DOI : 10.58993/ijh/2023.80.1.12

Indian J. Hortic. 80(1), March 2023: 80-85

Studies on fruit pedicel concerning fruitlet abscission in kinnow mandarin

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ABSTRACT

Fruit drop is a major constraint for the production of kinnow mandarin. The present investigation compared the physical, biochemical and anatomical dynamics in abscised and non-abscised fruit pedicels of Kinnow mandarin at physiological and pathological fruit drop stages. The pedicel parameters (length, girth and weight) were higher in non-abscised than the abscised fruits. The activities of polygalacturonase (4.01 µg D-glucose released g^{-1} FW min^{-1}) and cellulase (4.34 µg D-glucose released g^{-1} FW min^{-1}) enzymes were significantly low in non-abscised as compared to abscised fruit pedicels. The transverse sections of non-abscised healthy fruit pedicels revealed intact phloem and long xylem vessels in the vascular tissue. However, pedicels of abscised fruits showed degradation of cells and formation of the abscission zone. Fruit pedicel parameters positively correlated with mineral nutrients, total soluble sugars and total soluble proteins. However, non-abscised fruit pedicels were negatively correlated with cell wall degrading enzymes. The fruit pedicel health has been found essential and positively associated with the diminution in physiological and pathological fruit abscission in kinnow mandarin.

Keywords: Fruit drop, Citrus, Pedicel anatomy, Fruit stalk, Enzyme activity.

INTRODUCTION

In India, citrus fruits (mandarins, sweet oranges, grapefruits, lemons and limes) are cultivated on 1.03 million hectares with a total production and average productivity of 13.20 million metric tons and 12.76 tonnes per hectare, respectively (NHB, 1). Among mandarin cultivars, Kinnow occupies the largest area (44752 ha) in Punjab with an annual production of 1.17 million MT (Anon, 2). Kinnow production is limited by several causes *viz.,* fruit drop, nutrient deficiencies and occurrence of insect-pests and diseases. Of these, fruit drop is a serious concern that has become a restraining issue for increasing the fruit production. The fruit drop has been estimated to be ranged from 69.10 to 82.27 per cent in Abohar area of Punjab (Saini *et al.,* 19). Fruitlet drop or abscission is a natural separation of fruits from the plants, initiated from blooming and continues till harvesting. Citrus fruits have two abscission zones, first is between the peduncle of the fruit and the branch observable at the earlier stage of development, and second in the calyx region (Goren, 11). Fruits at fruit peduncle and branch normally abscise during the first eight weeks of fruit development and the region loses the capacity to abscise easily after eight weeks of fruit development. Fruits aged eight weeks or older abscise only at the calyx region; however, young fruits also abscise at the calyx region (Burns *et al*., 6). Formation of the abscission zone is the physiological process for the separation of plant organs from entire plant body, thus, the abscission zone is the cause of physiological fruit drop (Bonghi *et al*., 5). Anatomical studies done on peach, apple and sweet cherry proved the dissimilarity of abscission zone from the neighbouring tissues, not only anatomically, but also differ in terms of metabolism (Costa *et al*., 8)

Hydrolytic cell wall enzymes have a well-established role in leaf and fruit abscission in many fruit plants. A decrease in soluble protein content is correlated with the commencement of leaf abscission region cells in the seedlings of Starking Delicious apple (Pandita and Jindal, 17). Calcium (Ca) level in the abscission zone get diminished and both calcium and magnesium become absent from the cell wall during the process of abscission (Stosser *et al*., 21). Poor stalk health plays an imperative role in enhancing fruitlet abscission by depleting the nutritional supply from the source to the demanding sink. The information available on anatomical and biochemical dynamics occurring during the fruit drop in Kinnow is limited, so the present study was made in relation to Kinnow pedicel health with anatomical and biochemical dynamics, occurring during physiological and pathological fruit drops.

MATERIALS AND METHODS

The present research was carried out in the Division of Fruit Science Department, Punjab Agricultural University, Ludhiana during 2019-20 (Latitude 30^0 90'10" and longitude 75^0 80'70", 245m

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above mean sea level). The area represents sub-tropical along with semi-arid climatic conditions having very hot summer and cold winter. The soil was sandy loam with pH 8.0, organic carbon 0.28%, $CaCO_3$ 1%, EC 0.02 Sm^{-1}, available phosphorous 26.4 $kgha^{-1}$ and potash 171.8 4 $kgha^{-1}$. The Kinnow plants were of eight-year-old, budded on rough lemon rootstocks, and planted at a distance of 6m × 3 m. Standard cultural practices were followed during the study period.

