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Citrus Fruit-Cracking: Causes and Occurrence

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A B S T R A C T

Citrus fruit creasing or cracking is a complex pre-harvest physiological disorder that causes significant economic losses. Recent studies have indicated that citrus fruit creasing or cracking is caused not only by genetic factors but also by environmental factors. By reviewing the relationship between citrus fruit creasing or cracking and peel mechanical properties, the cellular wall ultrastructure, cellular wall metabolism and modification, this paper summarized the mechanism of citrus creasing or cracking and further explained the effects of genetic factors and environmental factors (light, temperature, humidity, mineral nutrition and plant growth regulators) on citrus fruit creasing or cracking rate. Further studies were proposed to provide a sound theoretical basis on citrus fruit creasing or cracking.

Keywords: citrus; fruit creasing; fruit cracking; cell wall metabolism

1. Introduction

Citrus fruit peel creasing or cracking is a serious pre-harvest physiological disorder. This phenomenon almost occurs on all citrus cultivars. The range of the fruit-cracking incidence is 10%–35%, which severely affects fruit quality and yield (Li, 2009). China is one of the main countries where citrus originated, and one of the world's largest citrus-producing countries; however, the causes and mechanisms of citrus fruit creasing or cracking have not been completely elucidated. Besides, there are many factors that might affect citrus fruit creasing and cracking which include cultivar characteristics (Agusti et al., 2003), weather conditions (Gambetta et al., 2000), rootstock (Storey et al., 2002), peel thickness (Holtzhausen, 1981), peel hardness (Li, 2009), and growth regulators (Li et al., 2016). This paper reviewed the physiological and molecular mechanisms and the influencing factors that underlie citrus fruit creasing or fruit cracking, in order to provide theoretical guidance for future researchers and citrus producers.

2. Occurrence period, types, and characteristics of citrus fruit cracking

The majority of fruit cracking in different citrus cultivars primarily occurs during the cell enlargement or the fruit maturity period; while in some cases, some cultivars can occur throughout the whole fruit growth and development period. The types of citrus fruit-cracking vary due to different varieties and fruit development stages. There are three citrus fruit-cracking patterns, including flavedo-splitting, inner-cracking and albedo-splitting (fruit creasing or pitting). For instance, flavedo-splitting of 'Jincheng' oranges begins with the cuticle splitting of flavedo, followed by the gradual distortion and breakdown from outside to inside of the flavedo cell, and ends with the disruption of albedo (Wang and Qin, 1987). The second pattern of citrus fruit-cracking is inner-cracking. The cracking of 'Duwei' pomelo starts from the central axis of fruit, then the fruit top, finally the fracture appears (Wu et al., 1987). The third pattern of citrus fruit cracking is creasing-fruit, also named pitting fruit. The creasing-fruit of 'Hongjiang'

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sweet orange starts from the cracking of albedo, then flavedo becomes pitted, finally the fruit cracks (Zou and Xu, 1995; Chen et al., 1999; Li et al., 2008).

Fruit cracking is not a burst but a gradual process. It can be divided into the period of normal fruit development, the critical period of fruit cracking, the initial period of fruit cracking, the middle stage of fruit cracking, and the later stage of fruit cracking. During the normal period, the fruit peel develops normally. The fruit peel surface is intact, and the oil glands have a regular shape and are arranged in order. During the critical fruit cracking period, a brown stripe starts to appear on the fruit peel surface, the cuticle fractures and a crack becomes visible, and the oil glands begin to deform. In the initial stage of fruit cracking, the peel cracking is aggravated, and the oil glands rupture. After that, due to the further injury of cells, the tissues of fruit surface are severely damaged and there is a larger space among cells of the broken albedo. In the later period of fruit cracking, as the fruit surface has been severely damaged, the tissue structures of the flavedo and albedo cannot be distinguished (Wang and Qin, 1987; Li, 2006b).

