STUDIES ON IDENTIFICATION OF BUD SPORTS IN APPLE (*MALUS × DOMESTICA* BORKH.)

Thesis

by

HEERENDRA PRASAD
(H-2014-10-D)

submitted to

Dr. YASHWANT SINGH PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY SOLAN (NAUNI) HP – 173 230 INDIA

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DOCTOR OF PHILOSOPHY
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Dr Krishan Kumar
Professor & Head (Retd.)

Department of Fruit Science
Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) - 173230, India

CERTIFICATE-I

This is to certify that the thesis titled "**Studies on identification of bud sports in Apple (*Malus* × *domestica* Borkh.)"** submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy Fruit Science** in the discipline **Horticultural Sciences** of Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 is a bonafide research work carried out by **Mr. Heerendra Prasad (H-2014-10-D)** son of Shri Girvar Prasad Sagar under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation has been fully acknowledged.

Place: Nauni-Solan
Dated:

Dr Krishan Kumar
(Major Advisor)

CERTIFICATE-II

This is to certify that the thesis titled, "**Studies on identification of bud sports in Apple (*Malus* × *domestica* Borkh.)**" submitted by **Mr. Heerendra Prasad (H-2014-10-D)** son of Shri Girvar Prasad Sagar to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry (Nauni) Solan (HP) – 173 230 India in partial fulfilment of the requirements for the degree of Doctor of Philosophy Fruit Science in the discipline of **Horticultural Sciences** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

10/01/2020

Dr Krishan Kumar
Professor & Head (Retd.)
(Major Advisor)

10/1/2020

Dr R M Sharma
(External Examiner)

10/1/2020

Dr Y R Shukla
(Dean's Nominee)

Members Advisory Committee

1. **Dr (Mrs.) R Kaur**
   Professor (Retd.)
   Department of Biotechnology
   10/01/2020

2. **Dr V S Rana**
   Principal Scientist
   Department of Fruit Science
   (Co-opted Member)
   10/01/2020

3. **Dr R K Dogra**
   Assistant Professor
   Department of Fruit Science
   10.1.2020

Head of the Department

Countersigned

Dean
College of Horticulture

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Needless to say, errors and omissions are solely mine.

Place : Nauni, Solan

Date : **(Heerendra Prasad)**

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ABBREVIATIONS USED

Abbreviation		Description
%	:	Per cent
µl	:	Microlitre
^{o}Brix	:	Degree Brix
^{o}C	:	Degree Celsius
AFLP	:	Amplified Fragment Length Polymorphism
cm	:	Centimetre
cv.	:	Cultivar
cm^2	:	Square centimeter
CTAB	:	Cetyl Trimethyl Ammonium Bromide
DNA	:	Deoxyribonucleic Acid
dNTP	:	Deoxynucleotide 5 triphosphate
DUS	:	Distinctness, Uniformity and Stability
EDTA	:	Ethylene diamine tetraacetic Acid
EST	:	Expressed Sequence Tag
et al.	:	Co-workers
g	:	Gram
H P	:	Himachal Pradesh
IBPGR	:	International Board for Plant Genetic Resources
i.e.	:	That is
ISSR	:	Inter Simple Sequence Repeat
kg	:	Kilogram
A.O.A.C	:	Association of Official Analytical Chemists
MAMSL	:	Metres above mean sea level
m	:	Metre
m^2	:	Metre square
mg	:	Milligram

$MgCl_2$	:	Magnesium Chloride
ml	:	Millilitre
mm	:	Millimetre
N	:	Normality
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide
PCR	:	Polymerase Chain Reaction
pH	:	Potential of Hydrogen
PVP	:	Polyvinyl pyrrolidone
RAPD	:	Random Amplified Polymorphic DNA
RFLP	:	Restriction Fragment Length Polymorphism
RNA	:	Ribonucleic Acid
RNase	:	Ribonuclease
SSR	:	Simple Sequence Repeat
S-SAP	:	Sequence-Specific Amplification Polymorphism
TE	:	Tris-EDTA
TAE	:	Tris Acetate EDTA
UPOV	:	International Union for the Protection of New Varieties of Plants
UV	:	Ultraviolet
viz.	:	*Videlicet*

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Chapter-1

INTRODUCTION

Apple (*Malus* x *domestica* Borkh.) is the most important deciduous temperate fruit cultivated globally. Botanically it is a pome fruit and belongs to family Rosaceae with somatic chromosome number (2n) = 34. Apple adapts to a wide range of climatic conditions extending from warmer areas of Indonesia to extremely cold Siberia (-40°C). So largely due to its wider adaptability coupled with the development of suitable cultivars, currently apple is grown in temperate, subtropical and tropical regions of the world on commercial scale.

Apple is a delicious fruit and carries excellent appearance and dessert quality. According to McGhie *et al.* (2005), its fruits are low in calorific value but rich in nutritional fibre (~2 g/100 g fruit). Owing to malic acid, its Vitamin content is lower (5-25 mg/100 g) as compared to other fruits (Davey *et al.,* 2006) but is a rich source of antioxidants particularly polyphenols. Additionally, apple fruit is a good source of Vitamin B complex (riboflavin, thiamine and pyridoxine) and minerals like calcium, phosphorus and potassium.

Apple is considered to be a native of Central Asia having primarily originated from *Malus sieversii,* a wild species which grows in abundance in Tian Shen mountains bordering Russia and China. It has spread to other parts of the world over centuries owing to migration of civilization during pre-historic times (Hancock *et al.,* 2008). 'An apple a day keeps the doctor away' was the slogan given by Royal Horticultural Society, London way back in 19th Century to promote its cultivation throughout the world. The exchange of germplasm introduction across the countries in 19th and 20th Century laid a strong foundation of cultivar base worldwide which paved the way for its large scale commercialization as is witnessed today (Way *et al*., 1991 ; Janick *et al*., 1996). India too witnessed introduction of apple in Kullu valley in 1865 followed by entry of 'Delicious' apples in Shimla hills in 1917. At present, apple is grown in north-western hilly regions and to some extent in North- East hilly areas. Low chill apple plantations are coming up in warmer areas of India.

Most of the earlier apple cultivars originated as chance selections and mutations, many of which are even grown today on commercial scale. To name a few are 'Delicious', 'Granny Smith', 'Golden Delicious', 'Jonathan' and many more. Later genetic improvement efforts in apple included controlled hybridization beside need-based introduction and selection of bud or limb sports (mutants). The latter method is still in vogue, rather preferred

as the use of traditional breeding methods to improve the quality, and quality of perennial fruit plants is affected by various factors, such as long generation cycle, heterozygosity, labour and cost intensive.

Comparatively apple is more prone to spontaneous mutations than other temperate fruit trees. Brown (1975) described that a bud sport or mutation is an outcome of variation occurring in a cell for a single inherited characters. The mutated cell develops into a bud, shoot or whole plant resulting into altogether a new character unlike the mother plant on which it is produced. Mutation can affect any part of the plant, but in apple, bud, limb or whole tree mutation occurs for fruit skin colour, spur type growth, russetting and ploidy differences (Pratt, 1983).

This is evident from today's popular red and spur bearing strains of 'Delicious' group which are the result of identification and selection from existing apple plantations/orchards (Fisher and Lapins, 1966 ; Fisher and Ketchie, 1989). Before 90s more than 100 strains or bud mutations were identified in cv. Delicious (Bougher *et al*., 1990) and the figure crossed 156 marks soon thereafter (Childers *et al*., 1995).

As a matter of fact, the identification and selection of bud sports in apple has worked as a handy tool to create novel genetic variability in otherwise a species with narrow genetic base. More importantly, these spontaneously occurring bud sports provide change in one or few characters only and retaining most of the parental characters.

Several other apple cultivars apart from Delicious such as 'McIntosh', 'Cox's Orange Pippin', 'Rome Beauty', Winesap, 'Golden Delicious', 'Gala', and 'Fuji' also mutate quite frequently as evident from large number of bud sports derived from them (Ferree and Warrington, 2003). Not only that, the so identified bud / limb / whole tree mutations in turn mutate many a times later on to result into still improved genotypes. Some of the classical examples are of 'Red Chief' Delicious, 'Starkrimson' Delicious, 'Red Fuji' and 'Royal' Gala. These bud sports, by and large, have been found to be commercially acceptable and are grown worldwide.

A notable mutant differing in plant architecture is the identification of a 'columnar' type of growth habit observed in 'Wijcik McIntosh' (Lapins, 1974), which has been used as a gene source for developing apples with columnar growth habit.

Recent molecular genetic studies suggest that the mutations arise due to small changes in DNA sequence or by epigenetic variations leading to altered gene expression without

changing the DNA sequences. Mostly point mutations, tandem repeats, small insertions, deletions and base mismatches occur due to DNA polymerase error leading to variability in simple sequence repeat (SSR) regions (Foster and Aranzana, 2018). Nevertheless, there have been cases where the characters or variation observed initially reverts and do not perpetuate. This phenomenon is called diplontic selection largely due to chimera formation wherein the bud or limb tissues are comprised of cell with different genetic constitution. To overcome this, Brown (1975) had earlier emphasized to propagate the identified bud sports for determining the stability and superiority of the character over its original clone.

Given the successful commercialization of apple strains, the isolation of naturally occurring bud sports has been regarded as one of the viable approaches of genetic improvement of apple by amateur and professional breeders. Even several nurseries have taken a lead in releasing tens of dozens of apple mutants, and the process continues. Even today's popular cultivars *viz*., Fuji, Gala and others have witnessed the development of several mutants which are fast attaining the commercial status (Yoshida, 1974 ; Kiddle, 1995 ; Janick *et al.,* 1996 ; Yi *et al*., 2006).

However, not much work has been done in this direction in India except reports of identification of bud sports in apple (Karkara, 1998 ; Kumar, 2011). Based upon past studies in other parts of the world, coupled with the high probability of occurrence of bud sports in existing orchards of apple growing areas of Himachal Pradesh, it was felt imperative to undertake a comprehensive study to identify bud sports in apple with major focus on horticulturally desirable characteristics such as compact growth habit, fruit skin colour, fruit shape, season of ripening, etc. Keeping in view the above, the present investigations "Studies on identification of bud sports in apple (*Malus* x *domestica* Borkh.)" were carried out with the following objectives :-

1. To undertake field surveys for identification of apple bud sports
2. To evaluate the identified apple bud sports for commercially important characters

Chapter-2

REVIEW OF LITERATURE

Sudden or marked variation in a bud, shoot or whole tree from its parental form in plants, especially vegetatively propagated plants is often termed as bud sport or bud mutation or somatic mutation. Darwin (1868) described such variations as bud sports later on superseded by De Vries (1906) as bud mutations. Over a considerable period, the terminology has varied but in all respects those signifying one or more commercially important altered features are horticulturaly termed as bud sports or somatic mutations or bud sport mutants occurring naturally if one goes by old as well as recent classical reports (Brown, 1975 ; Pratt, 1983 ; Lacey and Campbell, 1987 ; Ferree and Warrington, 2003 ; Wang *et al*., 2009 ; El-Sharkawy *et al*., 2015 ; Jiang *et al*., 2019). The very first report of occurrence of bud sports in plants was given by Gaspard Bauhin, a botanist way back in 1598 (Stubbe, 1963). He described an unusual type of leafy phenotype Celandine (*Chelidonium majus*) in a herbal garden. This was followed by reports of origin of some unusual fruits in citrus (Nati, 1674). Later on several accounts of occurrence of spontaneous mutations in plants were given by Darwin (1843, 1844, 1859) and De Vries (1906). Bud mutations are of common occurrence in fruit trees. According to Shamel and Pomeroy (1936) there were around 1664 known bud sports of the fruit crops for which patents were filed in USA. Apple is one of the striking example of occurrence of bud sports on a very large scale, be it 'Delicious', 'McIntosh', 'Gala' or 'Fuji'. Hundreds of bud sports are described in cv. Delicious alone (Fisher and Ketchie, 1989 ; Childers *et al*., 1995 ; Brooks and Olmo, 1997 ; Chadha, 2002) with first report of colour sports, 'Richared' Delicious in 1919. Keeping the above in background, the available literature relevant to present investigation "Studies on identification of bud sports in apple (*Malus* x *domestica* Borkh.)" is reviewed under the following sub headings :

2.1 BEARING HABIT

2.2 DEVELOPMENT OF FRUIT SKIN COLOUR

2.3 TIME OF FRUIT MATURATION

2.4 OTHER FRUIT QUALITY ATTRIBUTES .

2.5 MOLECULAR MARKER STUDIES

2.1 BEARING HABIT

A spur-type strain was discovered by Richard Bisbee on 1951 as 'Starkrimson' Delicious (Maas, 1970) followed by Morgan Spur, Oregon Spur, Red Spur, Ace Spur, Silver Spur, Hardi Spur, Sturdeespur and Scarlet Spur, all on cv. Delicious.

Bud sports in other apple cultivars having spur type growth over parents is also very common (Brown, 1975). Silvers (1977) disclosed an apple spur variety which was more dwarf than the parent and originated from mutation of cv. Hi Early.

Craig (1979) reported a new spur type apple which originated as a sport limb on cv. Oregon Spur. A new spontaneous mutation was identified which had dwarf growth habit and bearing on spurs on cv. Starking Delicious (Coke and Smith, 1982).

Spur-type mutants occurring spontaneously on cv. Red Delicious have been reported to occur besides having better fruit colour compared to parent cultivars (Evans and Snipes, 1982 ; Kemp, 1984 ; Valle, 1989). Similarly Starkspur Golden Delicious and Goldspur Delicious were discovered on cv. Golden Delicious (Gotz and Silbereisen, 1989).

Valle (1989) described a whole tree mutation 'Vallee Spur' of cv. Red Chief which showed quite early development of fruit colour, early maturity and distinctive 'blush' type colouring when ripe.

Sali (1990) registered a new spur type apple which originated from sport of cv. Gilbert. Sport was semi-spur in growth habit with purple fruit colour at maturity.

Occurrence of spur-type mutants (Granspur, Greenspur, Earee, Grannee) are reported in cv. Granny Smith (Brooks and Olmo, 1997).

Jeffrey (2000) identified 'CB515', a limb sport from apple cv. Empire. 'CB515' limb sport fruits developed early red colour some two and half a week before parent cultivar. 'CB515' had considerable improvements over parent cultivar as high colour intensity and spur density.

Several other compact spur mutants demonstrating typical columnar type of growth habit have been reported in apple (Jaroslav *et al.*, 2009 ; 2010, 2014 ; Brendon, 2015 ; Peter, 2017).

2.2 DEVELOPMENT OF FRUIT SKIN COLOUR

The earliest reports on origin of coloured sports over their parents belong to early 20th Century. Notable among these were in cvs. Twenty Ounce (Hedrick, 1915), Northern Spy

(Farmer, 1918), Gravenstein (Mead, 1916), Baldwin (Beach, 1905) and Williams (Castle, 1914). Shamel (1922) gave a comprehensive account of improvement of plants by bud selection. Origin of chimeral sports and cytochimeras exhibiting colour mutations were reported in apple (Dermen, 1948, 1955 ; Dayton, 1959). From middle of 20th Century, super colour bud sports were discovered on major commercial apple cultivars of the world such as Top Red, Starking, Royal Red and others from Delicious (Maas, 1970), Red Jonathan, Anderson Jonathan Jonared and Blackjon from Jonathan (Gotz and Silbereisen, 1989), Royal, Galaxy, Regal, Buckeye, Gale Gala and Gala Must from Gala (Kappel *et al*., 1992), Red Max, Imperial All Red McIntosh, Cornell McIntosh from McIntosh (Dzieciol *et al*., 1988 ; Kruczynska *et al*., 1999) and Red Braeburn, Joburn from Braeburn (Ferree and Warrington, 2003).

Yoshida (1974) reported two bud sports (mutations) in cv. Fuji. One exhibited early and red deep solid fruit skin colour development where as second bud sport had deep coloured but striped fruit skin in comparison to parent cultivar.

Craig (1979) reported a new spur type apple which originated as a sport limb on cv. Oregon Spur, characteristic for early colour development by two weeks.

Perleberg (1980) identified ‘Ace Strain’ as a limb sport from Red Delicious characterised by highly consistent better fruit skin colour development.

‘Rico Red’, an early colouring bud sport was identified (Gonzalez, 1985) which was, however, similar to parent cv. Sharp Red Delicious anatomically and morphologically for other characters.

Jenkins and Philip (1985) reported the occurrence of a limb mutation named ‘Jenred’ in Red Delicious having conspicuously dark red fruit over bright red ground colour.

Hanners (1985) identified a bud sport of apple on cv. Yellow Newton with striped/partially red coloured fruits.

A limb mutation (Hared) developed on cv. Red Delicious featuring solid dark red blushed fruits without stripes (Hare, 1985). Colour development was two to three weeks earlier in Hared as compared to parent cultivar.

Sandidge and Charles (1988) found a bud sport ‘Sandidge’ that originated on the Red Chief Campbell. ‘Sandidge’ was specifically identified for solid red fruit skin and early fruit colour formation over its parent.

Jolly and Lotze (1989) characterized a new bud sport ‘Royal Gala’ that originated from Gala (Kidd's Orange Red x Golden Delicious) cultivar. They found that ‘Royal Gala’ fruits were covered high percentage of red stripes with creamy white fruit flesh having excellent flavour.

Valle (1989) described a whole tree mutation of cv. Red Chief which showed extreme early maturity and distinctive ‘blush’ type colouring when ripe. Cultivar Gala is highly prone to emergence of red colour sports, both striped and blush types (Kappel *et al*., 1992).

Cooper and Brewster (1992) sought a patent for ‘T.A.C. # 114’ which originated as a single limb mutation of cv. Red Fuji. The fruits of bud sport ‘T.A.C. # 114’ were intense red in colour.

Van and Kennewick (1996) registered a new and distinct variety of Fuji apple tree (*Malus pumila*) ‘Myra’ with US Plant Patent Authority which was derived as a spontaneous mutation. Fruits of ‘Myra’ were uniform with bright pinkish red fruit skin colour having slightly darker pinkish-red stripes.

Denardi *et al.* (1997) found a bud sport ‘EPAGRI 407-Lisgala’on apple cv. Gala. The fruits of the bud sport were solid red in colour covering 80-95 per cent of the surface as compared to parent cv. Gala.

Auvil and Oronda (1997) registered bud sport named ‘Fuji 216’ which originated on cv. Fuji. Bud sport ‘Fuji 216’ fruits were characterized by high percentage of intense red coloration and early maturity over parent cv. Fuji.

Rotundo *et al.* (1998) found a bud sport named ‘Limoncella Rosa’ that got originated as a bud mutation of cv. Limoncella. Fruits of ‘Limoncella Rosa’ had bright red over colour with creamy white flesh as compared to those borne on the mother tree.

Wilhelmus and Antonius (1999) reported a bud sport ‘Red Jonaprince’ which originated as a bud mutation on cv. Jonagold. The fruits of the bud sport were distinctly dark red in colour in comparison to the poorly coloured fruits of mother tree.

Winkel (1992) identified a whole tree mutation named ‘Red Chief’ on cv. Delicious. The fruits of the mutant were characterized by full blush colour development in contrary to those of parent cultivar.

Several bud sports with improved colour e.g., Red Rome, Double Red Rome, Compact Red Rome originated from cv. Rome Beauty (Brooks and Olmo, 1997).

Deutscher (1999) identified a distinct sport of *Malus domestica* named 'Cumberland Spur' limb sport on cv. Oregon Spur. 'Cumberland Spur' fruits were characterized by intense 100 per cent red over colour as a blush at harvest time.

A new and distinct bud sport named 'Fiero' characterized by pinkish red blush colour and predominantly striped colour development pattern than Yataka Fuji was identified (Van and Kemewiek, 2000).

Alois (2001) obtained a U.S. patent for an apple limb sport originating on cv. Fuji with distinct red coloured fruits having yellowish dots.

Leslie and Linda (2002) registered 'Bull McIntosh' which originated as a whole tree mutation on cv. McIntosh. 'Bull McIntosh' fruits were having 100 per cent red blush with early colouring feature. Colour development started over a month earlier than the parent cultivar.

A limb sport named 'Burchinal Red Delicious' was identified on cv. Oregon Spur for uniform deep red colour which develops earlier than on fruits of parent cultivar (Robert, 2004).

Ira (2006) identified a sport of an apple 'Fugachee Fuji' on cv. Fuji. Fruits of 'Fugachee Fuji' were characterized by attractive blush and colour with early maturity.

Bob (2006) got patented a new colour sport of cv. Fuji having fruits with distinct dark red stripes over a lighter red ground colour as compared to parent cultivar.

