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Anatomical Aspects of Fruit Abscission in Local Tangerine (*Citrus Reticulata* Blanco)

Narjes S. Mohammadi Jahromi*, Homa Rajaei and Bahman Kholdebarin

Biology Department, Science College, Shiraz University, Shiraz, Iran

Corresponding author: Narjes S. Mohammadi Jahromi

ABSTRACT: Histochemical and structural studies on local tangerine (*Citrus Reticulata* Blanco) were carried out to identify abscission layers and extent of carbohydrates during fruit development. There are two abscission zones, the first, AZ-A, was visible morphologically but detectable histologically only at early stages of fruit development. AZ-C, the second abscission zone, was active throughout the year and could been seen by microscope. The cells in these two abscission zones were completely compact with no extracellular spaces and swollen cell walls which were easily distinguishable from neighboring parenchymatic cells. These cells underwent considerable anatomical changes with time, including increase in cell wall thickness, changes in cell shape, incidence of plasmolysis and formation of cracks just before abscission. The content of whole carbohydrate were decrease by time. Local tangerine has multiple abscission phase unlike many other citrus. Many structural studies have been done in citrus fruits abscission but rarely focused on“late summer” phase of drop. This study was carried out in order to better understanding of “late summer” phase of fruit abscission process, that was more intensive and showed more sever anatomical presages.

Keywords: Citrus; Abscission zone; AZ-A; AZ-C; carbohydrate.

INTRODUCTION

The so called local tangerine (*Citrus Reticulata* Blanco) is an important species of tangerine grown in Jahrom region, south of Iran. It has undesirable sever abscission in four steps. Some other kinds of citrus have severe fruit abscission in any time, too. 1) abscission in fruit set time which usually lasts for a month following full bloom also called as cleaning drop (Racsko , 2006). 2) period of intense fruit drop occurs at the onset of hot summer and is referred as “June-drop” (Saleem , 2007). 3) The third period of severe and undesirable fruit drop is in late summer (from September to October) when the fruitlets are immature and yellowish in color; and 4) called as ‘pre-harvest’ drop (Racsko , 2006). There are reports of some variety of oranges which their drop are not limited to mature fruit and may occur in each time of development (Stewart & Klotz, 1947). Some researchers limited post- June drop abscission to a little kinds of citrus that is because of plant nutrition, decrease in carbohydrate and carbon (Iglesias , 2003). Besides of June-drop, there are other incident fruit abscission in some citrus which occur in late summer and early autumn that is because of ethylene production and led to massive fruit drop (Goren, 1993).

Fruits were characterized by having two abscission zones; the first (AZ-A), between the branch and fruit peduncle, is visible morphologically, however histologically, is detectable only at early stages of fruit development. The second abscission zone (AZ-C), in calyx, is active throughout the year and detectable only by light microscope (Burns, 1998; Goren, 1983; Iglesias , 2007). These two abscission zones have small compact cells with no extracellular spaces and swollen cell walls, underwent considerable anatomical changes with time, such as increasing in cell wall thickness, changes in cell shape, incidence of plasmolysis at the final stage and formation of cracks among them which lead to fruit abscission at zone C (Huberman , 1983; Sexton and Roberts, 1982; Zanchin, 1995).

Because of heavy fruit drop specially in “late summer” phase , and existense of little updated studies on fruit abscission, it is scientifically important to study fruit drop in local tangerine. Identifying abscission layer characteristics, the content of cell wall carbohydrates during fruit development and abscission process were the aim of this research.

MATERIALS AND METHODS

In order to perusal abscission event, fruits of trees which were in equal condition and steady state in their age, were randomly selected and transferred to the laboratory. Sample preparing were done in the method of embedding in paraffin (Ruzzin, 1999). The process were fixation in FAA, dehydration in ethanole and TBA, influence in TBA-paraffin oil, embedding with paraffin and sectioning with rotatory microtome. After that, in order to histochemical studies Safranin-Fast green drying was done. PAS test was carried out to study cell wall changes during the time.

RESULTS AND DISCUSSION

The term “abscission” describes a distinct process culminates in the shedding of plant parts which occur by mechanical tearing or as a result of tissue death and decay (Burns, 1998). Fruit drop might be caused by several factors, such as nutrient deficiency, disturbances in embryogensis and/or embryo abortion, sink competition between fruits, and abiotic and biotic stressors (Chadha, 1993). The amount of available carbohydrate in fruit set time, environmental, hormonal and nutritional factors can effect citrus fruit abscission, too (Iglesias , 2003). Ethylene production is expressed as the reason of extra abscission in citrus and mango (Mangifera indica L.) fruit after June-drop in late summer (Goren, 1993; Malik , 2003).

