Chemical characteristics of Exotic Apple Cultivars in Kashmir's Temperate Conditions: Impact of Diverse Pollen Sources

Abstract

This investigation was conducted in the experimental fields of the Division of Fruit Science at Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar, Srinagar, Jammu & Kashmir, in 2018 to evaluate pollination dynamics in threeexotic apple cultivars—Gala Redlum, Fuji Zehn Aztec, and Super Chief Sandidge—grafted onM9-T337 rootstock. Six compatible pollinizers, Gala Redlum, Fuji Zehn Aztec, Super Chief Sandidge, Lal Ambri, Golden Clone-B, and Red Gold, were cross-pollinated, generating 18 cross combinations within a randomized complete block design. Observations revealed synchronized blooming behavior among the varieties, which is crucial for effective pollination. Gala Redlum exhibited early flowering (28th March), whereas Fuji Zehn Aztec flowered late (4thApril). Red Gold demonstrated the highest pollen viability (94.99%), with Red Gold and Golden Clone-B displaying superior pollen germination. Cross-compatibility studies identifiedRed Gold as an effective pollen source, yielding optimal initial fruit set in Gala Redlum and Super Chief Sandidge, whereas self-incompatibility was noted in Super Chief Sandidge and Fuji Zehn Aztec. Fruit set, drop, and retention varied across combinations, with Gala Redlum× Gala Redlum experiencing the maximum fruit drop (62.50%). Red Gold, as a pollen source, yielded the highest fruit weight, length, and seed number in certain combinations. Fuji Zehn Aztec x Red Gold exhibited maximum fruit firmness (8.79 Kg/cm^2), while Super Chief Sandidge x Red Gold showed elevated total sugars (12.54%) and reducing sugars (10.48%) with lower acidity (0.15%). Based on synchronization, fruit set, and retention, Red Gold and Golden Clone B were identified as effective pollinators for all exotic maternal cultivars, providing valuable insights for apple orchard management under temperate Kashmir conditions.

Keywords: Biochemical, Exotic, Pollen, Physiological and pollinizer

Introduction

The cultivated apple, *Malus × domestica* Borkh L., is an interspecific hybrid complex of allopolyploid origin (Korban and Skirvin, 1984). The progenitor species is thought to be *M. sieversii*. Apple was introduced into the country by the British in the Kullu Valley of the Himalayan State of H.P. as far back as 1865, while colored Delicious cultivars of apples were introduced to Shimla hills of the same state in 1917. The apple cultivar '*Ambri*' is considered indigenous to Kashmir and has grown long before Western introductions. Apple is the most produced temperate tree crop and is widely grown throughout the temperate zone, and its cultivation has been expanding into subtropical and tropical zones as well.

Apple production in India is currently 25.03 lakh metric ton on 3.32 lakh hectares of land (FAO, 2017). Jammu and Kashmir has a production of 17.27 lakh metric tons (Department of Horticulture, 2017), which represents approximately 62 percent of the nation's total production. As the Horticulture Sector is the backbone of the state economy, and apples are the major horticultural crops grown in the state, it is important to focus on all the measuresthat govern the yield and quality of apples. Apple production is a result of a series ofphysiological events, including fruit set (Sanzol and Herrero, 2001). One of the pressing problems currently in the state is poor quality and low production of apples. There could be a number of reasons for the decline in apple production in the state, but the most important factorthat needs quick attention is proper management of pollination in apples.

Pollination plays an important role in apple production. Pollination is the transfer of pollen from the producing anthers to the receptive stigma, and is an essential preliminary step for the sexual reproduction of flowering plants, including apples. The transportation of pollen from flowers of one variety to those of another is probably the most critical single process in the series of events leading to the production of a good-quality fruit. Indeed, pollination management should be regarded as a production factor in its own right for the apple crop as it can affect the agronomic and economic yields and their many components such as fruit set,fruit quality (e.g., size, shape, color, and storability), and seed content. Successful pollination and the formation of many healthy seeds contribute to the eventual size and quality of the fruit,and its failure can result in reduced yield, poor fruit set, pre-harvest fruit drop, and lower fruit yield.

weight, misshapen fruit, thereby reducing fruit quality, and finally, output (yield). Therefore,it is important to select a pollinizer variety with compatible pollen and an overlapping flowering period. An approximate estimate of Rs. 1600 crore loss is experienced every year due to the lack of pollination alone in the Kashmir Valley. Hence, pollination is undoubtedly the most critical event in apple production.

