



### **Full Length Article**

## **Anatomical Studies of Stone Cells in Fruits of Four Different Pear Cultivars**

Na Li<sup>1</sup>, Yanfeng Ma<sup>1</sup>, Yuqin Song<sup>1</sup>, Caifang Tian<sup>1</sup>, Li Zhang<sup>2</sup> and Liulin Li<sup>1\*</sup>

<sup>1</sup>College of Horticulture, Shanxi Agricultural University, Taigu 030801, China

<sup>2</sup>College of Agriculture, Shanxi Agricultural University, Taigu 030801, China

\*For correspondence: tgliulin@163.com; lnsxnydx@163.com

### **Abstract**

Stone cells are one of the most important factors affecting the fruit quality of pears (*Pyrus bretschneideri* Rehder, *P. communis* L., *P. pyrifolia* (Burm. f.) Nakai and *P. bretschneideri* × *communis*). Sections of pear fruits were examined during fruit development to analyze the content and diameter of stone cells in four different pear cultivars. At maturity, the abundance of stone cells in Dangshansuli pears was the highest at the peel, followed by core and flesh. In contrast, the stone cells of three other cultivars were more abundant at the core compared to the peel and flesh, and the percentage of stone cells with a diameter greater than 80 μm was significantly lower than in Dangshansuli. In addition, the initiation of stone cell formation in Dangshansuli pears was at 10 days after full bloom (DAFB) and peaked at 7 weeks after flowering. Contrarily in Whangkeumbae, Bartlett and Yuluxiangli pears, stone cells were initiated at 15 DAFB and peaked 4–6 weeks after flowering. In conclusion, the higher density, larger diameter, and radial distribution of stone cells in Dangshansuli pears resulted in a coarse texture compared to Whangkeumbae, Bartlett, and Yuluxiangli pears. These findings can help to improve the visualization of stone cells in pear fruit and help to improve the fruit quality by reducing the formation of stone cells. © 2017 Friends Science Publishers

**Keywords:** Pear; Fruit development; Paraffin sections; Dyeing; Stone cells distribution

### **Introduction**

Stone cells are formed by the deposition of lignin on primary cell walls, following secondary thickening of cell walls, and can occur singly or in groups in the flesh. Their size and distribution directly affects fruit quality, so they are important for evaluating the quality of pear fruits. Many scholars have conducted extensive research on stone cell size and content (Tao *et al.*, 2009; Jin *et al.*, 2013; Wang *et al.*, 2013; Yan *et al.*, 2014), development (Lee *et al.*, 2007; Cai *et al.*, 2010), morphology (Tao *et al.*, 2009), and the effects of cultivation techniques on the formation of stone cells (Lee *et al.*, 2007; Wang *et al.*, 2013).

At present, the primary ways of analyzing stone cells are the method which make use of hydrochloric acid (Tao *et al.*, 2009), natural mold (Wu and Shen, 1985), freezing (Cai *et al.*, 2010), enzymatic hydrolysis (Mou *et al.*, 1996), and freeze-H<sub>2</sub>SO<sub>4</sub> processing (Zhang *et al.*, 1998). The enzymatic method (Mou *et al.*, 1996) obtains cleaner stone cells, which allows for more accurate analysis of the stone cell content. However, the duration of the enzymatic hydrolysis affects the separation of flesh from stone cells; and if the duration is too long, it can change the structure of stone cells. There are also differences in the time required for enzymatic hydrolysis for each pear cultivar. Other

methods do not readily remove the stone cells attached to the flesh, leading to different results.

Stone cell distribution and size are highly important to fruit quality. Gu *et al.* (1989) in 72 pear varieties found that stone cells that clustered had a diameter of 150–200 μm and they were more common than stone cells with a diameter greater than 200 μm. Tian *et al.* (2014) researched stone cell size and percentage in 319 mature pear varieties. They also compared the texture of the flesh of different cultivars, and concluded that the content of stone cells with a diameter greater than 250 μm was an important factor affecting the texture of the flesh.