All parameters of abscised and non-abscised fruit pedicels of Kinnow mandarin were recorded twice in the year *i.e.,* May (physiological) and October (pathological) fruit drops. The pedicel weight, and length and girth were measured using weighing balance and vernier caliper, respectively. The activity of cell wall degrading enzymes was expressed as µg D-glucose released g^{-1} fresh weight min^{-1} (Malik and Singh, 15).

Total soluble sugar (Dubois *et al*., 9) and total soluble protein (Lowry *et al*., 14) content of abscised and non-abscised fruit pedicels was estimated. Nitrogen content (%) was estimated using oven-dried and ground sample in Kel Plus Nitrogen Estimation System (Pelican Equipment, India). Flame photometer method was used for the estimation of potassium content (AOAC, 3). Atomic absorption spectrophotometer (A Analyst 200, Perkin Elmer) was used for the estimation of calcium content in the samples.

Abscised and non-abscised fruit pedicels of Kinnow mandarin were collected during the peak period of fruit drop for their anatomical studies. The experimental fruit pedicels were cut into sections using potato pith (Schiff *et al*., 20). The transverse sections of fruit pedicels were mounted on the slides and examined using axio vision software under the microscope equipped with digital camera and computational imaging systems. Tukey's LSD test was used to statistically examine the data. Differences were considered statistically significant at the level, $p \leq 0.05$ and correlation coefficients between these evaluations were obtained through Pearson`s correlation coefficients ($p \leq 0.05$) using statistical analysis software SAS (Version 9.3 for Windows).

RESULTS AND DISCUSSION

The data depict that the pedicel parameters were significantly higher in non-abscised as compared to the abscised fruit pedicels of Kinnow mandarin at physiological and pathological fruit drop stages (Table 1). The pedicel length of non-abscised fruits was measured as 7.01 mm and 10.03 mm in May and October, respectively, while the pedicel length of abscised fruits was observed to be significantly shorter *i.e*., 4.88 mm and 7.84 mm in May and October, respectively. This trend was similar for the girth and weight also. The pedicel parameters (length, girth and weight) were observed to follow an increasing trend with the advancement of fruit growth. The increase in the length, girth, and weight of fruit pedicel during development occurs due to the secondary growth and deposition of other metabolites (Bustan *et al*., 7). The decrease in pedicel length, girth, and weight of abscised Kinnow fruits might be attributed to the cell distortion and cell wall degradation at the end of abscission process. Roongsatthaz *et al*. (18) also reported the shorter pedicel length of abscised fruits in oil palm.

The results revealed that there was a significant variation for polygalacturonase, cellulase, total soluble sugars (TSS) and total soluble proteins (TSP). The data (Fig. 1) demonstrated that the non-abscised had 4.01 µg D-glucose released g^{-1} FW min^{-1} PGA (Fig 1a) over 4.84 µg D-glucose released g^{-1} FW min^{-1} in abscised fruit pedicels during physiological fruit drop. The similar tendency was experienced at pathological fruit drop stage, where it was found to be significantly lower in non-abscised fruit pedicels (5.12 µg D-glucose released g^{-1} FW min^{-1}) as compared to the abscised ones (6.90 µg D-glucose released g^{-1} FWmin^{-1}). In abscised fruit pedicels, PGA activity was 1.20 and 1.34 times higher than non-abscised pedicels at physiological and pathological stages, respectively.

The activity of cellulase enzyme (Fig. 1b) was observed to increase substantially in abscised

Table 1. Pedicel parameters among non-abscised and abscised Kinnow mandarin at physiological and pathological fruit drop stages

Treatment	May			October		
	Pedicel length (mm/stalk)	Pedicel girth (mm/stalk)	Pedicel weight (g/stalk)	Pedicel length (mm/stalk)	Pedicel girth (mm/stalk)	Pedicel weight (g/stalk)
Non- abscised	7.01[a]	2.20[a]	0.31[a]	10.03[a]	3.61[a]	1.59[a]
Abscised	4.88[b]	1.57[b]	0.13[b]	7.84[b]	2.49[b]	0.95[b]
LSD (p≤0.05)	0.65	0.22	0.04	0.12	0.05	0.03

