Effects of Artificial De-astringency Treatment on the Fruit Storability of Wild Persimmon Germplasm from Guangxi (Postprint)

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Abstract

Using wild persimmon germplasm 'Youshi'(YS), 'Farmers'cultivated persimmon' (ZP), and the main cultivar 'Gongcheng Yueshi' from Guangxi as experimental materials, we analyzed changes in titratable acid content, soluble sugar content, firmness, ethylene biosynthesis amount, color difference, soluble tannin content, pectin, and cell wall degrading enzyme activities in artificially de-astringent ethephon-treated fruits during postharvest storage, to investigate the postharvest fruit softening mechanisms of different wild persimmon germplasms. The results demonstrated that, compared with Gongcheng Yueshi, Youshi exhibited higher titratable acid content, lower soluble sugar content, slower color development, and easier fruit softening. Among them, YS-4 was the least storage-tolerant, with firmness decreasing to 1.620 N on the 4th day of storage after ethephon treatment, soluble tannin content dropping to 2.398 $mg \cdot g^{1}$ on the 6th day, the slowest decline in protopectin content throughout the storage period, and the lowest final soluble pectin value of 0.832%. In contrast, YS-2 was the most storage-tolerant, with firmness on the 8th day after ethephon treatment being 3.6 times that of YS-4, and the highest firmness among all Youshi at the end of storage. In Farmers' cultivated persimmon, ZP-2 showed the highest titratable acid content, lowest soluble sugar content, slowest color development, smallest reduction in protopectin, and highest soluble pectin content at the end of ethephon-treated storage; conversely, ZP-3 fruits exhibited rapid color development and easy softening, with the lowest titratable acid content, low soluble sugar content, largest protopectin reduction, and low soluble pectin content. Gongcheng Yueshi showed PG activity and Cx activity far exceeding those of Youshi, and the correlation between fruit softening degree and cell wall degrading enzyme activities varied among different Youshi germplasms, with -D-galactosidase and Cx enzymes functioning during the early storage stage of Farmers' cultivated persimmon, while PG enzymes contributed to fruit softening during both early and late storage stages. This indicates that different

persimmon germplasms and cultivars have different dominant enzymes during the softening process. In summary, compared with Gongcheng Yueshi, 'Youshi' persimmon exhibited poor storage tolerance, with YS-4 being extremely non-storable, whereas 'Farmers' cultivated persimmon' ZP-2 was extremely storable. These results can provide basic germplasm materials for studying persimmon fruit softening mechanisms.

Full Text

Effects of Artificial Deastringency Treatment on the Storage Characteristics of Wild Persimmon Germplasm Fruits from Guangxi

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Abstract: This study investigated the postharvest softening mechanisms of different wild persimmon germplasms by analyzing changes in titratable acid content, soluble sugar content, fruit firmness, ethylene biosynthesis, color difference, soluble tannin content, pectin composition, and cell wall degrading enzyme activities during storage following artificial deastringency treatment with ethephon. Three distinct persimmon types were examined: the wild *Diospyros oleifera* germplasm 'Youshi' (YS), a farmer-cultivated landscape persimmon (ZP), and the main commercial cultivar 'Gongcheng Yueshi' .

The results demonstrated that, compared with Gongcheng Yueshi, *D. oleifera* fruits exhibited higher titratable acid content, lower soluble sugar content, slower color development, and greater susceptibility to softening. Among the *D. oleifera* accessions, YS-4 showed the poorest storage tolerance, with firmness decreasing to 1.620 N by day 4 and soluble tannin content dropping to 2.398 $mg \cdot g^{1}$ by day 6 after ethephon treatment. Throughout the storage period, YS-4 also showed the slowest decline in protopectin content and the lowest final soluble pectin value at 0.832%. In contrast, YS-2 demonstrated the best storage tolerance, maintaining a firmness 3.6 times higher than YS-4 on day 8 and showing the highest final firmness among all *D. oleifera* accessions.

For the farmer-cultivated persimmons, ZP-2 exhibited the highest titratable acid content, lowest soluble sugar content, and slowest color change at the end of ethephon treatment storage, with the smallest decrease in protopectin and highest soluble pectin content. Conversely, ZP-3 fruits showed rapid color development and easy softening, with the lowest titratable acid content and relatively low soluble sugar content, accompanied by the largest decrease in protopectin and low soluble pectin content.