A bud sport 'CABp Fuji' was identified on 'Nagafu-6' having distinctly large and attractive fruits with superior colour over parent (Chris *et al.*, 2007).

A new bud sport of apple named 'GUN-300' was identified and selected for notable fruits having even and early colour development over its parent (Hugh, 2007).

Another bud mutation 'El Nino' was selected (Steven, 2008) for having fruits with intense dark red stripes over a bright red background of its parent cultivar.

Robert (2008) found a new and distinct variety of apple tree named 'McLaughlin Gala,' which originated as a limb mutation on 'Kidds D-8'. Fruits of McLaughlin Gala were distinctly different from its parent having 100 per cent clear yellow fruit colour development with no red stripes or blush.

Glynn (2008) described a new apple bud mutant ‘Alvina’. Bud sport fruits carried very high level of over colour with solid flush and stripes as compared to parent cultivar.

David (2008) reported a new and distinct apple tree named ‘Candy’ that originated from limb sport of Aztec. Fruits of ‘Candy’ were distinct in having 95 to 100 per cent intense red colour development with stripes.

Another apple colour sport (limb mutation) observed on cv. Fuji was identified and reported by Thomas (2008). The distinguishing character of limb sport was dark ruby red skinned fruits with large yellow dots as compared to mother plant.

Michelangelo and Carlo (2008) reported another apple limb sport ‘FUJIKO’. Fruits of ‘FUJIKO’ were characterized by intense red skin colour with high over coloured development on the unexposed side of the fruits unlike in parent cultivar.

A bud sport named ‘Tianhong 2’ was selected from ‘Nagano Fuji 2’. The fruits of ‘Tianhong 2’ had good colour, smooth finish and strong fragrance. The single fruit weight of ‘Tianhong 2’was more than 260g (Shao *et al.*, 2008).

Bernard (2009) identified a new and distinct sport tree named ‘B. Thome McIntosh’ originated as limb mutation of the *Malus domestica* cv. ‘Starling’. This strain ‘B. Thome McIntosh’ was unique from its parent for greenish colour of flesh.

A unique limb mutation ‘Lentz Jonagold’ bearing fruits developing early colour with high intensity of colouring pattern was identified on cv. Jonagold (William, 2009).

A distinct limb mutation of cv. Jonathan named ‘Campbell Jonathan’ was identified (Jeffrey, 2010). The fruits of ‘Campbell Jonathan’ were different from its parent having very bold pattern of dark red and bright red stripes on the skin.

Several other bud sports of cv. Gala have been reported which were distinctly different from their mother trees in intensity and pattern of colouration. To name a few are ‘Delicia’ (Roduit and Roduit, 2011), ‘Monalisa’ (Anisio and Frederico, 2012), ‘Taishan Gala’ (Li *et al*., 2012) and ‘Galiwa’ (Markus, 2012).

Richard (2012) characterised a limb sport ‘Southfield’ having new and distinct characters. Fruits of ‘Southfield’ were dark red with colour development in the form of blush having attractive appearance.

An apple sport, ‘Gaia’ was identified as a branch mutation having characteristic yellowish green ground colour with striped red over colour than parent (Michelangelo *et al.,* 2013).

Siegfried (2013) registered ‘Gala Perathoner’ which originated as a bud mutation. ‘Gala Perathoner’ was distinguished by intense red over colour that covered 95 to 100 per cent of the fruit surface. The bud sport fruit exhibited an attractive pattern of light and partially overlapped dark red stripes with prominent lenticels over mother plant.

Robert (2014) registered ‘Foxtrot’ sport which originated as a bud mutation from Gala. ‘Foxtrot’ fruit was selected for its intense red colour.

Bernard (2014) received a U. S. patent for ‘B. Thome Gala’ which originated as a limb mutation bearing uniquely different fruits from its parent having prominently wide stripes.

A distinct colour bud sport was identified and named ‘FUCIV-51’ on cv. Fuji ‘NAGAFU-12’. ‘FUCIV-51’ apple fruits exhibited intense purple red over colour as compared to parent (Michelangelo and Alessio, 2016).

Christopher and Julia (2015) reported a bud sport named ‘Antietam Blush’ of *Malus domestica*. ‘Antietam Blush’ fruit was distinguished by attractive fruit colour.

Werner (2015) reported that a sport of *Malus domestica* ‘Stark Gugger’ was identified on ‘Starking Delicious’. Fruits of ‘Stark Gugger’ showed colour development some 10 days early having solid flush purple red colour over the whole surface over parent Starking Delicious.

Jurgen (2016) reported ‘Gala 0502’ apple, a notable sport from ‘Gala’ for fully coloured fruits before harvest with distinctive solid purple red over colour.

A bud sport ‘Gala 2013’ was identified which distinguished from the original mother plant ‘Gala Standard’ by solid flush purple red over colour that covers 100 per cent of the surface at harvesting time than mother tree fruits (Alois, 2016).

Beau and Jesse (2016) identified an apple bud sport that was unique in combination of colour and red stripe.

Derek *et al.* (2017) reported new and distinct apple tree named ‘GALA BIGBUCKS’ particularly characterised by dark red over colour on 90-100 per cent of fruit skin surface.

Richard (2017) identified a sport ‘RKD’ originated from branch mutation on Gala cultivar. ‘RKD’ apple was distinguished by early coloured formation in fruits.

Jurgen (2017) reported the grant of a U.S. patent to apple bud sport ‘Fuji VW’ from cv. ‘Fuji’. ‘Fuji VW’ fruit skin was covering solid purple red over colour on whole fruit.

An apple sport ‘Gala Schnico Red’ was identified and developed for distinctly intensive red over colour fruit skin with higher number of lenticels (Andreas *et al*., 2017).

Danay (2018) received a U. S. patent for ‘Duke Fuji’ apple that was limb mutation of ‘Brak’ Fuji. ‘Duke Fuji’ fruits were distinguished by solid red flush with even and early colour development.

Frederic and David (2018) identified an apple sport named ‘Gala Surf’ from ‘Gala’ apple. The fruits of ‘Gala Surf’ were distinctly different for early colouring and very dark purple red over colour than parent.

Lionel (2018) selected ‘Galafab’ from branch mutation on cv. Gala. Fruit of ‘Galafab’ was characterised by red over colour unlike striped fruits in parent cultivar.

Aldo (2018) registered a new and distinctive variety of a *Malus domestica* apple tree, named ‘RS103-110’ that was distinguished by fruits distinctly dark red in colour over and yellow-green ground colour.

2.3 TIME OF FRUIT MATURATION

Silvers (1977) reported early maturity of fruits in a mutation from cv. Hi Early which also recorded long fruits with high sugar content and good keeping quality.

Valle (1989) described a whole tree mutation ‘Vallee Spur’ of cv. Red Chief which showed early maturation of fruits.

An early maturing limb spot ‘TAC# 14’ occurring on cv Red Fuji was reported by Cooper and Brewster (1992).

Winkel (1992) identified a whole tree mutation named Redchief™’ on cv. Delicious. The fruits of sport were characterized by early fruit maturity with full blush colour development in contrary to those of parent cultivar.

Kiddle (1995) registered a spontaneous bud mutation named ‘Galaxy’ occurring on cv Royal Gala to Plant Variety Rights Authority in Australia. ‘Galaxy’ bud sport was identified

for early ripening fruit as compared to parent cultivar besides having medium vigour and spreading habit.

Van and Kennewick (1996) registered a new and distinct variety of Fuji apple tree (*Malus pumila*) 'Myra' at US plant patent authority which was derived as a spontaneous mutation. Fruits of 'Myra' were having early fruit maturity.

Auvil and Oronda (1997) registered a bud sport named 'Fuji 216' who identified for early maturing fruits intensely red in colour as compared to parent.

Wilhelmus and Antonius (1999) reported a bud sport 'Red Jonaprince' with early ripening fruits.

Deutscher (1999) identified a distinct sport of *Malus domestica* named 'Cumberland Spur' limb sport on cv. Oregon Spur in which fruits exhibited two weeks early maturity as compared to the parent cultivar.

A new and distinct bud sport named 'Fiero' characterized by early maturity (10 to 14 days earlier than Yataka Fuji) was identified (Van and Kennewick, 2000).

Dennis and Sterling (2002) reported the grant of a U. S. patent for a limb mutation named 'Swedes Fuji' which originated on cv. Fuji. The fruits of Swedes Fuji were late in maturation as compared to standard.

Rankin (2002) registered a sport 'Rankin Red' with U.S. plant patent authority which was derived as a spontaneous mutation. 'Rankin Red' was a new and distinct strain originated as a limb sport on Yataka apple tree. Its fruits matured five days earlier than Yataka. Fruit skin of 'Rankin Red' had higher percentage of red colour, colour uniformity, less russet and smoother finish.

Teague (2003) identified the sport 'Irene' from limb sport of Fuji. 'Irene' was distinguished by early harvest date, large fruit and resistance to powdery mildew and fire blight.

Guy (2005) reported a new sport 'Dalitoga' a whole tree mutation of cv. Imperial Gala. The fruits of 'Dalitoga' ware early in maturity and intensely coloured as compared to parent cv. Imperial Gala.

Denardi and Seccon (2005) reported 'Castel Gala' a bud mutant which was a low chilling apple with very early fruit ripening and having a blooming time of 20-25 days earlier than parent. Fruits of 'Castel Gala' matured much earlier than Gala.

Yi *et al*. (2005) identified a new apple sport 'Wangshanhong' characterised by early maturation, red colour and a long storage with disease resistance. Fruits of 'Wangshanhong' matured 15 days earlier having flushed red stripes on yellowish-green ground.

A sport 'Burkitt Gala' was identified on a limb maturation on 'Tenroy' Gala which was distinguishable from its parent by early maturity with 100 per cent cherry red coloured fruits. 'Burkitt Gala' matured 10 days earlier than parent 'Tenroy' Gala (Edward, 2006).

Ira (2006) registered 'Fugachee Fuji' which originated as a limb sport apple on cv. Fuji. Fruits of the 'Fugachee Fuji' were characterised by early fruit maturity and attractive blush colour formation.

Zhu *et al.* (2006) reported that 'Hongjiangjun', a bud sport of Japanese apple cv. Yataka. After observation and evaluation, it was revealed that the bud sport has resistance to diseases and hardiness better than its maternal cultivar. Its maturity time was around 30 days earlier than that of cv. Fuji.

Robert (2008) found a new and distinct variety of apple tree named 'McLaughlin Gala,' which originated as a limb mutation on 'Kidds D-8', which demonstrated maturation of fruits at least one week before than in fruits of parent cultivar.

Yan *et al*. (2010) characterized 'Jinxiu Hong' that was mid to late ripening apple selected from mutation of Huaguan. Fruits of 'Jinxiu Hong' were covered with full red blush having green yellow skin maturing 15 days early than parent Huaguan. An early maturing bud sport 'Jugala' on cv. Gala has also been reported (Max, 2010).

Several other workers have reported the occurrence of bud or limb sports in apple which showed early maturity in fruits over parents (Werner, 2015 ; Jurgen, 2016 ; Richard, 2017 ; Thierry, 2018).

2.4 OTHER FRUIT QUALITY ATTRIBUTES

Bud sports in apple with varying fruit size and shape are reported to occur (Perleberg, 1980 ; Jenkins and Philip, 1985 ; Hare, 1985 ; Hanners, 1985).

Bai *et al.* (1997) discovered 'Shaanxi Province' a spur type mutant on cv. Red Fuji. Average fruit weight was 272g but reached upto 700g having bright red to deep red colour. The flesh was creamy white, fine, crisp and juicy with a pleasant acid-sweet flavour.

Meng *et al*. (2003) observed a sport ‘Zaoxiangyu’ on cv. Hongyu. ‘Zaoxiangyu’ was characteristically sweet and sour in taste with aromatic flesh, good quality and resistance to aphids than parent Hongyu.

Khanizadeh *et al.* (2003) reported a unique bud mutation named ‘Reinette Russet’ on cv. Reine des Reinettes. ‘Reinette Russet’ fruits were recommended for the production of cider and fruit wine due to complexity of flavours.

A bud mutation named ‘Hongjiangjun’ was identified and selected for large sized (300g) and firm fruits (9.15-9.55 kg/cm^2) having TSS ranging 13.90 to 15.80 per cent with pleasant blend of acid-sweet flavour and excellent dessert quality (Liu *et al.*, 2003).

Edgar (2004) registered a new and distinct strain of apple designated ‘Weaver’ which originated as a limb sport on Fulford Gala tree. ‘Weaver’ fruit was 1.5 to 2.5 cm larger with improved fresh fruit qualities over parent tree fruits.

Zhao (2005) reported ‘Changhong’ which originated as a sport of Yanfu 10 Fuji apple cultivar. ‘Changhong’ fruits were good in quality with large fruit size (287 g) and bright red colour. Flesh of ‘Changhong’ was light yellow in colour, fine crisp, juicy with having total soluble solid content (17.5%), titratable acidity (0.38%) and fruit firmness (8.0-8.4 kg/cm^2) and better eating quality than those of parent cultivar.

A bud sport ‘CABp Fuji’ was identified on cv. ‘Nagafu-6’ having distinctly large and attractive fruits as compared to parent (Chris *et al.*, 2007).

Bernard (2009) identified a novel and distinct sport tree named ‘B. Thome McIntosh’ which had unique fruits differing from its parent in respect of greenish coloured flesh in fruits.

Allan (2010) received U. S. Patent for ‘PremA153’ which was a new and distinct apple tree (*Malus domestica* Mill.) having originated as a bud mutation. Fruits of ‘PremA153’ were attractive blush yellow coloured with excellent texture and unique flavour.

A spontaneous mutant ‘Grand Gala’ has been identified from cv. Gala which had exceptionally large and heavier fruits than in parent (Malladi and Hirst, 2010).

Wang *et al.* (2010) reported ‘Pinkish Qingguan’, a mutant of apple cv. Qingguan. ‘Pinkish Qingguan’ fruits matured in mid-October, were large weighing 300g to 420g with pinkish skin colour and attractive. Flesh of ‘Pinkish Qingguan’ fruits was yellow white, fine

and crispy, juicy having good amount of total soluble solids (16^{o} Brix) and good quality of eating. Fruits exhibited long keeping quality.

Li *et al*. (2012) found a sport 'Taishan Gala' which was large and red bud mutation of Royal Gala. Fruits of 'Taishan Gala' were yellow-green with bright red skin having large size. It yielded higher and showed strong resistance to various diseases.

Markus (2012) identified new Gala type apple variety named 'Galiwa' which originated as a limb mutation. 'Galiwa' fruits were distinguished by large orange red fruits, high fruit sugars content and resistance to scab over mother cultivar.

Pat (2010) reported a new apple 'Burnett Cultivar' which resulted as a bud mutation. 'Burnett Cultivar'. Fruits were early in maturity, had less sugars and less starch than 'Ultrared Gala'.

Bruce (2010) identified a new bud sport 'WA 2', that was distinguished by fruits having attractive blush, outstanding texture, firmness, crispy and juicy. 'WA 2' fruits were suitable longer periods of storage.

Inge *et al.* (2011) identified a new and distinct apple limb sport named 'B3F45'. Its fruits were particularly characterised by firm flesh, long storability and shelf life, conic fruit shape with strong aroma.

Michelangelo *et al*. (2013) registered a new sport of apple 'Gemini' which originated as a limb mutation. Fruit quality of 'Gemini' was very attractive, big fruit size with conical shape. Fruit ground colour of 'Gemini' was green with uniform ruby red blush covering large area over colour. However fruit flesh was creamy, crispy and juicy with slightly acidic taste.

Inge *et al.* (2016) registered a new and distinct bud sport of *Malus domestica* tree named 'KIZURI'. 'KIZURI' fruit was characteristicly aromatic and sweet with solid red flush, firm with dense texture and good storage quality.

Andreas *et al*. (2017) identified *Malus domestica* 'Gala Schnico Red' which originated from cv. Gala. 'Gala Schnico red' was distinguished by unique fruit quality characteristics including an intensive red over colour fruit skin.

2.5 MOLECULAR MARKER STUDIES

Several studies on characterization in apple have been conducted to detect genetic variations between synonym cultivars and their sports using SSR markers (Urrestarazu *et al.*, 2016 ; Lassois *et al*., 2016 ; Larsen *et al*., 2018).

Use of molecular (DNA) markers help in determining genetic diversity and relatedness in crop plants, especially in situations where morphological variations are deceptive and largely influenced by environment. Several type of marker *viz*., RAPDs, RFLPs, AFLPs, ISSRs and SSRs are known but differ from each other in their reproducibility and generation of polymorphism.

Random amplified polymorphic DNA (RAPD) markers are received increased attention in molecular fingerprinting. Zhu *et al.* (1997) used three sequences selected from 72 primers for identification of mutants, among 14 different apple varieties. Based upon consistency and reproducibility, RAPD markers were considered useful not only for varietal identification but also for mutant differentiation (Pancaldi *et al*., 1999: Tignon *et al*., 2000).

SSR markers are more stable and generate strong primer specificity and high level of polymorphism. In addition, the SSR markers have been an important tool for exploring genetic diversity and relationship (Zietkiewicz *et al.,* 1994; Guarino *et al.,* 2006).

Genetic diversity and genetic relationship studies in apple have been carried out using SSR markers (Hokanson *et al.,* 1998; Song *et al.,* 2006). Through several researchers disregard the screening of SSR primers, considering primer polymorphism as the sole criterion of primer screening without taking into account amount and representativeness of primers. Efficiency and representativeness of primers studied through series of comparative analysis on primers reveals differences to a great extent.

AFLPs were used (Li *et al.,* 2009) to validate early maturing sport and parent plant in ‘Changfu 2. 64 primer pairs were used to analyse as many as 2700 clear bands which were detected by 43 pairs of AFLP primers. As many as seven clear band differences under 4 pair primers between early-maturing sport and mother plant were found. Differences were ascertained to some extent between the early maturing bud sport and parent 'Changfu 2' through this study.

Four marker systems (SSR, RAPD, ISSR and S-SAP) were employed to determine genetic diversity and identify bud sports of ‘Antonovka’. A set of 10 accessions which were different in morphological characters were studied. Twenty SSR markers were used to reveal only one out of ten accessions differed in the allele composition at the loci studied thus indicating its cross pollination origin. ISSR analysis using 10 primers or RAPD analysis using 27 primers did not result in any differences between the accessions studied. In S-SAP

analysis using 33 combinations of primers were applied to yield different polymorphic fragments between sport mutations and accessions of ‘Antonovka’ (Urbanovich *et al*., 2013).

An attempt was made by Kuras *et al*. (2013) to apply five different DNA techniques to detect genetic variation between apple cultivars and their sports. These included cvs. Gala, Idared, Sampion, Golden Delicious, Jonared and as many 10 sports namely Gala Must, Gala Schinga, Idaredest, Red Idared, Szampion Reno Malinowy, Szampion Arno, Jonagered, Jonagold Excel, Golden Delicious Reiders Goldrosio. Out of the five marker systems (SSR, ISSR, AFLP, S-SAP and iPBS) used, the combined use of ISSR, AFLP, iPBS and S-SAP markers not only identified all the sports studied but also distinguished them.

More recent works are concentrated to develop deeper insight into red colour pigmentation and early ripening in apple bud sports (Wang *et al*., 2009). Ever since the publication of apple genome (Daccord *et al*., 2017) gene expression studies using advanced biochemical and molecular approaches are being undertaken to draw differences between coloured and early ripening mutant / and their parent cultivars (El-Sharkawy *et al*., 2015 ; Li *et al*., 2018 ; Jiang *et al*., 2019).

Chapter-3

MATERIALS AND METHODS

The present investigation entitled "**Studies on identification of bud sports in apple (*Malus* x *domestica* Borkh.)**" was carried out during 2016-18. The details of experimental sites, material and methodology adopted for field surveys and evaluation studies are described as under :

3.1 STUDY AREA AND CLIMATE

The study was conducted covering districts of Shimla, Kullu, Kinnaur and Lahaul & Spiti of Himachal Pradesh, India. The region opted for present research particularly falls in high hill wet temperate zone to high hill dry temperate zone which stretched from a latitude between 31°12' to 32°09' North and a longitude between 77° 35' to E78° 38' East (Fig 1a to 1d). The elevation ranged from 1816 metres to 3280 metres above mean sea level (MAMSL). Winter season extends from October to April and summer season starts from May upto September. The region receives an annual rainfall of about 1600 mm and annual temperature varies from −45°C to 34ºC. A major portion of precipitation is received during monsoon period from June to September. The months of July and August are the wettest. Morpho-physical, biochemical and marker analysis was carried out under Nauni (Solan) conditions in the University headquarters located at 1276 MAMSL having typical humid and mild winter climate with temperature extremes of as low as 0°C to high as 37°C.

