AATCC Review

03 September 2024: Received
06 October 2024: Revised
11 November 2024: Accepted
28 November 2024: Available Online

https://aatcc.peerjournals.net/

Original Research Article

Open Access

Expediting Apple Breeding: Integrating Bud Sport Identification and Genetic Profiling in North Western Himalayan Orchards

Suman Bodh*[1&2], Rajesh Kumar Dogra[2] and Praveen Verma[1]

[1]Department of Horticulture, School of Agriculture, Lovely Professional University Jalandhar, Punjab, India-144411, India
[2]Department of Fruit Science, College of Horticulture, Dr Yashwant Singh Parmar University of Horticulture & Forestry, Solan, Himachal Pradesh, India-173230, India

ABSTRACT

Apple breeding is traditionally a lengthy process due to the prolonged juvenile phase, making the development of new cultivars time-consuming. However, the occurrence of bud sport (spontaneous mutations in specific branches) offers a more rapid alternative for variety development compared to conventional breeding. Bud sports allow for vegetative propagation of the mutated part and molecular approaches enable early detection of genetic variation during growth. The North Western Himalayan region, particularly in areas such as Shimla, Kinnaur and Lahaul & Spiti in Himachal Pradesh, is exposed to high levels of ultraviolet (UV) radiation from sun, which increases the likelihood of bud sport formation. However, the study faced challenges, such as distinguishing true bud sports from environmental or developmental variations that can mimic mutations, complicating the identification process. Additionally, the high UV exposure, while promoting bud sport formation, also risks causing detrimental mutations that could affect fruit quality or tree health. A preliminary survey conducted in these orchards identified six bud sports. Samples were collected from these trees for both fruit characterization and molecular analysis of the leaves. Simple Sequence Repeat (SSR) primers were employed for the molecular study. Among the six bud sports, two exhibited significant genetic variations, while the remaining four displayed morphological differences from the mother trees. The genetically distinct bud sports were vegetatively propagated and maintained under controlled conditions for further study. This approach highlights the potential of bud sport selection, combined with molecular techniques, to accelerate apple breeding.

Keywords: *Bud sport, Apple, Variety, Himalayan region, Molecular analysis, SSR*

Introduction

New apple cultivars are created primarily to accommodate changing environmental conditions and customer preferences. It takes a lot of work to breed new apple varieties with enhanced quality attributes. Because apples have a long juvenile phase and are extremely heterozygous, the process of evaluating, selecting and releasing a new cultivar can take a long time (1). Apple cultivars and its novel varieties frequently result from genetic changes in meristematic tissues. A 'bud sport', which is propagated vegetatively, is the product of these natural occurrences. Apple bud sports have the potential to produce apple fruit with a genotype distinct from the parent plant (2). Many perennial crops are known to have bud sports, which can be a significant source of novel phenotypes and have given origin to numerous plant cultivars that are now widely produced (3).Certain gene mutations impact their functioning, leading to changes in phenotype (4). It is possible to create new variations of these bud sports by employing techniques like grafting to propagate them. Due to their ability to overcome traditional breeding problems like self-incompatibility and a long juvenile phase, bud sports in fruit crops have a high commercial value (5).

*Corresponding Author: ***Suman Bodh***

DOI: https://doi.org/10.21276/AATCCReview.2024.12.04.438

The long-term somatic variety preservation is made possible through the selection and breeding of clonal varieties made possible by the vegetative propagation of bud sports (6).

One of the most significant characteristics influencing the appearance and nutritional value of fruits is skin colour, which has a high anthocyanin content. The various colour phenotypes in the skin are caused by its composition and concentration of anthocyanins. Because the apple cultivar 'Red Delicious' is most prone to bud mutation, phenotypic observation can be used by researchers to select for fruits that possess superior qualities like flavour and colour (7).The selection of the 'Red Delicious' bud sport mutants is usually based on the short-branch characteristics and fruit skin coloring (8).

The Himalayan region of India experience high ultra-violate rays which can cause somatic mutation in apple. Due to this apple bud sports may result in desirable traits like enhanced color, early or late maturity, improved flavor, size and qualities. This study aims to identify and stabilize these traits to develop new apple cultivars that meet consumer preferences or market demands.

