Study on the Difference of EXP and PsEXPA10 in Ripening Period of ‘jinmi’ plum and ‘qingcui’ plum

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Abstract. The softening of plum fruit is an important factor affecting its promotion in production. In order to explore the changes of extension (EXP) during the ripening and softening stage of plum fruit, ‘jinmi’ plum and ‘qingcui’ plum were used as experimental materials to study the difference of EXP activity and PsEXPA10 expression during plum maturation and softening period. The results showed that as the fruit matured, the hardness of the fruit decreased, but there was a difference in the magnitude and rate of decrease between the two varieties, and the decrease of ‘Jinmi’ plum was even greater. The expression trends of EXP and PsEXPA10 were basically the same. At 81 d after flowering, the expression of PsEXPA10 in peel and flesh of ‘jinmi’ plum was the highest, and the activity of EXP reached the highest at 88 d after flowering. The expression levels of EXP and PsEXPA10 in peel and flesh of plum were gradually increased, and the highest values of PsEXPA10 expression in flesh of plum were all at 109 d after flowering, except that the expression level of PsEXPA10 in flesh of plum was at 102 d after flowering. The results showed that EXP was closely related to plum fruit ripening and softening. And this study also laid a foundation for further exploring the molecular mechanism of EXP's role in plum fruit softening.

1. Introduction

Plum (Prunus SPP.) belongs to the genus Prunus in Rosaceae. Plum fruit is easy to soften, and the softened fruit is susceptible to mechanical damage and pathogen infection11], which also affects the harvest time, storage life and other issues, so that it can not give full play to its due economic value. Expanin (EXP) is a class of cell wall proteins that regulate the relaxation and extension of the cell wall. Recent studies have shown that EXP is involved in almost all stages of plant growth and development, and plays an important role in fruit ripening and softening. It has been proved that EXP is related to ripening and softening in tomato22], kiwi33], loquat44] and other fruits. In order to understand the ripening and softening mechanism of plum fruit more comprehensively, it is necessary to study EXP.

‘Jinmi’ plum and ‘qingcui’ plum have different textures during their development and maturation. Compared with ‘jinmi’ plum, ‘qingcui’ plum is harder when fully matured. This study aims to determine the activity and gene expression of EXP during the fruit development of plum, to explore the correlation between EXP and plum softening, and to supplement the research blank of EXP.
2. Materials and methods

2.1. Test Materials
The test materials were collected from Plum Industrial Park, Da yi County, Chengdu City, Sichuan Province. The climate in this area is warm and humid, with sufficient heat. The orchard spacing is 4 m×5 m, the tree age is 8 years, the tree is medium, and the plant yield is 50-60 kg. The tested varieties were ‘jinmi’ plum and ‘qingcui’ plum with the same management level and basically the same growth.

2.2. Experimental design
Samples were collected from 70 days after flowering and every 7 days for a total of 8 times until the fruits were completely ripe. 3 trees are 1 plot, 3 repeats, 9 trees of each species. Two plum fruits were selected in the east, south, west, north, and center of the canopy, and a total of 90 plum fruits were picked from each variety at a time. The fruits were stored in the ice box immediately after picking and brought back to the laboratory within 3 hours. Under the ice bath condition, the pulp and peel were separated, frozen with liquid nitrogen, and stored in -80 ℃ refrigerator for subsequent determination of various indicators.

2.3. Test methods

2.3.1. Determination of fruit firmness. Peel hardness and pulp hardness were completed on a texture analyzer (TMS-PRO, USA) using a cylindrical probe. Parameters: The test speed is set to 40 mm/min, the starting force is set to 0.75 N, and the puncture distance is set to 25% of the average value of the transverse and lateral diameters. During the measurement, avoid the side where the suture line is located, and select the other three directions on the equatorial plane of the fruit for measurement. Repeat 30 times, every 10 times is a repetition.