3. Mechanisms of citrus fruit cracking

3.1. Citrus peel mechanical properties

The thickness and hardness of fruit peel are important indicators to measure fruit strength, and they are directly associated with citrus fruit peel creasing and fruit cracking. Li et al. (2011a) and Li and Chen (2011b) showed that the changes of fruit peel in mechanical property were closely associated with the fruit creasing occurrence during the late period of citrus fruit development; the cultivar with a low fruit creasing rate (‘Newhall’ navel orange) showed significantly higher fruit peel thickness and hardness, compared to the cultivar with a high fruit creasing rate (‘Cara Cara’ navel orange). Phiri (2010) also reported that during the thickness transition period of the citrus peel, the thicker the peel, the later when the peel becomes thin so that the fruit creasing rate would be lower. Ali et al. (2000) showed that fruit peel thickness was negatively correlated with the fruit creasing rate. The effect of the evenness of fruit peel thickness on fruit creasing was greater than that of the fruit shape index on fruit creasing rate (Ye et al., 2002; Zhu et al., 2006). Therefore, when the citrus fruit peel is thicker, the possibility that it will be stretched and deformed is lower and the crack resistance is also stronger. Fruit peel hardness is also an important factor that influences citrus fruit creasing and cracking. Under the same condition, the fruit peel hardness of the ‘Anliu’ sweet orange is significantly higher than that of the ‘Hongjiang’ sweet orange, and its fruit cracking rate is also lower than that of the ‘Hongjiang’ sweet orange (Li, 2006b).

3.2. Citrus peel ultrastructure

The fruit-cracking occurrence is closely associated with the number of cell layers and the structure of the flavedo (Li et al., 2011c). Li (2006a) posited that large oil glands might allow the cells surrounding the oil glands to bear higher pressures. The ‘Newhall’ navel orange is less likely to become cracked, and it has a higher density of oil gland layer cells than that of the ‘Bonanza’ navel orange. The albedo is a sensitive part for fruit cracking (Cohen et al., 1972; Erner et al., 1975; Zou and Xu, 1995; Li, 2006b). Li (2006b) observed that during fruit creasing, the mesocarp cell wall structure of the ‘Anliu’ sweet orange was basically intact and its cellulose fibres were dense, regular and arranged in order, while in the ‘Hongjiang’ sweet orange, the cell wall structure of the fruit peel had poor integrity, the structure was loose, the cell layers were deformed, the primary cell wall broke down and presented larger spaces, and the cell wall fibres were gradually separated. Therefore, the ultrastructural changes of the peel cell wall also influence fruit peel creasing or fruit cracking.

3.3. Citrus peel cell-wall modification

Citrus fruit maturation is closely associated with cell wall modification of fruit peel (Brownleader et al., 1999). The cell wall metabolism and the fruit peel structure determine the occurrence of fruit cracking and stiff (Monselise et al., 1976; Jona et al., 1989; Huang et al., 2006). Cell wall hydrolases and oxidoreductases play critical roles in fruit creasing or cracking (Abeles and Takeda, 1990; Xue and Shu, 2004). Researchers have found that in navel orange (Li et al., 2006), ‘Hongjiang’ sweet orange (Chen et al., 1999), and ‘Shatangju’ mandarin (Li et al., 2013), cell wall hydrolases reduced the fruit peel strength through the degradation of cellular polysaccharides, while cell wall oxidoreductases, especially cell wall peroxidases, could catalyse lignification and the formation of phenolic cross-linking among cell wall structural components (structural protein, hemicellulose, and pectin), which led to the cell wall hardening and a reduction in its extensibility (Campa, 1991). These modifications led to changes in the peel developmental status and mechanical properties and fruit creasing or even cracking.

3.4. Molecular mechanism

At the molecular level, the biochemical modification of the cell wall structure is closely associated with gene expression (Caffall and Mohnen, 2009); while reports on the molecular mechanism associations with fruit creasing occurrence are almost absent due to the perennial nature of woody fruit trees. Edgar et al. (2003) reported that since *TGB6* was inhibited, the cuticle thickness and the number of cracked tomato fruits were increased. *AsExp-1,3* (Shen, 2006), *Lc Exp-1,2* (Wang et al., 2006), and *LcXET1* (Lu et al., 2006) were closely associated with fruit cracking. Li (2009) analysed the gene expression differences between normal fruit peel and cracking fruit peel and obtained 20 specific bands with qualitative differences, which primarily consisted of cell division cycle proteins, chlorophyll synthesis proteins, and other unknown proteins. These results indicated that the occurrence of the fruit cracking might be the result of the regulation of many metabolic pathways during peel development. The fruit creasing-related gene *Cs-cdc48* and cell wall-related gene *Ct-Exp1* are specifically expressed in normal fruit peels, and they are not expressed in creasing fruit peels. In addition, zinc fertilizer treatment during the fruit enlargement period can promote the upregulation of *Cs-cdc48* expression (Li, 2009). The expressionof *Ct-Exp1* with high fruit creasing rate is stronger than that in normal fruit, and its expression in normal fruit is stronger during the middle and late fruit ripening period (Zhang, 2007; Li et al., 2009).