Material and Methods

Experimental site and plant materials

The study conducted in the temperate anddry temperate zone of Shimla, Kinnaur, Lahaul and Spiti districts of Himachal Pradesh, India. The altitude ranges from 2618 to 2860 m amsl. A preliminary survey was conducted in the existing orchards of those areas (table 1). Six bud sports KP 1, KP 2, KP 3, KK, ULS 1 and ULS 2 were Identified on the basis of phenotypic characters from the parent cultivar Royal Delicious.

The identified parent plants were marked and various morphological (Figure 1), phenological and biochemical characters were recorded. Further leave and fruit samples were collected for biochemical and quantitative analysis.

Table 1 Geographical locations of apple orchards selected after field survey

Sr. No.	Location	Sample code	Latitude	Longitude	Altitude (m amsl)	Mother cultivar
1	Kharapathar 1	KP 1	31.19°	77.62°	2642	Royal Delicious
2	Kharapathar 2	KP 2	31.19°	77.62°	2629	Royal Delicious
3	Kharapathar 3	KP 3	31.19°	77.62°	2627	Royal Delicious
4	Kalpa	KK	31.53°	78.27°	2860	Royal Delicious
5	Udaipur 1	ULS 1	32.72°	76.66°	2618	Royal Delicious
6	Udaipur 2	ULS 2	32.72°	76.66°	2618	Royal Delicious

