

## RESEARCH ARTICLE

# Chimeric Apple: A Study of Morphological and Biochemical Variations

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### Abstract

Chimeras are generally derived from spontaneous bud sport mutations in plants whose tissues are made up of two or more types of cells with different genetic makeup. The apple, one of the most well-known fruits, is very prone to these spontaneous mutations or chimeras. In this study, we discovered a half-red and half-green colouring spontaneous apple chimera 'JCS' from the cultivar 'Royal Delicious' in the Kinnaur district of Himachal Pradesh, India. Apple chimera 'JCS' also showed variation in different morphological and biochemical characters as compared to its parent tree. Spontaneous chimeras with different colour combinations may attract consumers and also may be a vital resource for revealing molecular mechanisms, and might be employed in future apple breeding programmes.

**Keywords:** Apple, Bud sports, Chimera, Conservation, Fruit colour, Genetic variation

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### Introduction

The Apple (*Malus × domestica* Borkh.) is among the most consumed fruits throughout the world (Kumar *et al.*, 2024a; Kumar *et al.*, 2024b; Sharma *et al.*, 2025). It is a member of the Rosaceae family having chromosome number  $2n = 2x = 34$ , which originated in Central Asia (Kumar *et al.*, 2022a; Kumar *et al.*, 2022b). Apple is known for its various health benefits and helps to reduce the risk of several diseases like diabetes, lung cancer and asthma. It is also rich in flavonols, polyphenols, vitamins and minerals (Preti and Tarola, 2021). Apple breeding for qualitative and quantitative traits is the main concern for fruit breeders due to its nutritional and health benefits (Kumar *et al.*, 2022a). The apple's fruit colour and maturity are the most desirable attributes because they influence the fruit's quality, price, and market acceptance, making the fruit attractive, desirable, and edible for consumers (Goncalves *et al.*, 2017).

Chimeras are generally derived from spontaneous bud sport mutations in plants whose tissues are made up of two or more types of cells with different genetic makeup (Frank and Chitwood, 2016), triggered by genetic variation in somatic cells and such chimeras can give rise to mature tissues of different phenotypes (Chevreau *et al.*, 1989), eventually resulting in numerous phenotypic alterations in qualitative and quantitative traits including variations in fruit colour and

maturity (Kumar *et al.*, 2022a). Trees with chimeras are known for having a mixture of both wild-type and mutant cells (Li, 2024), which produce cultivars like Cytisus Adami and Bizzaria with horticulturally valuable traits (Frank and Chitwood, 2016). Generally, three types of chimeras have been found in Nature (Pratt, 1960) i.e. Periclinal Chimeras (when whole of the L<sub>1</sub>, L<sub>2</sub>, or L<sub>3</sub> histogenic layer is affected), Mericlinal Chimeras (when only a part of the meristem layer is affected) and Sectorial Chimeras (when only a part of the L<sub>1</sub>, L<sub>2</sub>, or L<sub>3</sub> layer is affected).

Apple, particularly the 'Delicious' group, is prone to these spontaneous mutations or chimeras, for example, the globally known cultivar 'Red Delicious', which is a sport mutant of 'Delicious'. However, old crop improvement methods in apple are laborious and complicated due to the complex nature of the crop. Spontaneous Chimeras are rare, and identification & characterization of such Spontaneous bud sport Chimeras in apple is important as they might be a major source of unique cultivars as well as vital resources for revealing molecular mechanisms (Wu *et al.*, 2021). However, commercial utilization of such spontaneous chimeras requires phenotypic stability still it can be used as a rapid method of breeding in apples (Reichert, 2021, Kumar *et al.*, 2022a) and this can be achieved by either eliminating chimerism by mutagenesis, by selecting a chimeral pattern with an acceptable level of stability and separate chimeras into pure types (Chevreau *et al.*, 1989).

Taking this into account, as well as the increased likelihood of spontaneous apple chimeras at higher elevations, the present investigation was carried out, and a Spontaneous apple chimera 'JCS' was located from Shong village of the district Kinnaur in Himachal Pradesh, India. Fruits of identified chimera and fruits of its parent plants were evaluated for various morphological and biochemical parameters, and bud wood from bud sports was collected, conserved and propagated for further investigations.