Gongcheng Yueshi displayed significantly higher polygalacturonase (PG) and cellulase (Cx) activities than *D. oleifera*. The correlation between fruit softening

degree and cell wall degrading enzyme activity varied among germplasms, with -D-galactosidase (-D-Gal) and Cx playing important roles during early storage of farmer-cultivated persimmons, while PG contributed to softening during both early and late storage stages. These findings indicate that different enzymes dominate the softening process in different persimmon germplasms.

In summary, compared with Gongcheng Yueshi, *D. oleifera* showed poor storage tolerance, with YS-4 being extremely intolerant to storage, while the farmer-cultivated ZP-2 showed excellent storage tolerance. These results provide a valuable germplasm foundation for investigating persimmon fruit softening mechanisms.

Keywords: *Diospyros* germplasm; deastringency treatment; storage; softening; enzymatic activity

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Persimmon (*Diospyros* L.), a deciduous tree belonging to the family Ebenaceae, is primarily cultivated in tropical and temperate regions. As the country of origin, China possesses the world' s largest persimmon cultivation area and production, with over 50 species across two genera (Gao Zhiqiang, 2008). Guangxi, a major persimmon-producing region in China, is rich in persimmon germplasm resources, including multiple varieties such as *Diospyros kaki*, *D. lotus*, *D. oleifera*, *D. cathayensis*, *D. strigosa*, and *D. kaki* var. *silvestris*. In rural Guangxi, numerous wild *D. oleifera* varieties grow around farmhouses, characterized by non-cutinized leaves lacking luster and bearing pubescence. As fruits approach maturity, they develop a sticky surface coating that persists at full ripeness, with most showing brown spots and typically containing large, numerous seeds. These wild types exhibit broad adaptability and are commonly used as rootstocks (Deng Libao, 2012). Farmer-cultivated landraces, also found around houses, feature leathery leaves that are dark green and glossy on the adaxial surface and light green with pubescence on the abaxial surface (Deng Libao, 2012). Their fruits are medium-sized with good appearance quality, seedless or with few seeds, but generally exhibit poor comprehensive traits due to lack of management (Deng Libao, 2013). Genetic relationship analysis of 189 persimmon germplasm accessions from 12 Guangxi regions revealed that germplasms from Leye, Xilin, Tianlin, and Youjiang areas share close genetic relationships, as do those from Quanzhou, Huanjiang, Zhongshan, Luzhai, Wuxuan, Qintang,

and Hengxian (Deng Libao, 2015). Using SCoT molecular markers, Deng Libao (2013) divided the endemic *D. oleifera* into two subgroups, with 35 accessions of subgroup III further divided into four smaller subgroups at Euclidean distance D=12.09, and 17 accessions of subgroup IV divided into three smaller subgroups at D=9.59.

Persimmon is a climacteric fruit that undergoes a series of internal and external changes during storage (Smith et al., 2002), with softening being a major and visually apparent transformation (Ahmed & Labavitch, 1980). This surface softening results from the combined action of various substances, with alterations in cell wall structure and components having the most direct impact (Huber, 1983). The cell wall comprises cellulose, pectin, and other materials. As a primary cell wall component, pectin transforms from protopectin to soluble pectin during fruit maturation and storage, leading to fruit softening and firmness decline (Femenia, 1998; Fischer & Bennett, 1991). Naturally ripening Gongcheng Yueshi fruits showed a 3.5-fold increase in softening degree by day 5 of storage compared to day 0 (Fan Lingjiao, 2016), while postharvest Anxi *D. oleifera* fruits decreased to 6.0 N in firmness by day 33, with a respiratory peak occurring during storage, high decay rates in later stages, and substantial quality deterioration (Wang Hui, 2018).

Softening is a complex process involving not only macromolecular transformations but also synergistic enzyme activities. Polygalacturonase (PG), cellulase (Cx), pectin methylesterase (PME), -galactosidase (-D-Gal), and pectate lyase (PL) regulate fruit softening, as demonstrated in persimmon (Kang et al., 1994; Kang et al., 1998; Luo Zisheng, 2005), avocado (Buse & Laties, 1993; De Veau et al., 1993), apple (Abeles & Biles, 1991; Shen Shuguang, 1991), banana (Prabha & Bhagyalakshmi, 1998), and strawberry (Jiménez-Bermúdez et al., 2002).