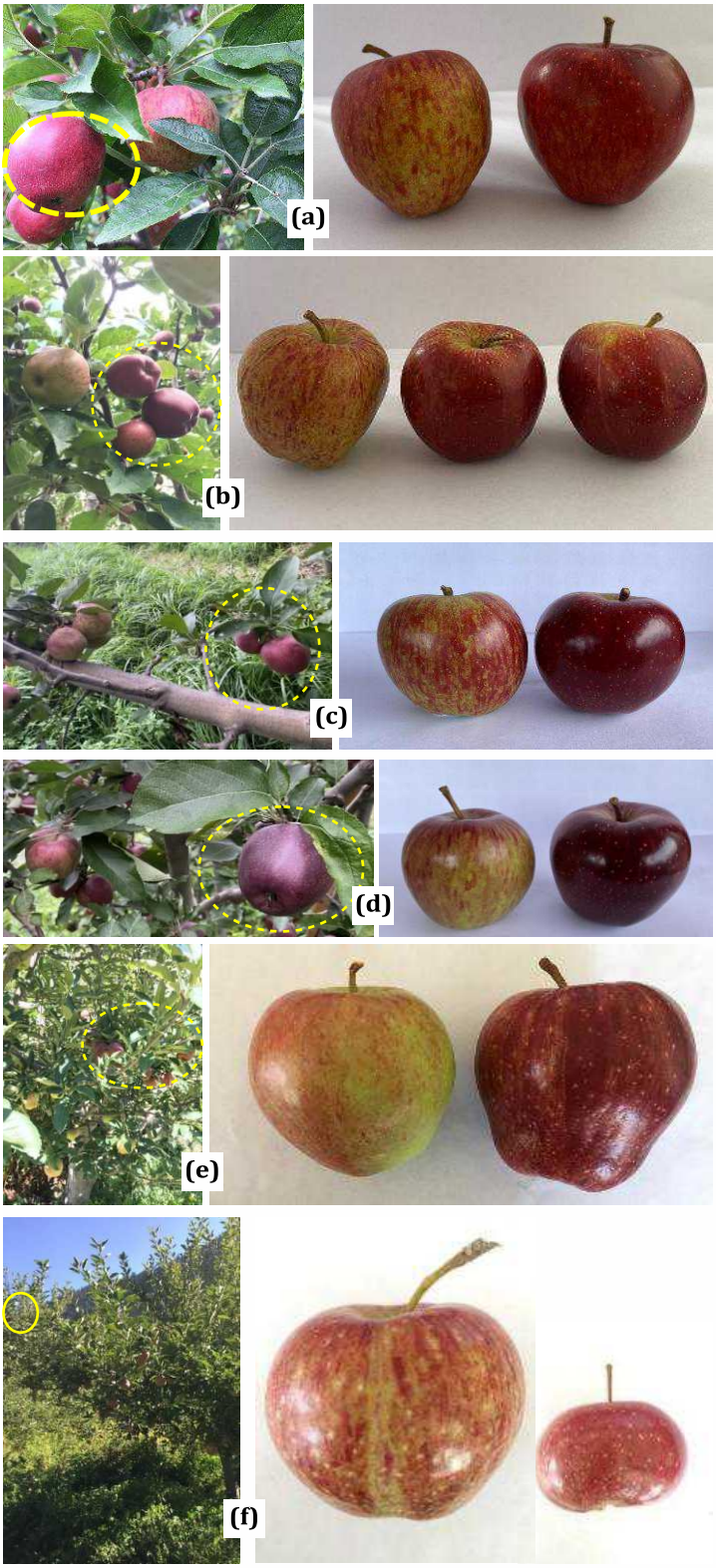

Figure 1. *Early and solid fruit peel colouring bud sports and parent tree. (a) Bud sport KP 1; (b) Bud sport KP 2;(c) Bud sport KP 3 ;(d) Bud sport KK;(e) Bud sport ULS 1;(f) Bud sport ULS*

Phenotyping

Morphological characters like phenological characters likethe time of initiation of leaf bud burst was determined by the unfolding of leaf primordia from the shoot axis in at least 3-4 buds.The observations on leaf fall were recorded as the date on which there was 80-85 per cent leaf fall.

Morpho-physical fruit characters were measured by fruit weight, length, volume, firmness, shape texture, colour, number of lenticels and organoleptic characters by taste, appearance and texture of fruit. Biochemical characters like TSS, titratable acidity, totals sugars, reducing sugar, and anthocyanin characters were also measured.

SSR Molecular marker

Fresh young green, disease free leaves were collected from the selected plants and further molecular analysis were done in the molecular laboratory. DNA isolation was done by CTAB method. After that qualitative and quantitative data were collected. The quality of the extracted DNA was assessed by agarose gel electrophoresis and quantity was estimated using spectrophotometer.38 genomic simple sequence repeats (SSR) markers were used to identify the difference between bud sport and mother plant.

Results and Discussion

Comparison of fruiting characteristics of mother plant and bud sport

The analysis of the data (Table 2) reveals, fruits of bud sport 'KP 1'varied in various traits and fruit weight (151.34 g), fruit length (6.50 cm), fruit breadth (6.91 cm), fruit firmness (12.54 kg/cm^2), relative area of over colour (100.00 %), the fruit skin lenticels (4 per cm^2), pedicel length (1.50 cm), fruit cavity depth (1.21), fruit base cavity length (1.67 cm), number of seeds (3), TSS (13.00°B), total sugars (8.00 %), reducing sugars (6.20 %), juice content (70.00 %) and anthocyanin content (1.78 OD). The rest of the recorded characters, on the other hand, were more or less similar to the parent plant.

Apart from this fruits of bud sport 'KP 2'varied in various traits and the leaf area (35.48 cm^2), fruit weight (168.98 g), fruit length (6.25 cm), fruit breadth (6.66 cm), fruit firmness (12.36 kg/cm^2), relative area of over colour (95 %), fruit skin lenticels (14 per cm^2), pedicel length (1.50 cm), fruit cavity depth (1.27 cm), fruit base cavity length (1.34 cm), number of seeds (6), TSS (12.20°B), total sugars (7.50 %), reducing sugars (5.95 %), juice content (68.00 %) and anthocyanin content (1.92 OD). The rest of the characters were more or less identical to the parent plant.

The fruits of bud sport 'KP 3'varied in various traits and the leaf area (36.27 cm^2), fruit weight (145.06 g), fruit length (6.01 cm), fruit breadth (6.62 cm), firmness (10.85 kg/cm^2), relative area of over colour (100.00%), fruit skin lenticels (8 per cm^2), pedicel length (2.00 cm), fruit cavity depth (1.43 cm), fruit base cavity length (1.51 cm), number of seeds (5), TSS (12.90°B), total sugars (7.85 %), reducing sugars (6.10 %), juice content

(51.00 %) and anthocyanin content (1.85 OD) were recorded. Figure 2 showing the biochemical difference between mother and bud sport.