Citrus fruit abscission has attached the attention of many researchers for many years (Goren, 1983, 1993; Burns, 1998; Iglesias , 2007). Citrus fruit have two abscission zones, AZ-A and AZ-C. AZ-A which is between the stem and the pedicel, and AZ-C which is in the calyx area (Burns, 1998).

In our study two abscission zones were identified, too (fig. 1 & 2). first abscission zone (A) were morphologically observable in early months of fruit development as a very narrow and a little prominent band near calyx on the peduncle. This zone was gradualy lignified during fruit development as it was not detectable easily in late phases of its development. The second abscission zone (C) was not detectable morphologically and only by microscope could been seen. It was active all the year. Although abscission from AZ-C was observed in young fruitlets, but most of them abscised from AZ-A. abscission from AZ-A in young citrus fruitlets in early months of fruit development were also reported by some other authers (Burns, 1998; Goren, 1983; Iglesias , 2007).

Fruits can abscise in any time of their development, as we pointed before, in four phases. Most of citrus fruits prefer to undergo one or two phases of abscission, but Local tangerine trees had undesirable fruit drop in all four phases; fruit set, June-drop, late-summer drop and preharvest drop; which dissapointed Citrus growers.

Generally, the cell separation process does not involve the entire AZ. The cells within the AZ that are involved in the abscission process by rapid reduction in cell integrity (Sexton and Roberts, 1982) have been identified as separation layer (Roberts , 2000). Huberman (1983) notify these characteristic for AZ-A cells and reported their number about 100 layers in shamuti oranges. In mango (Mangifera indica L.), just the separation layer is mentioned to initiate fruit drop without precise information on how many cell rows are involved (Nunez-Elisea and Davenport, 1986); however, in cherry fruit and leaflet of olive 2 to 8 longitudinally rows of cells define the fracture line (Polito and Lavee, 1980).

Our structural Microscopic observations showed that cells in the abscission zones were very compact with no extracellular spaces which are smaller than neighboring cells (fig. 1, 2) and swollen cell wall for abscission zone A (fig. 1B). Reports on AZ charecteristics were in accordance with our report (Sexton and Roberts, 1982; Huberman , 1983; Zanchin , 1995). Cells in sbscission zone C had the same characteristics in the small size, dense cytoplasm and spherical shape, unless cell wall swolling was more in AZ-C (fig. 2C). These features had notified before by some researchers (Huberman , 1983; Zanchin , 1995; Burns, 1998; Brown & Burns, 1998). In our study no specific layers of abscission in zone C were detected because these cells were spread in whole calyx which was widespread increasingly into fruit during fruit development and lead to abscission(fig. 2). Burns also showed that AZ-C was dispered in the calyx (Burns, 1998).

Vascular systems become intense gradually toward fruit (fig. 2B & 3). It seems there is a relation between extension of vascular bundles and fruit abscission, as in January and February that there are the most extensive vascular bundle in the fruits, the abscission became decrease. Cooper and his co workers and Burns pointed to this note, too (Cooper , 1968; Burns, 1998).

In the final stages of fruit development we observed sever plasmolysis (fig. 4A & B), and after that deep cracks were visible which led to fruit abscission (fig. 5A & B). Weis pointed to plasmolysis occurance as a natural phenomon during abscission in olive (Weis , 1991).

We also used periodic acid-schiff reagent to study cell wall carbohydrate changes during fruit development. The intensity of dye became decrease gradually (fig. 6 A, B & C) which is confirmed with Image Java program (Table 1), would show decrese in cell wall thickness during time, that could be because of carbohydrate content of cell wall because of cell wall hydrolyses enzymes activity. This could be the result of ethylene productivity which led to loose starch in the cells. Some other reserchers reported this, before (Huberman , 1983; Weis , 1991; Khurnpoon , 2007).

Thereupon, The "late summer" phase of fruit drop in Local tangerine has the most severity in both amount of fruit drop and structural and histochemical symptoms in comparison with phases before and after, despite similarity in structural features, itself. It showes the symptoms in rapid process. AZ-C cells during development retain their compactiveness, but become larger, cell wall become more swollen. Cells start to go through plasmolysis from almost July, which lead to appearance of cracks where/when it decides to abscise.

