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Ejaz Ahmad Parray
Division of Fruit Science, SKUAST-Kashmir, India

Munib Ur Rehman
Division of Fruit Science, SKUAST-Kashmir, India

Mohammed Towseef Ali
Division of Fruit Science, SKUAST-Kashmir, India

Mohammad Saleem Dar
Division of Plant Pathology, SKUAST- Kashmir, India

Ishaq A Bhat
Division of Fruit Science, SKUAST-Kashmir, India

Ishfaq Rafiq
Division of Entomology, SKUAST- Kashmir, India

Muzamil Ahmad Hajam
Division of Vegetable Science, SKUAST- Kashmir, India

Mohammad Asif Wani
Division of Fruit Science, SKUAST-Kashmir, India

MA Hajam
Division of Fruit Science, SKUAST-Kashmir, India

Correspondence
Ejaz Ahmad Parray
Division of Fruit Science, SKUAST-Kashmir, India

Effect of stigma excision on the Bio-Chemical characteristics of apple (*Malus × domestica* Borkh.) cv. Red Delicious

Ejaz Ahmad Parray, Munib Ur Rehman, Mohammed Towseef Ali, Mohammad Saleem Dar, Ishaq A Bhat, Ishfaq Rafiq, Muzamil Ahmad Hajam, Mohammad Asif Wani and MA Hajam

Abstract
An investigation was carried out at Commercial orchard, Shalimar (Jammu & Kashmir) during 2016-17 to find out the effect of different stigma excision levels (incomplete pollination) on the biochemical characteristics of Apple fruit (*Malus × domestica* Borkh.) cv. Red Delicious. The six treatments were pinching off 0, 1, 2, 3, 4, or 5 styles below the stigma from each of 40 flowers per side. The remaining stigmata were hand-pollinated on the second day following treatment. Flowers were pollinated with Golden Delicious pollens. Only flowers with five stigmata were used and each flower was considered an experimental unit. The results revealed that the maximum Total Soluble Solids (14.55ºBrix), acidity (0.32%) and calcium content (2.17mg/100g) were recorded in fruits from flowers with no stigma pinched whereas Total soluble solids/acidity ratio was maximum in fruits from flowers with 4 stigmata excised. Therefore, it may be concluded that that improved seed set (proper pollination) provides a inexpensive and efficient practice to improve the biochemical quality attributes of apple.

Keywords: apple, stigma excision, biochemical characteristics, quality, improvement

Introduction

Apples (genus Malus, a member of the Rosaceae or rose family) have been part of the human diet for thousands of years (Hancock et al., 2008) [7], as demonstrated by the discovery of 'fossilized' fruit (presumably Malus sylvestris, the European wild apple) in human dwellings in Switzerland (Elzebroek and Wind, 2008) [5]. Indeed, the importance of apples to human culture and nutrition over the millennia is demonstrated by their presence in art, mythology and the religions of many regions, including Norse, Greek and European Christian traditions, as well as in the Muslim world (Hancock et al., 2008) [7]. It is estimated that by 2050 the world population will increase by approximately 30 percent to 9.1 billion people, which will invariably increase the demand on global food supply. Meeting this challenge of addressing current and future global food demands is reliant upon our ability to sustain, or increase, agricultural food production from the same area of land in a way that does not intensify the negative impacts on the environment. To add to this, the influence of climate change on agricultural production systems and associated ecosystem services, such as nutrient cycling, pest regulation and pollination, is largely unknown. Many innovative and feasible opportunities and methods exist that can increase food production sustainably. In this context, the management of agricultural landscapes, so as to optimize the use of natural ecosystem services, will contribute to sustainable agricultural production while maintaining and encouraging biodiversity. Pollination provides an essential ecosystem service by ensuring production of good quality fruits, seeds, nuts and vegetables. Apple, as one of the most widely cultivated fruit tree crops in the world, unsurprisingly, is a top global commodity. China produces approximately half of the total apple production, followed by the United States, Turkey and Poland. Apple production depends on insect pollination - therefore, understanding role of pollination in improving bio-chemical attributes of apple fruit will allow for better pollination management strategies and increased production of good quality apple fruits