Therefore, this different expression is not only regulated by the internal mechanism within hereditary, but also induced by environmental factors (such as light, temperature and environmental stress). The application of molecular methods has provided new thoughts for the study of citrus creasing or cracking.

4. Factors affecting citrus fruit cracking

4.1. *Tree factors*

4.1.1. *Fruit size and shape index*

The fruit size and shape index also have certain effects on citrus fruit cracking. The larger fruit is more likely to experience the cracking phenomenon. However, extra large fruit (diameter >79 mm) has a lower incidence of fruit creasing than that of large fruit (diameter 62–79 mm) (Treeby et al., 1995). Cracking fruit is more oblate than normal fruit during the same period (Garcia-Luis et al., 2001). High round, round, and high oblate pomelos experience more fruit creasing than that of normal fruit (Dai et al., 1987). Oblate navel oranges are more likely to exhibit fruit cracking, because navel oranges peel rapidly turns thinner from the fruit waist to the fruit top (Qian, 1997); while the 'Newhall' navel oranges have low fruit cracking rate because of the little difference among the fruit top, equator, and pedicle (Li, 2006a).

4.1.2. *Rootstock*

The influence of the rootstock on citrus fruit peel creasing and fruit cracking is indirect. In citrus production, citrus fruits with different citrus rootstocks developed on the same cultivar presented a different fruit-cracking resistance. Different rootstocks have different influences on tree nutrition conditions and fruit quality; the fruit cracking conditions are also different (Treeby et al., 2000, 2007). In Australia, the creasing fruit rate of trees on rough lemon rootstock is higher than that of trees on sweet orange rootstock (Treeby et al., 1995, 2000). Some reports also showed that the citrus fruit creasing rate on 'Sour orange' rootstock was lower than that of 'Carrizo' citrange rootstock (Agusti et al., 2003). Therefore, the selection of proper rootstocks has important significance for the reduction of citrus fruit creasing.

4.2. *External factors*

4.2.1. *Light*

Daily variations in the light exposure intensity positively correlate with the daily fruit creasing rate (Li, 2009). Fruits growing on the exposed surface of trees were significantly lower in fruit creasing incidence than those growing on the shaded surface of trees and the inner portion of the canopy (Treeby et al., 2000). The fruit creasing rate of 'Shatangju' mandarin in the inner part of the tree canopy was significantly higher than that of the outer tree canopy (Li, 2009). According to Chen et al. (2005), the shaded surface of 'Hongjiang' sweet orange had more total pectin and soluble pectin and higher pectin methylesterase activity than those of the exposed surface; thus, fruit creasing or cracking is more likely to occur on the shaded surface of the fruit. During the fruit cell enlargement period, reflective film treatment under inner tree canopy could significantly decrease the fruit creasing rate (Li, 2009).

4.2.2. *Temperature*

The fruit creasing rate positively correlates with the daily variation values in the temperature. During the high fruit creasing incidence period of the 'Cara Cara' navel orange, there are 3 times of the sharp rise and fall of temperature (Li, 2009). The highest and the lowest temperatures in February have changed the citrus creasing rate during the harvest period (Ali et al., 2000). Treeby et al. (1995) also found that the summer temperature was closely related to the occurrence of creasing fruit. Some studies also found that citrus fruit creasing was affected by the highest and lowest temperatures but was not affected by the average temperature (Gambetta et al., 2000; Huang et al., 2006; Li, 2009).

4.2.3. *Humidity*

The daily fruit creasing rate positively correlates with daily variation values in light intensity. A higher average relative humidity before physiological fruit dropping period will increase the occurrence of fruit creasing (Gambetta et al., 2000). The daily variation values in the relative humidity during the high fruit creasing period of 'Cara Cara' navel orange have more than 3 times of sharp rise and fall (Li, 2009). The vapour pressure deficit (VPD) value is a comprehensive index of temperature and relative humidity. When the VPD falls sharply, the fruit cracking rate also rises sharply. This phenomenon is associated with the inhibition of transpiration, which causes an influx of a large amount of water into the fruit (Gao et al., 1994). Cosgrove and Cleland (1983) noted that water stress enhanced the fruit growth potential and did not inhibit dry matter accumulation. Once the condition was suitable, fruits enlargement growth would be more significant than that of unstressed fruit; at this time, fruit cracking occurred easily. A sudden change in the relative humidity during the fruit colouring period will also result in fruit creasing (Gonzalez-Altozano and Castel, 1999). With the increase in water stress during the late fruit development period, the activity of hydrolases related to cell wall metabolism increases and soluble pectin increases, which caused changes in the ultrastructure of the cell wall and cell loosening, thus resulting in fruit creasing or even fruit cracking (Li et al., 2008). A survey conducted by Li (2009) showed that the fruit creasing rate in trees that were planted on mountains was significantly higher than that from paddy fields. Another study showed that an insufficient water supply in September and October affected the fruit size and increased the incidence of fruit creasing (Gonzalez-Altozano and Castel, 1999). Water indirectly affects fruit development and absorption of mineral elements. Water insufficiency has significantly inhibitory effects on the absorption of calcium (Ca), boron (B), and ferrum (Fe) (Fan et al., 2012). Therefore, proper water management could be an effective measure to reduce the fruit creasing rate.