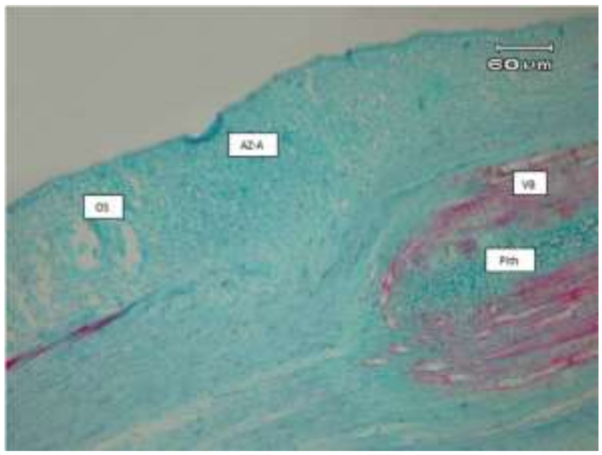

Figure 1A. AZ-A in the peduncle in May (×100). New sac is appearing right behind the epidermis. VB= Vascular boundle; OS= Oil Sac

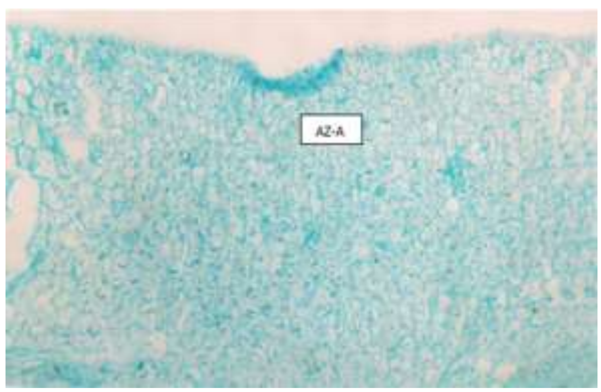

Figure 1B. AZ-A in the peduncle (×400). Cells stand vetically toward the peduncle. A concavity is creating right above AZ-A

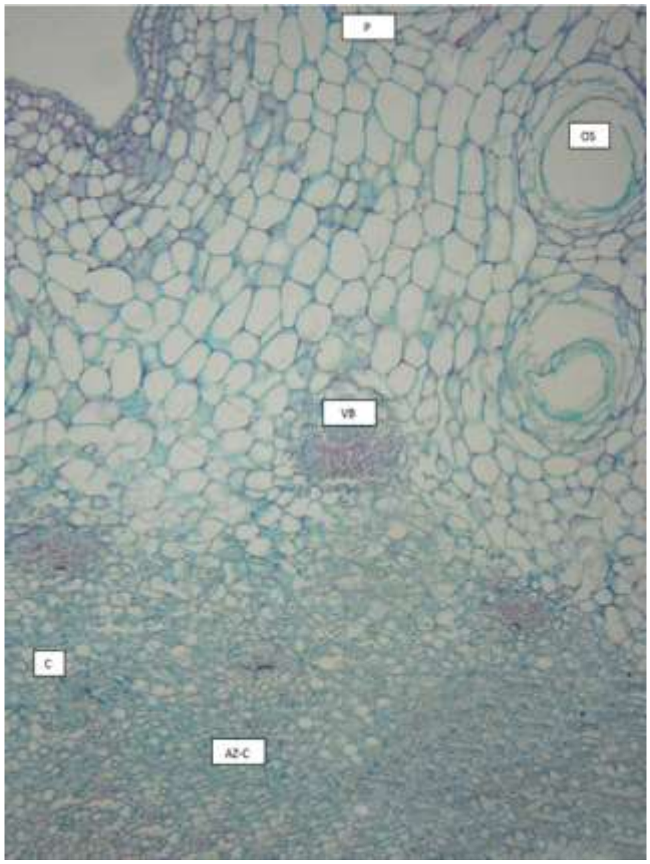

Figure 2.(A). AZ-C in May (×400). AZ-C cells are outspread in the calyx. They are still small but very compact. P= Peduncle; OS= Oil Sac; VB= Vascular Bundle; C= Calyx