Material and Methods

The present investigation on "Effect of pollination and spatial pattern of flowers on retention of Apple fruit (*Malus* × *domestica* Borkh.) cv. Red Delicious." was carried out at Commercial orchard, Shalimar (Jammu & Kashmir) during 2016-17. Full bloom of Red Delicious occurred on 7-10th April. Clusters containing five flowers were selected and petals were removed from unopened ("popcorn" stage) flowers, which were tagged to receive one of six treatments in a completely Randomized Block Design replicated four times. Only flowers with five stigmata were used and each flower was considered an experimental unit. The six treatments were pinching off 0, 1, 2, 3, 4, or 5 styles below the stigma from each of 40 flowers per side. The remaining stigmata were hand-pollinated on the second day following treatment. Flowers were pollinated with Golden Delicious pollens.A hand refractrometer ranging from 0-33 (°Brix) was used to determine TSS and values were corrected at 20 °C**.** Titrable Acidity (%) was determined by taking a known weight of fruit sample (10 g), crushed and added to 100 ml distilled water, then filtered through Whatman's No. 1 filter paper. 10 ml of aliquot was titrated against 0.1N NaOH using phenolphthalin indicator and end point was determined by pink colouration. The total titrable acidity was calculated in terms of malic acid on the basis of 1ml of 0.1N NaOH solution equivalent to 0.0067 and expressed in terms of per cent acidity (A.O.A.C., 1990) [1]. Acidity was calculated as malic acid by using the following formula:

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Dilution} \times 67}{\text{Weight of sample} \times \text{Volume of aliquot taken} \times 1000} \times 100$$

Total soluble solids/acid ratio was determined by using the formula:

$$\text{TSS/Acidity ratio} = \frac{\text{Total soluble solids (\%)}}{\text{Acidity}} \times 100$$

Calcium content was determined by collecting fruit samples as per procedure suggested by Waller (1980) [14]. Fruits were washed in tap water and later dipped in dilute hydrochloric acid. Further, washings were given with single and double distilled water. The moisture was wiped with filter paper muslin cloth. The fruits were cut into thin slices, the central core and seeds were removed. The samples were dried in an oven at a temperature of 60 to 85 °C for 48 hours and then crushed to pass through 2mm mesh and stored in polythene bags for subsequent analysis. Fruit samples after collection and processing, were digested in acid mixture of HNO_3 and $HClO_4$ in the ratio of 10:4. The digest was dissolved in double distilled water and filtered in 100ml volumetric flask. The calcium was estimated by E.D.T.A. method as described by Jackson (1973) [9].

Results and Discussion

Total Soluble Solids

Studies revealed that the maximum fruit TSS (14.55°brix) was recorded in treatment T_0 (no stigma pinched) and the minimum fruit TSS (11.91°brix) in fruits from flowers with 4 stigmata excised (Table-1, Figure 1). This increase in TSS with the higher seed content may be due to increased sink strength of the fruits containing higher number of seeds and thus greater attraction of photosynthates coming from other parts of the tree (Luckwill *et al.*, 1969) [13]. Fruit sink strength potential is greater in seeded than in seedless fruits (Abbott, 1984). Photosynthates produced by apple leaves are in form of sorbitol and to a lesser extent sucrose, sorbitol being the main transport carbohydrate.

Acidity

The results indicated that there was significant difference in the acidity of fruits under different treatments (Table-3). The maximum acidity (0.32%) was obtained in T_0 (no stigma pinched).This increased acidity in T_0 (no stigma pinched) might be due the decreased rate of respiration in fruits containing more seeds. As the seed number increases, respiration decreases hindering the metabolism of malate (organic acid) and vice versa. The metabolism of malate by tissue slices increases as apples pass through the climacteric (Hulme and Rhodes, 1971; Knee, 1993) [8, 10]. The other reason may be attributed to the ability of calcium to maintain cellular integrity by controlling membrane permeability as a result of which H+ ions cannot leach out from the cytosol, thus maintaining higher levels of acidity. This is in conformity with the results of Coombe and Hale (1989) [4] and Koblet *et al.* (1987) [11] who found a significant relationship among seed number, acidity and soluble solid content.

TSS/acidity ratio

The present investigation revealed that significantly maximum TSS/acidity ratio (52.28) was recorded in fruits with less number of seeds from flowers with 4 stigmata excised (Table-3). This increase in TSS/Acidity ratio with the increased number of stigma excised may be attributed to the decrease in acidity. This is in conformity with the results of Coombe and Hale (1989) [4] and Koblet *et al* (1987) [11] who found a significant relationship among seed number, acidity and soluble solid content.