## Materials and Methods

### Experimental locations

To identify apple spontaneous bud sports and chimeras, apple-growing areas were surveyed during this study. An identified chimera in apple was located at Shong village of district Kinnaur in Himachal Pradesh, India (Latitude: 31.44°, Longitude: 78.20°) with an altitude of 2423 metres above mean sea level. However, morpho-biochemical analyses were performed at Dr Yashwant Singh Parmar University of Horticulture &

Forestry, Nauni, Solan, Himachal Pradesh, India.

### Morpho-metric studies

After the identification of a spontaneous apple chimera, the mother trees as well as the identified chimera were tagged and studied extensively. Standard apple descriptor (IBPGR, 1982) and DUS test guidelines (UPOV, 2005) were followed to record the variations in leaf, flower and fruit characters. However, the bark colour was observed visually and presented as grey and reddish-brown. The LI-COR 3100 leaf area meter was used to measure the leaf area, and the average was displayed in cm<sup>2</sup>. A digital pressure tester (FHP-802) was used to determine fruit firmness, i.e. expressed in kg/cm<sup>2</sup>.

Fruit colour, overcolour and ground colour were determined using the colour chart of the Royal Horticultural Society. The relative area of fruit overcolour, grooves, lenticels, calyx persistence and. However, the quantum of fruit russet was visually assessed and recorded as 0%, 12%, 25%, 37%, 50%, 62%, 75%, 87% and 100%. Whereas the intensity of russet was visually assessed using standard descriptors and indicated as extremely fine, very fine, intermediate, coarse, scaly and cracked. Fruit volume (cc) and specific gravity were determined through the water-displaced method (Mazumdar *et al.*, 2003). Organoleptic characteristics were assessed by five panellists, who scored the samples using the 'Hedonic Rating Test'.

### Biochemical analysis

Biochemical parameters such as total sugars (%), titratable acidity (%), non-reducing sugars (%) and reducing sugars (%) were obtained using the standard method given by Ranganna (1995). While total soluble solids (°Brix) and ascorbic acid content were assessed using the method given by the Association of Official Analytical Chemists. To assess the presence of anthocyanin in apple fruit, the procedure outlined by Herborne (1973) was used. Apple fruits from both the mother plants and the bud sports were investigated for starch iodine stain area, measured using the 'Hedonic Rating Test' scale (Smith *et al.*, 1979).

### Germplasm conservation

To raise the plants for future studies and conserve the germplasm, bud wood of an identified chimera was collected and propagated on M-9 (T337) rootstock and maintained in the fruit nursery of the Dr YS Parmar University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh, India.

### Statistical analysis

The experimental data for each parameter under study were statistically analysed. For morphometric and biochemical analysis, three replications were used. Results from spontaneous chimera and mother plant were compared, and data were collected in triplicate and presented as the mean  $\pm$  standard error of the mean.

### Results and Discussion

Exploration of existing apple orchards led to the identification of a half-red and half-green colouring apple chimera 'JCS' originating from 'Royal Delicious', which can be used to exploit variability and new



**Fig. 1:** Identified half red and half green colouring apple chimera 'JCS' and their parent cultivar 'Royal Delicious'.

genotypes with one or more commercially useful traits. Extensive phenological, floral, and fruit evaluations were conducted on the spontaneous apple chimera 'JCS', and its parent is detailed in Table 1. Here, we reveal and discuss the results of our study.

Spontaneous apple chimera 'JCS' was primarily identified and isolated for a distinct half red and half green colouring pattern with respect to its mother cv. Royal Delicious is located at Shong, district Kinnaur (Latitude: 31.44°, Longitude: 78.20°). Apart from its unique colouring pattern, spotted and varied in fruit shape, which was cylindrical compared to conic fruits of the mother tree (Fig. 1).

Half red and half green colouring apple chimera 'JCS' also showed variation in different characters and revealed higher fruit weight (138.00 g), fruit breadth (6.37 cm), fruit base cavity (eye) length (1.22 cm), fruit base (eye) cavity depth (1.25 cm), juice content (56.00%), titratable acidity (0.21%), TSS (12.10 ° Brix), total sugars (5.56%), non-reducing sugars (2.76%), ascorbic acid/ vitamin – C (11.20 mg/100 g), anthocyanin content (0.39 OD), fruit firmness (12.00 kg/cm<sup>2</sup>), fruit peel lenticels (6 per cm<sup>2</sup>), lesser stained area (70%). Less fruit length (6.79 cm) and pedicel length (1.67 cm), leaf area (26.00 cm<sup>2</sup>), reducing sugars (2.66 %), fruit cavity depth (1.32 cm), number of seeds (5), and relative area of overcolour (50.00 %) were observed. Other characters examined were more or less similar in parent tree and spontaneous apple chimera 'JCS' (Table 1).