Apple varieties are generally self-unfruitful and do not fruit by their own pollen because ofthe antagonism that prevents pollen grains from growing on stigmas of the same variety. Genetic apples show gametophytic self-incompatibility (Thompson *et al.*, 1992) which necessitates pollen transfer from another pollinizer variety to set fruit in marketable quantities. For cross-pollination to be effective, it is important that the cultivars bloom atapproximately the same time and produce a sufficient quantity of viable and compatible pollen.

The fact that compatibility is controlled by an interaction between the pollen and pistil is fundamental to all modern studies of self incompatibility. It is now known that separatebut closely linked genes resolve the S-specificity in the pistil and pollen. Thus, the current convention is to use the term S-haplotype to describe S-locus variants. In gametophytes, self-incompatible pollen is rejected when there is a match between the single S-haplotype in the haploid pollen and either of the two S-haplotypes in the diploid pistil. Although early workers appreciated that this genetic interaction is mediated by the ‘constituents’ of the pollen and pistil (Darwin, 1877), the relationship between these constituents and the genetic interaction revealed by progeny analysis was unknown.

Pollination plays an important role in determining the chemical attributes of apples, and the difference in the levels of pollination and the difference in the pollen source can directly affectthe chemical characteristics of apples, such as TSS, acidity, total sugars, and reducing sugars. The pollensource influences the chemical characteristics of ‘Long Red B’ Wax apple, pollen of ‘Black,’ ‘Thyto’ resulted in high total sugars and better total soluble solids as compared with other pollens (Tuan and Chung-Ruey 2013). The effect of pollinizers on fruit soluble solids and acidcontent was influenced by the pollen source in a trial with 32 crosses (Davarynezhad *et al.*1993). Hence, pollination is essential for maintaining the final quality attribute that is profitable.

Material and Method

The present investigation was conducted in the experimental fields of the Division of Fruit Science, Sher-e-Kashmir University of Agricultural Science & Technology of Kashmir, Shalimar, Srinagar, Jammu & Kashmir in 2018.

The experiment consisted of three exotic cultivars of apple grafted on M9-T337of uniform age, *viz.* Gala Redlum, Fuji Zehn Aztec, and Super Chief Sandidge, which were used as femaleparents. The female cultivars were cross-pollinated with six compatible pollinizers: Gala Redlum, Fuji Zehn Aztec, Super Chief Sandidge, Lal Ambri, Golden Clone-B, and Red Gold. The experiment was designed using RCBD, with three replications comprising 18 cross combinations.

Table 1: The experimental framework

Maternal Parent	Source of Pollen	Treatments	Detail
M_1	P_1	M_1P_1	Gala Redlum × Gala Redlum
	P_2	M_1P_2	Gala Redlum × Super Chief Sandidge
	P_3	M_1P_3	Gala Redlum × Fuji Zehn Aztec
	P_4	M_1P_4	Gala Redlum × Lal Ambri
	P_5	M_1P_5	Gala Redlum × Golden Clone-B
	P_6	M_1P_6	Gala Redlum × Red Gold
M_2	P_1	M_2P_1	Super Chief Sandidge × Gala Redlum
	P_2	M_2P_2	Super Chief Sandidge × Super Chief Sandidge
	P_3	M_2P_3	Super Chief Sandidge × Fuji Zehn Aztec
	P_4	M_2P_4	Super Chief Sandidge × Lal Ambri
	P_5	M_2P_5	Super Chief Sandidge × Golden Clone-B
	P_6	M_2P_6	Super Chief Sandidge × Red Gold
M_3	P_1	M_3P_1	Fuji Zehn Aztec × Gala Redlum
	P_2	M_3P_2	Fuji Zehn Aztec × Super Chief Sandidge
	P_3	M_3P_3	Fuji Zehn Aztec × Fuji Zehn Aztec
	P_4	M_3P_4	FujiZehn Aztec× Lal Ambri
	P_5	M_3P_5	Fuji Zehn Aztec × Golden Clone-B
	P_6	M_3P_6	Fuji Zehn Aztec × Red Gold