Four different pears, *Pyrus bretschneideri* Rehder ‘Dangshansuli’, *P. pyrifolia* (Burm. F.) Nakai ‘Whangkeumbae’, *P. communis* L. ‘Bartlett’, and *P. bretschneideri* × *communis* ‘Yuluxiangli’, are cultivated in China, and their fruit quality is quite different. The aim of this study was to analyze the difference in fruit quality by observing the formation and distribution of stone cells in four different pear cultivars. We located and examined stone cells in these pear cultivars using Safranin. Morphological analysis software was used to analyze the size and distribution of stone cells. Paraffin sectioning was used to minimize the incomplete separation of parenchyma cells during stone cell extraction.

## Materials and Methods

### Materials

Fresh pear fruits were harvested from *Pyrus bretschneideri* Rehder ‘Dangshansuli’, *P. pyrifolia* (Burm. F.) Nakai ‘Whangkeumbae’, *P. communis* L. ‘Bartlett’, and *P. bretschneideri* × *communis* ‘Yuluxiangli’ trees grown at the Pomology Institute (Shanxi Academy of Agricultural Sciences, Shanxi, China). Trees were 10 years old and cultivated according to current regulations for integrated fruit production. Starting from the day after full bloom (DAFB), fruits were sampled at 5 d intervals until 35 DAFB, at weekly intervals until 70 DAFB, and at 10 d intervals until mature.

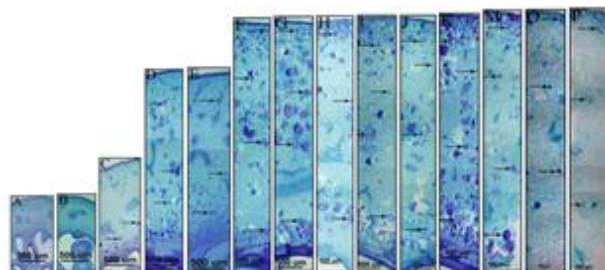
### Measurement of Stone Cell Content and Diameter

Uniformly developed fruits that were free of visual defects were selected. A slice of 1–2 mm width was cut from the peel to the core vertically. The sample was fixed with Farmer’s fixative (3:1 anhydrous alcohol: glacial acetic acid, v/v). The fixed tissues were embedded in paraffin using conventional method (Wang, 2015) and cut at a thickness of 8–15 µm. The samples were stained with Safranin O (Ourchem, Shanghai, China) and Fast Green FCF (Ourchem, Shanghai, China) and coverslips were mounted with neutral balsam (Biotopped, Beijing, China). The samples were observed with a digital microscope at 4× (Olympus BX50F-3; Tokyo, Japan) and digitized calibrated images were taken with a camera (Olympus; Tokyo, Japan). Images were merged using Photoshop CS6 (The set of images for each cultivar are presented in Fig. 1–4). Subsequently, the merged images were divided into five equal sections from the peel to the core (Fig. 5; Peel; OM: outer mesocarp; MM: middle mesocarp; IM: inner mesocarp; Core). Ten fields of view including the pericarp to the fruit core were observed for each cultivar and five images, as well as divided, were selected and analyzed by using the Image-Pro Plus software.

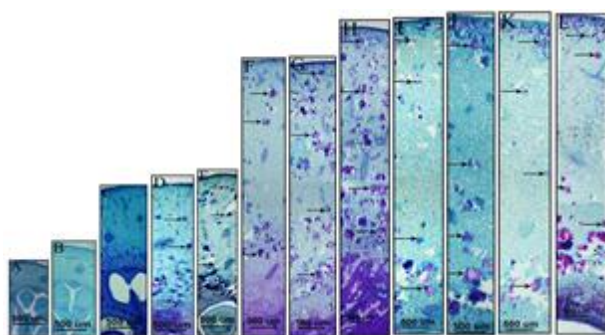
The stone cell diameter and quantity per unit area (width: 500 µm ; length: from peel to core) were recorded in Excel, and SAS 9.2 was used to analyze the data. Differences between five replications were evaluated by one way analysis of variance (ANOVA). Duncan’s multiple range test was used to determine significant differences among results. The differences with  $P < 0.05$  were considered statistically significant.