Table 2. Phenological, fruit and morpho-physical characters of bud sport 'KP 1', 'KP 2' and 'KP 3' and parent tree 'Royal Delicious'

Characters	Parent cultivar: Royal Delicious	Bud sport: KP 1	Parent cultivar: Royal Delicious	Bud sport: KP 2	Parent cultivar: Royal Delicious	Bud sport: KP 3
Phenological studies						
Time of leaf bud burst	1st Week of March	1st Week of March	1st Week of March	1st Week of March	1st Week of March	1st Week of March
Time of floral bud burst	2nd -3rd Week of March	2nd -3rd Week of March	2nd – 3rd Week of March	2nd – 3rd Week of March	2nd Week of March	2nd Week of March
Time of leaf fall	1st Week of December	1st Week of December	1st Week of December	1st Week of December	4th Week of October	4th Week of October
Morpho-Physical Fruit Characters						
Fruit weight (g)	168.18	151.34	134.49	168.98	155.75	145.06
Fruit length (cm)	6.00	6.50	6.30	6.25	6.26	6.01
Fruit breadth (cm)	6.76	6.91	6.51	6.66	6.71	6.62
Fruit volume (cc)	183.50	165.00	157.50	183.00	167.50	155.75
Fruit firmness (kg/cm^2)	11.23	12.54	12.25	12.36	10.73	10.85
Fruit shape	Conical	Conical	Globose	Globose	Flat-globose	Flat-globose
Fruit texture	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
Fruit ribbing	Absent	Absent	Absent	Absent	Absent	Absent
Fruit colour	Red Group-45-A	Red Group-46-A	Red Group-45-A	Red Group-46-A	Red Group-44- A	Red Group-46-A
Fruit over colour	Red Group-46-A	Red Group-53-A	Red Group-46-A	Red Group-53-A	Red Group-46-A	Red Purple Group-59-A
Relative area of fruit over colour (%)	65.00	100.00	65.00	95.00	60.00	100.00
Intensity of fruit over colour	Medium	Dark	Medium	Dark	Medium	Dark
Fruit ground colour	Yellow Group-4-A	Yellow Group-4-A	Green Yellow Group-1-A	Green Yellow Group-1-A	Green Yellow Group-1-A	Orange Red Group-34-A
Fruit skin lenticels (per cm^2)	9	4	7	14	3	8
Number of locules	5	5	5	5	4	5
Persistency of calyx	Present	Present	+	+	+	+
Pedicel length (cm)	2.00	1.50	1.80	1.50	1.90	2.00
Pedicel breadth (cm)	0.33	0.35	0.21	0.19	0.25	0.21
Fruit cavity depth (cm)	1.25	1.21	1.24	1.27	1.30	1.43
Fruit base cavity (eye) length (cm)	1.65	1.67	1.40	1.34	1.53	1.51
Fruit base cavity (eye) depth (cm)	1.28	1.21	1.04	1.06	1.08	1.09
Flesh colour	Greenish	Creamish	Greenish	Greenish	Green	Green
Number of seed/fruit	4	3	3	6	3	5
Seed colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Average seed weight (g)	0.053	0.051	0.038	0.054	0.027	0.045
Harvest time	3rd Week of September	1st Week of September	3rd Week of September	1st Week of September	1st Week of September	3rd Week of September
Eating quality (dessert)	Good	Good	Very good	Good	Good	Intermediate
Organoleptic analysis						
Pulp taste	6	7	7	7	6	5
Juiciness	6	6	6	6	6	4
Fruit Attractiveness	Good	Very good	Good	Very good	Good	Average

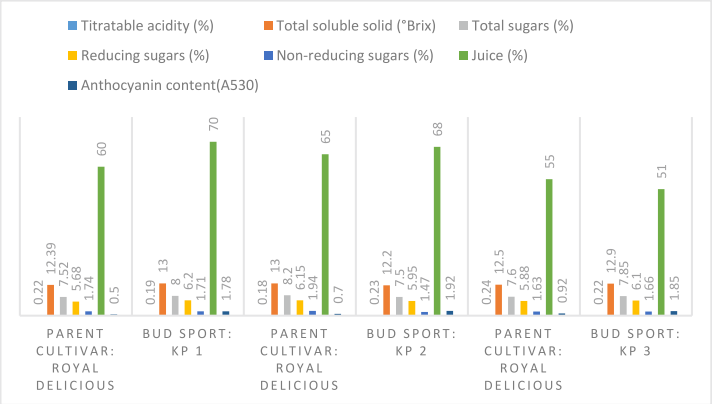

Figure 2. Comparison ofbiochemical characters of mother plant and bud sport KP 1, KP 2 and KP 3