Total sugars (%)

Total sugars were determined by taking a known weight of fruit sample of 25 g, crushed,and mixed with distilled water, and the volume was made up to 250 ml in a volumetric flask and neutralizedwith 1N NaOH. To this 250 ml solution, 2 ml of 45% lead acetate was added. After 5-10 min,2 ml of 42% potassium oxalate was added to precipitate the excess lead acetate and filtered. The filtrate (50 ml of filtrate was hydrolyzed by adding 10 ml of hydrochloric acid (1:1).

was allowed to stand overnight to complete the reaction. Excess hydrochloric acid was neutralizedwith a saturated NaOH solution the next day. The hydrolyzed aliquot was taken in a burette and titratedagainst boiling solution containing 5 ml each of Fehling A and B using methylene blue as an indicator (A.O.A.C., 1990). The endpoint was indicated by the appearance of a brick red color.

$$\textbf{\textit{Total Sugar}}\ (\%) = \frac{\textit{Glucose equivalent of Fehling's solution x Total volume made x Volume made up after inversion}}{\textit{Titre Value x Wt. of pulp taken x Aliquot taken for inversion}}\ x\,100$$

Reducing sugars (%)

The filtrate remaining after removing 50 ml of total sugars was used for the estimation of reducing sugars. To this end, 10 ml of mixed Fehling solution (5 ml Fehling A+ 5 ml Fehling B) was added to the titration flasks. About 30-40 ml distilled water was added to the solution. This solution was then titrated against the fruit juice extract using methylene blue as an indicator until a brick red color (endpoint) appeared.

$$\textbf{\textit{Reducing Sugar}}\ (\%) = \frac{\textit{Fehlings factor x Dilution}}{\textit{Titrate value x weight of sample}}\ x\,100$$

Titratable acidity (%)

The percent titratable acidity (as malic acid) was estimated by titrating a known quantity of homogenized juice against 0.1 N NaOH solution using phenolphthalein as an indicator. Acidity was expressed in terms of malic acid as a percentage of total titratable acidity.

$$\textbf{Titrable acidity}\ (\%) = \frac{Titrate\ value\ x\ 0.1\ x\ Equivalent\ weight\ of\ acid\ x\ dilution\ factor}{Weight\ of\ sample\ x\ Volume\ of\ filtrate\ for\ estimation\ x\ 1000}\ x\ 100$$

Statistical analysis

The observations recorded during the course of the investigation were subjected to statistical analysis according to the method of Analysis of Variance (Gomez and Gomez, 1984). The significance and non-significance of treatment effects were judged using OPSTAT software. A significant difference in the means was tested against the critical difference at a 5% significance level.

Result and Discussion

TSS

The data inscribed in Table 2 clearly indicates that mean total soluble solid content was was significantly higher (13.76^{o}Brix) in cv. 'Super Chief Sandidge' compared to (12.70^{o}Brix) in cv. 'Gala Redlum' and (11.72^{o}Brix) in cv. 'Fuji Zehn Aztec'.The influence of pollen source on maternal parent was significant, highest mean total soluble solid content (13.31^{o}Brix) was obtained with pollinizer 'Red Gold' followed by (13.20^{o}Brix) and (13.10^{o}Brix) with pollinizer 'Golden Clone B' and 'Lal Ambri' respectively and lowest (11.73^{o}Brix) with pollinizer 'Super Chief Sandidge. ' In addition, all pollinizers were significantly different from each other, and the interaction effect of pollen source and maternal parent was significant regarding the total soluble solid content of fruits. It is evident from the data documented in Table 2 that the maximum TSS (14.10^{o}B) was recorded in 'Super Chief Sandidge x Red Gold' and the minimum TSS of (11.20^{o}B) was recorded in 'Fuji Zehn Aztec x Super Chief Sandidge'. Significant differences were observed among the various crosses according to the crossing plan. These variations in soluble solid content may be due to varietal characteristics, or it could be due to activities of the enzyme systems initiated by the metaxenic effect and later passed into extracellularsites, dissolve readily into water, and invert the sugar. Hydrolytic enzymes such as polygalacturonase and cellulase may also be involved in these biochemical changes by solubilizing pectin and cellulose in the cell wall (Hasegawa and Smolensky, 1971; Ghnaim and Al-Muhtaseb, 2006). The axenic effect of pollen sources on fruit soluble solid content and total sugars has also been reported by many scientists (Militaru *et al.*, 2015; Gupta *et al.*, 2017).