### Results

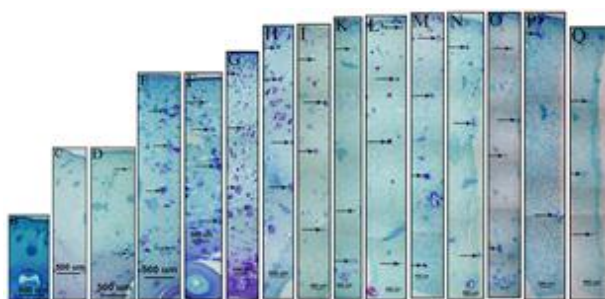
To identify morphological changes in stone cells, the prepared slides were examined (Fig. 1–4). Stages in the whole fruit development of each variety were labeled from A to S depending on the day of examination. Positions A and B represent Dangshansuli pears at 0 and 5 DAFB. Stone



**Fig. 1:** Development of Dangshansuli pear stone cells. Fruits were examined at (A) 0, (B) 5, (C) 10, (D) 15, (E) 20, (F) 25, (G) 30, (H) 35, (I) 42, (J) 49, (K) 56, (M) 70, (O) 90 and (P) 100 days after full bloom. Stone cells in the images are indicated with the arrows



**Fig. 2:** Development of Whangkeumbae pear stone cells. Fruits were examined at (A) 0, (B) 5, (C) 10, (D) 15, (E) 20, (F) 25, (G) 30, (H) 35, (I) 42, (J) 49, (K) 56 and (L) 63 days after full bloom. Stone cells in the images are indicated with the arrows



**Fig. 3:** Development of Bartlett pear stone cells. Fruits were examined at (B) 5, (C) 10, (D) 15, (E) 20, (F) 25, (G) 30, (H) 35, (I) 42, (K) 56, (L) 63, (M) 70, (N) 80, (O) 90, (P) 100 and (Q) 110 d after full bloom. Stone cells in the images are indicated with the arrows

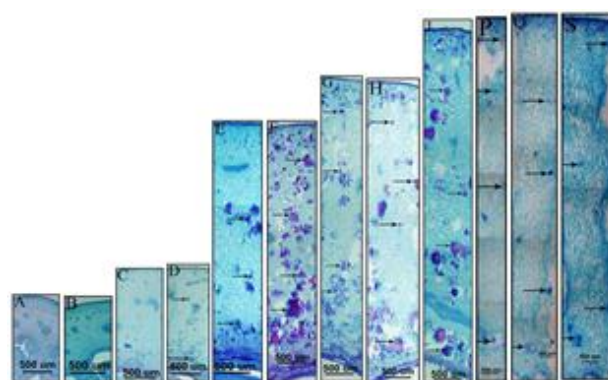
cell formation was initiated at 10 DAFB in Dangshansuli (Fig. 1C) and at 15 DAFB in Whangkeumbae, Bartlett, and Yuluxiangli (Fig. 2D, 3D and 4D). Stone cells of Dangshansuli, Bartlett, and Yuluxiangli were initially single. In Whangkeumbae pears, clusters formed from a few stone cells, and their peels contained more single stone cells than

those of the other cultivars. In Dangshansuli pears, irregular and differently shaped stone cell clusters were observed at the peel and core from 25 to 70 DAFB (Fig. 1F–M). In Whangkeumbae pears, abundant stone cell clusters were observed between 20 and 35 DAFB (Fig. 2E–H). After 49 DAFB (Fig. 2J), small single stone cells were observed at the peel and irregular stone cell clusters at the core. Many irregular stone cells were found in Bartlett pears between 22 and 35 DAFB (Fig. 3E–H), but their abundance decreased after 42 DAFB (Fig. 3I). In Yuluxiangli pears, the abundance of irregular stone cells decreased after 100 DAFB (Fig. 4P). The abundance of stone cells first increased and then decreased during fruit development in all four cultivars. Moreover, three cultivars had more abundant stone cells at the core than at the peel as compared to Dangshansuli, in which the stone cells were more abundant at the core than at the peel.