3.2 FIELD SURVEYS

Field visits were carried out for identification of the bud sports in some existing apple orchards in the districts of Shimla, Kullu, Kinnaur and Lahaul & Spiti of Himachal Pradesh. The details of the selected apple orchards / sites are presented in Table 3.1. A preliminary survey was undertaken in each of the selected orchards of all the four districts. Interactions were held with the orchardist / farmers and local inhabitants to collect feedback information on a questionnaire as under:

Table 3.1: Details of apple orchards surveyed in Himachal Pradesh

S.No.	Location	Name of the Farmers	Latitude		Major Cultivar (s)	Altitude (MAMSL)	No. of Plants in Orchard
			North	East			
District Shimla							
1	Talai, Fagu	Roshan Lal	31°04.997’	077°18.399’	Starking Delicious, Red Delicious	2195	86
2	Fagu	Dani Ram Rajta	31°05.171’	077°18.252’	Starking Delicious	2201	128
3	Matiana	Kamlesh Sharma	31°12.311’	077°24.043’	Starking Delicious, Red Delicious	2252	90
4	Matiana	Jogendra Chauhan	31°12.316’	077°24.235’	Starking Delicious	2245	152
5	Ban Kuffer Mahasu, Kotkhai	Chandra Mohan	30°05.557’	077°30.214’	Starking Delicious	2312	198
6	Azadpur station, Mahasu, Kotkhai	Prem Singh	30°06.557’	077°30.997’	Starking Delicious, Red Delicious	2316	220
7	Ban Kuffer Mahasu, Kotkhai	Roshan Lal	31°05.403’	077°30.177’	Starking Delicious	2304	185
8	Halaila Mahasu, Kotkhai	Pratap Bhandari	31°05.412’	077°30.169’	Starking Delicious	2310	160
9	Ban Kuffer Mahasu, Kotkhai	Brij Sharma	31°05.446’	077°30.190’	Starking Delicious	2308	200
10	Ban Kuffer Mahasu, Kotkhai	Moj Ram	31°05.420’	077°30.205’	Starking Delicious, Red Delicious	2311	167
11	Ban Kuffer Mahasu, Kotkhai	Moj Ram	31°05.431’	077°30.200’	Starking Delicious	2307	145
12	Bakhol Mahasu, Kotkhai	Hetram	31°06.844’	077°29.777’	Starking Delicious	2324	156
13	Bakhol Mahasu, Kotkhai	Jiyalal	31°06.024’	077°29.674’	Starking Delicious, Red Delicious	2320	174
14	Bakhol Mahasu, Kotkhai	Brijmohan	31°06.090’	077°29.834’	Starking Delicious	2321	180
15	Bakhol Mahasu, Kotkhai	Santosh	31°06.248’	077°29.673’	Starking Delicious	2325	120
16	Manjoli, Theog	Jogindra Singh	31°09.376’	077°22.034’	Red Delicious	2320	210
17	Mashobra	RHR&TS	31°10.672’	077°23.894’	Vance delicious	2274	140
18	Sillu, Theog	Ramesh Verma	31°08.264’	077°20.161’	Starking Delicious	2272	215
19	Sillu, Theog	Suresh Verma	31°08.216’	077°20.115’	Starking Delicious, Red Delicious	2271	310
20	Sillu, Theog	Hemchand Mehta	31°08.296’	077°20.359’	Starking Delicious	2273	140
21	Mandhak, Kharapathar	Sardar Singh	31°06.659’	077°38.083’	Starking Delicious	2507	250
22	Mondhak, Kharapathar	Ajay Bracta	31°06.721’	077°38.076’	Starking Delicious	2548	200
23	Mondhak, Kharapathar	Vikram Bracta	31°06.723’	077°38.984’	Starking Delicious	2555	160
24	Padshal, Kharapathar	Harish	31°06.439’	077°35.614’	Starking Delicious	2388	180
25	Jathela, Kharapathar	Pradeep Kumar	31°06.656’	077°37.921’	Starking Delicious	2572	150
26	Jathela, Kharapathar	Hardik Chauhan	31°06.731’	077°37.999’	Starking Delicious	2584	168
District Kullu							
27	Jana Naggar Block	Manish Thakur	34°04.841’	077°09.370’	Starking Delicious, Red Delicious	1830	230
28	Jana Naggar Block	Manish Thakur	32°04.867’	077°09.305’	Starking Delicious	1825	195
29	Jana Naggar Block	Manish Thakur	32°04.876’	077°09.346’	Starking Delicious, Red Delicious	1823	80
30	Jana Naggar Block	Manish Thakur	32°04.833’	077°09.351’	Starking Delicious	1816	110
31	Banot, Jana Naggar Block	Duni	32°05.929’	077°14.335’	Starking Delicious	1957	400
32	Banot, Jana Naggar Block	Duni	32°05.938’	077°14.342’	Starking Delicious	1955	350
33	Banot, Jana Naggar Block	Duni	32°05.934’	077°14.356’	Starking Delicious	1962	500
34	Kasada Barada Jari, Manikaran	Sudhir Thakur	31°58.902’	077°15.362’	Starking Delicious	1821	260
District Lahaul & Spiti							
35	Tabo	KVK	32°05.576’	078°23.139’	Starking Delicious	3280	180
District Kinnaur							
36	Kalpa, Kinnaur	Ramesha Kumari	31°32.425’	078°15.390’	Starking Delicious	2727	60
37	Kalpa, Kinnaur	Ramesha Kumari	31°32.407’	078°15.374’	Starking Delicious	2741	80
38	Kalpa, Kinnaur	Chering Dejhe	31°32.608’	078°15.341’	Red Delicious	2747	65
39	Kalpa, Kinnaur	Yogesh Negi	31°32.512’	078°15.337’	Starking Delicious	2755	125
40	Kalpa Kinnaur	Suresh Negi	31°32.621’	078°15.389’	Starking Delicious	2739	140

SURVEY INQUIRY FORM (QUESTIONNAIRE)

1. **Date of Visit** :- _____
2. **Location** :- _____
3. **Locality** :- _____
4. **Name of the Farmer/Owner** :- _____
5. **Contact No.** :- _____
6. **Age of Tree** :- _____**Years**
7. **Cultivar Name** :- _____
8. **Preliminary Observations** :- _____
9. **Notation for Plant** :- _____

The above feedback information served as a base for marking of apple mother trees exhibiting bud sports on the basis of pre-selection criteria as follows :

3.3 PRE-SELECTION CRITERIA FOR IDENTIFICATION OF THE APPLE BUD SPORTS

The following characters were observed on existing apple plantations in the selected orchards particularly to record variation (if any) in any bud, shoot or whole tree in respect of :

a) Bearing habit

b) Time of skin colour development

c) Time of harvesting of fruits

d) Fruit shape

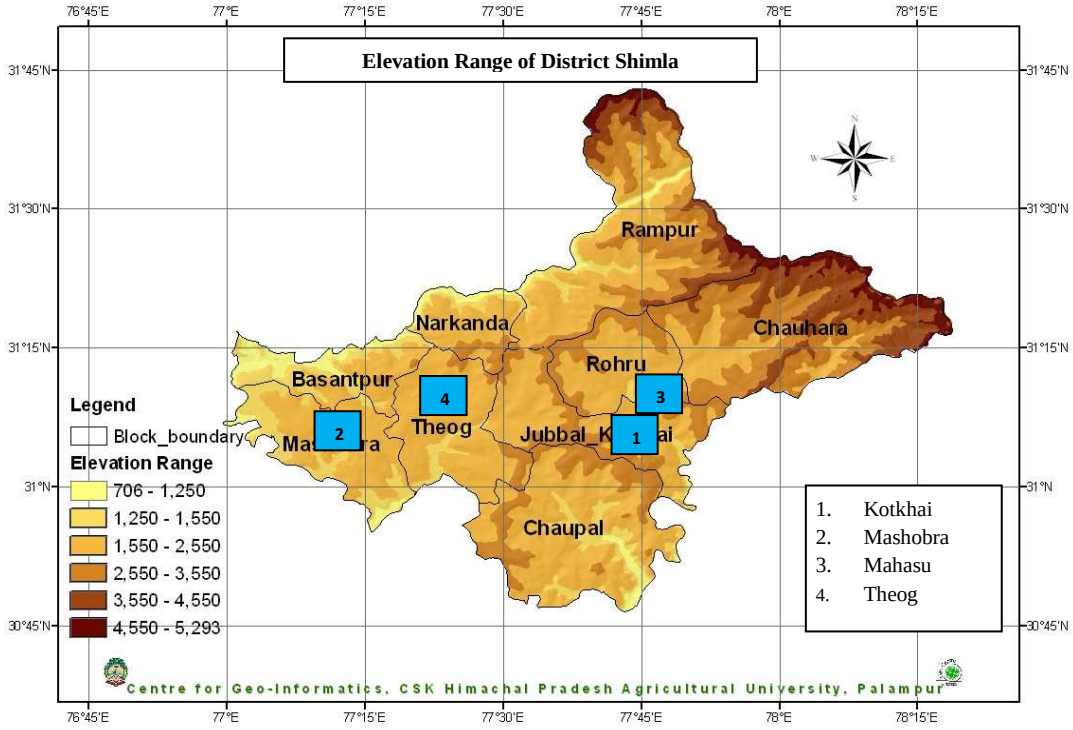


Fig. 1a. Locations surveyed in district Shimla

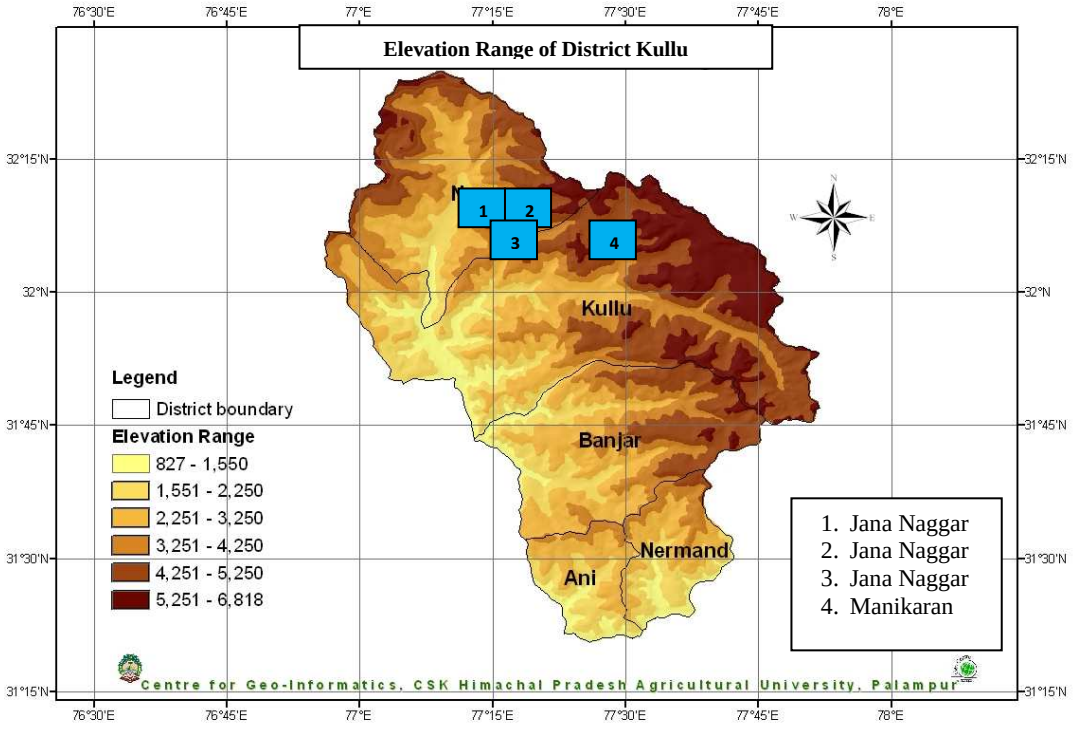


Fig. 1b. Locations surveyed in district Kullu

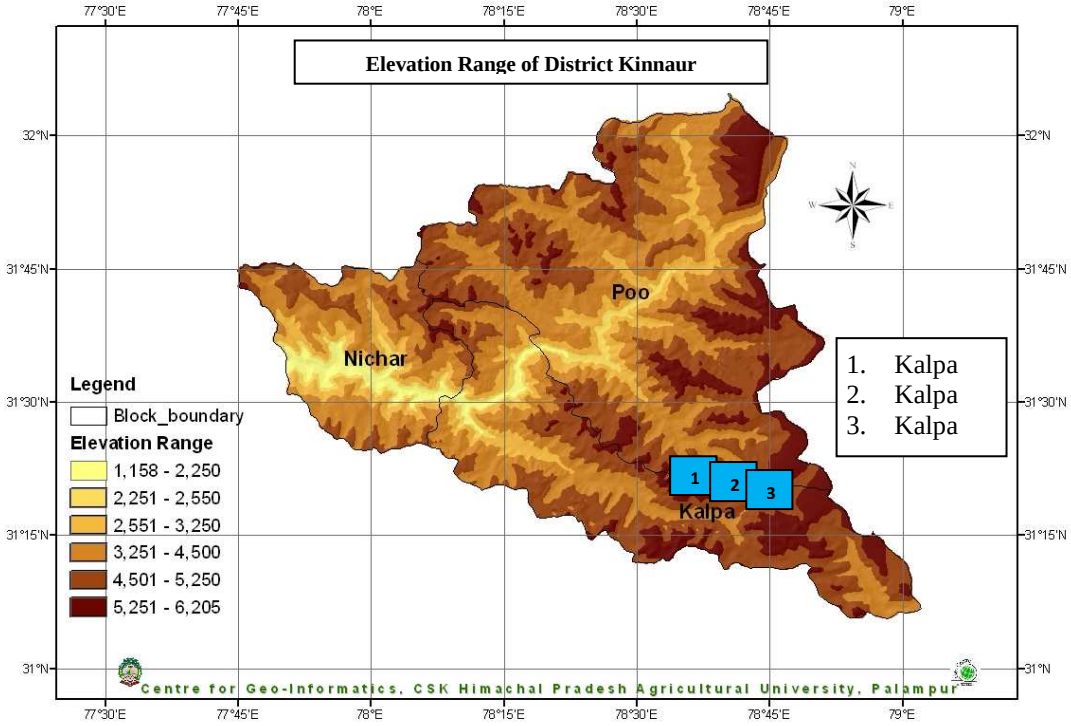


Fig. 1c. Location surveyed in district Kinnaur

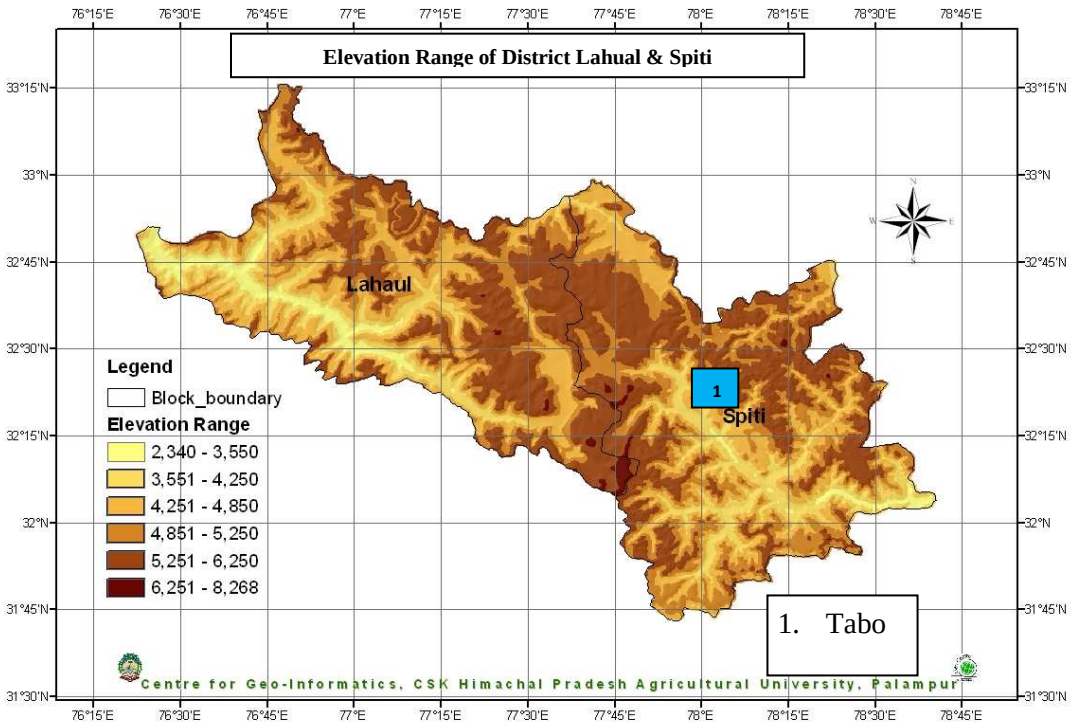


Fig. 1d. Location surveyed in district Lahaul & Spiti

3.4 EVALUATION STUDIES

All the marked mother trees as well as the identified bud sports (variants) in one respect or the other were subjected to evaluation studies. These were studied in detail for recording various observations as per standard apple descriptors (IBPGR, 1982) and DUS test guidelines (UPOV, 2005) which are as follows :

3.4.1 Phenological Characters

3.4.1.1 Time of leaf bud burst (initiation)

Unfolding of leaf primodia from shoot axis in 3-4 buds at least was taken as the criterion to record the time of initiation of leaf bud burst.

3.4.1.2 Time of floral bud burst

Opening its developmental stage of the flower bud up to the calyx cracking stage was recorded as time of floral bud burst.

3.4.1.3 Time of leaf fall

Peak stage of leaf fall (indicated by more than 80 % shedding of leaves) was recorded as time of leaf fall.

3.4.2 Inflorescence and Flowering Characters

3.4.2.1 Bearing habit (standard/spur)

This was assigned on the basis of physical observation and categorized following standard descriptors.

On spurs only On spurs and long shoots On long shoots only

3.4.2.2 Time of flowering

The time of opening of more than 75 per cent flowers was recorded as time of full-bloom.

3.4.3 Fruit Characters

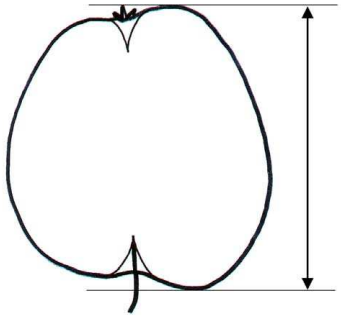
Fruits from all the variants and mother trees were observed for various morpho-physical characters :

3.4.3.1 Fruit weight (g)

Fruits were weighed on an electronic balance and average fruit weight (g) was worked out.

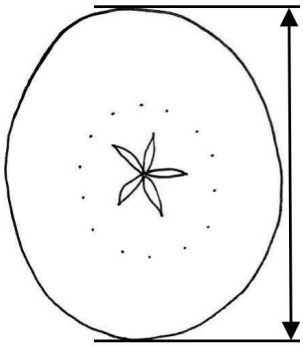
3.4.3.2 Fruit length (cm)

The length of the fruits as illustrated below was measured with the help of digital Vernier callipers and the average length was worked out and expressed in centimetres (cm).



3.4.3.3 Fruit breadth (cm)

The breadth of the fruits was recorded as illustrated below with the help of digital Vernier callipers and the average breadth was determined and expressed in centimetres (cm).