No previous studies have reported on fruit softening in Guangxi wild persimmon germplasm resources. This experiment grouped endemic *D. oleifera* and farmer-cultivated persimmon germplasms by genetic relationship to investigate relationships between fruit softening indicators during postharvest natural ripening and ethephon deastringency treatment. By comparing storage tolerance differences with the main Guangxi cultivar 'Gongcheng Yueshi' , we explored the causes of softening in wild persimmon germplasm to provide both a germplasm foundation for studying persimmon fruit softening mechanisms and a theoretical basis for future development and utilization of wild persimmon germplasm resources.

1.1 Materials

The experimental materials included *D. oleifera*, farmer-cultivated persimmon, and 'Gongcheng Yueshi' , all harvested from the persimmon germplasm repository at the Specimen Garden of Guangxi University College of Agriculture. Farmer-cultivated persimmons and corresponding 'Gongcheng Yueshi' samples were harvested on October 21, 2017, and stored until November 6, 2017. *D. oleifera* and corresponding 'Gongcheng Yueshi'samples were harvested on Novem-

ber 12, 2017, and stored until November 26, 2017. Uniform fruits with similar maturity, free from pests, diseases, and obvious mechanical damage were selected and stored in a constant temperature warehouse at 25°C (with strict control of error within 1°C) and relative humidity maintained between 60% and 70%.

Following Deng Libao (2013), the *D. oleifera* accessions were divided into four subgroups based on genetic relationships and designated as 'YS-' , while farmer-cultivated varieties were divided into three subgroups designated as 'ZP-' .

1.2 Experimental Treatment

Ethephon deastringency treatment was applied by completely immersing persimmon fruits in 500 $mg \cdot L^{1}$ ethephon solution for 5 minutes. After immersion, fruits were quickly removed, air-dried in a ventilated shade area, sealed in plastic bags for 24 hours, and then placed in the constant temperature warehouse for storage. Control fruits received identical treatment using deionized water instead of ethephon solution. Physiological indicators were measured at one-day intervals. After measurement, the flesh surrounding the fruit core was chopped, frozen in liquid nitrogen, and stored at -80°C.

1.3 Measurement Methods

1.3.1 Firmness Determination Firmness was measured following the method of Wang et al. (2010). Nine persimmon fruits were randomly selected from both treatment and control groups, divided into three replicates of three fruits each. The fruit skin was removed before measurement, and firmness was determined using a texture analyzer.

1.3.2 Total Color Difference Determination Total color difference was measured by randomly selecting nine fruits from each group (three replicates of three fruits), with measurements taken on two opposite surfaces of each fruit using a colorimeter.

1.3.3 Ethylene Biosynthesis Measurement Ethylene production was measured following the method of Liu et al. (2018). Fifteen fruits were randomly selected (three replicates of five fruits), placed in a 4 L sealed jar for 6 hours, after which five gas samples were extracted and analyzed by gas chromatography.

1.3.4 Titratable Acid Determination Titratable acid content was determined by sodium hydroxide titration following Cao et al. (2007).

1.3.5 Soluble Sugar Determination Soluble sugar content was measured using the anthrone reagent method according to Cao et al. (2007).

1.3.6 Soluble Tannin Determination Soluble tannin content was determined by the Folin-Denis method following Taira (1996).

1.3.7 Protopectin and Soluble Pectin Determination Protopectin and soluble pectin contents were measured by the carbazole colorimetric method according to Cao et al. (2007).

1.3.8 Enzyme Extraction and Activity Assay Enzyme extraction followed the method of Liu et al. (2018) with modifications: (1) 2.0 g of flesh was ground in liquid nitrogen and placed in a 10 mL centrifuge tube; (2) 4.0 mL of 0.2% sodium sulfate solution was added and mixed thoroughly; (3) The mixture was centrifuged at 4°C for 30 min, the supernatant was removed, and 4.0 mL of 100 mmol · L ^{1} sodium acetate buffer was added and mixed; (4) After centrifugation at 4°C for 30 min, the supernatant was collected as crude enzyme extract.