4.2.4. *Mineral nutrition*

Insufficient nutrients in the partial peel will cause developmental and metabolic disorders in the peel; therefore, the stimulation from an external adverse environment will result in fruit creasing and cracking (Fawcett and Lee, 1936; Erickson, 1957; Monselise et al., 1986; Wang and Qin, 1987; Almela et al., 1990). The creasing fruit rate could decrease significantly with the spraying treatments of potassium (K) fertilizer, calcium (Ca) fertilizer, boron (B) fertilizer, and zinc (Zn) fertilizer during the fruit cell enlargement period; therefore the Zn, B, and K contents of the peel increased, the hardness and thickness of the peel on the shaded and exposed surfaces increased accordingly (Li, 2009) .

K can maintain high osmotic pressure and turgor pressure, which can provide power for cell division, cell wall extension, and cell expansion to accelerate cell growth. A high K content could increase the fruit size and make peel thick and smooth; on the contrary, when the K content of peel is low, fruit creasing and dropping will occur easily, resulting in smaller fruit, thinner peel and the reduction of the soluble solids content (SSC), organic acids, and vitamin C (Vc) (Alva et al., 2006). However, some studies also showed that the K content in the peels of creasing fruit was higher than that of normal fruit peels (Storey et al., 2002). K can promote cell division in citrus fruit pulp and peels, and reduce fruit cracking. Applying K fertilizer in spring or during the early fruit development period can promote fruit peel development, increase peel thickness, enhance the fruit cracking resistance ability, and reduce pre-harvest fruit cracking (Ali et al., 2000). However, the additional application of K fertilizer during the late fruit development period has little effect on the reduction of pre-harvest fruit cracking (Koo, 1961).

Ca participates in the whole development of citrus fruit, especially in modifying the metabolism of the cell wall (Storey et al., 2002; Dong et al., 2009; Tam et al., 2012). Ca^{2+} has interactive functions with cell wall composition and has important physiological functions such as the maintenance of the stability of the cell wall structure and the regulation of cell wall metabolic enzyme activity (White and Broadley, 2003; Jain et al., 2011). The Ca^{2+} in the intracellular space interacts with the cell wall to synthesize the Ca^{2+}-pectin crosslinking polymer, which helps to facilitate extrusion among pectin polymers to form the cell wall network, enhance mechanic strength, and limit access by cell wall hydrolases (White and Broadley, 2003), thus reducing the incidence of citrus fruit creasing (Treeby and Storey, 2002). Ca treatment can significantly increase the cell-wall Ca^{2+} content in peel; in particularly, it can significantly increase the content of bound calcium in the peel cell wall. Besides, it can inhibit the degradation of pectin, cellulose and hemicellulose, reduce arabinose and galactose, and increase water-soluble pectin in the peel cell wall (Alvaro et al., 2010). Wen and Shi (2012) showed that the Ca content in the leaves, cracking peel, and pulp of 'Jincheng' sweet orange was significantly lower than that in normal fruit. A study conducted by Chen (1993) showed that the Ca content of normal 'Hongjiang' sweet orange fruit (high fruit-cracking rate cultivar) was higher than that of the cracking ones; the Ca content in the exposed peel was higher than that in the shaded side, and the fruit cracking rate and Ca content showed a highly negative correlation. It has proved that the fruit creasing rate could significantly decrease by applying the long-term treatment of 5% and 10% calcium acetate solutions during the fruit growing period (Zhang, 2007).

