610 U/gFW·min. Another increase lasted from 80 to 90 DAFB and reached to a maximum of 3.9141U/gFW·min at 90 DAFB. The similar changing trend of Cx activity existed in pulp of Whangkeumbae pear. The rapid increment appeared for three times during fruit growth and development. The first one was from 20 to 30 DAFB (from 0.3333 U/gFW· min to 0.9837U/gFW· min). The second one lasted from 50 to 70 DAFB as corresponding increment as 1.1 times. And the period from 80 to 120 DAFB was the last rapid increase with an increment of half-fold. A maximum of 3.2428 U/gFW·min was detected at 120 DAFB, which was not significantly different from that of Zaomi pear at 90 DAFB. As a whole, the Cx activity in pulp of Whangkeumbae pear was lower than that of Zaomi pear at the most time of fruit growth and development.

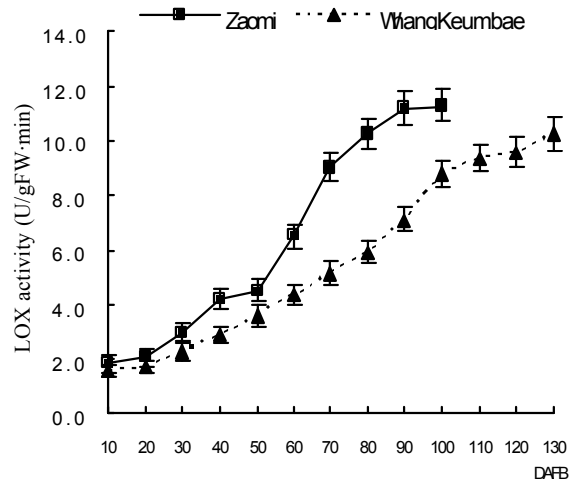

Fig. 2: Changes in LOX activity in pulp during fruit growth and development of Zaomi and WhangKeumbae pear

Changes in activity of LOX in pulp during fruit growth and development: Seasonal changes in activity of LOX in pulp of Zaomi and WhangKeumbae pears are shown in Fig. 2.

It was obvious that LOX activity in pulp of these two cultivars had similar changing trend during fruit growth and development as showed in Fig. 2. A little low level was determined at initial stage and it increased gradually along with fruit development. However, the LOX activity of Zaomi pear was higher than that of Whangkeumbae pear at the same developing stage and it increased remarkably during two different phases. The first one was from 20 to 40 DAFB, with the corresponding value being from 2.07 OD_{234}/gFW·min to 4.18 OD_{234}/gFW·min. The second one lasted from 50 to 90 DAFB, with the value being from 4.54 OD_{234}/gFW ·min to 11.22 OD_{234}/gFW·min as increment as 1.5 times. It reached to a maximum of 11.31 OD_{234}/gFW·min at harvest.

For Whangkeumbae pear, the LOX activity increased slightly during 20 DAFB, with only increment of 0.19 OD_{234}/gFW·min. After then it increased up to maturation in equable and slow rate. However, an appreciable increment lasted from 90 to 100 DAFB with the value being from 7.11 OD_{234}/gFW·min to 8.76 OD_{234}/gFW·min. It reached to a maximum of 10.25 OD_{234}/gFW·min at harvest, which had not statistically significant differences from that of Zaomi pear at the same stage.

DISCUSSION

Activity of PG, PE, Cx and fruit development of pears: A many of research work have been focused on the role of PG in fruit maturating and softening since Hobson firstly found that PG activity of tomato fruits increased gradually along with fruit growth and development and was associated with decrease in fruit firmness in 1956. During fruit maturation activities of pectinases in pulp such as PG, PE increase and many pectins are dissolved, which result in fruit softening. PG is the primary enzyme playing a significant role in pectin dissolution and fruit maturating of pears [5]. In this experiment the PG activity in pulp of Zaomi pear was comparatively higher than that of Whangkeumbae pear at the middle stage of fruit growth and development, but it was not significantly different at late stage. This result might explain to some extent that cell wall of Zaomi pear fruits started to decompose much earlier and fruit softening was triggered more rapidly than Whangkeumbae pear. However, the direct correlation between PG and softening of strawberry fruits was not found clearly yet [12]. On the other hand, only the activity of endo-PG in pulp was measured during fruit development in this experiment, so it is very necessary of determining the activity of exo-PG and total PG in next research for confirming their effects on fruit development, maturation and softening. The past researches showed that PE played a vital role in producing polygalacturonase. So it seems that PG might play effects on fruit maturation only after PE worked actively. In this experiment PE activity in pulp of these two pear cultivars decreased gradually along with fruit development, but it returned to increase before fruit maturation again. And Zaomi pear contained more increment of PE activity than Whangkeumbae pear and higher level of activity at middle and late stage of fruit development. Especially the statistically significant differences were measured between these two cultivars at harvest, which might be advantageous of storing well for Whangkeumbae pear fruits. At the same time three peaks of PE activity of these two experimental pear cultivars were determined at early, middle and late stage of fruit development, respectively. At these peaking time cell wall might be vigorously going on relaxing and extending with concomitant cell division and expansion in pulp. And it also might result in increase of cell number and pectin molecular in cell wall. This conclusion was coincided with the results of Zeng [10] and Li [13].