2.3.2. Determination of EXP activity. EXP activity is measured by plant expansion protease-linked immunoassay kit (Mbio Company), and the operation is carried out in strict accordance with the kit instructions. Grind the flesh and skin into fine powder with liquid nitrogen, weigh 0.5g, add 4.5ml PBS (pH7.4), shake well and centrifuge for 20 min (2000 RPM), and carefully collect the supernatant for later use. Then add samples, add enzymes, warm breeding, mixing liquid, washing, color, termination, determination, calculation.

2.3.3. Determination of PsEXPA10 expression. With reference to the method of Shao Yi et al.[5], the improved perchlorate method was used to extract total RNA from plum fruits, and the optimization was based on the perchlorate method of Boss et al.[6]. Take the total RNA of fruit tissue as a template, refer to the cDNA reverse transcription kit instructions (ReverTraAce4-w (ToYoBo Japan)) for cDNA synthesis, and 3 biological replicates. Design primers based on the transcriptome data sequence measured by our group. According to Xu Qiu Hong's[7] method, protein elongation factor (EB2) was selected as the housekeeping gene. The primers were designed according to the housekeeping gene protein elongation factor EF-2 registered on NCBI, and the primers are shown in Table 1.

| Target genes | Primer sequences (5’-3’) |
|--------------|--------------------------|
| EXPA10       | AGGAGGGCCAGAAGTTTGTGG    |
|              | GGTGTTGCAGGAGGTGCTT      |
| EF-2 (housekeeping gene) | GGTGTGACGATGAAGAGTGATG |
|              | TGAAGGAGGGAAAGTGAAA     |

Table 1. Fluorescent quantitative primer sequence.
Quantitative analysis uses ABI7500 fluorescent quantitative PCR instrument (Applied Biosystems, USA), uses the 2–ΔΔCT formula to calculate the relative gene expression, converts the internal reference gene value in the experiment to 1, as a calibration sample, and compares with other samples, to obtain the relative expression value.

2.4. Data processing
Excel2016 and SPSS 23.0 software were used for data processing and analysis.

3. Results and analysis

3.1. Changes in peel hardness
It can be seen from Figure 1 that as the fruit matures, the peel hardness of ‘jinmi’ plum and ‘qingcui’ plum have a decreasing trend, but the decreasing amplitude and rate are different. The hardness of the peel of ‘Jinmi’ plum decreased the most from 81 d to 88 d after flowering. The hardness of the peel of ‘Jinmi’ plum was the highest at 64.99 N at 80 d after flowering, and the hardness was only 7.74 N at 109 d after flowering, and its hardness decreased 85.47%. From 60 d to 109 d after flowering, the hardness of the green crisp plum peel was less than that of the golden honey plum. At 109 d, the peel hardness of the green crisp plum reached the lowest value of 34.43 N, which was 58.6% lower than the initial hardness.

3.2. Changes in pulp hardness
It can be seen from Figure 2 that as the fruit matures, the firmness of the ‘jinmi’ plum pulp first slowly increases, and the firmness of the pulp gradually decreases at 74 d after flowering. The firmness of the pulp reaches the minimum value of 0.5 N at 109 d after flowering, which is 98.24% lower than the initial value. The flesh hardness of ‘qingcui’ plums showed an overall downward trend, and the degree of flesh hardness decreased the most from 67 d to 74 d after flowering. On 109 d after flowering, the firmness of the flesh of the green crisp plum reached the lowest value of 19.05 N, which was 66.41% lower than the initial value.
3.3. Changes in EXP activity in peel

It can be seen from Figure 3 that the EXP activity in the peel of ‘Jinmi’ plum gradually increased as the fruit matured, and it began to decrease after reaching the maximum value at 88 d after flowering. The EXP activity in the peel of the ‘Jinmi’ plum 109 d after flowering was 15.59 times the initial value. The activity of expanded protein in ‘qingcui’ plum peel showed an overall upward trend as the fruit matured. On 109 d after flowering, the EXP activity in the ‘qingcui’ plum peel was 18.64 times the initial value.