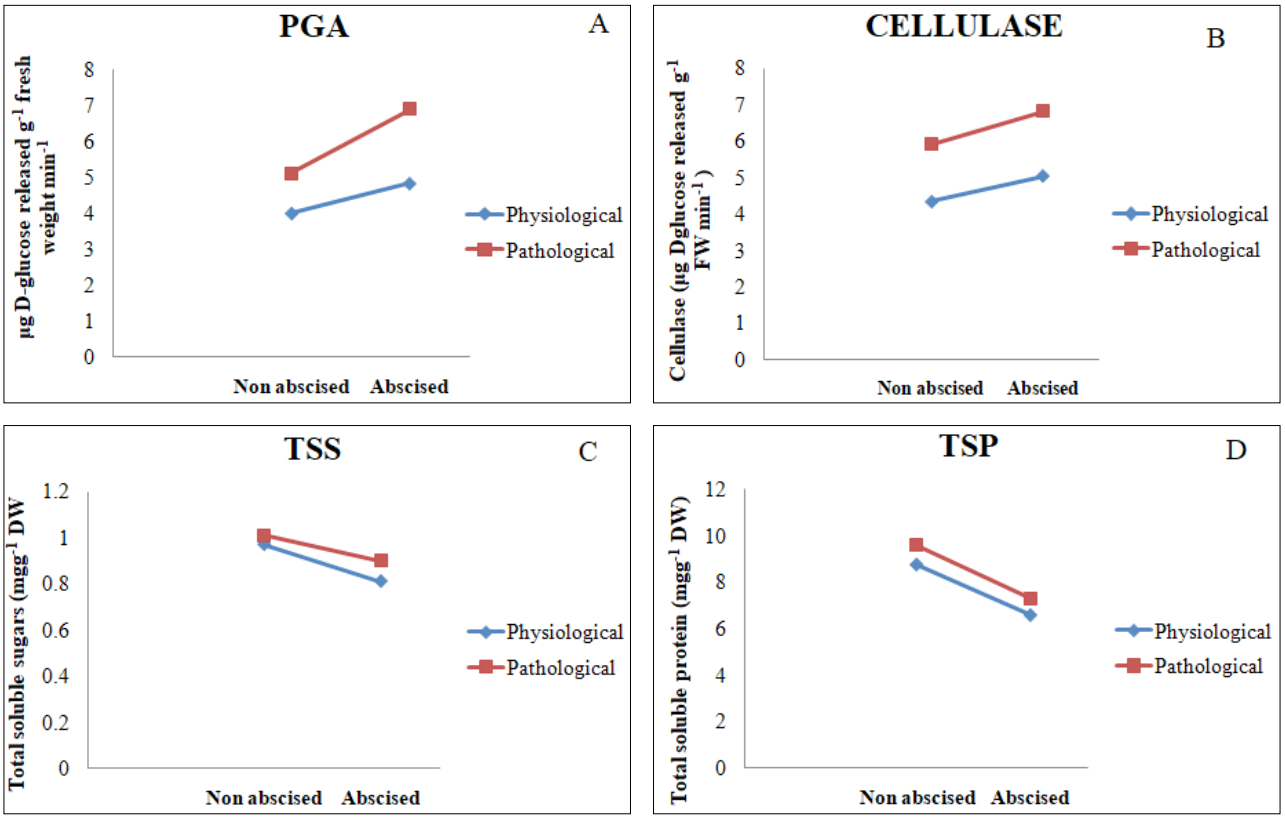

Fig. 1. Graphical representation of different parameters of non-abscised and abscised fruit pedicels of Kinnow mandarin during physiological and pathological fruit drop. (A) PGA (B) Cellulase (C) TSS, D) TSP; PGA: Polygalacturonase TSS: Total soluble sugars, TSP: Total soluble proteins