Calcium concentration

The present investigation revealed that maximum fruit calcium (2.17 mg/100g) was obtained in T_0 (no stigma pinching) while as minimum fruit calcium (1.25 mg/100g) was obtained in fruits from flowers with 4 stigmata excised (Table-2, Figure 2). Fruits with more seeds showed a higher calcium concentration, in spite of the dilution effect due to their larger size. The influence of seed number of fruit on calcium concentration could be explained by the fact that calcium transport in the plant is acropetal and is closely linked to basipetal auxin transport (Guzman and De La Fuente, 1984) [6]. Indeed apple seeds, by producing auxin, can affect calcium transport towards the fruit (Luckwill, 1953) [12] and a high seed number may result in greater auxin transport, which, in turn, may enhance calcium transport into fruit. Parthenocarpic apple fruits tend to have lower calcium concentrations (Bangreth, 1993) [3] hence for this species seed number may be one of the factors affecting fruit calcium content.

Table 1: Effect of stigma excision on the TSS (°Brix) of apple cv. Red Delicious.

Treatment	TSS (°Brix)
(0 stigma pinching)	14.55
(1 stigma pinching)	14.42
(2 stigmata pinching)	13.80
(3 stigmata pinching)	12.93
(4 stigmata pinching)	11.91
(5 stigmata pinching)	-
C.D($p \leq 0.05$)	0.03

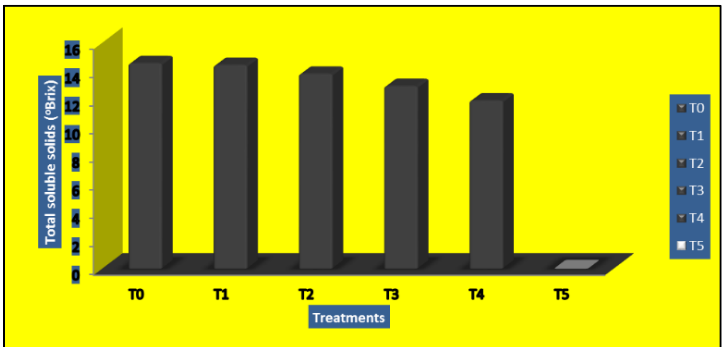

Fig 1: Relationship between the number of stigma excised and TSS for cv. Red Delicious

Table 2: Effect of stigma excision on the calcium content (mg/100g) of apple cv. Red Delicious.

Treatment		Calcium (mg/100g)
T_0	(0 stigma pinching)	2.17
T_1	(1 stigma pinching)	1.95
T_2	(2 stigmata pinching)	1.87
T_3	(3 stigmata pinching)	1.57
T_4	(4 stigmata pinching)	1.25
T_5	(5 stigmata pinching)	-*
	C.D($p \leq 0.05$)	0.25

*No fruit set thus no reading available

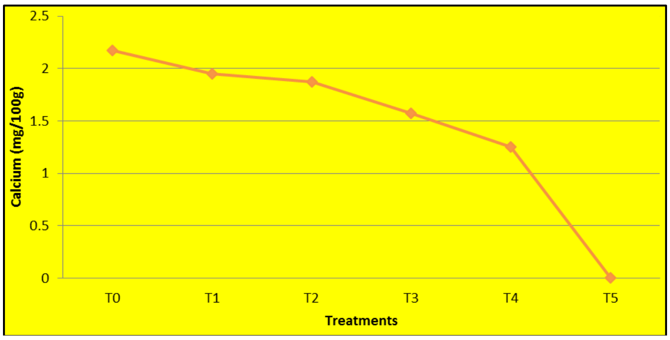

Fig 2: Relationship between the number of stigma excised and calcium content for cv. Red Delicious.

Table 3: Effect of stigma excision on the Acidity (%) and TSS/acidity ratio of apple cv. Red Delicious.

Treatment		Acidity (%)	TSS/acidity ratio
T_0	(0 stigma pinching)	0.32	45.47
T_1	(1 stigma pinching)	0.31	46.53
T_2	(2 stigmata pinching)	0.29	47.12
T_3	(3 stigmata pinching)	0.25	51.15
T_4	(4 stigmata pinching)	0.22	52.28
T_5	(5 stigmata pinching)	-	-
	C.D($p \leq 0.05$)	0.12	1.07

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