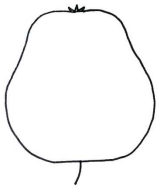


3.4.3.4 Fruit shape

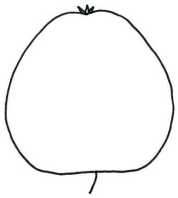
Shape of the fruits was described following standard descriptors as illustrated below :

1. Cylindrical waisted
2. Conic

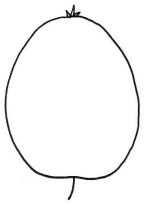
3. Ovoid
4. Cylindrical
5. Ellipsoid
6. Globose
7. Obloid



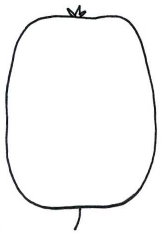
1
Cylindrical waisted



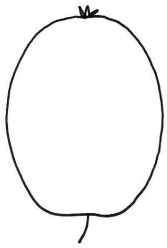
2
Conic



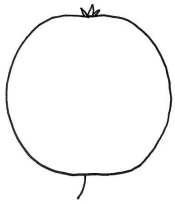
3
Ovoid



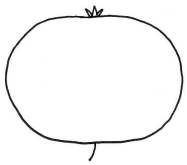
4
Cylindrical



5
Ellipsoid



6
Globose



7
Obloid

3.4.3.5 Surface of fruit

Physical examination of the fruit was done to categorise the outer surface of the fruits :

1. Smooth
2. Rough

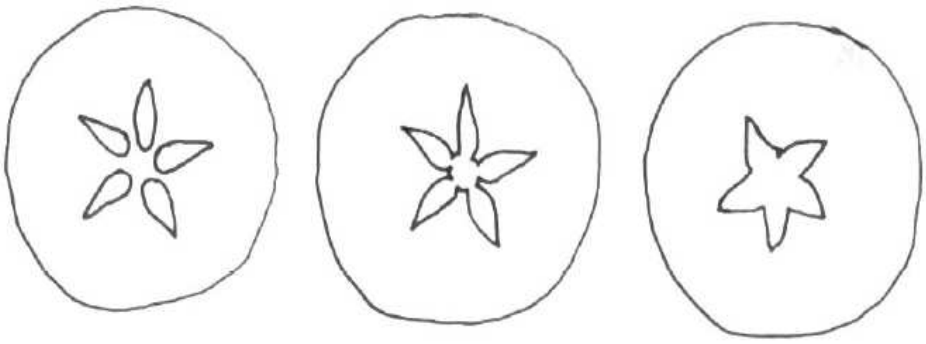
3.4.3.6 Fruit base

Base of the fruits was visually observed to categorise into:

1. Narrow
2. Intermediate
3. Broad

3.4.3.7 Fruit : aperture of locules (in transverse section)

The fruits were cut horizontally to observe the aperture of locules and categorisation was done following standard descriptors as illustrated below :



Closed or Slightly Open Moderately Open Fully Open

3.4.3.8 Fruit base cavity depth

Depth of fruit base cavities was measured with the help of digital Vernier callipers at fruit base. The average depth was worked out and expressed in centimetres (cm).

3.4.3.9 Fruit apex

Fruit apex was observed externally to describe as per the standard descriptors :

1. Smooth
2. Wrinkled
3. Grooved
4. Other

3.4.3.10 Fruit ground colour

Skin ground colour of fruit was assigned as per colour chart of Royal Horticultural Society.

3.4.3.11 Fruit over colour

Fruit skin over colour was assigned as per colour chart of Royal Horticultural Society.

3.4.3.12 Fruit skin lenticels

The abundance of lenticels on fruits was estimated visually to classify as under :

1. Few
2. Medium
3. Many

3.4.3.13 Type of over colour

Visual observations with respect to over colour on fruits were recorded to assign a category as per the standard descriptors :

1. Striped
2. Streaked
3. Mottled
4. Splashed
5. Slightly blushed
6. Washed-out (faded)
7. Complete over colour
8. Other

3.4.3.14 Fruit attractiveness

The attractiveness of fruits based on physical appearance was judged by five penalists and accordingly standard categorisation was made :

1. Extremely poor
2. Poor
3. Intermediate
4. Good
5. Extremely good

3.4.3.15 Flesh colour

Freshly harvested fruits were cut into slices to record the flesh colour and categorisation was done as given below :

1. White
2. Cream
3. Yellowish

4. Greenish
5. Pinkish
6. Reddish

3.4.3.16 Fruit firmness

Flesh firmness of fruit was measured after removing the skin (0.8 cm) and using Effigy Penetrometer (Model FT 327) with plunger of 11 mm diameter and was expressed as kg/cm^2.

3.4.3.17 Number of seeds

Fruits were cut to remove the seeds from the core. Seed number was counted after discarding chaffy and shriveled seeds.

3.4.3.18 Number of locules

Fruits were cut from the core to count the number of locules in a fruit.

3.4.3.19 Persistency of calyx

Persistency of calyx was assigned on the basis of physical observation of presence of calyx on fruit and categorization was done as follows :

\+ = presence of persistency in matured fruits

\- = absence of persistency in matured fruits

3.4.3.20 Harvest time

The time of picking of fruits by the farmers / growers was taken as harvest time.

3.4.3.21 Eating quality (dessert)

Eating quality of fruits based on combined assessment of flavour, acidity, sweetness, aroma judged by five penalists at optimum eating time and categorisation was made as under

1. Extremely poor
2. Very poor
3. Poor
4. Poor/intermediate
5. Intermediate
6. Intermediate/good
7. Good

8. Very good
9. Extremely good

3.4.4 Organoleptic analysis

Organoleptic characters of the sampled fruits were judged by five penalists.

3.4.4.1 Sensory analysis

Fruit samples were subjected to sensory evaluation for characters viz. pulp texture, pulp taste and juiciness following 'Hedonic Rating Test'. Each character was given a separate score out of a scale of 10 points. The panelists evaluated the samples as per hedonic scale described by Ranganna (2000) in format detailed below:

Sample No.	Pulp Texture (10)	Pulp Taste (10)	Pulp Juiciness (10)

3.4.5 Visual observations

3.4.5.1 Russet amount

Russet amount on skin of fruits was observed visually and categorisation was done as under :

1. 0%
2. 12%
3. 25%
4. 37%
5. 50%
6. 62%
7. 75%
8. 87%
9. 100%

3.4.5.2 Russet type

Russet type was assigned on the basis of fruit surface covered by russeting and categorisation was made as follows :

1. Few
2. Medium
3. High

3.4.6 Biochemical analysis

Harvested fruits were subjected to biochemical analysis following standard methods :

3.4.6.1 Total soluble solids (°Brix)

The total soluble solid (TSS) contents of fruits from mother trees as well as bud sports were estimated with help of Erma Hand Refractrometer (0-32˚Brix) by placing few drops of juice on the prim and reading was recorded (A.O.A.C, 1990).

3.4.6.2 Titratable acidity (%)

The same fruit samples of bud sports as well as mother plants were used for estimation of titratable acidity (A.O.A.C, 1990). Twenty five grams of fruit of each sample was crushed to make volume upto 250 ml in a volumetric flask by adding distilled water. Out of this, 50 ml extract (solution) was taken for the estimation of acidity and the rest was used for estimation of total and reducing sugars. The juice extract (10 ml) was titrated against N/10 NaOH using phenolphthalein as an indicator, change of the solution colour to light pink indicated the end point. The total titratable acidity was then calculated in terms of malic acid in percentage.

$$\text{Per cent titratable acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times \text{Equiv. Wt. of malic acid} \times 100}{\text{Weight of sample taken for estimation} \times \text{Aliquot taken} \times 100}$$

3.4.6.3 Total sugars (%)

For estimation of total sugars, 200 ml of the stock solution left from titratable acidity was taken in a conical flask, adding 10 ml of saturated lead acetate solution, the final volume was made to 250 ml. This solution was shaken and filtered into a flask containing 10 ml potassium oxalate and filtered again. This solution was hydrolyzed by adding 3 ml of concentrated hydrochloric acid in 250 ml volumetric flask having 100 ml of the filtrate and leaving it overnight. Added 10 per cent of sodium hydroxide solution to neutralize the excess of the hydrochloric acid.

Hydrolysed solution was taken in a burette and titrated against boiling mixture of Fehling A and Fehling B solutions (5 ml each), using methylene blue as an indicator (A.O.A.C, 1990). The end point of titration was indicated by appearance of brick red colour and total sugar content was expressed in percentage.

$$\text{Total sugars (\%)} = \frac{\text{Fehling factor} \times \text{Dilution} \times 100}{\text{Titre} \times \text{weight of sample}}$$

3.4.6.4 Reducing sugars (%)

Boiling solution mixture containing Fehling A and Fehling B (5 ml each) was titrated against the remaining unhydrolysed but deleaded and clarified pulp solution to determine the reducing sugars (A.O.A.C., 1990). The content of reducing sugars was expressed in percentage.

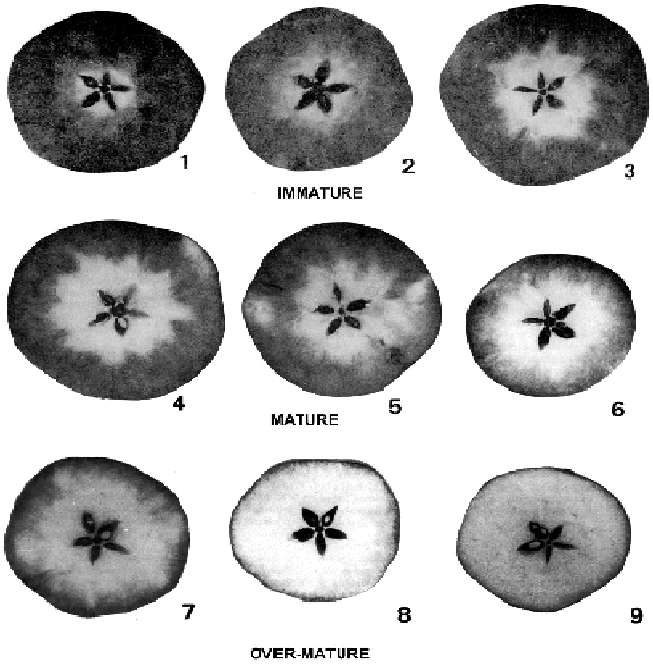
3.4.6.5 Non-reducing sugars (%)

Non-reducing sugars were calculated by subtracting the amount of reducing sugars from the total sugars and multiplied by the standard factor 0.95 and were expressed in percentage.

Non reducing sugars (%) = (Total sugars – Reducing sugars) x 0.95

3.4.6.6 Starch-Iodine Test Stained Area (%)

Apple fruits of mother plants as well as bud sports were subjected to starch-iodine test given by Smith *et al*. (1979). The test was performed with the help of iodine solution prepared by potassium iodide (10 g) in distilled water (30 ml) and then adding iodine (3 g). When the iodine has dissolved, the mixture was made up to 1000 ml by adding distilled water at room temperature. Each fruit of apple (mother plant fruits as well as bud sport fruits) was sliced crosswise using a sharp knife in half. Surface of each sample was evenly coated with iodine solution and left for minimum one minute before results were assessed. After reaction of iodine on starch, half surface was compared with iodine starch chart as illustrated below :



3.5 MOLECULAR MARKER STUDIES

3.5.1 Collection of leaves

Fresh green and disease free leaves were separately excised from all trees of the identified 10 apple mother and bud sports, and were brought to Molecular Breeding Laboratory, Department of Fruit Science, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.). Before plucking, leaves were wiped off the soil with paper towels, wrapped in moist blotting paper and carefully put in transparent sealable polythene bags after proper labeling and were then transferred to the laboratory in icebox and stored in deep freezer at -80ºC till further use.

3.5.1.1 Isolation of genomic DNA

CTAB method given by Doyle and Doyle (1987) was used with minor modification to extract genomic DNA from the above collected leaf samples.

Reagents:

1.	10% CTAB	CTAB (10 g) was dissolved in distilled water and warmed at 65ºC to make final volume of 100 ml.
2.	0.5 M EDTA (pH8.0)	18.61g of EDTA was dissolved in 80 ml distilled water. The pH of the solution was adjusted to 8.0 by addition of 1N NaOH. Final volume of solution was made to 100 ml with distilled water and the solution was sterilized by autoclaving.
3.	4M (Sodium Chloride) NaCl	23.37 g of NaCl was dissolved in minimum amount of distilled water and the final volume was made to 100 ml with distilled water.
4.	1 M Tris (pH8.0)	15.76 g of TrisHCl was dissolved in 80ml distilled water. The pH was adjusted to 8.0 with 1N NaOH. The final volume was made to 100 ml with distilled water and the solution was sterilized by autoclaving.
5.	DNA extraction buffer	100 ml of extraction buffer contained i. 10 ml 1 M Tris HCl ii. ml 0.5 M EDTA iii. 20 ml 10% CTAB iv. 35 ml 4 M NaCl v. 1 g PVP vi. 31 ml distilled water vii. 0.2% β-mercapto ethanol
6.	Chloroform : Isoamyl	96 ml of chloroform and 4 ml of isoamyl alcohol (24:1,v/v) were mixed together and the solution was kept in a closed container at room temperature.

7.	70% Ethanol	70 ml of absolute alcohol was mixed with 30 ml of distilled water to make it 100 ml.
8.	TE buffer	0.1576 g of TrisHCl and 0.0372 g of EDTA were dissolved in 100 ml of distilled water. The pH was adjusted to 8.0 with 1N NaOH.

Procedure :

Various steps followed to extract genomic DNA from apple leaf samples are described below ;

Young and healthy leaves (2 g) were homogenized completely to a fine powder with liquid nitrogen using pre-chilled pestle and mortar.

↓

The leaf powder was transferred to 50 ml centrifuge tubes containing 20 ml of pre-warmed (at 65ºC) DNA extraction buffer. Care was taken that leaf powder did not get moist because under moist conditions DNase digests DNA.

↓

The tubes were incubated at 65ºC for one and a half hour in a water bath. Samples were mixed during the period of incubation by inverting the tubes after every five minutes.

↓

Equal volume of chloroform: isoamyl alcohol (24:1, v/v) was added to each tube and the contents mixed gently by hand inversions till the colour in the lower portion of the tube turned dark green.

↓

The above suspension was then centrifuged at 12,000 rpm for10 minutes at room temperature (25ºC).

↓

Aqueous phase was transferred to fresh autoclaved centrifuge tubes without disturbing the interphase.

↓

Two –third volume of pre-chilled isopropanol was added, the contents were mixed gently by hand inversions and incubated at -20ºC for 1 hour or overnight so that DNA got precipitated.

↓

Precipitated DNA was pelleted by centrifugation at 10,000 rpm for 10 minutes at 4ºC.

↓

DNA pellet was then washed with 500 µl of 70 per cent ethanol and centrifuged at 5,000 rpm for 5 minutes at 4ºC.

↓

Supernatant was discarded off and DNA pellet was dried overnight to completely evaporate the alcohol.

↓

DNA pellet was then dissolved in 500 µl of TE buffer.

3.5.1.2 Purification of Genomic DNA

DNA was purified by successive RNase treatment followed by phenol : chloroform extraction.

Reagents :

a)	Preparation of RNase	RNase was dissolved in autoclaved distilled water at a concentration of 10 mg/ml.
b)	Phenol : chloroform (1:1v/v)	50 ml of phenol and 50 ml of chloroform were mixed together and stored at 4ºC in closed container.
c)	Chloroform : Isoamyl (24:1,v/v)	96 ml of chloroform and 4ml of Isoamyl alcohol were mixed together and the solution was kept in a closed container at room temperature
d)	3 M Sodium Acetate	24.609 g of sodium acetate was dissolved in 80 ml distilled water and the pH was adjusted to 4.8 using glacial acetic acid. The final volume was then made to100 ml with distilled water.
e)	Ethanol (70%)	70 ml of absolute alcohol was mixed with 30 ml of distilled water to make it 100 ml.

Procedure :

Step-1 0.2 µl of RNase (10 mg/ml) was added to 100 µl DNA and incubation was carried out at 37ºC for one hour.

Step-2 Equal volume of phenol: chloroform (1:1,v/v) was added and mixed gently.

Step-3 Suspension was centrifuged at 11,000 rpm for two minutes at room temperature and the aqueous phase was transferred to fresh microfuge tubes.

Step-4 Extraction with equal volume of chloroform : isoamyl alcohol (24:1, v/v) was done twice, each followed by centrifugation at 11,000 rpm for two minutes.

Step-5 The aqueous phase was separated out into fresh microfuge tubes. $1/10^{th}$ volume of 3M sodium acetate and 2.5 volume of absolute ethanol was added. The contents were mixed gently and incubated at 4ºC for one hour.

Step-6 The DNA was pelleted by centrifugation at11,000 rpm for five minutes.

Step-7 The supernatant was decanted off carefully and the pellet washed with 70% ethanol.

Step-8 The pellet was air dried and resuspended in 100 µl TEbuffer.

3.5.2 Qualitative and Quantitative Assessment of DNA

The quality of the extracted DNA was assessed by agarose gel electrophoresis and quantity was estimated using UV-VIS spectrophotometer.

3.5.2.1 Agarose gel electrophoresis

The isolated genomic DNA was electrophoresed on 0.8 per cent agarose gel for 1.5 hr at 5 V cm^{-1} in 1X TAE buffer. The gel was stained with ethidium bromide (0.25µg/ml) and then observed on UV-transilluminator. Quality of DNA samples was judged on the basis of whether sample DNA formed a single high molecular weight band or smear.

3.5.2.2 DNA quantification

Concentration of DNA was quantified spectrophotometrically on the basis of absorbance at 260 nm using formula :

$$\text{DNA (µg/ml)} = \frac{\text{OD260 x dilution factor x50}}{1000}$$

The ratio of absorbance at 260 nm and at 280 nm was measured to check the contamination of proteins (Table 3.2).

Table 3.2: Purity check of DNA on the basis of A_{260} : A_{280} ratio

S. No.	A_{260}:A_{280} ratio (Absorbance ratio)	Indication
1	Above 1.8	Protein contamination
2	1.4 to 1.8	Good quality DNA
3	Below 1.4	RNA contamination

3.5.3 Simple Sequence Repeat (SSR) Studies

Thirty SSR primers, including 20 genic and 10 genomic were used. The details of the primer pairs are provided in Table 3.3 and 3.4.

Table 3.3: List of 20 EST-SSR primer pairs used

Sr. No	Source Sequence ID	Repeat Motif	No. of Repeats	SSR start	SSR End	Sequence Length	Primer Name	Sequence	Base pairs
1.	gi226857985	GT	6	367	378	837	E1	F: CGGTGCATGTACAACACGTA R: TCCAGCAGAAGAGGTGATGA	20 20
2.	gi226857903	ATG	5	663	677	912	E2	F: GTATTGGCTCCACCACAACC R: GAAACCCGGTCGTGTAAAAA	20 20
3.	gi226857895	TGC	5	574	588	717	E3	F: AAAGTGGCGTAAGGTTGTGG R: CACGCTGGACACATACATCC	20 20
4.	gi226857752	AGA	5	287	301	689	E5	F: GGCCCAAATGTCATCGAATA R: TATTCCAAATTCCCGGACTG	20 20
5.	gi226814813	CCT	5	557	571	796	E6	F: AACATATGGGGCAGCGTATT R: TTGTGATGCTTGTGGGGATA	20 20
6.	gi226811802	GAA	5	488	502	698	E7	F: TCCTCTGGAGTAGCGAGCAC R: ACCGTCAACGAGGCTCAG	20 18
7.	gi226805503	GAA	5	391	405	894	E8	F: CAACAAAGCCTTTTCCCAGT R: TTATTCGGCCTTTGTTTTGG	20 20
8.	gi226796317	TCG	9	300	326	378	E10	F: TCATCGCCACCTTGATGATA R: AGCTCAAAGAGGCCGTCATA	20 20
9.	gi226794476	GCA	5	113	127	540	E11	F: CACTTGGGCCAATTTCTGAC R: CCAAGGAGTGAGTGGAGGAG	20 20
10.	gi226814826	TTG	6	415	432	818	E12	F: TTCCAACGACACCAACCTTT R: GCGATGATGTATGGCACAGA	20 20
11.	gi226812665	GTC	5	514	528	774	E13	F: TCCGACAAATCAACATTGGA R: CTGACGGACGCTCATAACAA	20 20
12.	gi226810461	AGA	5	408	452	706	E14	F: TGATGATGACGATGACGATG R: GAGCAAAAGTTGAAACCCTCA	20 20
13.	gi226810487	ATA	9	208	234	403	E15	F: GGGCTGCTTGATTTGAACTT R: GAAGCTAAAACTTCACCCTTGA	20 22
14.	gi226810743	TTG	6	573	590	631	E16	F: TGTTCATGTCGGTGCTCAAT R: GACGATGATCAGGCCATTCT	20 20
15.	gi226811551	CCAAT C	5	626	655	836	E17	F: ACTGAGGGCTTGTGTTGGTC R: GCTTCTGAACGAGGAAGACG	20 20
16.	Contig220-1	GCA	5	245	259	944	E21	F: AGCCCAAAAGGCAGTCTACA R: ACCCCCATTTTCAGCCTATC	20 20
17.	Contig139-2	AAC	6	263	280	872	E22	F: CGCTCCAGAAGTCAACATCA R: GGAGCTCCAATTCCATTTCA	20 20
18.	Contig10-1	ACC	5	124	138	579	E23	F: TGTTAGATCCGGACCCACTC R: GATGGAAGGGGAGAAGAGC	20 20
19.	Contig127-1	GTG	5	342	356	645	E24	F: TGAGGTGACCACAAGCAAAG R: CAATTCTTTTGTGCGCGTAT	20 20
20.	Contig27-1	ACAT	6	47	70	533	E25	F: TTTTCCCCCTCAAGAACAAA R: TGAAGCCGAAGGTTCATCAT	20 20

Table 3.4: List of genomic SSR primer pairs used

S. No.	Primer Name	Sequence	Tm (^{o}C)
1.	CH01f02	F: ACC ACA TTA GAG CAG TTG AGG R: CTG GTT TGT TTT CCT CCA GC	59 58
2.	CH01g12	F: CCC ACC AAT CAA AAA TCA CC R:TGA AGT ATG GTG GTG CGT TC	58 56
3.	CH01d08	F: CTC CGC CGC TAT AAC ACT TC R:TAC TCT GGA GGG TAT GTC AAA G	60 60
4.	CH01h01	F: GAA AGA CTT GCA GTG GGA GC R:GGA GTG GGT TTG AGA AGG TT	60 58
5.	CH02a04	F: GAA ACA GGC GCC ATT ATT TG R:AAA GGA GAC GTT GCA AGT GG	56 58
6.	CH01e12	F: AAA CTG AAG CCA TGA GGG C R:TTC CAA TTC ACA TGA GGC TG	57 56
7.	CH01a07b	F: AAC CCA TGA AAC ACA ATC CC R:GGA ACG ATC CAT AGG TGG TG	56 60
8.	CH01f03b	F: GAG AAG CAA ATG CAA AAC CC R:CTC CCC GGC TCC TAT TCT AC	56 63
9.	CH05e03	F: CGA ATA TTT TCA CTC TGA CTG GG R:CAA GTT GTT GTA CTG CTC CGA C	53 55
10.	CH01f07a	F: CCC TAC ACA GTT TCT CAA CCC R:CGT TTT TGG AGC GTA GGA AC	61 58

3.5.3.1 Standardization of SSR-PCR

PCR protocol was standardized for different concentrations of primers, template DNA, dNTPs, $MgCl_2$ and Taq DNA polymerase (Table 3.5).