Enzyme activities were determined as follows: pectinase activity by the method of Jiang et al. (2010), cellulase activity by the 3,5-dinitrosalicylic acid method (Opigo & Ying, 2010), and -D-galactosidase activity by the method of Itamura et al. (2013).

2.1.1 Changes in Soluble Tannin Content

During storage, soluble tannin content decreased progressively in all persimmon germplasms [Figure 1: see original paper]. In the control group, *D. oleifera* fruits initially contained higher soluble tannin levels than Gongcheng Yueshi, with YS-2 showing the slowest decline and YS-5 the fastest, reaching a minimum of 0.440 mg · g ^{1} by day 10. At the end of storage, YS-2 maintained the highest tannin content at 21.722 mg · g ^{1} with the smallest reduction, followed by Gongcheng Yueshi with an 11.946 mg · g ^{1} decrease.

Following ethephon treatment, soluble tannin content in *D. oleifera* decreased dramatically during the first 10 days before stabilizing, with all values falling below 5 mg · g ^{1} by day 10. At storage termination, Gongcheng Yueshi showed the lowest soluble tannin content at 0.002 mg · g ^{1}, while *D. oleifera* maintained approximately 1 mg · g ^{1}.

For farmer-cultivated persimmons, Gongcheng Yueshi initially had the lowest soluble tannin content while ZP-2 had the highest. ZP-2 showed rapid tannin decline between days 12-14, ZP-3 declined rapidly during days 0-4, and ZP-1 exhibited greater reduction in later storage stages. ZP-3 showed the greatest overall decrease at 13.13 mg · g ^{1}.

Ethephon treatment induced sharp tannin reductions during days 12-16 in farmer-cultivated persimmons. At storage end, Gongcheng Yueshi had the lowest content (6.75 mg · g ^{1}), followed by ZP-3 (7.45 mg · g ^{1}), while ZP-2 maintained the highest levels. ZP-3 showed the largest decrease at 17.99 mg · g ^{1}. These results indicate that wild persimmon germplasms contain higher tannin levels than Gongcheng Yueshi, and ethephon treatment accelerates tannin degradation.

Note: A. Control of Diospyros oleifera*; B. Ethephon treatment of* D. oleifera*; C. Control of cultivated persimmon; D. Ethephon treatment of the cultivated*

persimmon. The same below.

2.1.2 Changes in Soluble Sugar Content

Soluble sugar content in control *D. oleifera* showed varied patterns: YS-5 and YS-6 increased continuously, while YS-2 and YS-4 increased initially then decreased, and Gongcheng Yueshi decreased overall [Figure 2: see original paper]. Initial soluble sugar content in Gongcheng Yueshi was approximately double that of *D. oleifera*. During days 12-14, soluble sugar content dropped sharply to 0.6-0.8% in all accessions.

Ethephon treatment caused soluble sugar content to decrease during days 0-10 before increasing, with a rapid decline to below 1% during days 8-10 followed by substantial increases.

In farmer-cultivated persimmons, Gongcheng Yueshi showed increasing soluble sugar content during storage, while the landraces generally decreased, ending at 6.7% and approximately 4.0%, respectively. Ethephon treatment inhibited sugar accumulation in Gongcheng Yueshi but had minimal effect on farmer-cultivated types. These findings demonstrate that *D. oleifera* has lower soluble sugar content than Gongcheng Yueshi, with ethephon treatment accelerating initial sugar decline but promoting later increases, while slowing titratable acid decline in farmer-cultivated persimmons.

2.1.3 Changes in Titratable Acid Content

Titratable acid content decreased overall during storage [Figure 3: see original paper]. Initially, Gongcheng Yueshi had the lowest content (1.10%), followed by YS-5 (1.16%), while YS-6 had the highest (1.85%). By storage end, YS-4 showed the greatest reduction (1.19%), YS-2 the smallest (0.34%), and YS-5 and Gongcheng Yueshi decreased by 0.66% and 0.55%, respectively.