over the non-abscised fruit pedicels. Cellulase enzyme was recorded to be 5.05 and 6.82 µg D-glucose released g^{-1} FW min^{-1} at physiological and pathological fruit abscission stages, respectively. However, the activity of cellulase enzyme was significantly lower in non-abscised than abscised fruit pedicels during physiological (4.34 µg D-glucose released g^{-1} FW min^{-1}) and pathological (5.91 µg D-glucose released g^{-1} FW min^{-1}) fruit drop stages. In abscised fruit pedicels, cellulase activity was 1.16 and 1.15 times higher than non-abscised at physiological and pathological stages, respectively. The cell wall degrading enzymes play a significant role in abscission of citrus fruits, and their activity is greatly enhanced at calyx end during the process of abscission. These enzymes participate in the degradation of adhesive components of the cell wall, that bind the cells together in abscission zone (Huberman *et al.* 12). The total soluble sugars (Fig. 1c) were significantly higher in non- abscised, when compared to abscised fruit pedicels at both the stages of fruit separation. The total soluble sugars were 0.97 and 1.01 mgg^{-1} DW in non-abscised, while these were recorded to be 0.81 and 0.90 mgg^{-1} DW from the abscised fruit pedicels at physiological and pathological stages, respectively. The deficiency of sugars in the pedicel accelerates fruit abscission. Total soluble proteins (Fig. 1d) were also observed to be significantly higher in non-abscised pedicels as compared to abscised pedicels during the months of drop. The same trend was observed in total soluble proteins from non-abscised and abscised pedicels. There are evidences to advocate that the plant's nutritional status is of paramount importance in determining the percentage of fruits reaching maturity. Insufficient nutrient supply leads to embryo abortion which drastically reduces the auxin supply to the fruits leading to the abscission. The current data indicated that non abscised fruit pedicels contained the higher amount of nutrients (Calcium, Nitrogen and Potassium) than the pedicels of abscised fruits (Table 2). The calcium content of non-abscised fruit pedicels was registered as 1.38% and 1.69% at physiological and pathological fruit detachment phase, respectively, as compared to 1.01% in physiological and 1.13% in pathological in abscised fruit pedicels. Calcium has a vital function in fruit set, fruit retention and cause significant increase in the final yield and fruit quality

Table 2. Mineral content (Ca, N, K) from non-abscised and abscised fruits pedicels of Kinnow mandarin at physiological (May) and pathological (October) fruit drop stage.

Treatment	May			October		
	Ca (%)	N (%)	K (%)	Ca (%)	N (%)	K (%)
Non abscised	1.38[a]	1.49[a]	0.80[a]	1.69[a]	1.32[a]	0.72[a]
Abscised	1.01[b]	1.22[b]	0.73[b]	1.13[b]	1.20[b]	0.66[b]
LSD (p≤0.05)	0.11	0.12	0.04	0.09	0.06	0.04

(Huberman *et al.,* 12). Calcium influences the process of abscission by delaying, and maintaining the cell wall integrity and strengthens it. Calcium pectate, an indispensable module of middle lamellae holds the cells together in abscission zone. The middle lamellae loose substantial calcium before abscission, which leads to the loss of binding sites in the region of abscission due to cell wall degradation (Tao *et al.,* 22). The same trend was observed for nitrogen and potassium content. In Punjab's Hoshiarpur and Abohar, fruit drop was discovered to be related to low levels of nitrogen, calcium, cobalt, phosphorus, zinc, and potassium in Kinnow mandarin leaves (Saini *et al.*, 19). Potassium plays an important role in the synthesis and processing of proteins, chlorophyll, and the regulation of stomata opening and closing, and storage and translocation of freshly formed carbohydrates. It also causes the heavy dropping of flowers and fruits leading to the low yield of poor quality (Bailey and Gwathmey, 4).

The correlation coefficients establishing the correlation between the parameters relating to pedicel (pedicel length, girth and weight), biochemical (PGA, cellulase, total soluble sugars and total soluble proteins) and mineral nutrients (Ca, N and K) are presented in Table 3. Fruit pedicel length was significant correlated positively with TSS (r=0.68), TSP (r =0.81), Ca (r =0.92), N (r = 0.92) and K (r = 0.94). Sufficient supply of nutrients to the fruit pedicel ensures a constant supply of auxins resulting in increased the pedicel strength and reduced fruit abscission in Kinnow mandarin. A negative correlation was observed between fruit pedicel parameters and cell wall degrading enzymes, PGA (-0.91) and cellulase (-0.87). It is evident from the current investigation that pedicel health has negative association with enzymes that break down cell walls and *vice-versa* with mineral nutrients and biochemical traits. Cell wall hydrolyzing enzymes are involved in the disintegration of cell wall, resulting in the reduction of fruit pedicel strength (González-Carranza *et al.,* 10).

The abscission zone in case of the fruit pedicel of Kinnow mandarin was studied with reference to the structural changes in cell wall during abscission process. No degradation of cells and abscission zone formation were observed in the section of non-abscised fruit pedicels of Kinnow mandarin at physiological (Fig. 2a) as well as pathological fruit drop stages (Fig. 2b) in phloem region as shown by red encircled area.