Table 2: Effect of pollen source on TSS (^{o}B) of exotic apple cultivars

Cultivars \ Pollen source	Gala Redlum	Super Chief Sandidge	Fuji Zehn Aztec	Lal Ambri	Golden Clone B	Red Gold	Mean
Gala Redlum	11.88	12.26	12.44	12.87	13.32	13.39	12.70
Super Chief Sandidge	13.37	-	13.76	13.56	14.01	14.10	13.76
Fuji Zehn Aztec	11.46	11.20	-	11.26	12.27	12.43	11.72
Mean	**12.24**	**11.73**	**13.10**	**12.56**	**13.20**	**13.31**	**12.73**

CD ($P \leq 0.05$)

Cultivar (M)	**:**	**0.61**
Pollen source (P)	**:**	**0.12**
M x P	**:**	**0.74**

Total sugars

The data shown in Table 3 reveal that total sugars were significantly influenced by different pollen sources (11.92%) in cv. 'Super Chief Sandidge' as compared to and (10.65%) in cv. 'Gala Redlum' and (10.09%) in cv. 'Fuji Zehn Aztec' .The pollinizer 'Red Gold' resulted in significantly higher mean total sugars (11.42%) followed by(11.32%) and (10.86%) with pollinizers 'Golden Clone B' and 'Fuji Zehn Aztec' respectively,and lowest (10.29%) was obtained with ' Gala Redlum, ' irrespective of the cultivars under study, Also all the pollinizers were significantly different with each other.It is evident from the data documented in Table 3 among the various crosses made the maximum total sugars (12.54%) was recorded in 'Super Chief Sandidge x Red Gold' and the minimum total sugars (9.47%) was recorded in 'Fuji Zehn Aztec x Gala Redlum'. Significant differences were observed among the various crosses according to the crossing plan. These variations in total sugars maybe due to varietal characteristics, or it could be due to activities of the enzyme systems initiated by the metaxenic effect and later passed into extracellular sites, get dissolved readilyinto water, and invert the sugar. Hydrolytic enzymes such as polygalacturonase and cellulase may also be involved in these biochemical changes by solubilizing pectin andcellulose in the cell wall (Hasegawa and Smolensky, 1971; Ghnaim and Al-Muhtaseb, 2006). The axenic effect of pollen sources on fruit soluble solid content and total sugars has also been reported by many scientists (Militaru *et al.*, 2015; Gupta *et al.*, 2017).

Table 3: Effect of pollen source on total sugars (%) of exotic apple cultivars

Cultivars \ Pollen source	Gala Redlum	Super Chief Sandidge	Fuji Zehn Aztec	Lal Ambri	Golden Clone B	Red Gold	Mean
Gala Redlum	10.11	10.88	10.54	10.25	11.00	11.15	10.65
Super Chief Sandidge	11.30	-	11.19	12.05	12.51	12.54	11.92
Fuji Zehn Aztec	9.47	10.14	-	9.83	10.44	10.58	10.09
Mean	**10.29**	**10.51**	**10.86**	**10.71**	**11.32**	**11.42**	**10.89**

CD ($P \leq 0.05$)