B plays an important role in the promotion of cell division and the synthesis of cell wall (Li, 2009). B could help with the maintenance of cell wall integrity and toughness because of the association of cell wall composition (Goldbach and Wimmer, 2007; Dong et al., 2009). Wu et al. (1987) indicated that B could increase the regulatory function of endogenous growth regulators. Spraying boron fertilizers 3–4 times during flowering and early fruit development periods can reduce fruit cracking in 'Duwei' pomelo. Wang and Qin (1987) reported that the difference in the effective B content between normal fruit peels and cracking fruit peels was not significant; however, a higher effective boron content in leaves could help to reduce fruit cracking. Although Tanq et al. (2007) showed that spraying boron on leaves could make the fruit peel softer and thinner; it was reported that spraying boron on leaves did not have significant effects on the peel thickness of sweet orange (Maurer and Truman, 2000).

The phosphorus (P) level of peel during fruit cracking period positively correlates with fruit cracking rate. When the P level is higher, the fruit peel is thinner. When citrus fruit lacks of P, the fruit becomes smaller, and the fruit peel becomes rougher. Erickson (1957) reported that the P and N contents of cracking navel orange peel were higher than that of normal fruits. Wang and Qin (1987) reported the nitrogen (N) and P contents did not significantly correlate with 'Jincheng' sweet orange fruit cracking. Gambetta et al. (2000) reported the P content in the creasing fruit was higher than that in the normal fruit. Chen et al. (2002) showed that the correlation between the Mg content and the creasing fruit rate was not significant.

Other mineral nutritions also have a correlation with fruit cracking rate. Eman et al. (2007) and Zekri and Obreza (2003) reported that two continuous foliar sprays of chelated zinc during the early fruit development stage could stimulate cell enlargement and promote the acceleration of fruit growth. Li (2009) and Liang (2013) reported that there was a significant correlation between fruit creasing occurrence and peel zinc content. Excessive manganese (Mn) will cause the deficiencies of Ca, ferrum (Fe), and magnesium (Mg), and Mn^{2+} will result in an increase in the indoleacetic acid (IAA) oxidase activity, IAA decomposition and reduction, and the blocking of cell wall extension (Guan and Maxsaure, 2003). The lack of Mn in citrus fruit will cause the low fruit quality.

4.2.5. *Plant growth regulators*

Gibberellin (GA_3) can increase cell wall plasticity so that cell growth and cell extension can be promoted, which could inhibit the occurrence of fruit creasing (Holtzhausen, 1981). Spraying GA_3 during the young fruit development period can significantly increase citrus fruit size, peel thickness, and fruit quality (Eman et al., 2007). Fruit creasing was also reported to be associated with the pectin level in the fruit peel, while GA_3 could reduce the pectin methylesterase activity, thus decreasing the incidence of fruit creasing (Jona et al., 1989). When it comes to the long drought season during the fruit rapid enlargement period, spraying 0.3% urea + 30 $mg \cdot L^{-1}$ GA on fruit in the evening once a week for 3 times could significantly enhance the prevention of fruit cracking (Wang and Qin, 1987).

2,4-Dichlorophenoxyacetic acid (2,4-D) is an artificially synthesized plant growth regulator. The spraying of 2,4-D on the leaf surface during the flowering period could increase the fruit peel thickness so that citrus fruit cracking can be effectively reduced (Garcia-Luis et al., 2001). In Spain, spraying a 2,4-D and GA_3 mixture 30 days and 60 days before the anticipated fruit cracking time shows better effects in reducing navel orange cracking (Agusti et al., 1994).

In citrus production, naphthylacetic acid (NAA) was applied to regulate peel development during the fruit enlargement stage (Greenberg et al., 1995, 1996, 2006; Nawaz et al., 2008). Greenberg et al. (1995, 1996, 2006, 2010) reported that in summer after the physiological fruits drop, the application of NAA significantly improved the fruit set rate, reduced the small fruit rate and increased the peel hardness.

5. Outlook

Citrus fruit creasing or cracking is a very complex process. Even with in-depth studies, the mechanisms and influencing factors related to fruit cracking are still uncertain. The previous research results have shown the relationship between the cell wall metabolism and biochemical modification of peel and the occurrence of fruit creasing which have provided important guidance for increasing citrus production and storage quality. For further studies and to build up a stronger theoretical base for preventing fruit cracking or pitting during the citrus production, the investigation of peel mechanic properties and cell wall metabolism at the molecular level are encouraged, the changes in ultrastructure between normal peel and creasing citrus peel with the nutritional and plant growth regulator (PGR) treatments before the cell enlargement are to be clarified, and the mechanisms of fruit creasing and cracking have to be elucidated.

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