

## **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 the year 2018 aimed to evaluate pollination dynamics in three exotic apple cultivars—Gala Redlum, Fuji Zehn Aztec and Super Chief Sandidge—grafted on M9-T337 rootstock. Six compatible pollinizers, including 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 behaviour among varieties, crucial for effective pollination. Gala Redlum exhibited early flowering (28<sup>th</sup> March), while Fuji Zehn Aztec flowered late (4<sup>th</sup> April). Red Gold demonstrated the highest pollen viability (94.99%), with Red Gold and Golden Clone-B displaying superior pollen germination. Cross compatibility studies identified Red Gold as an effective pollen source, yielding optimal initial fruit set in Gala Redlum and Super Chief Sandidge, while self-incompatibility was noted in Super Chief Sandidge and Fuji Zehn Aztec. Fruit set, drop, and retention varied across cross combinations, with Gala Redlum x Gala Redlum experiencing 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<sup>2</sup>), 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 in 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 the colored Delicious cultivars of apple were introduced to Shimla hills of the same State in 1917. The apple cultivar 'Ambri' is considered to be indigenous to Kashmir and had been grown long before Western introductions. The 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 tonnes on 3.32 lakh hectares of land (FAO, 2017). Jammu and Kashmir has a production of 17.27 lakh metric tonnes (Department of horticulture, 2017) which represents approximately 62 percent of the nation's total production. As Horticulture Sector is the backbone of States economy, and apple is the major horticultural crop grown in the State, it is therefore important to focus on all the measures which govern the yield and quality of apple. Apple production is a result of series of physiological events including fruit set (Sanzol and Herrero, 2001). One of the pressing problems nowadays in the state is poor quality and low production of apple. There could be a number of reasons behind declined apple production in the state, but the most important factor which needs a quick attention is proper management of pollination in apples.

Pollination plays a key role in profitable apple production. Pollination is the transfer of pollen from the producing anthers to the receptive stigma, and it is essential preliminary step for the sexual reproduction of flowering plants including apple. 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 by many components such as fruit set, fruit quality (e.g. size, shape, colour and storability) and seed content. Successful pollination and the formation of many healthy seeds contribute to the eventual size and quality of that fruit and its failure can result in reduced yield, poor fruit set, pre-harvest fruit drop, lower fruit

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weight, misshapen fruit, thereby reducing fruit quality and finally the output (yield). Therefore, it is important to select a pollinizer variety which has compatible pollen and an overlapping flowering period. A roughly estimate of Rs. 1600 crore loss is experienced every year due to lack of pollination alone in Kashmir valley. Hence pollination is, without any doubt, the most critical event in the production of apples.

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

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The fact that compatibility is controlled by an interaction between the pollen and the pistil is fundamental to all modern studies of self incompatibility. It is now known that separate, but closely linked genes resolve S-specificity in the pistil and pollen. Thus, the current convention is to use the term S-haplotype to describe S-locus variants. In gametophytic self-Incompatibility 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.

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Pollination plays an important role in developing the chemical attributes of apple the difference in the levels of pollination and the difference in the pollen source can directly affect the chemical characters of apple like TSS, acidity, total sugars and reducing sugars. The pollen source 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 acid content were influenced by the source of pollen in a trial with 32 crosses (Davarynezhad *et al.* 1993). Hence pollination is essential in maintaining the final quality attribute profitable.

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## Material and Method

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

The experiment consisted of three exotic cultivars of apple grafted on M9-T337 of uniform age *viz.* Gala Redlum, Fuji Zehn Aztec and Super Chief Sandidge were taken as female parent. Female cultivars were cross pollinated with six compatible pollinizers *viz.* Gala Redlum, Fuji Zehn Aztec, Super Chief Sandidge, Lal Ambri, Golden Clone-B and Red Gold. The design of experiment was RCBD with three replications comprising of 18 cross combinations.