According to data shown (Table 3), the fruits of bud sport 'KK' varied in various traits and the leaf area (35.95 cm^2), fruit weight (109.27 g), fruit length (5.54 cm), fruit breadth (6.73 cm), fruit firmness (12.97 kg/cm^2), relative area of fruit over colour (100.00 %), fruit skin lenticels (12 per cm^2), pedicel length (1.67 cm), fruit cavity depth (1.27 cm) , fruit base cavity length (1.53 cm), number of seeds (6), TSS (12.60°B), total sugars (7.70 %), reducing sugars (5.99 %), juice content (50.00 %) and anthocyanin content (2.34 OD) (Figure 3). The rest of the characters recorded were similar to the parent plant.

The early coloration and early maturing characteristics of a new bud sport 'ULS 1'in comparison to its mother cv.

Royal Delicious found in Udaipur, district Lahaul & Spiti at altitude of 2618 m above mean sea level (Latitude: 32.72°N, Longitude: 76.66°E) drew attention. This fresh and distinct bud sport had complete over colour and matured 10-15 days earlier than the mother tree. Apart from their early maturity, the fruits of bud sport 'ULS 1'differ in fruit over colour (Red Group-46-A) and shape (Oblong-conical) as compare to fruits of parent tree having colour (Red Group-45-A) and shape (Conical). Except for this, the fruits of bud sport 'ULS 1' differed in terms of leaf area (36.72 cm^2), fruit weight (117.35 g), fruit length (6.63 cm), fruit breadth (6.84 cm), fruit firmness (11.30 kg/cm^2), relative area of over colour (100 %), fruit skin lenticels (5 per cm^2), pedicel length (1.34 cm), fruit cavity depth (0.75 cm), fruit base cavity length (0.68 cm), number of seeds (7), TSS (13.00°B), total sugars (8.00 %), reducing sugars (6.00 %), juice content (62.00 %) and anthocyanin content (1.92 OD). On the other hand the remaining of the recorded characters were almost similar to the parent plant (Table 2).

Besides this, the fruits of bud sport 'ULS 2' differed in terms of leaf area (35.90 cm^2), fruit weight (82.00 g), fruit length (4.56 cm), fruit breadth (5.58 cm), fruit firmness (11.34 kg/cm^2), relative area of fruit over colour (100.00 %), fruit skin lenticels (6 per cm^2), pedicel length (2.86 cm), fruit cavity depth was (1.08 cm), fruit base cavity length (0.98 cm), number of seeds (6), TSS (12.20°B), total sugars (7.45 %), reducing sugars (5.21 %), juice content (35.00 %) and anthocyanin content (0.67 OD). However, rest of the characters recorded were more or less similar to parent plant.

Table 3. Phenological, fruit and morpho-physical characters of bud sport 'KK', 'ULS 1' and 'ULS 2' parent tree 'Royal Delicious'