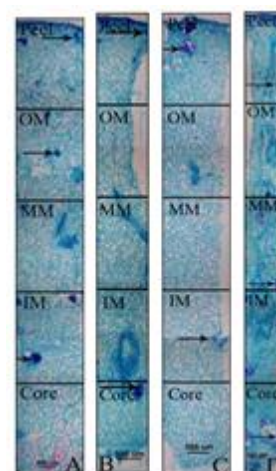
The number of stone cells per unit area in Dangshansuli pears increased rapidly from 10 to 15 DAFB, reached a maximum at 49 DAFB, decreased after 70 DAFB, and was stable after 90 DAFB (Fig. 6). The number of stone cells per unit area in Whangkeumbae pears increased from 15 to 35 DAFB, when it reached a maximum, and decreased after 100 DAFB, later stabilizing at 110 DAFB. The number of stone cells per unit area in Bartlett pears increased rapidly from 15 to 20 DAFB, reached a maximum after 30 DAFB, and stabilized after 100 DAFB. The number of stone cells per unit area in Yuluxiangli pears increased gradually from 15 to 25 DAFB, reached a maximum after 42 DAFB, and stabilized after 120 DAFB.

The number of stone cells per unit area in Dangshansuli and Whangkeumbae pears was significantly higher than that in Bartlett and Yuluxiangli pears at 15 DAFB ( $P<0.05$ ). However, there were no significant differences in the number of stone cells per unit area among the four cultivars between 20 and 35 DAFB ( $P<0.05$ ). The number of stone cells per unit area in Yuluxiangli pears was significantly higher than that of the other three cultivars at 42 DAFB and from 80 to 110 DAFB ( $P<0.05$ ). The number of stone cells per unit area in Dangshansuli pears was significantly higher than that in 'Bartlett' and Yuluxiangli pears from 49 to 70 DAFB ( $P<0.05$ ).

The size distribution of stone cells in different mature pear cultivars is shown in Fig. 7. During fruit ripening, the percentage of stone cells with a diameter less than 20  $\mu\text{m}$  per unit area was significantly higher in Whangkeumbae than in Bartlett and Yuluxiangli ( $P<0.05$ ), both of which had significantly higher percentage ( $P<0.05$ ) than that of Dangshansuli pears. The percentage of stone cells with a diameter between 20 and 80  $\mu\text{m}$  per unit area for Yuluxiangli was significantly higher than that of Dangshansuli and Whangkeumbae ( $P<0.05$ ), both of which had significantly higher percentage than that of Bartlett pears ( $P<0.05$ ). The percentage of stone cells with a diameter greater than 80  $\mu\text{m}$  per unit area for Dangshansuli pears was significantly higher than that of the other three



**Fig. 4:** Development of Yuluxiangli pear stone cells. Fruits were examined at (A) 0, (B) 5, (C) 10, (D) 15, (E) 20, (F) 25, (G) 30, (H) 35, (J) 49, (P) 100, (Q) 110 and (S) 130 days after full bloom. Stone cells in the images are indicated with the arrows

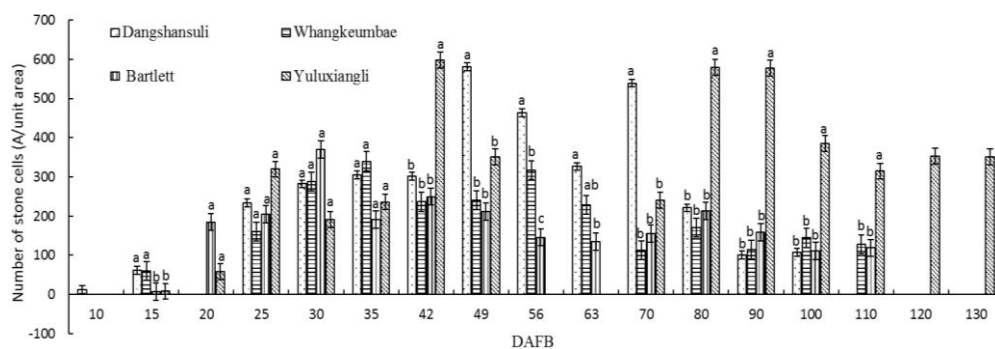


**Fig. 5:** Four pear varieties at maturity. (A) Dangshansuli at 90 days after full bloom (DAFB), (B) Whangkeumbae at 110 DAFB, (C) Bartlett at 100 DAFB, (D) Yuluxiangli at 120 DAFB. (OM: outer mesocarp; MM: middle mesocarp; IM: inner mesocarp)