Ethephon treatment resulted in the smallest reduction in YS-2 (0.22%) and the largest in YS-6 (1.11%), with Gongcheng Yueshi decreasing by 0.59%. In farmer-cultivated persimmons, all except ZP-1 showed declining trends, with ZP-2 having the highest initial content (0.86%) and ZP-3 the lowest (0.58%). ZP-1 showed a sharp increase on day 12. Ethephon treatment slightly slowed acid decline in Gongcheng Yueshi and caused increases in later storage stages. These results indicate that *D. oleifera* has higher titratable acid content than Gongcheng Yueshi, with deastringency treatment having minimal effect on *D. oleifera* but slowing acid decline in farmer-cultivated types.

2.2.1 Changes in Fruit Firmness

Fruit firmness decreased continuously during storage [Figure 4: see original paper]. Among *D. oleifera* accessions, YS-4 showed the most rapid decline, dropping 27.08 N during days 0-6 before stabilizing, followed by YS-5 which decreased 25.40 N during days 0-8. After day 8, Gongcheng Yueshi maintained

higher firmness than all *D. oleifera* accessions, showing the smallest overall reduction at 16.57 N.

Ethephon treatment caused dramatic firmness losses during days 0-4, with YS-4 decreasing 26.67 N and YS-5 24.01 N. After day 6, Gongcheng Yueshi maintained higher firmness than *D. oleifera*, though it reached the lowest final value of 0.39 N. All *D. oleifera* accessions fell below 5 N by day 8.

In farmer-cultivated persimmons, Gongcheng Yueshi had higher initial firmness than the landraces, showing significant reductions during days 0-4 and 12-16 with a stable middle period. ZP-1 and ZP-2 began softening after day 4, with maximum losses during days 12-16, while ZP-3 softened slowly until day 12 then dropped sharply to 8.57 N. Gongcheng Yueshi showed the greatest total reduction (15.91 N), followed by ZP-3 (15.36 N), while ZP-2 had the smallest decrease (6.98 N).

Ethephon treatment accelerated softening more than the control, particularly during days 0-4. ZP-3 declined sharply after day 8, ZP-1 showed uniform reduction throughout storage, and Gongcheng Yueshi decreased rapidly except during days 8-12. Final firmness values were lowest in Gongcheng Yueshi (6.09 N), followed by ZP-3 (7.66 N), with ZP-2 maintaining the highest firmness. These results indicate that ethephon treatment accelerates fruit softening, particularly in *D. oleifera*, with YS-4 softening fastest and YS-2 slowest among *D. oleifera*, while ZP-3 softened most readily and ZP-2 was most resistant among farmer-cultivated types.

2.2.2 Changes in Ethylene Biosynthesis

Ethylene production showed fluctuating patterns with multiple peaks [Figure 5: see original paper]. In control *D. oleifera*, YS-2, YS-4, and Gongcheng Yueshi peaked on day 4 (3.88, 3.58, and 1.39 $\mu L \cdot g^{-1} \cdot h^{-1}$, respectively), while YS-5 and YS-6 peaked on days 8 and 10 (9.94 and 7.51 $\mu L \cdot g^{-1} \cdot h^{-1}$, respectively).

Ethephon treatment increased peak ethylene production, with YS-2 and Gongcheng Yueshi peaking on day 4 (9.76 and 3.44 $\mu L \cdot g^{-1} \cdot h^{-1}$), YS-4 and YS-5 on day 8 (12.60 and 10.36 $\mu L \cdot g^{-1} \cdot h^{-1}$), and YS-6 on day 10 (10.23 $\mu L \cdot g^{-1} \cdot h^{-1}$).

In control farmer-cultivated persimmons, ethylene fluctuations were relatively small, with landraces peaking on day 12 and Gongcheng Yueshi showing peaks on days 4 and 12. Ethephon treatment advanced peak appearance times, with ZP-1 peaking first on day 4 (2.94 $\mu L \cdot g^{-1} \cdot h^{-1}$), followed by Gongcheng Yueshi and ZP-2 on day 8 (2.34 and 2.00 $\mu L \cdot g^{-1} \cdot h^{-1}$), and ZP-3 latest and highest on day 12 (2.56 $\mu L \cdot g^{-1} \cdot h^{-1}$). These results demonstrate that *D. oleifera* produces much higher ethylene peaks than farmer-cultivated and Gongcheng Yueshi persimmons, with ethephon treatment amplifying ethylene production, particularly in *D. oleifera*, and accelerating peak appearance in ZP-1, ZP-2, and Gongcheng Yueshi.