**Table 1: The experimental framework**

| Maternal Parent | Source of Pollen | Treatments                    | Detail                                      |
|-----------------|------------------|-------------------------------|---|
| M <sub>1</sub>  | P <sub>1</sub>   | M <sub>1</sub> P <sub>1</sub> | Gala Redlum × Gala Redlum                   |
|                 | P <sub>2</sub>   | M <sub>1</sub> P <sub>2</sub> | Gala Redlum × Super Chief Sandidge          |
|                 | P <sub>3</sub>   | M <sub>1</sub> P <sub>3</sub> | Gala Redlum × Fuji Zehn Aztec               |
|                 | P <sub>4</sub>   | M <sub>1</sub> P <sub>4</sub> | Gala Redlum × Lal Ambri                     |
|                 | P <sub>5</sub>   | M <sub>1</sub> P <sub>5</sub> | Gala Redlum × Golden Clone-B                |
|                 | P <sub>6</sub>   | M <sub>1</sub> P <sub>6</sub> | Gala Redlum × Red Gold                      |
| M <sub>2</sub>  | P <sub>1</sub>   | M <sub>2</sub> P <sub>1</sub> | Super Chief Sandidge × Gala Redlum          |
|                 | P <sub>2</sub>   | M <sub>2</sub> P <sub>2</sub> | Super Chief Sandidge × Super Chief Sandidge |
|                 | P <sub>3</sub>   | M <sub>2</sub> P <sub>3</sub> | Super Chief Sandidge × Fuji Zehn Aztec      |
|                 | P <sub>4</sub>   | M <sub>2</sub> P <sub>4</sub> | Super Chief Sandidge × Lal Ambri            |
|                 | P <sub>5</sub>   | M <sub>2</sub> P <sub>5</sub> | Super Chief Sandidge × Golden Clone-B       |
|                 | P <sub>6</sub>   | M <sub>2</sub> P <sub>6</sub> | Super Chief Sandidge × Red Gold             |
| M <sub>3</sub>  | P <sub>1</sub>   | M <sub>3</sub> P <sub>1</sub> | Fuji Zehn Aztec × Gala Redlum               |
|                 | P <sub>2</sub>   | M <sub>3</sub> P <sub>2</sub> | Fuji Zehn Aztec × Super Chief Sandidge      |
|                 | P <sub>3</sub>   | M <sub>3</sub> P <sub>3</sub> | Fuji Zehn Aztec × Fuji Zehn Aztec           |
|                 | P <sub>4</sub>   | M <sub>3</sub> P <sub>4</sub> | Fuji Zehn Aztec × Lal Ambri                 |
|                 | P <sub>5</sub>   | M <sub>3</sub> P <sub>5</sub> | Fuji Zehn Aztec × Golden Clone-B            |
|                 | P <sub>6</sub>   | M <sub>3</sub> P <sub>6</sub> | Fuji Zehn Aztec × Red Gold                  |

#### Total sugars (%)

Total sugars were determined by taking known weight of fruit sample of 25g, crushed and mixed with distilled water and volume made to 250 ml in a volumetric flask and neutralized with 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. 50 ml of filtrate was taken and hydrolyzed by adding 10 ml of hydrochloric acid (1:1) and was

allowed to stand overnight for complete reaction. The excess hydrochloric acid was neutralized with saturated NaOH solution next day. The hydrolysed aliquot was taken in burette and titrated against boiling solution containing 5ml each of Fehling A and B using methylene blue as indicator (A.O.A.C., 1990). The end point was indicated by the appearance of brick red colour.

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

### Reducing sugars (%)

The filtrate left after taking 50 ml for total sugars was used for estimation of reducing sugars. For this, 10 ml of mixed Fehling solution (5ml Fehling A+ 5 ml Fehling B) was taken in titration flasks. About 30-40 ml of distilled water was added to it. This solution was then titrated against the fruit juice extract, using methylene blue as indicator, till brick red colour (end point) appears.

$$\text{Reducing Sugar (\%)} = \frac{\text{Fehlings factor} \times \text{Dilution}}{\text{Titrate value} \times \text{weight of sample}} \times 100$$

### Titrateable acidity (%)

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

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

### Statistical analysis

The observations recorded during the course of investigation were subjected to statistical analysis as per the method of 'Analysis of Variance' (Gomez and Gomez, 1984). The significance and non-significance of treatment effects were judged with the help of software OPSTAT. The significant difference on the means was tested against the critical difference at 5% significance.