Characters	**Parent cultivar:** Royal Delicious	**Bud sport:** KK	**Parent cultivar:** Royal Delicious	**Bud sport:** ULS 1	**Parent cultivar:** Royal Delicious	**Bud sport:** ULS 2
Phenological studies						
Time of leaf bud burst	1st Week of March	1st Week of March	1st Week of March	1st Week of March	1st Week of March	1st Week of March
Time of floral bud burst	2nd -3rd Week of March	2nd -3rd Week of March	2nd – 3rd Week of March	2nd – 3rd Week of March	2nd Week of March	2nd Week of March
Time of leaf fall	1st Week of December	1st Week of December	1st Week of December	1st Week of December	4th Week of October	4th Week of October
Morpho-Physical Fruit Characters						
Fruit weight (g)	127.22	109.27	104.84	117.35	126.89	82.00
Fruit length (cm)	5.68	5.54	5.94	6.63	6.46	4.56
Fruit breadth (cm)	6.31	6.73	6.62	6.84	6.53	5.58
Fruit volume (cc)	142.25	137.50	125.50	135.75	135.00	105.90
Fruit firmness (Kg/cm^2)	12.37	12.97	11.32	11.30	10.33	11.34
Fruit shape	Globose-conical	Globose-conical	Conical	Oblong-conical	Conical	Flat
Fruit texture	Intermediate	Fine	Fine	Fine	Fine	Fine
Fruit ribbing	Absent	Absent	Absent	Absent	Absent	Absent
Fruit colour	Red Group-46-A	Red Purple Group-59-B	Red Group-45-A	Red Group-45-A	Red group-45-C	Red group-46-A
Fruit over colour	Red Group-46-B	Red Purple Group-59-A	Red Group-45-A	Red Group-46-A	Red group-45-C	Red group-46-A
Relative area of fruit over colour (%)	60.00	100.00	60.00	100.00	70.00	100.00
Intensity of fruit over colour	Medium	Dark	Medium	Dark	Medium	Dark
Fruit ground colour	Green Yellow Group-1-A	Green Yellow Group-2-A	Yellow Green Group-23-A	Orange Red Group-34-A	Yellow Green Group- 146-A	Yellow Green Group- 146-A
Fruit skin lenticels (per cm^2)	7	12	3	5	4	6
Number of locules	5	5	5	5	6	6
Persistency of calyx	+	+	+	+	+	+
Pedicel length (cm)	1.80	1.67	1.45	1.34	1.97	2.86
Pedicel breadth (cm)	0.25	0.24	0.28	0.32	0.29	0.21

Fruit cavity depth (cm)	1.34	1.27	1.56	0.75	1.05	1.08
Fruit base cavity (eye) length (cm)	1.45	1.53	1.63	0.68	1.54	0.98
Fruit base cavity (eye) depth (cm)	1.05	1.07	1.38	0.84	1.02	0.76
Flesh colour	Greenish	Creamish	Greenish	Greenish	Greenish	Greenish
Number of seed/fruit	6	6	6	7	6	6
Seed colour	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown
Average seed weight (g)	0.059	0.60	0.051	0.057	0.035	0.024
Harvest time	1st Week of October	2nd Week of September	2nd Week of October	3rd Week of September	1stWeek of October	4thWeek of September
Eating quality (dessert)	Good	Intermediate	Good	Very good	Good	Good
Organoleptic analysis						
Pulp taste	6	5	6	7	6	7
Juiciness	6	6	6	6	6	6
Fruit Attractiveness	Good	Very good	Good	Very good	Good	Intermediate

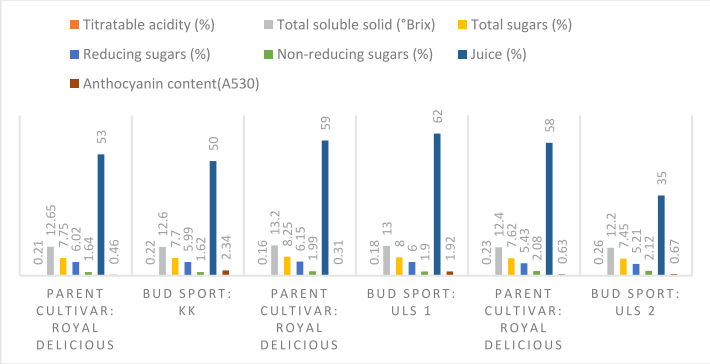

Figure 3. Comparison ofbiochemical characters of mother plant and bud sport KK, ULS 1 and ULS 2

As per the results, bud sports 'KP 1', 'KP 2', 'KP 3', KK', 'ULS 1' and 'ULS 2' demonstrated solid red colour and early fruit development. Fruit skin with increased anthocyanin concentration is widespread and several well-known varieties have been created as red sports. The new variety of apple tree 'SF118', resulted from a spontaneous limb sport mutation of 'Scifresh'. Its fruit skin over colour made it a suitable for additional examination.Fruit of the SF118 type is medium in size, with skin that is more about colour and pattern than anything else. The over colour is red (almost dark red 46B), with a big relative area and a solid flush-over colour pattern (9).