2.2.3 Changes in Total Color Difference

Total color difference increased continuously during storage [Figure 6: see original paper]. In control *D. oleifera*, the most significant increase occurred during days 0-4, inversely corresponding to the rapid firmness decline during the same period. At storage end, Gongcheng Yueshi showed the highest color difference value (25.28), followed by YS-4 (14.77) and YS-5 (14.44).

Ethephon treatment resulted in higher color difference values than the control, with both groups stabilizing during days 8-10. Gongcheng Yueshi exceeded *D. oleifera* from day 6 onward, showing large increases during days 0-8, stabilization, then another sharp rise during days 12-14, while *D. oleifera* showed much smaller increases during the final period.

In farmer-cultivated persimmons, color difference increased sharply during days 0-4, stabilized, then increased significantly again. ZP-3 showed the fastest color development, reaching the highest final value (18.6), followed by ZP-2 (15.8), while Gongcheng Yueshi had the lowest value (13.0). Ethephon treatment produced higher values than the control, with both groups stabilizing during days 8-12 and showing significant increases in early and late storage. Final values were highest in ZP-3 (25.0), followed by ZP-2 (20.0), with ZP-1 lowest (15.2). These results indicate that persimmon fruits gradually yellow during storage, with ethephon treatment accelerating ripening and color development.

2.2.4 Changes in Protopectin Content

Protopectin content decreased overall during storage [Figure 7: see original paper]. In control *D. oleifera*, initial values were higher than in Gongcheng Yueshi, with greater reduction rates. YS-2 showed the largest decrease (2.47%), followed by YS-4 (1.84%), while Gongcheng Yueshi had the smallest reduction (0.14%). Ethephon treatment caused YS-2 to decrease by 2.81%, YS-4 by 2.54%, and Gongcheng Yueshi by only 1.03%, indicating that *D. oleifera* has higher protopectin content than Gongcheng Yueshi, with substantial decreases during postharvest ripening.

In farmer-cultivated persimmons, initial protopectin values exceeded those of Gongcheng Yueshi, with significant declines after day 8. ZP-3 showed the greatest reduction (1.12%), while ZP-2 had the smallest (0.54%). Ethephon treatment induced sharp protopectin decreases during days 0-4, with ZP-3 showing the largest final reduction (1.37%), followed by Gongcheng Yueshi (1.05%), and ZP-2 the smallest (0.79%). These results demonstrate that ethephon treatment promotes rapid protopectin degradation during early storage.

2.2.5 Changes in Soluble Pectin Content

Soluble pectin content increased during storage [Figure 8: see original paper]. In control *D. oleifera*, YS-2 showed the greatest increase (0.57%), followed by YS-4 (0.41%), while Gongcheng Yueshi had the smallest increase (0.09%). Ethephon

treatment produced higher values than the control, with YS-2 reaching the highest final content (1.00%) and greatest increase (0.68%), while Gongcheng Yueshi had the lowest content (0.70%) and smallest increase (0.34%).

In farmer-cultivated persimmons, ZP-2 had the highest final soluble pectin content (0.66%), followed by ZP-1 (0.57%), with Gongcheng Yueshi lowest (0.44%). Ethephon treatment caused rapid increases during days 0-4, substantially enhancing the increase in Gongcheng Yueshi but having minimal effect on ZP-1 and ZP-2. Final values were highest in ZP-2 (0.62%) and lowest in ZP-3 (0.53%). These results indicate that ethephon treatment promotes soluble pectin accumulation during early storage and accelerates the conversion of protopectin to soluble pectin.

2.3.1 Changes in PG Enzyme Activity

PG, Cx, and -D-Gal play crucial roles in fruit softening. Gongcheng Yueshi maintained higher PG activity than *D. oleifera* throughout storage, showing an initial increase followed by decrease, while *D. oleifera* generally decreased [Figure 9: see original paper]. Gongcheng Yueshi reached maximum PG activity on day 8 (24.67 mg · h ^{1} · g ^{1}). Among *D. oleifera*, YS-5 maintained the highest activity, while YS-4 showed elevated activity on days 6 and 12.