**Table 1:** Phenological, flower, fruit, morpho-physical and biochemical characters of half red and half green colouring apple chimera 'JCS' and their parent cultivar 'Royal Delicious'

Location: Shong, Kinnaur (Latitude: 31.44°, Longitude: 78.20°) (Altitude: 2423 m)

Characters	Parent Cultivar: Royal Delicious	Bud Sport: JCS
<i>Plant morphological characters</i>		
Bark colour	Grey	Grey
Internodal length (cm)	2.50	Yet to be ascertained
<i>Phenological characters</i>		
Time of leaf bud burst	1 <sup>st</sup> week of March	1 <sup>st</sup> week of March
Time of floral bud burst	3 <sup>rd</sup> week of March	3 <sup>rd</sup> week of March
Time of peel colour development	4 <sup>th</sup> week of July	4 <sup>th</sup> week of July
Time of leaf fall	3 <sup>rd</sup> week of December	3 <sup>rd</sup> week of December
<i>Foliage characters</i>		
Colour of young leaf	Light Green	Light Green
Leaf pubescence	Medium	Medium
Leaf margin	Serrate type 1	Serrate type 1
Leaf apex shape	Acuminate	Acuminate
Leaf base shape	Acute	Acute
Leaf area (cm <sup>2</sup> )	33.00	26.00

*Inflorescence and flowering characters*

Bearing habit	On spurs and long shoots	
Yet to be ascertained Flower colour	Pinkish white	Pinkish white
Season of flowering	Early	Early
Duration of flowering	12-15 days	Yet to be ascertained

*Morpho-physical fruit characters*

Fruit weight (g)	121.50	138.00
Fruit length (cm)	6.82	6.79
Fruit breadth (cm)	6.19	6.37
Fruit volume (cc)	141.50	180.00
Specific gravity	0.86	0.77
Fruit firmness (kg/cm <sup>2</sup> )	10.50	12.00
Fruit shape	Conic	Cylindrical
Fruit shape index	1.10	1.07
Fruit texture	Intermediate	Intermediate
Fruit ribbing	Weak	Weak
Number of grooves	Moderate and 5 in number	Moderate and 5 in number
Surface of fruit	Smooth	Smooth
Fruit colour	Red group 45-A	Red group 45-A
Fruit overcolour	Red group 45-A	Red group 45-A
Relative area of fruit overcolour (%)	80.00	50.00
Type of overcolour	Striped	Striped
Intensity of fruit overcolour	Medium	Medium
Fruit ground colour	Yellow green group 149 B	Yellow green group 149 B
Stripes (Present/Absent)	Present	Absent
Width of stripes	Medium	Medium
Lenticels	Few	Medium
Fruit peel lenticels (per cm <sup>2</sup> )	3	6
Number of locules	5	5
Fruit: aperture of locules	Moderately open	Moderately open
Persistency of calyx	+	+
Pedicle length (cm)	1.75	1.67
Pedicle breadth (cm)	0.43	0.30
Fruit cavity depth (cm)	1.53	1.32
Fruit base cavity (eye) length (cm)	1.04	1.22
Fruit base cavity (eye) depth (cm)	1.07	1.25
Flesh colour	Greenish	Greenish
Pulp texture	5	5
Number of seeds/fruit	6	5
Seed colour	Light brown	Light brown
Average seed weight (g)	0.048	0.052
Harvest time	1 <sup>st</sup> week of September	1 <sup>st</sup> week of September
Organoleptic analysis		
Pulp texture	5	5
Pulp taste	5	5
Pulp juiciness	5	5
Fruit attractiveness	Intermediate	Intermediate
Eating quality (dessert)	Intermediate	Intermediate
Visual observation		
Russetting (%)	Nil	Nil
Russet type	Absent	Absent