The bud sport 'KK' was noticed because of its solid and distinctive fruit colour (Red Purple Group-59-B) than that of parent fruit colour (Red Group-46-A). This type of study similarly observed by Brisset and Brisset (10), they identified 'Gala Surf' sport mutation which evolved naturally from the 'Gala'. Early colorations and very dark purple red over colour were the characteristic feature of the fruit. In one more similar study (11), It was found 'Pinkish Qinguan' a sport (mutation) of Qinguan. Bud sport fruits have a nice reddish colour on the top, making them quite appealing. They mature in mid-October, are large in size, weight between 300 and 420 g, yellow white flesh, fine soft texture, crisp, juicy, 16 per cent soluble solids and good eating quality. Fruit had a lengthy shelf life and trees are young and prolific. Variations in MdMYB/transcript levels, governs fruit skin colour. Fruit shape in bud sport was cylindrical as compared to conical in parent plant, a negative market attribute (12).

Similarly, a different apple cultivar was developed as a result of limb mutation (14). The new cultivar's fruits coloured around 2-3 weeks earlier, were spherical and conical in shape and had a solid rich red blush with no stripes or russet. On the other hand, another rich red mutant of the 'Fuji' apple cultivar with high precocity, productivity and hardiness that ripened 20 days' sooner. The fruits were large, weighing around 300 g, with a bright red colour (90 %) on the surface, fruit firmness of 9.15-9.55 kg/cm^2, soluble solids content of 13.9-15.8 per cent, pleasant acid sweet flavour and great eating quality (15). Hormones, which are thought to play a role in controlling anthocyanin accumulation, altered highly flushed colour patterns in apple bud sports (16). One limb of the 'PremA17' tree bore fruit that matured earlier and was more intensely colored as compared to the rest of the tree (17). Furthermore, no solid cause for smaller and lighter fruits of bud sports can be properly attributed until the bud sport is propagated, duplicated, and field tested as grown up trees. Despite the fact that it is widely understood that early, solid red colour and early fruit maturation are crucial from a consumer and business point of view. The current findings are incredibly important qualities across apple cultivars at various sites that are among orchardists' top priorities, particularly in places affected by colour development.

Molecular marker

The polymorphism was measured between two bud sports and their mother tree using the primer CH01H01 (mother plant KP 2, bud sport KP 2, mother plant ULS 1 and bud sport ULS 1). Primer CH01H01 produced two polymorphic bands, revealing genetic variations between identified bud sports and their parent trees.

Use of such molecular (DNA) markers successfully established itself as an effective genetic tool for differentiating spontaneous (bud) mutations from their own parent cultivars (18), and it was clear from the above observations that three bud sports (KP 2 and ULS 1) showed genetic differences between recognized bud sports and their mother plants. Whereas, the remaining 4 of the 6 found bud sports exhibited significant morphological and biochemical differences between bud sport and parent cultivars. These bud sports may need more precise SSRs to validate their differences, or they might just be epigenetic modifications, which generate phenotypic diversity due to inheritance of traits in gene function without changing the DNA sequence (19). In apple fruits, the patterns of universal and genic DNA methylation are yet unclear. DNA methylation is the modification that develop throughout the span of a life cycle of plants may be passed on to subsequent generations since plant germ cells are generated from somatic cells (20).

14 SSR primers in order to characterize a mutant grape early developing bud and discovered that just one of the primers showed polymorphism (21).

However, because of identical genotypes, actual bud mutants are challenging to differentiate using SSRs from original cultivars (22).While the S-SAP methodology distinguishes between apple sport mutants and their parent plant, SSR approaches only distinguish between plants, cultivars, and hybrids, not between sport mutants (23).

Conclusion

In conclusion, the comprehensive analysis of all six bud sports, encompassing morpho-physical, biochemical and organoleptic assessments, reveals substantial genetic diversity within the apple gene pool of Himachal Pradesh. This diversity holds significant potential for the development of new apple cultivars.

Future scope of the study: These identified bud sports, following systematic evaluation, could be introduced into future breeding programs. However, the release of these variants as new cultivars will require rigorous testing and validation through multi-location field trials across apple-growing regions to assess their performance for key horticultural traits.

Acknowledgment: We appreciate Department of Fruit Science, Dr YS Parmar University of Horticulture & Forestry, Solan, Himachal Pradesh, India for giving us opportunity to conduct this research.