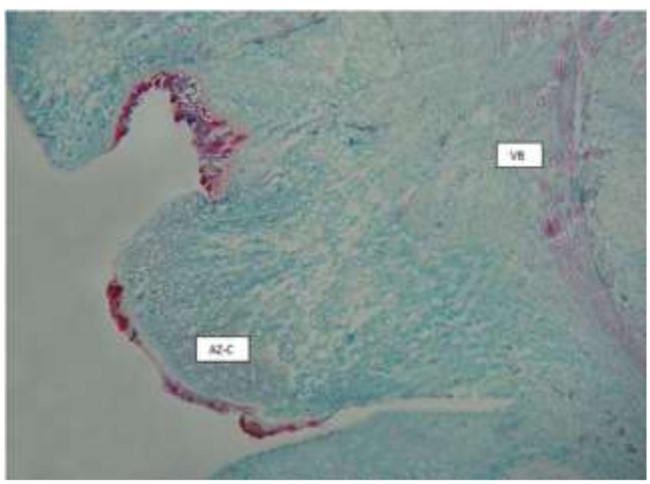

Figure 2 (B). AZ-C in July (×100). AZ-C is expanding through calyx toward the peduncle. A vascular bundle (VB) can be seen which is going to enter the calyx. Cells starts to reduce their integrity

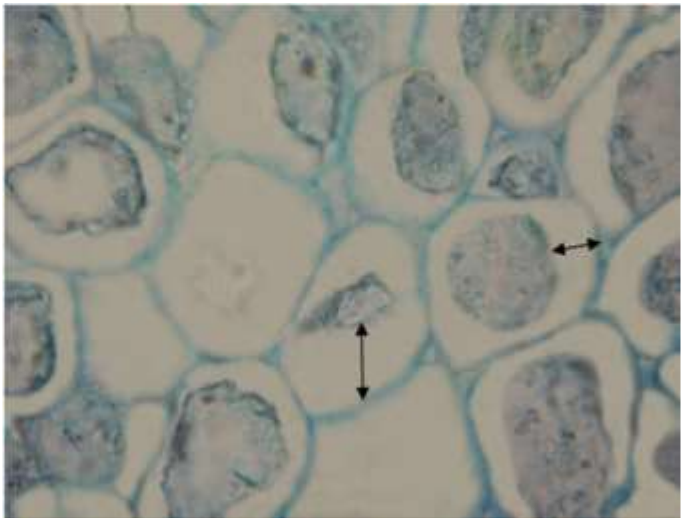

Figure 2 (C). AZ-C cells (×1000). Cell wall swelling is high

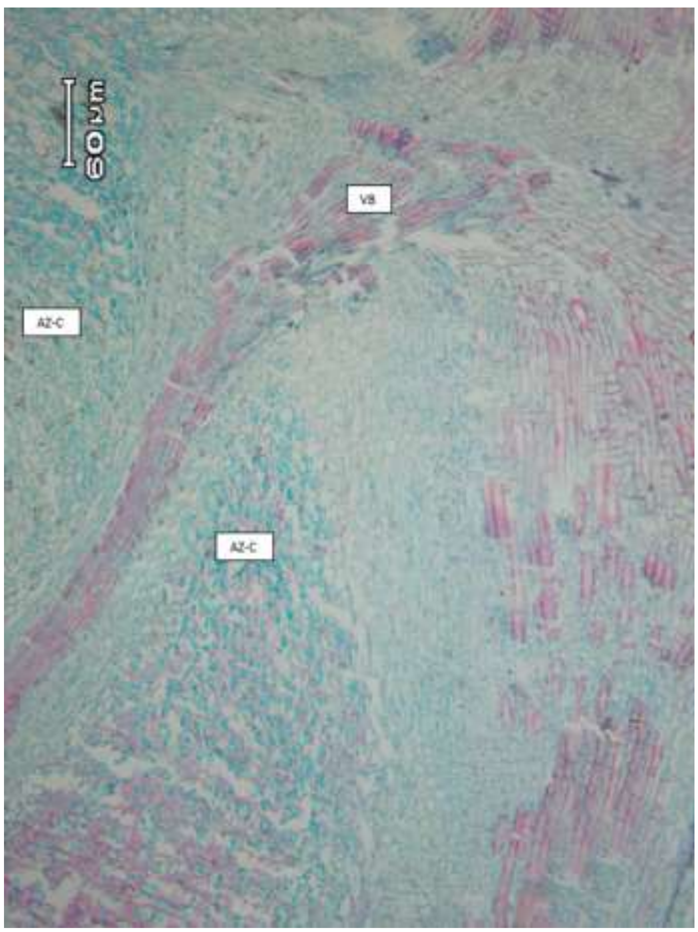

Figure 3. Vascular bundle (VB) entering the fruit. AZ-C cells can be seen on both sides of vascular bundle. (×100)