*Biochemical parameters*

Titrateable acidity (%)	0.18	0.21
Total soluble solids (°Brix)	11.80	12.10
Total sugars (%)	4.96	5.56
Reducing sugars (%)	3.21	2.66
Non-reducing sugars (%)	1.66	2.76
Juice content (%)	55.00	56.00
Anthocyanin content (A <sub>530</sub> )	0.37	0.39
Ascorbic acid / Vitamin C (mg/ 100 g)	10.55	11.20
Starch content (%)	75.00	70.00

Now days farmers are adapting to new cultivars with earlier and more intense colour, to increase market price. The fruit colour and maturity in apples are the foremost desirable attributes because they influence the fruit's price and market acceptance. However, spontaneous bud sports and chimeras with different colours or colour combinations may attract consumers, and also, such spontaneous bud sports or chimeras might be a major source of unique cultivars as well as vital resources for revealing molecular mechanisms and might be employed in future apple breeding programmes.

A huge number of spontaneous chimeras are already found in different flower and fruit crops like pear (Chevreau *et al.*, 1989), and some chimeras of apple were reopted from the diploid apple cultivars 'Ontario', 'Wealthy', 'Jona-than' and 'Rome' (Blaser and Einset, 1948). Whereas, the structure of chimeras in the carnation and apple cultivar 'Giant Spy' has been modified through X-rays (Pratt, 1960). Initial chimerism in perennial trees can be efficiently reduced through regeneration (Malabarba *et al.*, 2020). Cytological study of diploid-tetraploid apple cytochimeras revealed that cytochimeral variation was caused by layer replacement, and apple cytochimeras were shown to be more stable under normal conditions (Pratt *et al.*, 1967). Periclinal chimera can be propagated through cuttings as they most stable due to completely colonising a cell layer (Sichel *et al.*, 2023). However, Graft chimeras may have the potential to improve the growth of plants, quality of fruits, and disease resistance in plants (Augstein and Melnyk, 2025).

The findings of the present study revealed variation in morphological and biochemical compositions of spontaneous chimera, and such type variations in chimera and their parents are quite common. The maturation of apples is observed by texture, firmness, starch index, soluble solids and titrateable acidity (Goncalves *et al.*, 2017). Fruit ripening and maturity influence fruit colour, texture, and aroma (Su *et al.*,

2025). This may be due to genetic changes or might be due to epigenetic modifications, which impact fruit ripening in apple (Kumar *et al.*, 2024c). Variations in the shape of chimera correspond to the results of Michelangelo *et al.*, (2013) and Kumar *et al.*, (2022a), who previously documented changes in the shape of the fruits in bud sports. However, Liu *et al.*, (2003) discovered a bud sport called 'Hongjiangjun' with a soluble solids content of 13.9-15.8%, a pleasant acid-sweet flavour, and excellent eating quality. The spur bud sport mutant of 'Nagano Fuji 2' resulted in the improvement of a new apple known as 'Tianhong 2' with a 14.5-16.8% of soluble solid content, a smooth finish, and a strong fragrance (Shao *et al.*, 2008). Similarly, Wang *et al.*, (2010) discovered 'Pinkish Qinguan' as a sport (mutant) of the Qinguan apple cultivar, with large fruits with a pretty pinkish colour on the surface, yellow-white flesh, fine, tender, crisp and juicy texture, having soluble solids 16%, and a very decent eating quality.

### **Germplasm collection and conservation**

To raise the plants for future studies and conserve the germplasm, bud wood of the identified apple chimera was collected and propagated on M-9 (T<sub>337</sub>) rootstock, and the germplasm was conserved to check the stability of the changed traits.

### **Conclusion**

Genetic improvement in apple through traditional methods is complicated and time-consuming. The fruit colour and maturity are the foremost desirable attributes in apples, as they influence the price and market acceptance. Nowadays, farmers are adapting to new cultivars with earlier and more intense colour, to increase market price. Spontaneous bud sports and chimeras with different colours or colour combinations may attract consumers, and also, such spontaneous bud sports or chimeras might be a major source of

unique cultivars as well as vital resources for revealing molecular mechanisms and might be employed in future apple breeding programmes. However, commercial utilization of such spontaneous chimeras requires phenotypic stability, which can be achieved by either eliminating chimerism by mutagenesis, separating chimeras into pure types or selecting a chimeral pattern with an acceptable level of stability.

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