Different quantities in "µl" of each reaction component were taken to prepare the reaction volume in a 2.0 ml microfuge tube. The final volume was made with autoclaved distilled water and all the reagents were mixed thoroughly and vortexed for few seconds. 17 µl of the prepared mixture was distributed to each 0.2 ml thin walled reaction tube (Axygen Scientific Pvt. Ltd., New Delhi, India) and then 3 (after standardizing) µl DNA was added separately to each tube to make 20 µl of reaction volume.

Table 3.5 : Concentrations of components in SSR studies

Components	Variable conditions
PCR buffer	1 X
$MgCl_2$	1.0mM – 4.0mM
dNTPs	1.0 mM – 5.0mM
Primer – Forward	30-50 pm
Primer – Reverse	30-50 pm
Taq DNA polymerase	0.5U – 1.0 U
Template DNA	20 – 60 ng
Autoclaved Distilled Water	To make up the final volume

The tubes with the reaction mixture were then placed in a thermal cycler (Applied Biosystems, Foster city, California, USA) for cyclic amplification.

The thermal profile followed for SSRs is given below.

Table 3.6 : Details of thermal profile followed for SSR amplification

Steps	**Temperature**	**Duration**
1. Initial denaturation	95°C	5 minutes
2. 40 cycles each of		
i) Denaturation	94°C	1 minute
ii) Annealing	Varied with Tm of primer pairs	1 minute
iii) Extension	72°C	2 minutes
3. Final Extension	72°C	5 minutes
4. Hold at 4°C		

3.5.3.2 Electrophoresis of amplified DNA

Thoroughly mixing of amplified DNA was done with 6X loading dye and then electrophoresed in 2.5 per cent agarose gel in 1X TAE buffer. The gel was run for about 2 hours at constant voltage at the rate of 5V/cm under submerged conditions. Ethidium bromide was incorporated in the gel at the rate of 0.25 µg/ml.

Co-electrophoresis of standard molecular weight marker (Hind III/ EcoRI, double digest, Bangalore Genei, India) was used to determine the size of amplified product. DNA profiles were visualised on UV Transilluminator and photographed using Gel Documentation System (Syngene, Cambridge, UK).

Chapter-4
RESULTS AND DISCUSSION

Breeding of perennial fruit crops is a labour-intensive and time consuming process with relatively low success rate as compared with annual crops. The major reasons assigned for low efficiency are long juvenile period, self-incompatibility (allogamy) and heterozygosity. Due to these reproductive peculiarities, the distinguishing characteristics of a genotype are lost during sexual reproduction due to segregation of alleles. Fortunately, the advent of vegetative propagation methods made it possible to maintain the genetic identity of the distinct variety as cultivar. In other words, clonal multiplication through asexual means *viz.*, cutting, grafting, budding and relatively more recently plant tissue culture came to the rescue of highly self-incompatible cross-pollinating fruit species including apple. According to an estimate, some 10000 apple cultivars are named today which have originated as chance selections, spontaneous mutations or as bred genotypes through hybridization. At the same time, it is well known that the available apple gene pool has a very narrow genetic base, and the entire apple industry is relying upon a handful of cultivars. This warrants the development of genetically diverse forms with improved horticultural characteristics to meet the consumer and market demands and to overcome the ill effects of abiotic and biotic stresses. Occurrence of spontaneous bud mutations in plants has often led to their subsequent development as commercial cultivars. Apple is a typical example of such a case, being highly prone to occurrence of bud sports as is evident from large number of bud, limb or whole tree mutations occurring naturally. Not all the apple cultivars demonstrates this, but classical examples are ‘Delicious’, ‘Golden Delicious’, ‘Rome Beauty’, ‘Jonathan’, ‘Fuji’ and ‘Gala’. And the process is continuing and newer and newer bud sports are being reported. With the above in consideration, it was felt worthwhile to explore selected apple plantations to identify bud sports not only with an aim of harnessing variability but also to look for genotypes with potential of having one or more commercially important characters.

The experimental findings resulting from the present study entitled “Studies on identification of bud sports in apple (*Malus* x *domesitica* Borkh.)” are presented and discussed under the following sub-headings :

4.1 IDENTIFICATION OF BUD SPORTS IN APPLE
4.2 EVALUATION STUDIES
4.3 MOLECULAR MARKER STUDIES
4.4 CLONAL PROPAGATION

4.1 IDENTIFICATION OF BUD SPORTS IN APPLE

Field surveys of existing apple plantations in orchards/sites located in four districts (*viz*, Shimla, Kullu, Kinnaur and Lahaul & Spiti) of Himachal Pradesh were undertaken during 2016-17. Apple cultivars grown in the selected orchards are Starking Delicious and Red Delicious, by and large, with Vance Delicious in only one orchard. A total 7154 bearing trees were screened to identify the bud sports on the basis of pre-selection criteria (for details see Section 3.2). This resulted in identification of as many as 10 bud sports exhibiting variation from their respective mother trees in one or the other character and are enlisted (Table 4.1) below :-

Table 4.1 : List of identified bud sports in apple

S. No	District	Location	Altitude (MAMSL)	Name of the Orchardist	Cultivar	Bud Sport	
						Code	Distinct Variation
	Shimla	Manjari, Theog	2320	Jogendra Singh	Red Delicious	Shimla 1	Early fruit skin colour development
2		Mashobra	2274	RHR&TS	Vance Delicious	Shimla 2	Early fruit skin colour development
3	Kullu	Jana Naggar Block	1830	Manish Thakur	Starking Delicious	Kullu	Solid fruit skin colour development
4		Jana Naggar Block	1825	Manish Thakur	Starking Delicious	Kullu 1	Solid fruit skin colour development
5		Jana Naggar Block	1823	Manish Thakur	Starking Delicious	Kullu 2	Solid fruit skin colour development
6		Kasada Barada, Jari, Manikaran	1821	Sudhir Thakur	Starking Delicious	Manikaran 5	Solid fruit skin colour development
7	Lahaul & Spiti	Tabo	3297	KVK	Starking Delicious	Tabo 1	Fruit bearing on spur
8	Kinnaur	Kalpa	2727	Ramesha	Starking Delicious	Kalpa 1	Late fruit skin colour development
9		Kalpa	2741	Ramesha	Starking Delicious	Kalpa 2	Solid fruit skin colour development
10		Kalpa	2747	Chering Dejhe	Red Delicious	Kalpa 3	Fruit bearing on spur

The perusal of Table 4.1 reveals that out of total of 10 bud sports as many two early colouring (Shimla 1, Shimla 2), five solid coloured (Kullu, Kullu 1, Kullu 2, Manikaran 5, Kalpa 2) two spur-type bearing (Kalpa 3, Tabo 1) and one late colouring (Kalpa 1) were observed in different orchards and marked for detailed evaluation studies.

The occurrence of such like bud sports in apple is very common and various workers have reported so very extensively in the past (Dermen, 1948 ; Dayton, 1959 ; Lapins and

Fisher, 1974 ; Fisher and Ketchie, 1981, 1989 ; Fallahi *et al*., 1994 ; Deutscher, 1999 ; Jeffrey, 2000 ; Khanizadch *et al*., 2003 ; Sansavini *et al*., 2005 ; Jarsolev *et al*., 2009, 2010 ; Michelangelo *et al*., 2013 ; Chen *et al*., 2015 ; Foster and Aranzana, 2018) providing support to the observations occurrence of bud sports in the present study. However, these variations to be regarded useful or commercially acceptable must undergo further testing and accordingly systematic evaluation, though preliminary, were undertaken and salient findings are as follows :

4.2 EVALUATION STUDIES

All the 10 identified bud sports and their mother trees were subjected to evaluation for phonological, flower and fruit characters as per UPOV (2005) test guidelines and IBPGR (1982) descriptors. Standard procedures were followed for biochemical and organoleptic (sensory) analysis. All the observations recorded in each of the identified bud sport are presented as follows :

4.2.1 Apple Bud Sport 'Shimla 1'

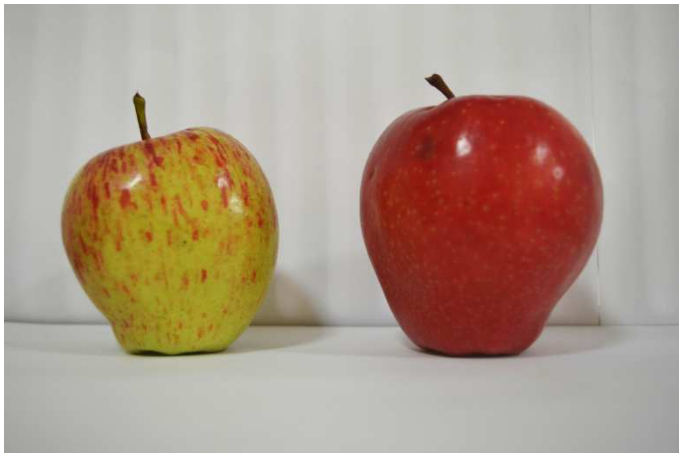
Bud sport 'Shimla 1' was identified in cv. Red Delicious in Theog, district Shimla primarily due to early colour development in the fruits with respect to its mother tree. Development of complete over colour in the bud sport was observed as early as on 9th July (Table 4.2) as compared to streaked fruits in the mother tree till harvesting time (Plate 1). Apart from early colouring, fruits of bud sport 'Shimla 1' recorded higher fruit weight (167.00 g) increased number of seed (5), less firm fruit (8.10 kg/cm^2), higher TSS (14.40 ^{o}Brix), total sugars (10.51 %), low acidity (0.37 %) and low stained area (60 %) as per starch-iodine test as compared to corresponding values in the mother tree (Table 4.2). The remaining characters recorded were almost similar in mother tree as well as in the bud sport. It is revealed from the above finding that the bud sport Shimla 1 demonstrated not only early fruit skin colour development but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by high level of TSS, low acidity, low stained area as per starch-iodine test, firmness, good eating quality, better pulp texture and pulp taste. This finding is in accordance with the reports of Dong *et al.* (2011). Early colouration of fruit skin in apple will always remain on top of the priority list amongst the orchardists, especially in areas where colour development is a serious problem.

Table 4.2 : Phenological, flower and fruit characters of early colouring bud sport 'Shimla 1' and mother tree in cv. Red Delicious

District : Shimla	**Location : Manjari, Theog**	
Characters	**Cultivar : Red Delicious**	**Bud Sport : Shimla 1**
Phenological characters		
Time of leaf bud burst	2^{nd} – 3^{rd} Week of March	-
Time of floral bud burst	4^{th} Week of March	-
Time of leaf fall	2^{nd} Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	2^{nd} Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**128.00**	**167.00**
Fruit length (cm)	6.89	7.38
Fruit breadth (cm)	6.69	7.29
Fruit shape	Conical	Conical
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.2	1.2
Fruit apex	Smooth	Smooth
Fruit ground colour	Yellow green group 150 B	Yellow green group 145 C
Fruit over colour	**Greyed purple group 185 A**	**Red group 45 C**
Fruit width of stripes	**Broad**	**Absent**
Type of over colour	**Streaked**	**Complete over colour (colour development started on 9^{th} July)**
Fruit skin lenticels	**Few**	**More**
Fruit attractiveness	**Intermediate**	**Good**
Flesh colour	**White**	**Cream white**
Fruit firmness (kg/cm^2)	**8.90**	**8.10**
Number of seeds	**2**	**5**
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Persistency of calyx	Present	Present
Harvest time	20^{th} August	20^{th} August
Eating quality (dessert)	**Intermediate**	**Good**
Russet amount (%)	12	12
Russet type	Few	Few
Organoleptic analysis		
Pulp texture	**5.5**	**7.5**
Pulp taste	**6**	**8.5**
Juiciness	8	8
Biochemical characters		
TSS (^{O}Brix)	**12.60**	**14.40**
Titratable acidity (%)	0.43	0.37
Total sugars (%)	9.32	10.51
Reducing sugars (%)	7.37	8.09
Non-reducing sugars (%)	1.86	2.30
Starch-Iodine Test Stained Area (%)	**80**	**60**

Plate 1 : Early colouring bud sport ‘Shimla 1’ in cv. Red Delicious

Developing Fruits



Mother Tree **Bud Sport**

Matured Fruits

4.2.2 Apple Bud Sport 'Shimla 2'

Bud sport 'Shimla 2' was identified in cv. Vance Delicious in Mashobra, district Shimla primarily due to early fruit skin colour development with respect to its mother tree. Development of complete over colour in the bud sport was observed as early as on 20th July (Table 4.3) as compared to streaked fruits in the mother tree till harvesting time (Plate 2). Apart from early colouring, fruits of bud sport 'Shimla 2' exhibited medium fruit weight (89.00 g), less firm fruit (7.60 kg/cm^2), higher TSS (13.20 ^{o}Brix), total sugars (9.77 %), low acidity (0.34 %) and low stained area (60 %) as per starch-iodine test as compared to corresponding values in mother tree (Table 4.3). The remaining characters recorded were almost similar in mother tree as well as in the bud sport except fruit weight of mother tree (158.50 g). It is revealed from the above finding that the bud sport 'Shimla 2' demonstrated not only early fruit skin colour development but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by high level of TSS, low acidity, low stained area as per starch-iodine test, firmness, good eating quality, better pulp texture and pulp taste.

The present finding of early fruit skin colouring and early maturing bud sports 'Shimla 1' and 'Shimla 2' in cv. Red Delicious and cv. Vance Delicious, respectively assumes greater significance from marketing point of view. Both the characters are always preferred because not only that it would avoid the use of colouring agents but also would fetch higher price. 'Shimla 1' is in particular quite early wherein colour development started some six weeks earlier to parent cv. Red Delicious while 'Shimla 2' was four weeks earlier to mother cv. Vance Delicious. Two to four weeks early colouring bud sports in apple have been reported earlier also (Craig, 1979 ; Hare, 1985 ; Leslie and Linda, 2002 ; Robert, 2004 ; Werner, 2015). Although early colour development cannot be directly corrected with early fruit maturation time, but both bud sports 'Shimla 1' and 'Shimla 2' demonstrated this. Similar reports of early colouring and early maturing bud sports were also made in past (Silvers, 1977 ; Valle, 1989 ; Winkel, 1992 ; Wilhelmus and Antonius, 1999 ; Deutscher, 1999 ; Ira, 2006 ; Robert, 2008 ; Richard, 2017).

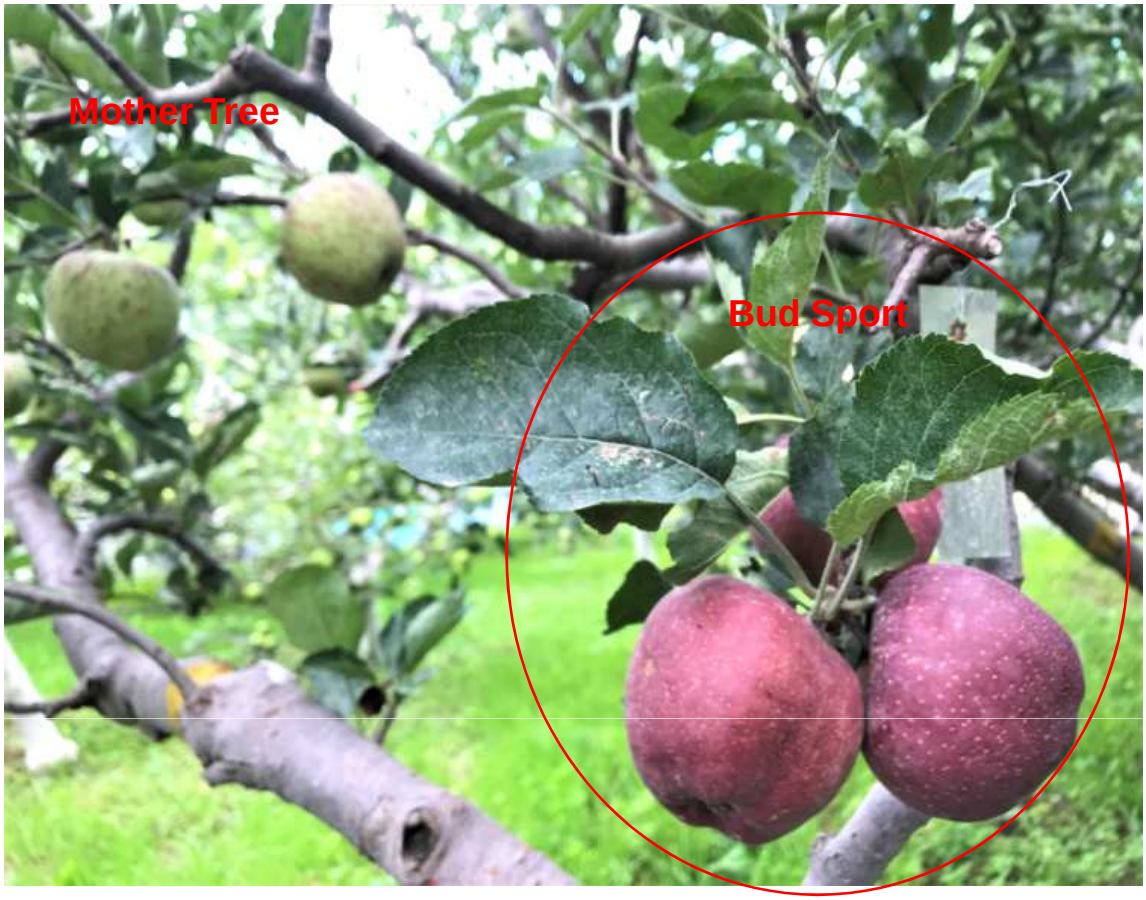
4.2.3 Apple Bud Sport 'Kullu'

Bud sport 'Kullu' was identified in cv. Starking Delicious in Jana Naggar, district Kullu primarily due to solid coloured with respect to its mother tree.