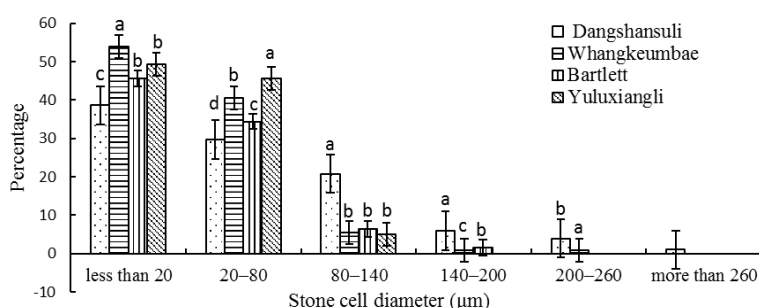
cultivars ( $P<0.05$ ).

The radial distribution of stone cells per unit area in Dangshansuli assumed a V-shape (Fig. 8A), with more stone cells at the peel and core than in the mesocarp. The peel had more stone cells than the core. Stone cells with a diameter less than 20  $\mu\text{m}$  and between 20 and 80  $\mu\text{m}$  were the most abundant at the peel. The number of stone cells with a diameter between 80 and 140  $\mu\text{m}$  and 140 and 200  $\mu\text{m}$  was similar at the peel.

A progressive increase was observed in the radial distribution of stone cells in Whangkeumbae pears (Fig. 8B). The lowest number of stone cells was noticed in the center of the mesocarp, while the core had the highest. The most common stone cells had a diameter less than 20  $\mu\text{m}$ , followed by those with a diameter between 20 and 80  $\mu\text{m}$ ,



**Fig. 6:** Change in number of stone cells per unit area in different pear cultivars over time (days after full bloom, DAFB). Different letters indicate significant differences ( $P < 0.05$ )



**Fig. 7:** Size distribution of stone cells at fruit maturity in different pear cultivars. Different letters indicate significant differences ( $P < 0.05$ )

and those with a diameter between 140 and 260  $\mu\text{m}$  were the least common.

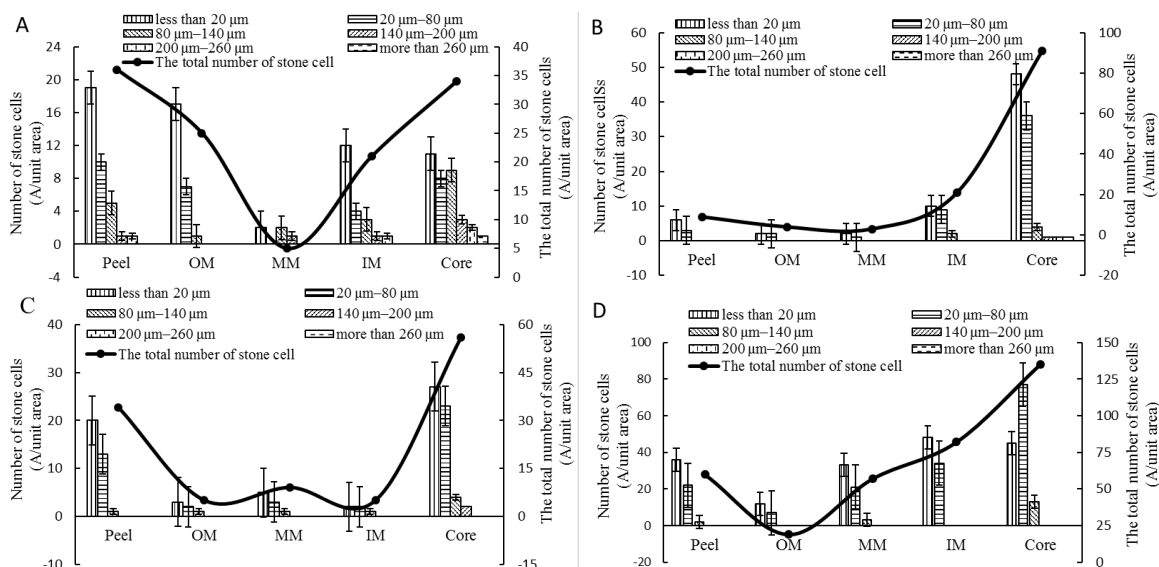
The radial distribution of stone cells per unit area in Bartlett had a W-shape (Fig. 8C), with the highest abundance at the core. Stone cells with a diameter less than 200  $\mu\text{m}$  were the most common. More stone cells were present in the middle part of the mesocarp than in the portion adjacent to the peel, which in turn, had more stone cells than the portion adjacent to the core. In Yuluxiangli pears, most of the stone cells were found at the core, and those with a diameter between 20 and 80  $\mu\text{m}$  were the most common. The area adjacent to the core had the least number of stone cells (Fig. 8D).