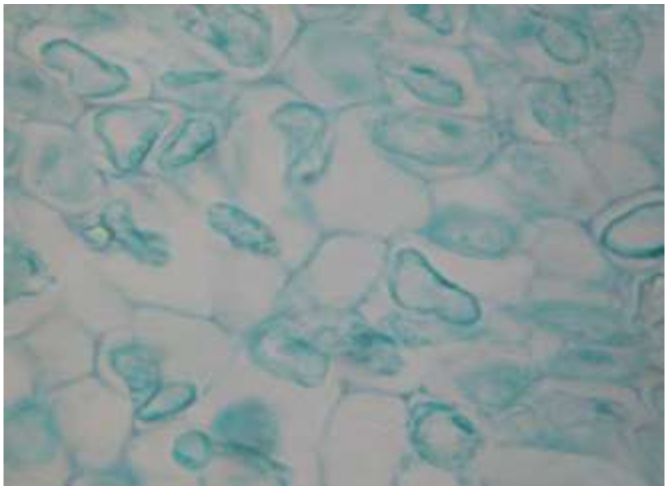

Figure 4A. AZ-C cells in October (×400)

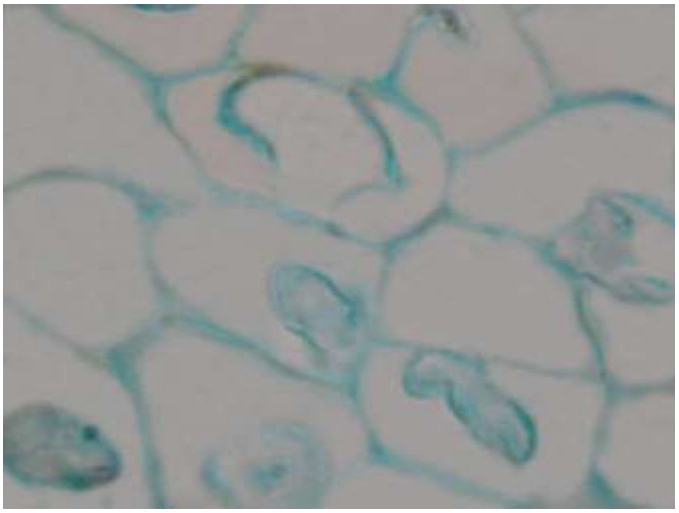

Figure 4B. AZ-C cells (×1000)

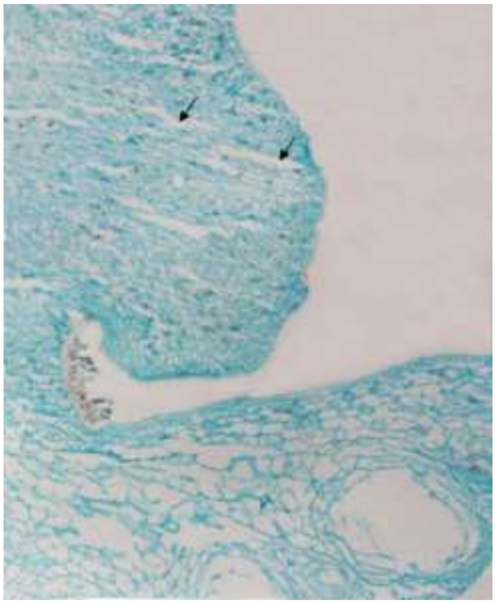

Figure 5A. Cracks (arrow) which lead to abscission in AZ-C (×100)

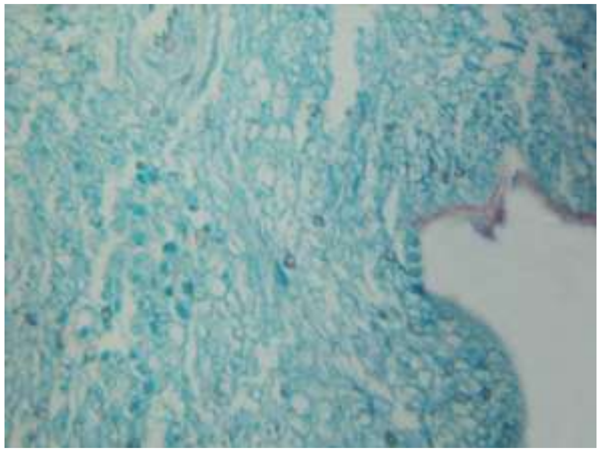

Figure 5B. AZ-C cells and created cracks in it prior to abscission(×400)