Cultivar (M) : 0.22

Pollen source (P) : 0.09

M x P : 0.32

Reducing sugars

The data shown in Table 4 reveal that reducing sugars were significantly influenced by different pollen sources (9.74%) in cv. 'Super Chief Sandidge' as compared to (8.57%) in cv. 'Gala Redlum' and (8.39%) in cv. 'Fuji Zehn Aztec'.The pollinizer'Red Gold' resulted in significantly higher mean reducing sugars (9.73%) followed by (9.48%)and (8.79%) with pollinizers 'Golden Clone B' and 'Fuji Zehn Aztec' respectively, and lowest(8.11%) was obtained with 'Super Chief Sandidge, ' irrespective of the cultivars under study, Also all the pollinizers were significantly different with each other.It is evident from the datadocumented in Table 4, among the various crosses made that the maximum reducing sugars (10.48%) was recorded in 'Super Chief Sandidge x Red Gold' and the minimum reducing sugars (7.10%) was recorded in 'Fuji Zehn Aztec x Gala Redlum'. Significant differences were observed among the various crosses according to the crossing plan. These variations in reducing sugars may be due to varietal characteristics, or it could be due to activities of theenzyme systems initiated by the metaxenic effect and later passed into extracellular sites, get dissolved readily into water, and invert the sugar. Hydrolytic enzymes such aspolygalacturonase and cellulase may also be involved in these biochemical changes bysolubilizing pectin and cellulose in the cell wall (Hasegawa and Smolensky, 1971; Ghnaim andAl-Muhtaseb, 2006). The axenic effect of pollen sources on fruit soluble solid content and totalsugars has also been reported by many scientists (Militaru *et al.*, 2015; Gupta *et al.*, 2017).

Table 4: Effect of pollen source on reducing sugars (%) of exotic apple cultivars

Pollen source / Cultivars	**Gala Redlum**	**Super Chief Sandidge**	**Fuji Zehn Aztec**	**Lal Ambri**	**Golden Clone B**	**Red Gold**	**Mean**
Gala Redlum	8.38	8.14	8.10	8.17	9.25	9.38	8.57
Super Chief Sandidge	9.38	-	9.49	9.29	10.07	10.48	9.74
Fuji Zehn Aztec	7.10	8.08	-	8.36	9.13	9.31	8.39
Mean	**8.29**	**8.11**	**8.79**	**8.61**	**9.48**	**9.73**	**8.90**
CD ($P \leq 0.05$)							
Cultivar (M)		:	**0.08**				
Pollen source (P)		:	**0.06**				
M x P		:	**0.15**				

Titratable acidity

The data in Table 5 clearly indicate that the mean fruit acidity content was the highest (0.33%) in cv. 'Fuji Zehn Aztec' 'Super Chief Sandidge' compared to (0.28%) in cv. 'Gala Redlum' and (0.19%) in cv. 'Super Chief Sandidge. ' The influence of pollen source on maternal parent was significant, highest mean fruit acidity content (0.31%) was obtained with pollinizer 'Super Chief Sandidge' followed by (0.29%) and (0.27%) with pollinizer 'Gala Redlum' and 'Lal Ambri' respectively and lowest (0.23%) with pollinizer 'Red Gold. ' In addition, all pollinizers differed significantly from each other. It is evident from the data documented in Table 5 that the maximum fruit acidity (0.36%) was recorded in 'Fuji Zehn Aztec x Gala Redlum' and the minimum fruit acidity (0.15%) was recorded in 'Super Chief Sandidge × Red Gold'. Significant differences were observed among the various crosses according to the crossing plan. These variations may be due to the effect of pollen donors on recipients or it could be due to low temperature conditions or due to varietal characters of the cultivars under study. The increase or decrease in acidity observed with some pollinizersis similar to the observations of Sulusoglu and Cavusoglu (2014), who reported a metaxenic effect of pollen source on fruit titratable acidity in cherry laurel.

Table 5: Effect of pollen source on fruit acidity (%) of exotic apple cultivars

Cultivars \ Pollen source	Gala Redlum	Super Chief Sandidge	Fuji Zehn Aztec	Lal Ambri	Golden Clone B	Red Gold	Mean
Gala Redlum	0.31	0.28	0.26	0.29	0.27	0.25	0.28
Super Chief Sandidge	0.20	-	0.22	0.23	0.19	0.15	0.19
Fuji Zehn Aztec	0.36	0.35	-	0.30	0.34	0.29	0.33
Mean	**0.29**	**0.31**	**0.24**	**0.27**	**0.26**	**0.23**	**0.27**
CD ($P \leq 0.05$)							
Cultivar (M)	**:**	**0.04**					
Pollen source (P)	**:**	**0.05**					
M x P	**:**	**0.09**					

Conclusion

The number of seeds per fruit, TSS, fruit acidity, and total sugars were affected by the source of pollen used in crossing, and the superiority was noticed with Red Gold, suggesting that Red Gold acts as a more effective pollen source for exotic cultivarsunder study in temperate conditions of Kashmir because it produces abundant numbers of viable and compatible pollens**.**

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