Funding: None

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Choi C, Dougherty L, and Ban S (2024). 'Tools and Techniques for Allele Mining in Apple'. *Allele Mining for Genomic Designing of Fruit Crops*. CRC Press 80-102.
2. Engelsma, Alexander J. 2024. *The Biological Mechanism Behind Early and Late Apple Sports*. MS thesis. Michigan State University.
3. Ritter, Eleanore J., et al. 2024. 'From buds to shoots: insights into grapevine development from the Witch's Broom bud sport'. *BMC Plant Biology* 24.1: 283.
4. Kobayashi, S., Goto-Yamamoto, N., and Hirochika, H. 2004, Retrotransposon-induced mutations in grape skin color, Science, 304, 982.
5. Ban, Seunghyun, et al. 2024. 'Comparative transcriptome analysis of persimmon somatic mutants (Diospyros kaki) identifies regulatory networks for fruit maturation and size'. *Frontiers in Plant Science* 15 : 1448851.
6. 'Fuji' apple clonal varieties reveals evolutionary history and genetic mechanism of the spur-type morphology." *bioRxiv* : 2024-05.
7. Huang, Yaping, et al. 2023. 'MdMYB66 Is Associated with Anthocyanin Biosynthesis via the Activation of the MdF3H Promoter in the Fruit Skin of an Apple Bud Mutant'. *International Journal of Molecular Sciences* 24.23: 16871.
8. Ben-Yehudah G, Korchinsky R, Redel G, Ovadya R, Oren-Shamir M, Cohen Y (2005) Colour accumulation patterns and the anthocyanin biosynthetic pathway in 'Red Delicious' apple variants. J Horticult Sci Biotechnol 80(2):187–192.
9. Essner, B., & Breen, K. C. (2024). Apple tree named 'SF118'. https://patents.google.com/patent/USPP36080P3/en
10. Brisset F and Brisset D. 2018. *Apple Tree*: 'Gala Surf'.https://patents.justia.com/patent/20180077833
11. TianLi W, Li Q,Hong C,MaoY Z, Yuan LW. 2010. Pinkish Qingguan, a promising apple selection. *China Fruits*2:69.
12. Jiang S, Wang N, Chen M, Zhang R, Sun Q, Xu H, Zhang Z, Wang Y, Sui X, Wang S and Fang H. 2020. Methylation of MAMYBI locus mediated by RdDM pathway regulates anthocyanin biosynthesis in apple. *Plant biotechnology*18:1736-1748.
13. Hare B (1985) Apple Tree: 'Hared'. https://www.freepatentsonline.com/PP05547.html
14. Liu Z, Yi K. Zhang JE, Yang W. Wang D M and Yang F. 2003. Cultural techniques for Hongjiangjun apple cultivar. *China Fruits*2:13-15.
15. Li W F, Mao J, Yang S J, Guo Z G, Ma Z H, Dawuda M M, Zuo C W, Chu M Y and Chen B H. 2018. Anthocyanin accumulation correlates with hormones in the fruit skin of "Red Delicious and its four generation bud sport mutants. *BMC Plant Biology*18:363.
16. CraneB. 2024. 'Apple Tree Named'SM102543'. https://patents.google.com/patent/US20240206354P1/en
17. Kai Y, Liu Z, Wang D M, Yan Z Y, Zhang J and Yang F. 2005 A new apple cultivar Wangshanhong. *Acta Horticulturae Sinica*32:753-758.
18. Eccleston A, DeWitt N, Gunter C, Marte B and Nath D. 2007. Epigenetics. *Nature* 7143: 7395.
19. Sekhon R S and Chopra S. 2008. Progressive loss of DNA methylation releases epigenetic gene silencing from a tandemly repeated maize *Myb* gene. *Genetics* 181:81-91.
20. Guo D L, Yu Y H, Xi F F, Shi Y Y and Zhang G H. 2016. Histological and molecular characterization of grape early ripening bud mutant. *International journal of genomics.*
21. Yamamoto T K, Mochida T, Imai T, Haji H, Yaegaki M, Yamaguchi N, Matsutal, Ogiwara and Hayashi T. 2003. Parentage analysis in Japanese peaches using SSR markers. *Breeding Science* 53:35-40.
22. Urbanovich O Y, Kuzmitskaya P V, Kartel N A, Kazlouskaya Z A. 2011. Identification of apple sport mutants of Antonovkaby molecular methods. In: *XIII Eucarpia Symposium on Fruit Breeding and Genetics* pp. 99-303.