The transverse sections of non- abscised fruit pedicels (Fig 2a and Fig b) revealed that there was no degradation of phloem fibre and long xylem vessels in vascular tissue of healthy fruit pedicels, while the red circle in Fig. 2 c - d exhibited the degradation of cells and formation of abscission zone in abscised

Table 3. Relationship between pedicel, biochemical and mineral content of Kinnow mandarin

	Pedicel length	Pedicel girth	Pedicel weight	PGA	Cellulase	TSS	TSP	Ca	N	K
Pedicel length	1.00									
Pedicel girth	0.924**	1.00								
Pedicel weight	0.939**	0.990**	1.00							
PGA	-0.911*	-0.729**	-0.787**	1.00						
Cellulase	-0.877*	-0.846*	-0.811*	0.659**	1.00					
TSS	0.686**	0.817*	0.831*	-0.626	-0.440	1.00				
TSP	0.812*	0.908*	0.887*	-0.674	-0.666	0.928**	1.00			
Ca	0.923**	0.921**	0.955**	-0.875	-0.667	0.875*	0.887*	1.00		
N	0.929**	0.904*	0.942**	-0.876	-0.781	0.777**	0.783	0.919**	1.00	
K	0.943**	0.783	0.820*	-0.908*	-0.850*	0.456*	0.576	0.798*	0.883*	1.00

*Correlation is significant at 0.05 level

**Correlation is significant at 0.01 level

PGA: Polygalacturonase, TSS: Total soluble sugars, TSP: Total soluble proteins, Ca: Calcium, N: Nitrogen, K: Potassium

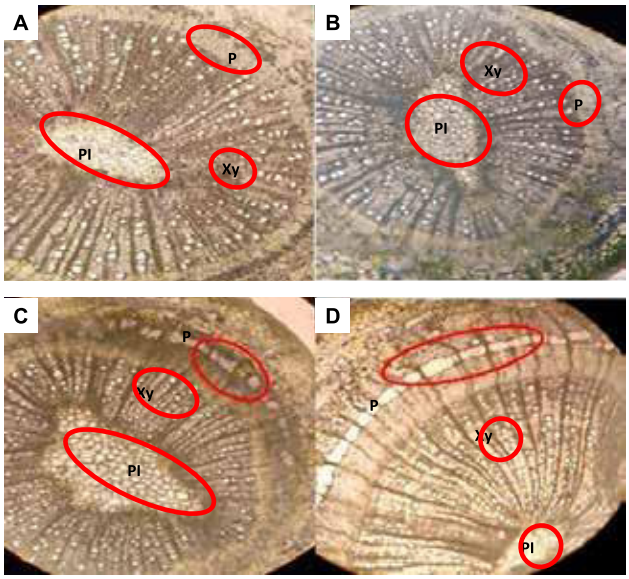

Fig. 2. Transverse sections of fruit stalk of Kinnow mandarin; (A&B): T.S. of non-abscised fruit stalk; (C&D): T.S. of abscised fruit stalk; PI= Pith, P= Phloem, Xy=Xylem

fruit pedicels at the physiological and pathological fruit drop stages, respectively. The sections of fruit pedicel showed that there would be smaller pith and large xylem area in non-abscised fruit pedicels (Fig. 2a) over the larger pith diameter, and less xylem area in abscised fruit pedicels (Fig. 2c). The healthy fruit pedicels retarded the abscission process automatically by means of providing the passage for photosynthates, nutrients and hormonal translocations to the developing fruits. These claims were strengthened by Lee (13) who also reported that the fruit growth in crop plants generally depends on the accumulation of water and assimilates which are transported through vascular tissues of pedicel towards fruits. A fissure develops across the abscission sector consequential in fruit detachment from the peduncle (Huberman *et al.,* 12). The cell wall disassembly, which would result in the efficient organ separation, would be caused by genes, encoding enzymes that hydrolyze the cell wall and middle lamella (Merelo *et al.,*16).

The present investigation clearly revealed that by understanding the mechanism of ongoing anatomical and biochemical changes underlying abscission in Kinnow may help in regularizing its fruit production.

AUTHORS' CONTRIBUTION

Conceptualization of research (MG, HSR); Designing of the experiments (MG, HSR, SS, NK); Execution of field/lab experiments and data collection (SS, MG); Analysis of data and interpretation (SS, GS, NK); Preparation of the manuscript (SS, GS, MG).

DECLARATION

All authors declare that they do not have any conflict of interest.

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Received : November, 2022; Revised : February, 2023; Accepted : February, 2023