![Fig.3 EXP activity in the peels of plum](image)

3.4. Changes of EXP activity in pulp

It can be seen from Figure 4 that the EXP activity in the ‘Jinmi’ plum pulp gradually increases with the maturity of the fruit, reaching a maximum value at 88 da after flowering and then starting to decrease. At 109 d after flowering, the EXP activity in ‘Jinmi’ plum plup is 2376.35 ng/ml, which is the initial value of 6.39 Times. Exp activity in plum pulp increased gradually with fruit ripening. At 109 d after flowering, the activity of EXP in plum flesh was 580.9733 ng/mL, which was 12.47 times of the initial value.

![Fig.4 EXP activity in the pulp of plum](image)

3.5. Changes of PsEXPA10 Expression in Peel

It can be seen from Figure 5 that the relative expression level of PsEXPA10 in the peel of the ‘jinmi’ plum gradually increased with the ripening of the fruit, the highest rate of increase was from 74 d to 81 d, and reached the maximum value at 81 d after flowering, and then gradually began to decrease. The relative expression of PsEXPA10 in the peel of Jinmi plum at 109 d after flowering was 3.67 times the initial value. PsEXPA10 in ‘qingcui’ plum peels showed an overall upward trend as the fruit matured. The expression level of PsEXPA10 in the peel of ‘qingcui’ was the highest at 109 d after flowering, which was 11.62 times the initial value.
3.6. Changes of PsEXPA10 Expression in pulp

It can be seen from Figure 6 that with the gradual maturity of the fruit, the relative expression level of \( \text{PsEXPA10} \) in ‘jinmi’ plum pulp gradually increased, and began to decrease after reaching the maximum value at 81 d after anthesis. The relative expression level of PsEXPA10 in ‘jinmi’ plum pulp was compared with that at 109 d after anthesis. There is no major difference in the initial value. The relative expression level of PsEXPA10 in ‘qingcui’ plum pulp showed an overall upward trend with fruit maturity. The relative expression level of \( \text{PsEXPA10} \) in the ‘qingcui’ plum pulp was the highest at 102 d after flowering, which was 8.27 times the initial value.

4. Discussion and conclusion

In recent years, more and more studies have shown that fruit softening is the result of a series of degrading enzymes, and EXP is one of the possible enzymes. It has been reported that EXP can play a role in the fruit softening process by breaking the non-covalent bonds between cellulose and hemicellulose in the cell wall, such as hydrogen bonds. The results of this study showed that with the maturity of the fruit, the peel and pulp hardness of the ‘jinmi’ plum and ‘qingcui’ plum showed a decreasing trend, and the decrease in pulp hardness was greater than that of the peel, which was in line with the law of fruit development. The change trend of EXP activity during fruit ripening showed good consistency with the relative expression of \( \text{PsEXPA10} \), and the change of \( \text{PsEXPA10} \) preceded the change of EXP activity. In addition, the ‘qingcui’ plum is a hard variety, the overall hardness is higher than that of the ‘jinmi’ plum, the hardness decreases slowly and uniformly, and the corresponding EXP activity and \( \text{PsEXPA10} \) also rise slowly. Meanwhile, ‘Jinmi’ plum is a soft variety, and its hardness decreases rapidly in the middle period. During this period, the EXP activity and \( \text{PsEXPA10} \) expression increase rapidly. This indicates that \( \text{PsEXPA10} \) is significantly differently expressed during the ripening and softening stage of ‘jinmi’ Plum and ‘qingcui’ Plum, indicating that EXP may be involved in the softening process of fruit ripening. This is consistent with the results of previous studies on strawberry\(^8\), apricot\(^9\) and other fruits, which proves that EXP is related to fruit ripening and softening.
Through the above analysis, it is speculated that EXP plays an important role in plum fruit ripening and softening. The expression of *PsEXPA10* is highly correlated with plum fruit softening, and its expression has tissue specificity. This laid a theoretical and experimental basis for further analysis of the mechanism of expansin in the process of fruit ripening and softening. In addition, to fully understand the regulation mechanism of EXP during plum fruit ripening and softening, especially its interaction with other cell wall hydrolases and its response to other plant hormones, more in-depth research is needed.

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