Ethephon treatment, except in YS-2, induced initial increases followed by decreases, with smaller reductions than the control. Gongcheng Yueshi peaked on day 4 (26.22 mg · h ^{1} · g ^{1}) and maintained high activity thereafter, while YS-4 showed minimal fluctuation during days 0-10 before a final peak.

In farmer-cultivated persimmons, PG activity initially increased, decreased, then increased again, with minimum activity on day 8. ZP-1 and ZP-2 showed first peaks on day 4 (14.50 and 12.50 mg · h ^{1} · g ^{1}), decreasing then rising to maximum values on day 16 (15.60 and 12.81 mg · h ^{1} · g ^{1}). ZP-3 peaked on day 4 (16.29 mg · h ^{1} · g ^{1}), while Gongcheng Yueshi peaked on day 12 (16.74 mg · h ^{1} · g ^{1}). Ethephon treatment delayed the activity minimum in ZP-1, ZP-3, and Gongcheng Yueshi while increasing peak values.

Gongcheng Yueshi consistently maintained higher PG activity than *D. oleifera*, remaining high during late softening stages. Significant correlations were found between firmness and PG activity in YS-2 (positive) and ZP-2 (negative) , indicating PG regulation in these accessions.

2.3.2 Changes in Cx Enzyme Activity

Gongcheng Yueshi Cx activity increased overall, while *D. oleifera* decreased [Figure 10: see original paper]. During days 0-4, Gongcheng Yueshi Cx activity increased rapidly by 9.33 mg · h ^{1} · g ^{1}, reaching minimum values during days 10-12 before rising again. YS-6 showed the greatest final reduction (6.70 mg · h ^{1} · g ^{1}), followed by YS-4 (5.50 mg · h ^{1} · g ^{1}).

Ethephon treatment effectively reduced the decline rate in *D. oleifera* but had minimal effect on Gongcheng Yueshi, which maintained high activity after an initial rapid increase on day 4. YS-4 showed a peak on day 12 (14.92 mg · h ^{1} · g ^{1}).

In farmer-cultivated persimmons, Cx activity patterns varied among accessions, with ZP-1 peaking on day 12, ZP-2 and Gongcheng Yueshi on days 4 and 16, and ZP-3 on day 8. Ethephon treatment promoted Cx activity, most significantly in Gongcheng Yueshi, followed by ZP-3, with minimal effect on ZP-2.

Significant positive correlations between firmness and Cx activity were observed in control YS-2, YS-4, and YS-6, while ethephon-treated Gongcheng Yueshi showed significant negative correlation, and *D. oleifera* showed extremely significant positive correlation , indicating enhanced Cx regulation after ethephon treatment. Different peak times among farmer-cultivated persimmons suggest that ethephon increases Cx activity peaks while inhibiting activity decline in *D. oleifera*.

2.3.3 Changes in -D-Gal Enzyme Activity

-D-Gal activity increased overall during storage [Figure 11: see original paper]. In control *D. oleifera*, YS-5 and YS-4 began rapid increases on days 6 and 10, reaching final activities of 89.03 and 55.50 mg · h ^{1} · g ^{1}, respectively. Ethephon treatment produced greater increases than the control, with different accessions showing rapid increases at different times: YS-5 and YS-6 after day 4, and YS-2 and YS-4 after day 10.

Significant negative correlations between firmness and -D-Gal activity were found in control YS-4 and in both control and ethephon-treated YS-2 and YS-4 . In farmer-cultivated persimmons, ZP-3 reached the highest final -D-Gal activity (22.95 mg · h ^{1} · g ^{1}), while ZP-1 had the lowest (18.06 mg · h ^{1} · g ^{1}). Ethephon treatment promoted activity increases in ZP-1 and Gongcheng Yueshi throughout storage but inhibited ZP-2 and ZP-3 after day 8. Significant negative correlations were observed between firmness and -D-Gal activity in ZP-1 and ZP-2 .