Table 4.3 : Phenological, flower and fruit characters of early colouring bud sport 'Shimla 2' and mother tree in cv. Vance Delicious

District : Shimla	**Location : Mashobra**	
Characters	**Cultivar : Vance Delicious**	**Bud Sport : Shimla 2**
Phenological characters		
Time of leaf bud burst	2^{nd} – 3^{rd} Week of March	-
Time of floral bud burst	4^{th} Week of March	-
Time of leaf fall	2^{nd} Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	2^{nd} Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**158.50**	**89.00**
Fruit length (cm)	6.51	4.92
Fruit breadth (cm)	7.66	6.12
Fruit shape	Globose Conical	Globose Conical
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.1	0.8
Fruit apex	Smooth	Smooth
Fruit ground colour	**Grayed yellow161 group A**	-
Fruit over colour	**Greyed red group 181 A**	**Greyed purple group 187 A**
Fruit width of stripes	**Intermediate**	**Absent**
Type of over colour	**Streaked**	**Complete over colour (Colour development started on 20^{th} July)**
Fruit skin lenticels	More	More
Fruit attractiveness	**Intermediate**	**Good**
Flesh colour	White	Cream white
Fruit firmness (kg/cm^2)	8.50	7.60
Number of seeds	5	5
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Persistency of calyx	Present	Present
Harvest time	24^{th} August	24^{th} August
Eating quality (dessert)	**Intermediate**	**Good**
Russet amount (%)	0	0
Russet type	Absent	Absent
Organoleptic analysis		
Pulp texture	6.1	7.2
Pulp taste	6.4	7.6
Juiciness	7.5	7.8
Biochemical characters		
TSS (^{O}Brix)	**11.80**	**13.20**
Titratable acidity (%)	0.41	0.34
Total sugars (%)	8.97	9.77
Reducing sugars (%)	7.08	7.52
Non-reducing sugars (%)	1.79	2.13
Starch-Iodine Test Stained Area (%)	**80**	**60**

Plate 2 : Early colouring bud sport 'Shimla 2 'in cv. Vance Delicious



Developing Fruits

Mother Tree **Bud Sport**

Matured Fruits

Development of solid (washed out) over colour in the bud sport was observed at early fruit development stage (Table 4.4) as compared to streaked fruits in the mother tree till harvesting time (Plate 3). Apart from solid coloured, fruits of bud sport 'Kullu' were also larger in size (150.83g), less firm (8.30 kg/cm^2) and had low stained area (70 %) as per starch-iodine test as compared to corresponding values in fruits of mother tree (Table 4.4). The remaining characters recorded were almost similar in mother tree as well as in the bud sport except higher TSS (13.10 ^{o}Brix), total sugars (9.96 %) and less acidity (0.18 %) in fruits of mother tree. It is revealed from the above finding that the bud sport 'Kullu' demonstrated not only solid fruit coloured development but also varied in fruit maturation time. Early maturity in solid coloured fruits of bud sport is indicated by low starch-iodine stained area, low firmness and better pulp texture. The present finding of a solid coloured over the entire fruit surface coupled with early colour development is again a much cherished character amongst the apple growers. A bud sport named 'Sandidge' on cv. Red Chief Compbell was specifically identified for having solid red fruit skin and early fruit colour formation over its parent (Sandidge and Charles, 1988). Similarly, Glynn (2008) described a bud sport carrying high level of over colour with solid flush on fruits as compared to parent tree. Even the observation of larger fruits in bud sport 'Kullu' as against its mother tree fruits as recorded in the present study get the support from the finding of Chris *et al*. (2007) and Shao *et al*. (2008). These workers also recorded similar observations of larger fruit size in coloured sports. The exact mechanism of cell enlargement leading to fruit larger and heavier in size and weight, respectively is not clearly understand as far as such observations of bud sports in apple are concerned (Mallardi and Hirst, 2010).

4.2.4 Apple Bud Sport 'Kullu 1'

Bud sport 'Kullu 1' was identified in cv. Starking Delicious in Jana Naggar, district Kullu primarily due to solid fruit coloured development with respect to its mother tree. Development of solid over colour in the fruits of the bud sport was observed at developing stage (Table 4.5) as compared to streaked fruits in the mother tree till harvesting time (Plate 4). Apart from solid fruit skin colour development, bud sport 'Kullu 1' also exhibited medium fruit weight (121.00 g), less firm fruits (8.23 kg/cm^2), higher TSS (12.90 ^{o}Brix), total sugars (9.55 %), low acidity (0.21 %), low stained area (60 %) as per starch-iodine test and fruit over colour (Grayed purple Group 185 B) as compared to corresponding values in mother tree (Table 4.5).

Table 4.4 : Phenological, flower and fruit characters of solid coloured bud sport 'Kullu' and mother tree in cv. Starking Delicious

District : Kullu	**Location : Jana Naggar**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Kullu**
Phenological characters		
Time of leaf bud burst	2nd Week of March	-
Time of floral bud burst	3rd -4th Week of March	-
Time of leaf fall	2nd Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	1st -2nd Week April	-
Morpho-physical fruit characters		
Fruit weight (g)	**115.75**	**150.83**
Fruit length (cm)	6.10	6.52
Fruit breadth (cm)	6.71	7.06
Fruit shape	Conic	Conic
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.4	1.4
Fruit apex	Smooth	Smooth
Fruit ground colour	Yellow green group 149 C	Yellow green group 149 B
Fruit over colour	Greyed red group 179 A	Greyed red group 181 A
Fruit skin lenticels	**Few**	**Medium**
Fruit attractiveness	Intermediate	Intermediate
Flesh colour	White	Cream
Fruit firmness (kg/cm^2)	8.60	8.30
Number of seeds	7	3
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Fruit width of stripes	**Medium**	**Narrow**
Type of over colour	**Streaked**	**Washed out (Faded)**
Persistency of calyx	Present	Present
Harvest time	11th September	11th September
Eating quality	Good	Intermediate/good
Russet amount (%)	0	12
Russet type	**Absent**	**Few**
Organoleptic analysis		
Pulp texture	5	6
Pulp taste	5	4
Juiciness	5	5
Biochemical characters		
TSS (^{O}Brix)	**13.10**	**12.80**
Titratable acidity (%)	0.18	0.25
Total sugars (%)	9.96	9.47
Reducing sugars (%)	7.87	7.39
Non-reducing sugars (%)	1.99	1.98
Starch-Iodine Test Stained Area (%)	**80**	**70**

Plate 3 : Solid coloured bud sport 'Kullu' in cv. Starking Delicious

Developing Fruits

Mother Tree **Bud Sport**

Matured Fruits

Fruit shape was cylindrical in bud sport as against conic fruits of the mother tree (Table 4.5 and Plate 4). The remaining characters recorded were almost similar in mother tree as well as in the bud sport. It is revealed from the above finding that the bud sport 'Kullu 1' demonstrated not only solid fruit skin colour but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by high level of TSS, low acidity, low starch-iodine test stained area, firmness, good eating quality, better pulp texture and pulp taste. The above observations are in line with the occurrence of 'Kullu' bud sport described previously. Both the horticulturally important characters i.e., development of solid red skin colour and early maturation of fruits are significant from consumer and market point of view. However, the shape of the fruits in bud sport is cylindrical as against conic in mother tree which otherwise may not be a preferred character. However, these are reports of change in the shape of the fruits in the bud sports (Inge *et al*., 2011 ; Michelangelo *et al*., 2013).

4.2.5 Apple Bud Sport 'Kullu 2'

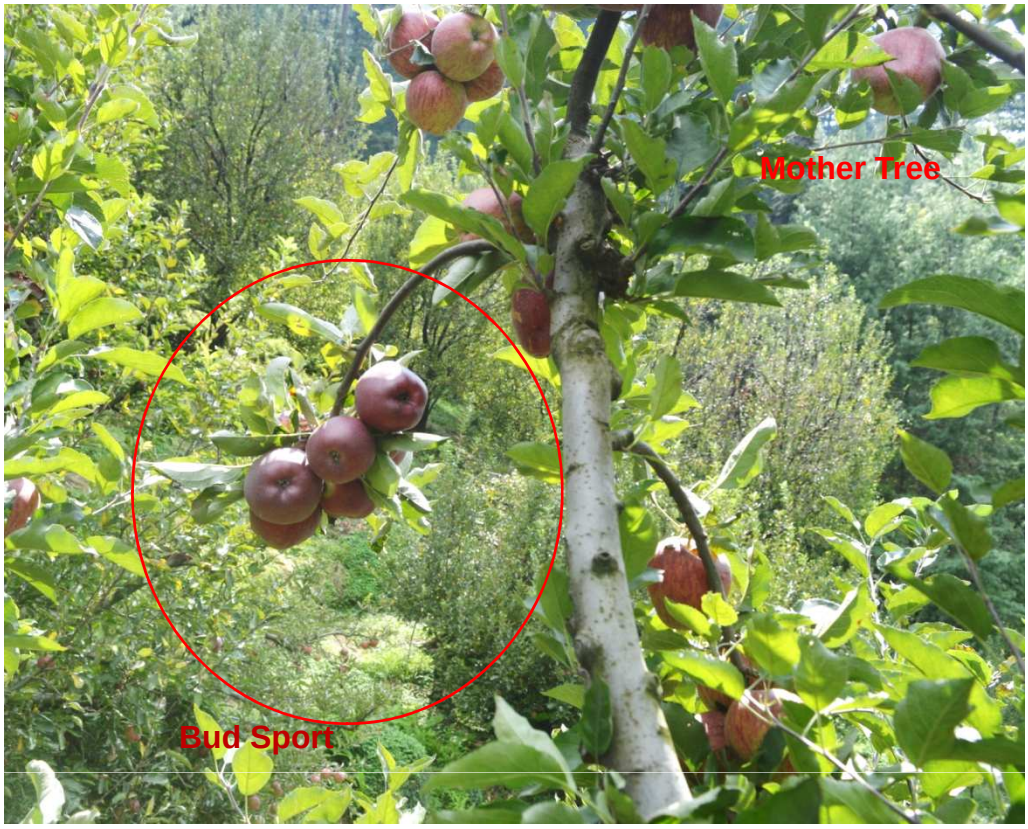
Bud sport 'Kullu 2' was identified in cv. Starking Delicious in Jana Naggar, district Kullu primarily due to solid colouring of fruit skin with respect to fruits on its mother tree. Development of solid colour over colour in the bud sport was observed at developing fruit stage (Table 4.6) as compared to streaked fruits in the mother tree till harvesting time (Plate 5). Apart from early colouring, fruits of bud sport 'Kullu 2' also recorded higher fruit weight (172.00 g), increased number of seed (10), less firm fruits (8.10 kg/cm^2), higher TSS (13.50 ^{o}Brix), higher total sugars (9.99 %) and low acidity (0.16 %) and low stained area (60 %) as per starch-iodine test as compared to corresponding values in mother tree (Table 4.6). Shape of the fruits in the bud sport were cylindrical waisted as against conic fruits in the mother tree. The remaining characters were recorded almost similar in mother tree as well as in the bud sport. It is revealed from the above findings that the bud sport 'Kullu 2' demonstrated not only solid fruit skin colour but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by high level of TSS, low acidity, starch-iodine test stained area, firmness, good eating quality, better pulp texture and pulp taste.

Besides the development of solid fruit skin in bud sport 'Kullu 2', other important characters like larger fruit size, higher TSS content and low acidity brings the identification of this bud sport with earlier described bud sports 'Kullu' and 'Kullu 1'. More so with 'Kullu 1' as it also recorded cylindrical waisted shape of the fruits against the conic fruits in the mother tree.

Table 4.5 : Phenological, flower and fruit characters of solid coloured bud sport 'Kullu 1' and mother tree in cv. Starking Delicious

District : Kullu	**Location : Jana Naggar**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Kullu 1**
Phenological characters		
Time of leaf bud burst	2nd Week of March	-
Time of floral bud burst	3rd -4th Week of March	-
Time of leaf fall	2nd Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	1st to 2nd Week April	-
Morpho-physical fruit characters		
Fruit weight (g)	**148.80**	**121.00**
Fruit length (cm)	6.72	5.92
Fruit breadth (cm)	7.06	6.53
Fruit shape	**Conic**	**Cylindrical**
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.2	1.2
Fruit apex	**Smooth**	**Grooved**
Fruit ground colour	Yellow green group 145 D	Yellow green group 144 D
Fruit over colour	**Greyed red group 180 A**	**Greyed purple group 185 B**
Fruit width of stripes	**Broad**	**Absent**
Type of over colour	**Streaked**	**Complete over colour**
Fruit skin lenticels	**Few**	**More**
Fruit attractiveness	**Intermediate**	**Good**
Flesh colour	**White**	**Cream**
Fruit firmness (kg/cm^2)	9.10	8.23
Number of seeds	**5**	**4**
Number of locules	5	5
Fruit aperture of locules	**Moderately open**	**Fully open**
Persistency of calyx	Present	Present
Harvest time	12th September	12th September
Eating quality	**Intermediate**	**Good**
Russet amount (%)	0	12
Russet type	**Absent**	**Few**
Organoleptic analysis		
Pulp texture	5	5
Pulp taste	4	6
Juiciness	5	6
Biochemical characters		
TSS (^{O}Brix)	**12.40**	**12.90**
Titratable acidity (%)	**0.26**	**0.21**
Total sugars (%)	9.42	9.55
Reducing sugars (%)	7.35	7.64
Non-reducing sugars (%)	1.97	1.81
Starch-Iodine Test Stained Area (%)	**75**	**60**

Plate 4 : Solid coloured bud sport 'Kullu 1' in cv. Starking Delicious



Developing Fruits

Mother Tree

Bud Sport

Matured Fruits

Plate 5 : Solid coloured bud sport 'Kullu 2' in cv. Starking Delicious

Developing Fruits

Mother Tree

Bud Sport

Matured Fruits

Table 4.6 : Phenological, flower and fruit characters of solid coloured bud sport 'Kullu 2' and mother tree in cv. Starking Delicious

District : Kullu	**Location : Jana Naggar**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Kullu 2**
Phenological characters		
Time of leaf bud burst	2nd Week of March	-
Time of floral bud burst	3rd -4th Week of March	-
Time of leaf fall	2nd Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	1st -2nd Week April	-
Morpho-physical fruit characters		
Fruit weight (g)	**146.50**	**172.00**
Fruit length (cm)	6.67	7.35
Fruit breadth (cm)	7.01	7.32
Fruit shape	**Conic**	**Cylindrical waisted**
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.6	1.7
Fruit apex	Grooved	Grooved
Fruit ground colour	**Yellow green group 149 D**	**No ground colour**
Fruit over colour	**Greyed red group 181 A**	**Greyed purple group 187 A**
Fruit width of stripes	**Medium**	**Absent**
Type of over colour	**Streaked**	**Completely over colour**
Fruit skin lenticels	**Few**	**Medium**
Fruit attractiveness	**Poor**	**Good**
Flesh colour	**White**	**Cream**
Fruit firmness (kg/cm^2)	8.80	8.10
Number of seeds	**6**	**10**
Number of locules	5	5
Fruit aperture of locules	**Moderately open**	**Fully Open**
Persistency of calyx	**Absent**	**Present**
Harvest time	12th September	12th September
Eating quality	Intermediate	Good
Russet amount (%)	12	12
Russet type	Few	Few
Organoleptic analysis		
Pulp texture	5	6
Pulp taste	5	6
Juiciness	6	5
Biochemical characters		
TSS (^{O}Brix)	**12.40**	**13.50**
Titratable acidity (%)	**0.28**	**0.16**
Total sugars	9.42	9.99
Reducing sugars (%)	7.35	7.99
Non-reducing sugars (%)	1.97	1.90
Starch-Iodine Test Stained Area (%)	**70**	**60**

4.2.6 Apple Bud Sport 'Manikaran 5'

Bud sport 'Manikaran 5' was identified in cv. Starking Delicious in Lari Manikaran, district Kullu due to solid colour development in the fruits with respect to its mother tree. Development of solid skin colour in fruits of the bud sport was observed at matured stage (Table 4.7) as compared to streaked fruits in the mother tree till harvesting time (Plate 6). Apart from solid fruit skin colour, bud sport 'Manikaran 5' also had medium sized fruits (147.16 g), less firm fruits (8.13 kg/cm^2), higher TSS (13.40 ^{o}Brix), higher total sugars (9.92 %), low acidity (0.19 %) and low stained area (70 %) as per starch-iodine test as compared to corresponding values in mother tree (Table 4.7). Bud sport also differed from mother tree in having grooved fruit apex, less number of seeds in the fruit and fruit over colour (Grayed purple group 185 A). The remaining characters recorded were almost similar in mother tree as well as in the bud sport. It is revealed from the above finding that the bud sport 'Manikaran 5' demonstrated not only solid fruit colour but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by high level of TSS, low acidity, low starch-iodine test stained area, firmness, good eating quality, better pulp texture and pulp taste. These observations are again somewhat similar to those observed for other identified bud sports in cv. Delicious growing in different orchards in district Kullu. Interestingly, the fruits of this bud sport were smaller and lighter against those of its mother tree unlike in other bud sports. Scientifically, no valid reason can be assigned to such an observation until the bud sport is propagated and multiplied and field tested as grown up trees.

4.2.7 Apple Bud Sport Kalpa 1

Bud sport 'Kalpa 1' was identified in cv. Starking Delicious in Kalpa, district Kinnaur primarily due to late colouring with respect to its mother tree. Development of slightly blush over colour in the bud sport was observed as late as on 15th September (Table 4.8) as compared to complete over colour in the mother tree before harvesting time (Plate 7). Apart from late colouring, fruits of bud sport 'Kalpa 1' also recorded differences in fruit weight (132.00 g), high fruit firmness (9.30 kg/cm^2), high stained area (80 %) as per starch-iodine test and fruit over colour (Grayed red group 181 B) as compared to corresponding values in mother tree (Table 4.8). The remaining characters recorded were almost similar in mother tree as well as in the bud sport. It is revealed from the above finding that the bud sport 'Kalpa 1' demonstrated not only late fruit skin colour development but also varied in fruit maturation time.

Plate 6 : Solid coloured bud sport 'Manikaran 5' in cv. Starking Delicious

Developing Fruits

Mother Tree

Bud Sport

Matured Fruits

Table 4.7 : Phenological, flower and fruit characters of solid coloured bud sport 'Manikaran 5' and mother tree in cv. Starking Delicious

District : Kullu	**Location : Lari Manikaran**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Manikaran 5**
Phenological characters		
Time of leaf bud burst	2^{nd} -3^{rd} Week of March	-
Time of floral bud burst	3^{rd} -4^{th} Week of March	-
Time of leaf fall	2^{nd} -3^{rd} Week of November	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	2^{nd} Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**187.16**	**147.16**
Fruit length (cm)	7.47	6.50
Fruit breadth (cm)	7.54	7.22
Fruit shape	Conic	Conic
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.6	1.5
Fruit apex	**Wrinkled**	**Grooved**
Fruit ground colour	Yellow green Group 145 D	Yellow green Group 145 D
Fruit over colour	**Greyed red group 181 A**	**Greyed purple group 185 A**
Fruit width of stripes	**Broad**	**Absent**
Type of over colour	**Streaked**	**Complete over colour**
Fruit skin lenticels	**Few**	**More**
Fruit attractiveness	**Intermediate**	**Good**
Flesh colour	**White**	**Cream**
Fruit firmness (kg/cm^2)	8.92	8.13
Number of seeds	**10**	**4**
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Persistency of calyx	Present	Present
Harvest time	15^{th} September	15^{th} September
Eating quality	**Intermediate**	**Good**
Russet amount (%)	12	12
Russet type	Few	Few
Organoleptic analysis		
Pulp texture	5	6
Pulp taste	6	6
Juiciness	6	6
Biochemical characters		
TSS (^{O}Brix)	**13.10**	**13.40**
Titratable acidity (%)	**0.23**	**0.19**
Total sugars (%)	9.83	9.92
Reducing sugars (%)	7.76	7.93
Non-reducing sugars (%)	1.96	1.88
Starch-Iodine Test Stained Area (%)	**85**	**70**

Table 4.8 : Phenological, flower and fruit characters of late colouring bud sport 'Kalpa 1' and mother tree in cv. Starking Delicious

District : Kinnaur	**Location : Kalpa**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Kalpa 1**
Phenological characters		
Time of leaf bud burst	1st Week of April	-
Time of floral bud burst	2nd -3rd Week of April	-
Time of leaf fall	1st Week of December	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	4th Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**145.75**	**132.00**
Fruit length (cm)	6.55	6.46
Fruit breadth (cm)	6.82	6.81
Fruit shape	Cylindrical	Cylindrical waisted
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.4	1.0
Fruit apex	Grooved	Grooved
Fruit ground colour	Yellow green Group 149 A	Yellow green Group 150 C
Fruit over colour	**Greyed purple group 185 B**	**Greyed red group 181 B**
Fruit width of stripes	**Absent**	**Broad**
Type of over colour	**Complete over colour**	**Slightly blushed**
Fruit skin lenticels	**Medium**	**Few**
Fruit attractiveness	Intermediate	Intermediate
Flesh colour	**White**	**Cream**
Fruit firmness (kg/cm^2)	9.10	9.30
Number of seeds	**7**	**6**
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Persistency of calyx	Present	Present
Harvest time	30th September	30th September
Eating quality	Good	Intermediate
Russet amount (%)	0	0
Russet type	Absent	Absent
Organoleptic analysis		
Pulp texture	5	6
Pulp taste	6	5
Juiciness	7	6
Biochemical characters		
TSS (^{O}Brix)	**13.50**	**13.10**
Titratable acidity (%)	0.18	0.23
Total sugars (%)	9.86	9.69
Reducing sugars (%)	7.49	7.46
Non-reducing sugars (%)	2.25	2.12
Starch-Iodine Test Stained Area (%)	**60**	**80**

Plate 7 : Late colouring bud sport ‘Kalpa 1’ in cv. Starking Delicious

Developing Fruits

Mother Tree **Bud Sport**

Matured Fruits

Late maturation in the fruits of bud sport is further indicated by low level of TSS, high acidity, high starch-iodine test stained area and fruit firmness.