## Discussion

In our study, Dangshansuli pears formed stone cells at 10 DAFB, which reached their maximum at 49 DAFB. Dangshansuli has been reported to form stone cells at 15 DAFB and reach a maximum at 51 DAFB (Liu *et al.*, 2006). This differences may be attributed to differences in the natural environment. Sterling (1954) reported that initiation of stone formation takes place randomly throughout the pear flash. However other reports show that the formation of stone cells may be determined by genetic traits (Li *et al.*, 2002). We found the stone cell content initially increased and then decreased in all four cultivars. An increase in cellulase and pectinase activity can destroy

the stone cell structure, thereby reducing the stone cell content and size.

Different reasons are attributed to sizes, content, and development of stone cell in different pear cultivars. Tian *et al.* (2014) analyzed the size of stone cells in 319 pear cultivars and concluded that the texture of the flesh was closely related to the size and density of stone cell clusters. When the diameter of stone cells is small and their number is high, the texture of the flesh will be relatively rough. Contrarily, when the diameter of stone cells is large and their number is low, the texture of the flesh will be relatively smooth. Significant differences in the stone cell content were observed among different pear cultivars. Especially, the number of stone cells with a diameter between 250 and 500  $\mu\text{m}$  lead to significant differences in fruit quality (Li *et al.*, 2004). Our findings showed that the number of stone cells per unit area in Dangshansuli pears was relatively low after the fruits reached maturity. A higher proportion of stone cells with a diameter greater than 80  $\mu\text{m}$  per unit area causes the flesh to become rough. The number of stone cells per unit area in Yuluxiangli pears was relatively higher after the fruits reached maturity. The lower proportion of stone cells with a diameter greater than 80  $\mu\text{m}$  per unit area causes the flesh to be smooth. In this study, the number of stone cells at the peel was higher than that at the core in Dangshansuli. This is inconsistent with Ermaimaitihasimu and Yixiamu (2011). In their work, Dangshansuli pear was divided into the outer, middle, and inner parts. They found



**Fig. 8:** Distribution of stone cells from the peel to the core in different pear cultivars. Different size classes are indicated. (A) Dangshansuli, (B) Whangkeumbae, (C) Bartlett, (D) Yuluxiangli. (OM: outer mesocarp; MM: middle mesocarp; IM: inner mesocarp)

the inner stone cell content was the highest and the middle stone cell content was the lowest. A higher stone cell content of the peel might make birds less likely to peck at the fruits and facilitate storage and transport of pears. The stone cell content of pear fruits showed quantitative trait inheritance controlled by multiple genes (Li *et al.*, 2002). Thus, the regulation of stone cell formation needs to be further studied at the molecular level.

## Conclusion

Experiments using Safranin dyeing and digital microscopy for the determination of the content and diameter of stone cells revealed that the higher density, larger diameter and radial distribution of stone cells in Dangshansuli pears resulted in a coarse texture compared to Whangkeumbae, Bartlett, and Yuluxiangli pears. It is hoped that the information presented here can help to improve the visualization of stone cells and to improve the fruit quality by reducing the stone cell content in different varieties of pear.

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