TABLE:1 Correlation analysis between fruit firmness and six other postharvest physiological indexes with ethephon treated and control of *Diospyros* Germplasm

Treatment	Soluble Tannin	Protopectin	Soluble Pectin	PG	Cx	-D-Gal
Control	0.854**	0.939**	-0.931**	0.666**	-0.585*	-0.471*
Ethephon	0.673**	0.839**	-0.869**	0.965**	-0.943**	0.740**

Treatment	Soluble Tannin	Protopectin	Soluble Pectin	PG	Cx	-D-Gal
···(table continues with other germplasms)						

Note: means significant correlation ($P < 0.05$); ** means extremely significant correlation ($P < 0.01$).*

3.1 Relationship Between Fruit Softening and Soluble Tannin Content During Artificial Deastringency

Tannins are the source of astringency in persimmon fruits. Freshly harvested astringent persimmons contain high tannin concentrations that decrease during ripening as aldehydes bind with soluble tannin molecules to form insoluble condensed tannin polymers, resulting in deastringency (Yin Xueren, 2011). During late storage stages when fruits are extremely soft, soluble tannin content declines more rapidly (Zhang Guixia et al., 2009). Additionally, interactions between condensed tannins and pectin can reduce astringency (Tuerxun Maimaiti, 2017). In this study, soluble tannin content decreased with firmness, showing extremely significant positive correlation. Ethephon treatment caused rapid firmness loss in *D. oleifera* during days 0-4, followed by sharp tannin decline after day 4, while farmer-cultivated persimmons showed accelerated firmness and tannin losses during days 12-16, confirming previous findings.

3.2 Relationship Between Fruit Softening and Pectin Content

Pectin is the primary component of the cell wall middle lamella. During fruit softening, middle lamella dissolution precedes cell wall breakdown, ultimately causing fruit softening (Wang Rencai, 2000). Before ripening, pectin exists as protopectin, and its conversion to soluble pectin during ripening leads to firmness decline. Studies have shown significant positive correlation between firmness and protopectin content, and significant negative correlation with soluble pectin (Tian Jianwen, 1994; Xia Chunsen, 1991). In this study, ethephon-treated *D. oleifera* showed substantial protopectin decline during days 0-4, corresponding to rapid firmness loss, with extremely significant correlations between firmness and protopectin (positive) and soluble pectin (negative) , consistent with previous research. However, studies on papaya found that rapid protopectin decline did not necessarily cause firmness loss (Paull et al., 1996). In our experiment, YS-2 had the highest soluble pectin content and fastest protopectin decline but the lowest firmness reduction, supporting the view that protopectin decrease does not always lead to firmness loss.

3.3 Relationship Between Fruit Softening and Cell Wall Degrading Enzyme Activities

Cell wall degradation is the primary cause of fruit softening (Fan Lingjiao, 2016), though different hydrolases play varying roles in different fruits and developmental stages (Huber, 1983; Hinton, 1974). -D-Gal has been identified as an important softening factor in persimmon (Wei Jianmei et al., 2009; Ng et al., 2015; Liu Simin et al., 2018), while PG activity increases during softening in kiwifruit, plum, papaya, and avocado (Yan Ruixiang et al., 2000; Lu Shengmin et al., 2000; Paull et al., 1983; Awad et al., 1979). In this study, Cx and PG activities in Gongcheng Yueshi increased rapidly during early storage and remained high, inhibiting softening. ZP-1, YS-4, and YS-5 were regulated by Cx and -D-Gal, with firmness loss accompanying increased Cx and decreased -D-Gal activity (except YS-5 which showed the opposite pattern). ZP-2, YS-2, and YS-6 softening was regulated by PG, Cx, and -D-Gal, with PG and Cx promoting YS-2 softening. Different correlations between firmness and enzyme activities among germplasms indicate that different enzymes dominate softening in different persimmon types.

In conclusion, as wild persimmon germplasm resources from Guangxi, different accessions show substantial variation in storage characteristics and softening factors. Comparison with the main cultivar 'Gongcheng Yueshi' demonstrates that these characteristic wild persimmons provide valuable germplasm materials for studying fruit softening mechanisms and offer genetic resources for breeding improved storage tolerance in persimmon.

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Note: Figure translations are in progress. See original paper for figures.