## Result and Discussion

### TSS

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

**Table 2: Effect of pollen source on TSS (°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        |
|                       | <b>Mean</b>              | <b>12.24</b> | <b>11.73</b>         | <b>13.10</b>    | <b>12.56</b> | <b>13.20</b>   | <b>13.31</b> | <b>12.73</b> |
| <b>CD (p&lt;0.05)</b> |                          |              |                      |                 |              |                |              |              |
|                       | <b>Cultivar (M)</b>      | :            | <b>0.61</b>          |                 |              |                |              |              |
|                       | <b>Pollen source (P)</b> | :            | <b>0.12</b>          |                 |              |                |              |              |
|                       | <b>M x P</b>             | :            | <b>0.74</b>          |                 |              |                |              |              |

## Total sugars

The data documented in Table 3 reveal that total sugars was influenced significantly by different pollen source being significantly highest (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’. There were significant differences among the various crosses made as per the crossing plan. These variations in total sugars may be due to the varietal characteristics or it could be due to activities of the enzyme systems initiated by the metaxenic effect and later on passed into extra cellular sites, get dissolve readily into water and invert the sugar. Similarly, the hydrolytic enzymes such as polygalacturonase and cellulase may also be involved in these biochemical changes by solubilizing the pectin and cellulose of cell wall (Hasegawa and Smolensky, 1971; Ghnaim and Al-Muhtaseb, 2006). Metaxenic effect of pollen source on fruit soluble solid content and total sugars was also reported by many scientists (Militaru *et al.*, 2015; Gupta *et al.*, 2017).

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

| Pollen source<br>Cultivars | Gala<br>Redlum | Super<br>Chief<br>Sandidge | Fuji<br>Zehn<br>Aztec | Lal<br>Ambri | Golden<br>Clone B | Red<br>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        |
| <b>Mean</b>                | <b>10.29</b>   | <b>10.51</b>               | <b>10.86</b>          | <b>10.71</b> | <b>11.32</b>      | <b>11.42</b> | <b>10.89</b> |
| <b>CD (P&lt;0.05)</b>      |                |                            |                       |              |                   |              |              |
| <b>Cultivar (M)</b>        | <b>: 0.22</b>  |                            |                       |              |                   |              |              |
| <b>Pollen source (P)</b>   | <b>: 0.09</b>  |                            |                       |              |                   |              |              |
| <b>M x P</b>               | <b>: 0.32</b>  |                            |                       |              |                   |              |              |

## Reducing sugars

The data documented in Table 4 reveal that reducing sugars was influenced significantly by different pollen source being significantly highest (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 data documented 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'. There were significant differences among the various crosses made as per the crossing plan. These variations in reducing sugars may be due to the varietal characteristics or it could be due to activities of the enzyme systems initiated by the metaxenic effect and later on passed into extra cellular sites, get dissolve readily into water and invert the sugar. Similarly, the hydrolytic enzymes such as polygalacturonase and cellulase may also be involved in these biochemical changes by solubilizing the pectin and cellulose of cell wall (Hasegawa and Smolensky, 1971; Ghnaim and Al-Muhtaseb, 2006). Metaxenic effect of pollen source on fruit soluble solid content and total sugars was also 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**

| Cultivars                            | Pollen source<br>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        |
| <b>Mean</b>                          | <b>8.29</b>                  | <b>8.11</b>          | <b>8.79</b>     | <b>8.61</b> | <b>9.48</b>    | <b>9.73</b> | <b>8.90</b> |
| <b>CD (<math>P \leq 0.05</math>)</b> |                              |                      |                 |             |                |             |             |
| <b>Cultivar (M)</b>                  | :                            |                      | <b>0.08</b>     |             |                |             |             |
| <b>Pollen source (P)</b>             | :                            |                      | <b>0.06</b>     |             |                |             |             |
| <b>M x P</b>                         | :                            |                      | <b>0.15</b>     |             |                |             |             |

#### Titrateable acidity

The data inscribed in Table 5 clearly indicate that mean fruit acidity content was significantly 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’. Also all the pollinizers were significantly different with 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 x Red Gold’. There were significant differences among the various crosses made as per 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 of the pollinizers is similar to the observations of Sulusoglu and Cavusoglu (2014) who reported metaxenic effect of pollen source on fruit titrable 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        |
|                                      | <b>Mean</b>              | <b>0.29</b>   | <b>0.31</b>          | <b>0.24</b>     | <b>0.27</b> | <b>0.26</b>    | <b>0.23</b> | <b>0.27</b> |
| <b>CD (<math>p \leq 0.05</math>)</b> |                          |               |                      |                 |             |                |             |             |
|                                      | <b>Cultivar (M)</b>      | <b>: 0.04</b> |                      |                 |             |                |             |             |
|                                      | <b>Pollen source (P)</b> | <b>: 0.05</b> |                      |                 |             |                |             |             |
|                                      | <b>M x P</b>             | <b>: 0.09</b> |                      |                 |             |                |             |             |

## 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, It is therefore suggested from this study that Red Gold act as more effective pollen source for exotic cultivars under study in temperate conditions of Kashmir since it produce abundant number of viable and compatible pollens.

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