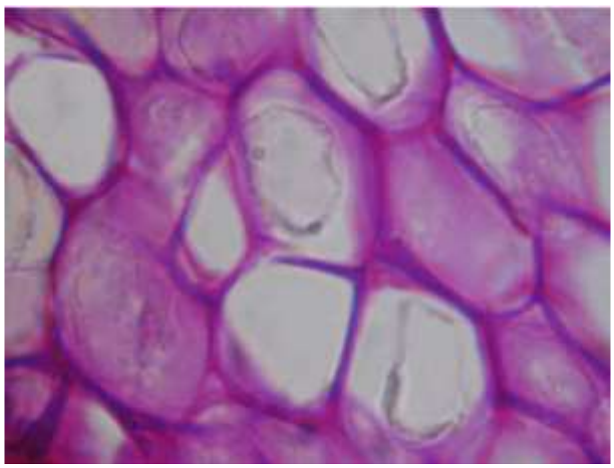

Figure 6A. AZ-C cell in May

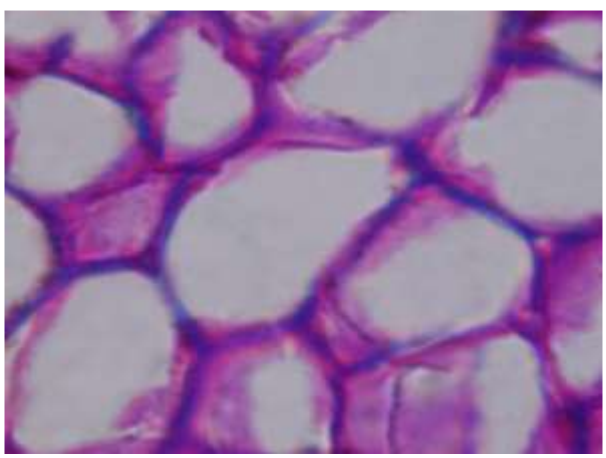

Figure 6B. AZ-C cell in July

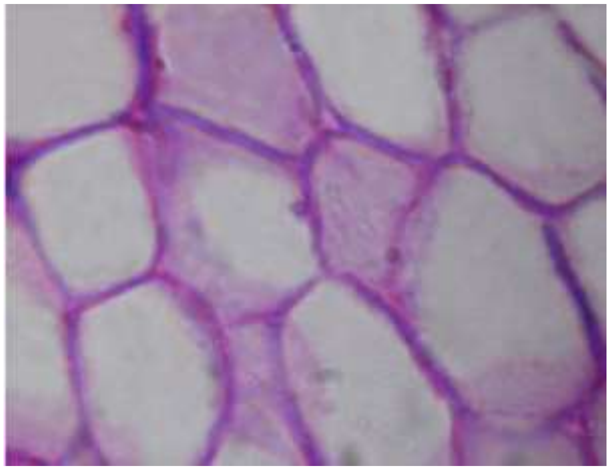
Figure 6C. AZ-C cell in February

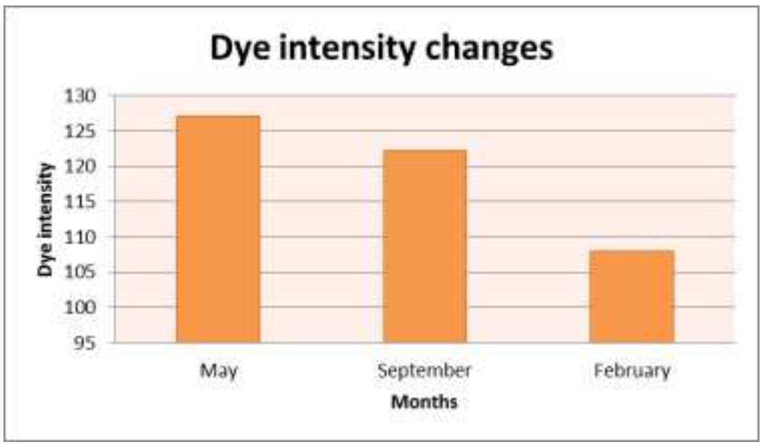

Table 1. Results from PAS staining intensity changes based on Java software

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