It is generally accepted that Cx is associated with fruit growth and development at initial stage and with fruit softening at late stage [14]. In this study Cx activity in pulp of Zaomi pear changed equably at the early stage of fruit development, but it rose dramatically from 20 to 30 DAFB for Whangkeumbae pear fruits. It was presumed that Cx could accelerate cell division and regulate growth and development of cell. In addition, Cx activity rose rapidly with concomitant fast increase in fruit growth for these two pear cultivars. It was speculated that Cx might accelerate relaxation and extension of cell wall and might stimulate synthesis of new substances during this phase. However, the rapid increase of Cx activity lasted for shorter time for Zaomi pear than that of Whangkeumbae pear and the higher level of activity was detected in Zaomi pear. It was deduced that Cx in pulp of Zaomi pear fruits worked more actively and seemed to be responsible for its early maturation. On the other hand Cx activity increased dramatically before maturation for the two experimental cultivars. It might cut off the crosslink points among the biological substances in cell wall, reduce the strength of cell wall and accelerate cell expansion and softening. And Zaomi pear contained higher level of Cx activity in pulp than Whangkeumbae pear at harvest. It might result in more complete degradation of cellulose in pulp, which would contribute to the tenderer, crisper, but not long keeping pulp texture for Zaomi pear.

In conclusion, cellulase is a multienzyme system composed of several enzymes. They take effects on different substances of sugar in different tissues of plant. Therefore, genes of cellulose can express differently in different tissues and developing stages of pear fruits.

Activity of LOX and fruit development of pears: Bousquest put forward an argument that LOX could catalyse the peroxidization reaction which would produce some free radicals [15]. These free radicals could badly destroy cell membrane and they might participate in the processes of biological synthesis of ethylene. As a result fruits went into maturating more rapidly. In this study LOX activity in pulp of the two experimental pear cultivars changed in similar trend. It was a little low at initial days and increased gradually along with fruit growth and development. But Whangkeumbae pear contained lower level than Zaomi pear and its LOX activity increased in equable rate

during the whole period of fruit growth and development. However, Zaomi pear showed two peaks of LOX activity. Especially it seemed to lead to accelerate fruit maturation for the second remarkable increase of activity.

Up to now much attention was focused on the relationship between LOX activity and ethylene releasing during fruit maturation and senescence. It is generally accepted that LOX could trigger peroxidization reactions of cell lipid and produce superabundant free radicals which positively participated in the biological synthesis of ethylene. As a result, a many of ethylene were produced and released [16]. However, this mutual relationship was not been established in this experiment because ethylene concentration was not determined for both the two pear cultivars during fruit growth and development. Therefore, it is an important research topic for LOX to elucidate its effects on fruit growth and development, especially on regulating fruit maturation. At the same time, LOX is a general enzyme existed almost in all the plants. They have different levels of activity in different tissues even in different stages of growth and development.

In conclusion, the activities of PG, PE, Cx and LOX in pulp of Zaomi pear were higher than that of Whangkeumbae pear at the same developing stage of fruits, respectively and they reached to peaks much earlier. Cell relaxation and degradation were correspondingly triggered earlier and fruits developed into maturation much faster for Zaomi pear. However, the process from growth to maturation of pear fruits is extremely complicated and is not influenced by only one enzyme, but many enzymes together. So it is desirable that significant breakthroughs in what make these enzymes active and taking effects on fruit growth and development will come forth in the near future.

On the other hand, recent advances in molecular biology have provided a better understanding of the biochemistry of fruit development as well as providing a hand for genetic manipulation of the entire development process [17]. It is very necessary for the related genes of these enzymes to be located and cloned in pear fruits, which could lead to considerable economic benefits.

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