Although late maturation time (Table 4.8 and Plate 7) in apple is largely considered an undesirable character from marketing point of view yet such genotypes can spread the season of fruit availability. At the same time, the perusal of literature suggest that there are hardly any reports of occurrence of late maturity bud sports in apple especially in cv. Delicious. However, there is a single report of a late maturing bud sport named 'Swedes Fuji' discovered on cv. Fuji (Dennis and Sterling, 2002). Perhaps this is obvious too as it is a least preferred character. Nevertheless, it can serve as a special genetic stock to look into the mechanism of fruit skin colouration in apple.

4.2.8 Apple Bud Sport 'Kalpa 2'

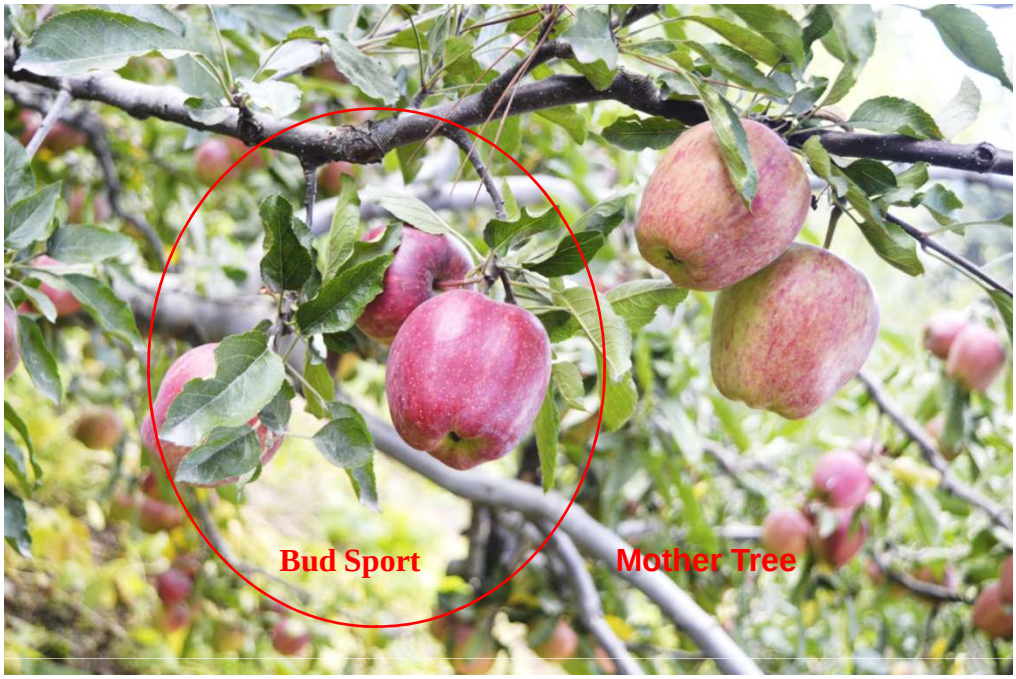
Bud sport 'Kalpa 2' was identified in cv. Starking Delicious in Kalpa, district Kinnaur primarily due to solid colour (washed out) with respect to its mother tree. Development of solid colour in the bud sport was observed at matured stage (Table 4.9) as compared to streaked fruits in the mother tree till harvesting time (Plate 8). Apart solid fruit skin colouring, fruits of bud sport 'Kalpa 2' differed from mother tree in having higher fruit weight (168.00 g), less firm fruits (8.40 kg/cm^2), low stained area (70 %) as per starch-iodine test and fruit over colour (Greyed red group 181 A) as compared to corresponding values in mother tree (Table 4.9). The remaining characters recorded were almost similar in mother tree as well as in the bud sport except TSS, total sugars and acidity. It is revealed from the above findings that the bud sport 'Kalpa 2' demonstrated not only solid fruit skin colour development but also varied in fruit maturation time. Early maturation in the fruits of bud sport is indicated by low starch-iodine stained area and firmness.

The occurrence of solid coloured (washed out) fruits in as many as five bud sports in the present study is a step forward towards developing intensely red coloured fruit cultivars as against streaked fruits in the mother cultivars. Fruit size was also higher in bud sport 'Kullu', 'Kullu 2' and 'Kalpa 2'. These findings are in confirmity with earlier reports on origin of solid coloured bud sports excelling in fruit size (Perleberg, 1980 ; Hare, 1985 ; Denardi *et al*., 1997 ; Leslie and Linda, 2002 ; Robert, 2004 ; Michelangelo and Carlo, 2008 ; Alois, 2016).

Table 4.9 : Phenological, flower and fruit characters of solid coloured bud sport 'Kalpa 2' and mother tree in cv. Starking Delicious

District : Kinnaur	**Location : Kalpa**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Kalpa 2**
Phenological characters		
Time of leaf bud burst	1st Week of April	-
Time of floral bud burst	2nd -3rd Week of April	-
Time of leaf fall	1st Week of December	-
Inflorescence and flowering characters		
Bearing habit	Standard	Standard
Time of flowering	4th Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**133.00**	**168.00**
Fruit length (cm)	6.52	7.09
Fruit breadth (cm)	6.67	7.11
Fruit shape	Cylindrical waisted	Cylindrical
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	0.6	0.8
Fruit apex	Grooved	Grooved
Fruit ground colour	Yellow green group 144 B	Yellow green group 149 B
Fruit over colour	**Greyed purple group 185 A**	**Greyed red group 181 A**
Fruit width of stripes	Medium	Absent
Type of over colour	**Streaked**	**Washed out (faded)**
Fruit skin lenticels	**Medium**	**Few**
Fruit attractiveness	**Intermediate**	**Good**
Flesh colour	**White**	**Cream**
Fruit firmness (kg/cm^2)	8.70	8.40
Number of seeds	7	8
Number of locules	5	5
Fruit aperture of locules	Fully open	Fully open
Persistency of calyx	Present	Present
Harvest time	29 September	29 September
Eating quality	**Good**	**Intermediate**
Russet amount (%)	**12**	**0**
Russet type	**Few**	**Absent**
Organoleptic analysis		
Pulp texture	6	5
Pulp taste	5	5
Juiciness	6	5
Biochemical characters		
TSS (^{O}Brix)	**13.60**	**12.90**
Titratable acidity (%)	0.17	0.21
Total sugars (%)	10.06	9.55
Reducing sugars (%)	7.95	7.73
Non-reducing sugars (%)	2.01	1.72
Starch-Iodine Test Stained Area (%)	**80**	**70**

Plate 8 : Solid coloured bud sport 'Kalpa 2' in cv. Starking Delicious



Developing Fruits

Mother Tree **Bud Sport**

Matured Fruits

4.2.9 Apple Bud Sport 'Kalpa 3'

Bud sport 'Kalpa 3' was identified in cv. Red Delicious in Kalpa, district Kinnaur primarily due to spur bearing with respect to its mother tree (Table 4.10 and Plate 9). Apart from spur bearing bud sport 'Kalpa 3' recorded higher fruit weight (250.00 g), increased number of seed (8) and higher TSS (13.30 ^{o}Brix) as compared to corresponding values in the mother tree (Table 4.10). The remaining characters recorded were almost similar in mother tree as well as in the bud sport. It is revealed from the above finding that the bud sport 'Kalpa 3' demonstrated not only spur bearing but also varied in fruit size. Occurrence of bud sports exhibiting spur-type bearing unlike standard bearing habit of mother cultivars is again very common in apple. It is preferred also because spur bearing genotype are dwarf in the tree size and as such extremely suitable for raising apple planting at a closer distance (plant-to-plant and row-to-row), and also are amenable to mechanization. As early as in 1921, first spur-type mutation 'Okanoma' was observed in cv. Delicious (Maas, 1970) and later on in other cvs. Golden Delicious, McIntosh, Granny Smith besides of course cv. Delicious (Meheriuk and Fisher, 1973).

The observations of larger fruits (Table 4.10 and Plate 9) in 'Kalpa 3' than in standard Red Delicious are in conformity with the similar observations recorded in 'Richared' (Sullivan, 1965).

4.2.10 Apple Bud Sport 'Tabo 1'

Bud sport 'Tabo 1' was identified in cv. Starking Delicious in Tabo, district Lahaul & Spiti primarily due to spur bearing habit with respect to its mother tree (Table 4.11 and Plate 10). Apart from spur bearing bud sport 'Tabo 1' differed in having higher fruit weight (200.00 g) increased number of seed (6), high fruit firmness (9.10 kg/cm^2) and over colour (Greyed purple group 185 A) in the form of slight blush as compared to corresponding values in mother tree (Table 4.11). The remaining characters recorded were almost similar in mother tree as well as in the bud sport except high TSS, total sugars and low acidity in the mother tree. It is revealed from the above finding that the bud sport 'Tabo 1' demonstrated not only spur bearing but also varied in fruit maturation time. Late maturation in the fruits of 'Tabo 1' bud sport is indicated by low level of TSS, high acidity, high starch-iodine test stained area and firmness.

Identification of spur-type bud sports 'Tabo 1' and 'Kalpa 3' in cv. Starking Delicious at Tabo and Red Delicious at Kalpa locations are suggestive of the fact that this particular

Table 4.10 : Phenological, flower and fruit characters of spur-type bud sport 'Kalpa 3' and mother tree in cv. Red Delicious

District : Kinnaur	**Location : Kalpa**	
Characters	**Cultivar : Red Delicious**	**Bud Sport : Kalpa 3**
Phenological characters		
Time of leaf bud burst	1st Week of April	-
Time of floral bud burst	2nd -3rd Week of April	-
Time of leaf fall	1st Week of December	-
Inflorescence and flowering characters		
Bearing habit	**Standard**	**Spur**
Time of flowering	4th Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**213.00**	**250.00**
Fruit length (cm)	7.93	6.89
Fruit breadth (cm)	7.87	8.67
Fruit shape	Conic	Conic
Surface of fruit	Smooth	Smooth
Fruit base	Broad	Broad
Fruit base cavity depth (cm)	1.0	0.9
Fruit apex	Grooved	Grooved
Fruit ground colour	Yellow green group 150 A	Yellow green group 150 C
Fruit over colour	Greyed purple group 185 A	Greyed purple group 185 B
Fruit width of stripes	Absent	Absent
Type of over colour	Washed out (faded)	Washed out (faded)
Fruit skin lenticels	Medium	Medium
Fruit attractiveness	Intermediate	Intermediate
Flesh colour	White	White
Fruit firmness (kg/cm^2)	8.10	8.34
Number of seeds	5	8
Number of locules	5	5
Fruit aperture of locules	Fully open	Fully open
Persistency of calyx	Present	Present
Harvest time	30 September	30 September
Eating quality	Intermediate/good	Intermediate/good
Russet amount (%)	**25**	**0**
Russet type	**Medium**	**Absent**
Organoleptic analysis		
Pulp texture	5	5
Pulp taste	5	6
Juiciness	6	6
Biochemical characters		
TSS (^{O}Brix)	13.10	13.30
Titratable acidity (%)	0.18	0.19
Total sugars (%)	9.83	10.11
Reducing sugars (%)	7.57	7.88
Non-reducing sugars (%)	2.15	2.11
Starch-Iodine Test Stained Area (%)	60	65

Plate 9 : Spur-type limb sport 'Kalpa 3 in cv. Red Delicious

Developing Fruits

Matured Fruits

Plate 10 : Spur-type limb sport ‘Tabo 1’ in cv. Starking Delicious

Developing Fruits

Matured Fruits

Table 4.11 : Phenological, flower and fruit characters of spur bearing bud sport 'Tabo 1' and mother tree in cv. Starking Delicious

District : Lahaul & Spiti	**Location : Tabo**	
Characters	**Cultivar : Starking Delicious**	**Bud Sport : Tabo 1**
Phenological characters		
Time of leaf bud burst	1st Week of April	-
Time of floral bud burst	2nd -3rd Week of April	-
Time of leaf fall	1st Week of December	-
Inflorescence and flowering characters		
Bearing habit	**Standard**	**Spur**
Time of flowering	4th Week of April	-
Morpho-physical fruit characters		
Fruit weight (g)	**168.33**	**200.00**
Fruit length (cm)	6.71	7.55
Fruit breadth (cm)	7.31	7.88
Fruit shape	Cylindrical	Cylindrical
Surface of fruit	Smooth	Smooth
Fruit base	Intermediate	Intermediate
Fruit base cavity depth (cm)	1.2	1.1
Fruit apex	Grooved	Grooved
Fruit ground colour	Yellow green group 144 C	Yellow green group 145 D
Fruit over colour	**Greyed red group 180 A**	**Greyed purple group 185 A**
Fruit width of stripes	**Absent**	**Medium**
Type of over colour	**Complete over colour**	**Slightly blushed**
Fruit skin lenticels	**High**	**Medium**
Fruit attractiveness	**Good**	**Intermediate**
Flesh colour	White	White
Fruit firmness (kg/cm^2)	8.90	9.10
Number of seeds	6	6
Number of locules	5	5
Fruit aperture of locules	Moderately open	Moderately open
Persistency of calyx	Present	Present
Harvest time	27th September	27th September
Eating quality	**Very good**	**Good**
Russet amount (%)	0	0
Russet type	Absent	Absent
Organoleptic analysis		
Pulp texture	7	6
Pulp taste	7	7
Juiciness	8	7
Biochemical characters		
TSS (^{O}Brix)	**13.70**	**13.20**
Titratable acidity (%)	0.16	0.19
Total sugars (%)	10.28	9.77
Reducing sugars (%)	7.91	7.62
Non-reducing sugars (%)	2.25	2.04
Starch-Iodine Test Stained Area (%)	**60**	**70**

cultivars are highly prone to spontaneous mutations. This has been contended by earlier workers also that 'Delicious' demonstrates large scale occurrence of bud sports as compared to other apple cultivars (Fischer and Ketchie, 1981 ; 1989; Childers *et al*., 1995 ; Ferree and Warrington, 2003). The occurrence of spur-type bud sports (Table 4.11 and Plate 10) are of paramount importance as these are generally compact in growth habit and would suit a great deal under high density planting.

Summarizing the morpho-physical, biochemical and organoleptic analysis of all the 10 identified bud sports are suggestive of existence of valuable gene pool of apple in Himachal Pradesh. As a matter of fact, all these observations are too preliminary and need to be validated by raising extensive multi- location field trials in apple growing areas of Himachal Pradesh. Further, the data with respect to fruit recovery from each of the mother tree from which the bud sports have been identified are moderate to high in fruit yield. Given the yield potential and adaptation of the mother trees, it would be all the more worthwhile to test the identified bud sports for most agronomic characters.

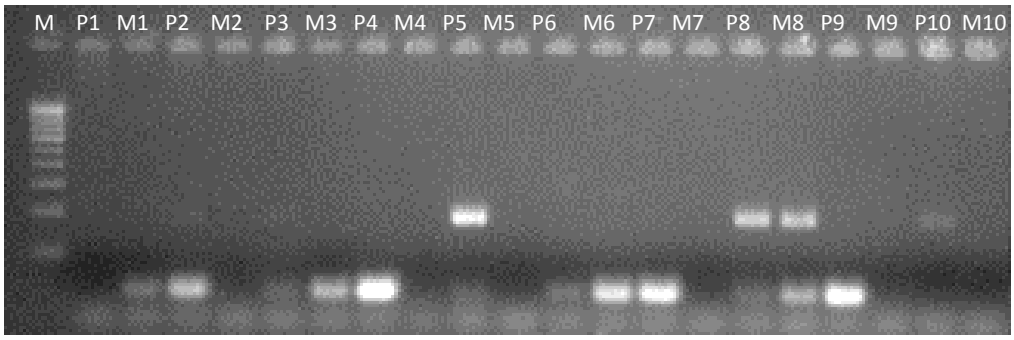
4.3 MOLECULAR MARKER STUDIES

Use of molecular (DNA) markers fast emerged as a viable genetic tool to ascertain differences between spontaneous (bud) mutations and their parent cultivars. Although some studies did not yield encouraging results (Ferree and Warrington, 2003) yet recent developments point towards usefulness of more robust and reliable DNA markers.

Both genomic and EST derived simple sequence repeats (SSR) markers were used in present study to determine differences between identified bud sports with their corresponding mother trees to validate the observed variations in the bud sports. The salient findings obtained are presented in Plate 11 to 30 and are discussed as under:

4.3.1 Genomic Simple Sequence Repeats (SSRs)

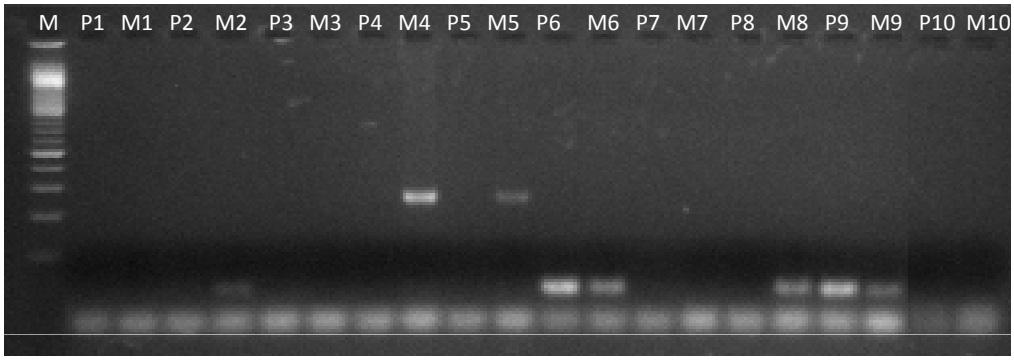
Out of a total of 10 genomic SSR primers used in the present study, as many as eight were found to be informative and yielded polymorphism to a considerable extent. The pattern generated by each of the informative genomic SSR primer is described as under:



SSR Pattern of apple mother trees and bud sports generated by primer CH01f02

F: ACC ACA TTA GAG CAG TTG AGG
R: CTG GTT TGT TTT CCT CCA GC

Plate 11

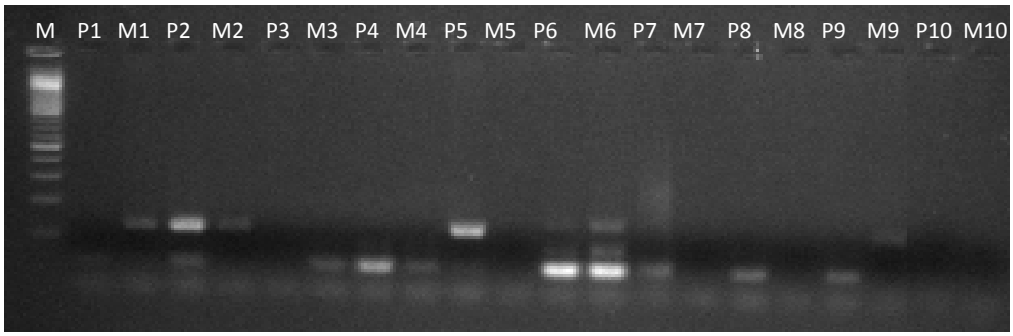


SSR Pattern of apple mother trees and bud sports generated by primer CH01d08

F: CTC CGC CGC TAT AAC ACT TC
R: TAC TCT GGA GGG TAT GTC AAA G

Plate 12

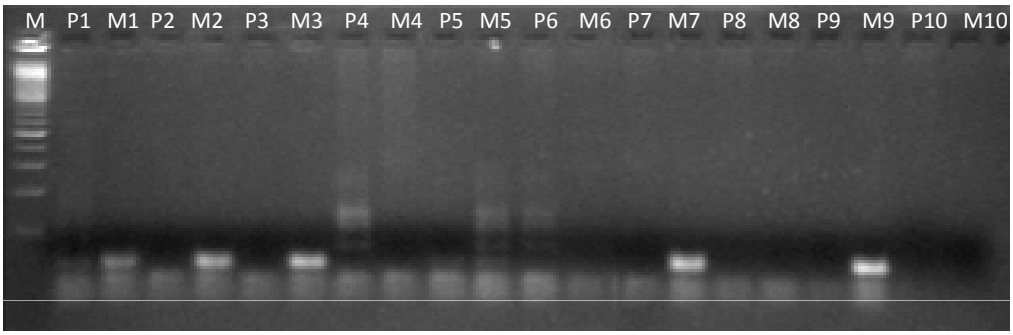
Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



SSR Pattern of apple mother trees and bud sports generated by primer CH01h01

F: GAA AGA CTT GCA GTG GGA GC
R: GGA GTG GGT TTG AGA AGG TT

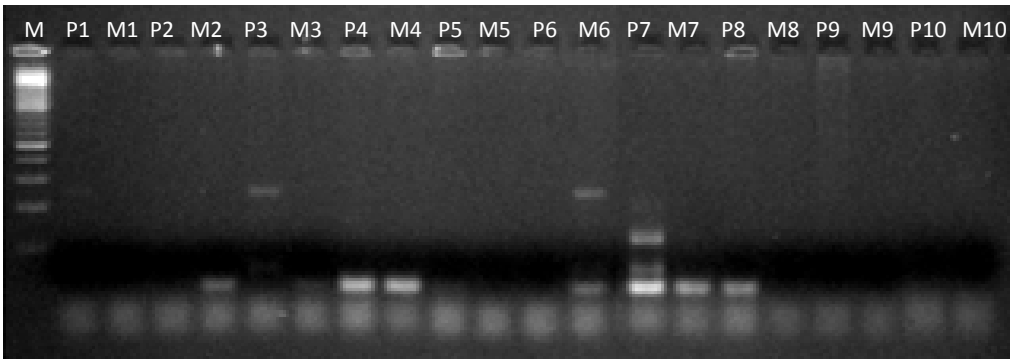
Plate 13



SSR Pattern of apple mother trees and bud sports generated by primer CH02a04
F: GAA ACA GGC GCC ATT ATT TG
R: AAA GGA GAC GTT GCA AGT GG

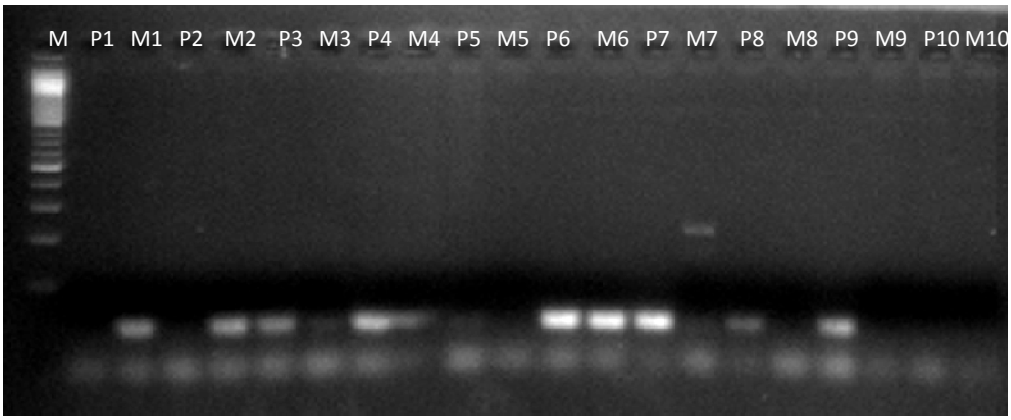
Plate 14

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



SSR Pattern of apple mother trees and bud sports generated by primer CH01e12
F: AAA CTG AAG CCA TGA GGG C
R: TTC CAA TTC ACA TGA GGC TG

Plate 15



SSR Pattern of apple mother trees and bud sports generated by primer CH01a07b
F: AAC CCA TGA AAC ACA ATC CC
R: GGA ACG ATC CAT AGG TGG TG

Plate 16

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2

4.3.1.1 Genomic SSR pattern with primer CH01f02

Primer CH01f02 generated seven polymorphic bands indicating therefore, that the variations in identified bud sports are the result of genetic differences *vis-à-vis* their mother trees. Primer CH01f02 showed polymorphism between seven bud sports and their mother trees. These are namely Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 ; Kullu and Kullu M ; Kullu 2 and Kullu M2 ; Shimla 1 and Shimla M1 ; Shimla 2 and Shimla M2 (Plate 11).

4.3.1.2 Genomic SSR pattern with primer CH01d08

CH01d08 amplified four bands which were polymorphic. CH01d08 primer showed polymorphism between four bud sports and their mother trees viz., Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 ; Kullu and Kullu M ; Manikaran 5 and Manikaran M5 (Plate 12).

4.3.1.3 Genomic SSR pattern with primer CH01h01

CH01h01 primer showed polymorphism in as many as seven bud sports and their mother trees. The polymorphism was observed in Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu and Kullu M ; Kullu 2 and Kullu M2 ; Manikaran 5 and Manikaran M5 ; Shimla 1 and Shimla M1 (Plate 13).

4.3.1.4 Genomic SSR pattern with primer CH02a04

Seven polymorphic bands were generated by Primer CH02a04 in seven different bud sports and their respective mother trees namely, Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kalpa 3 and Kalpa M3 ; Kullu and Kullu M ; Kullu 1 and Kullu M1 ; Kullu 2 and Kullu M2 ; Shimla 1 and Shimla M1 (Plate 14).

4.3.1.5 Genomic SSR pattern with primer CH01e12

Primer CH01e12 generated five bands which were polymorphic between bud sports and their corresponding mother trees. The polymorphism was found in Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu 1 and Kullu M1 ; Kullu 2 and Kullu M2 ; Manikaran 5 and Manikaran M5 (Plate 15).

4.3.1.6 Genomic SSR pattern with primer CH01a07b

Primer CH01a07b exhibited polymorphism in six different bud sports as against their respective mother trees. The polymorphism was observed in Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu 2 and Kullu M2 ; Manikaran 5 and Manikaran M5 ; Shimla 1 and Shimla M1 (Plate 16).

4.3.1.7 Genomic SSR pattern with primer CH01f03b

CH01f03b amplified eight bands which were polymorphic. CH01f03b primer was polymorphic in eight different bud sports vis-à-vis their mother trees namely, Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu and Kullu M ; Kullu 1 and Kullu M1 ; Manikaran 5 and Manikaran M5 ; Shimla 1 and Shimla M1 ; Shimla 2 and Shimla M2 (Plate 17).

4.3.1.8 Genomic SSR pattern with primer CH05e03

Primer CH05e03 generated seven polymorphic bands indicating genetic differences between seven bud sports and mother trees. Primer CH05e03 showed polymorphism in Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kalpa 3 and Kalpa M3 ; Kullu and Kullu M ; Kullu 1 and Kullu M1 ; Kullu 2 and Kullu M2 (Plate 18).

4.3.2 EST- derived simple sequence repeats (EST-SSRs)

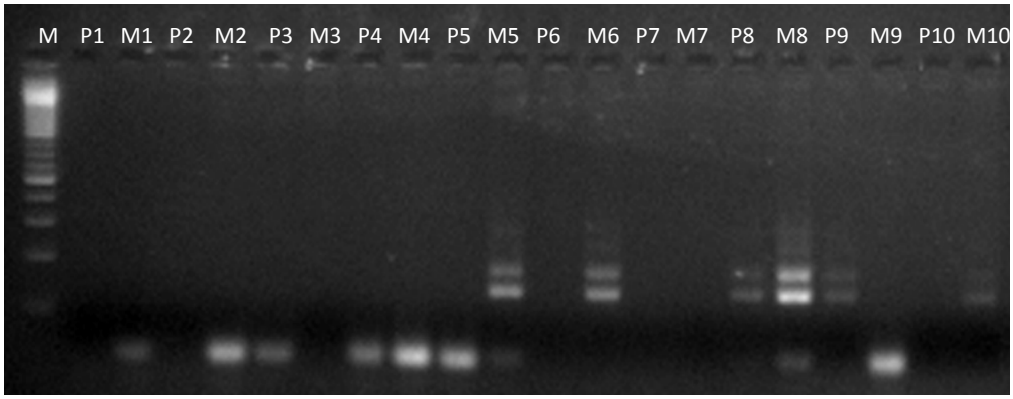
Out of a total of 20 EST-SSRs primers, 12 were found to be informative yielding polymorphism to determine differences between bud sports and their corresponding mother trees. The pattern generated by each of the polymorphic EST-SSRs primer is presented as under :

4.3.2.1 EST-SSR pattern with primer E1

As many as five polymorphic bands were generated by primer E1 to elucidate into differences between bud sports and their respective mother trees. The polymorphism was observed in Tabo 1 and Tabo M1 ; Kalpa 3 and Kalpa M3 ; Kullu 1 and Kullu M1 ; Kullu 2 and Kullu M2 ; Manikaran 5 and Manikaran M5 (Plate 19).

4.3.2.2 EST-SSR pattern with primer E2

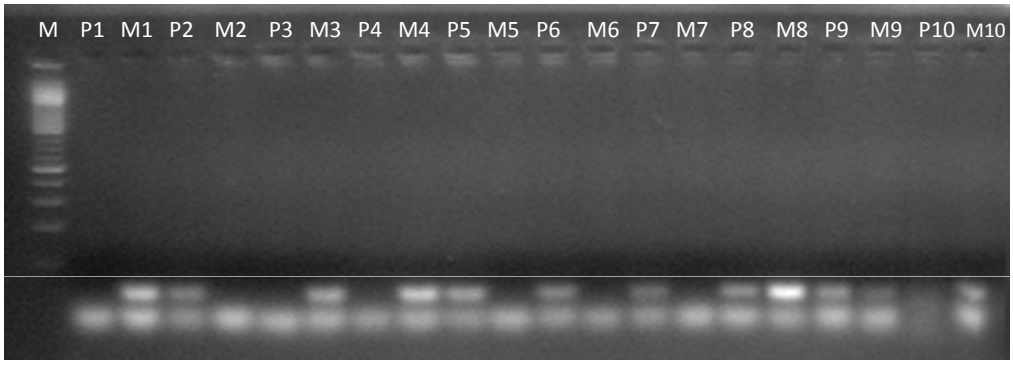
Primer E2 amplified six bands which were polymorphic. E2 primer showed polymorphism in six different bud sports as against their mother trees namely Tabo 1 and Tabo M1 ; Kalpa 2 and Kalpa M2 ; Kalpa 3 and Kalpa M3 ; Kullu 1 and Kullu M1 ; Shimla 1 and Shimla M1 ; Shimla 2 and Shimla M2 (Plate 20).



SSR Pattern of apple mother trees and bud sports generated by primer CH01f03b

F: GAG AAG CAA ATG CAA AAC CC
R: CTC CCC GGC TCC TAT TCT AC

Plate 17

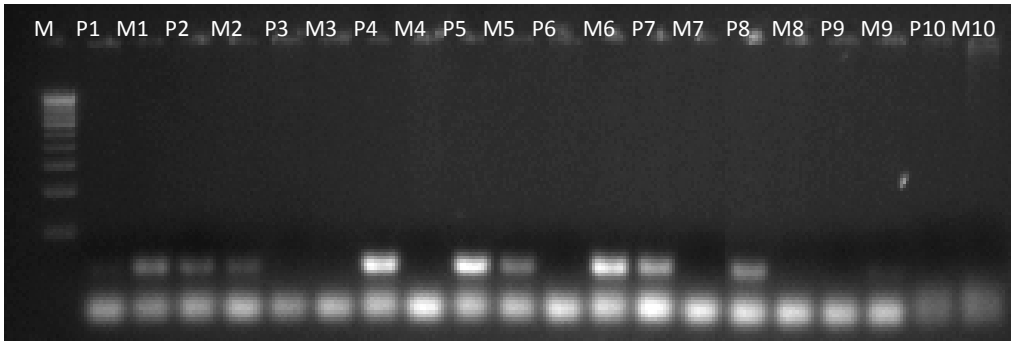


SSR Pattern of apple mother trees and bud sports generated by primer CH05e03

F: CGA ATA TTT TCA CTC TGA CTG GG
R: CAA GTT GTT GTA CTG CTC CGA C

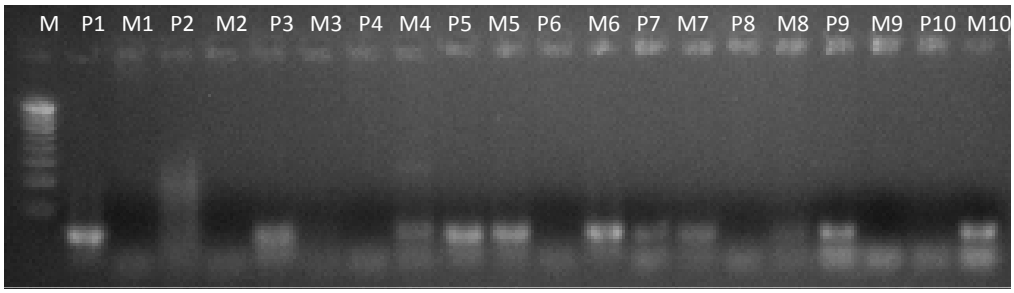
Plate 18

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



EST-SSR Pattern of apple mother trees and bud sports generated by primer E1
F: CGGTGCATGTACAACACGTA
R: TCCAGCAGAAGAGGTGATGA

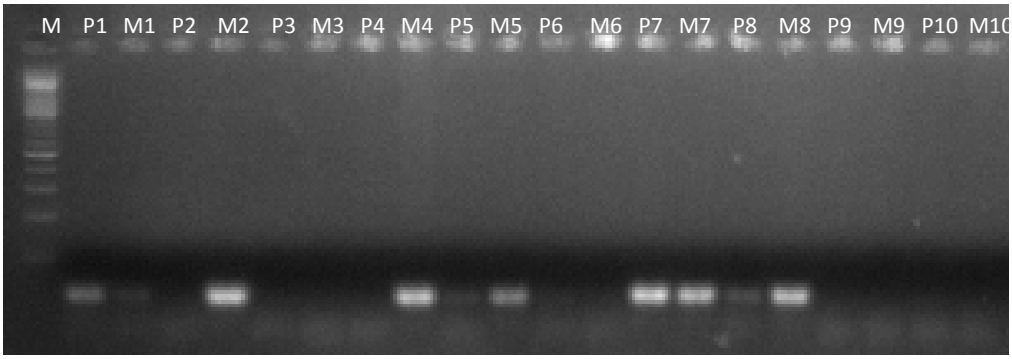
Plate 19



EST-SSR Pattern of apple mother trees and bud sports generated by primer E2
F: GTATTGGCTCCACCACAACC
R: GAAACCCGGTCGTGTAAAAA

Plate 20

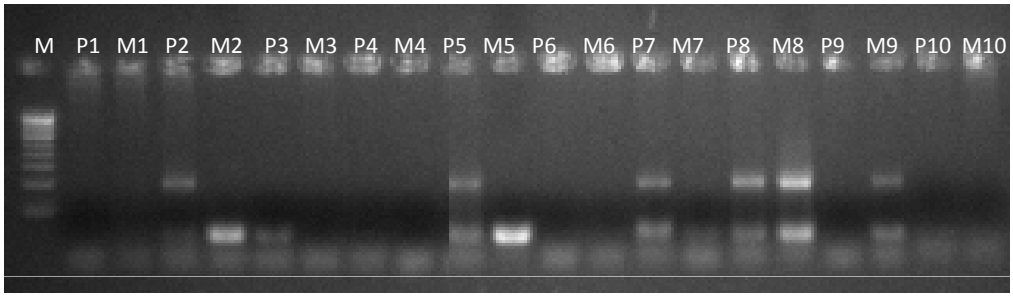
Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



EST-SSR Pattern of apple mother trees and bud sports generated by primer E3

F: AAAGTGGCGTAAGGTTGTGG
R: CACGCTGGACACATACATCC

Plate 21

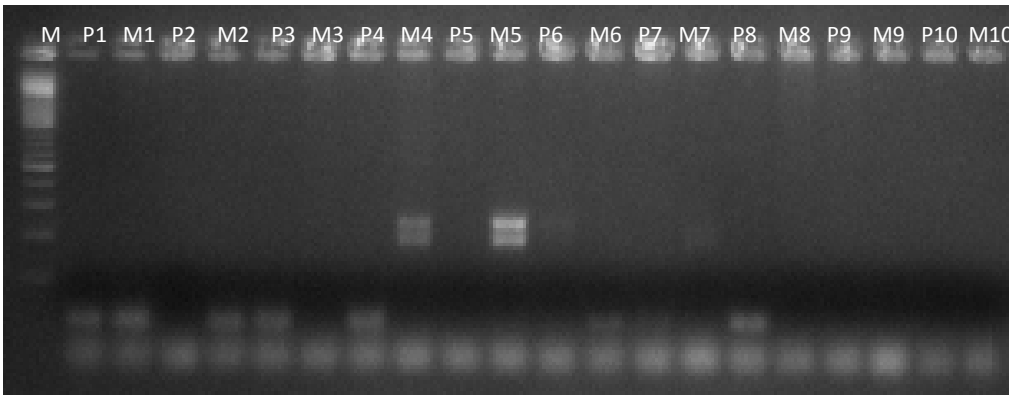


EST-SSR Pattern of apple mother trees and bud sports generated by primer E5

F: GGCCCAAATGTCATCGAATA
R: TATTCCAAATTCCCGGACTG

Plate 22

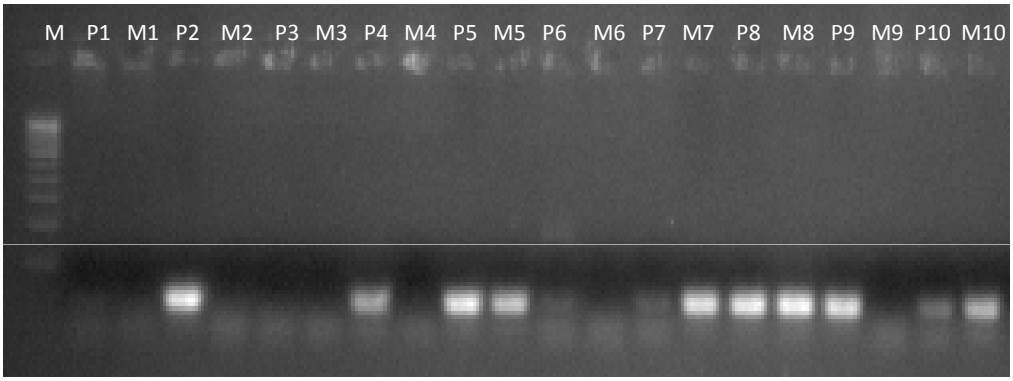
Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



EST-SSR Pattern of apple mother trees and bud sports generated by primer E6

F: AACATATGGGGCAGCGTATT
R: TTGTGATGCTTGTGGGGATA

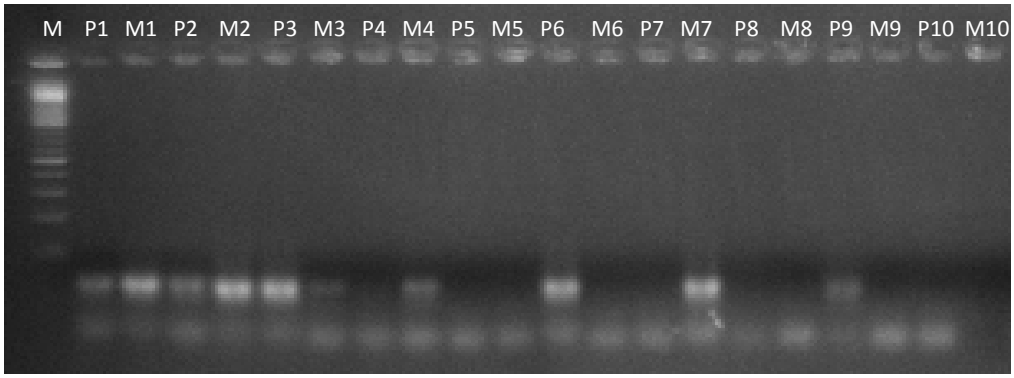
Plate 23



EST-SSR Pattern of apple mother trees and bud sports generated by primer E11
F: CACTTGGGCCAATTTCTGAC
R: CCAAGGAGTGAGTGGAGGAG

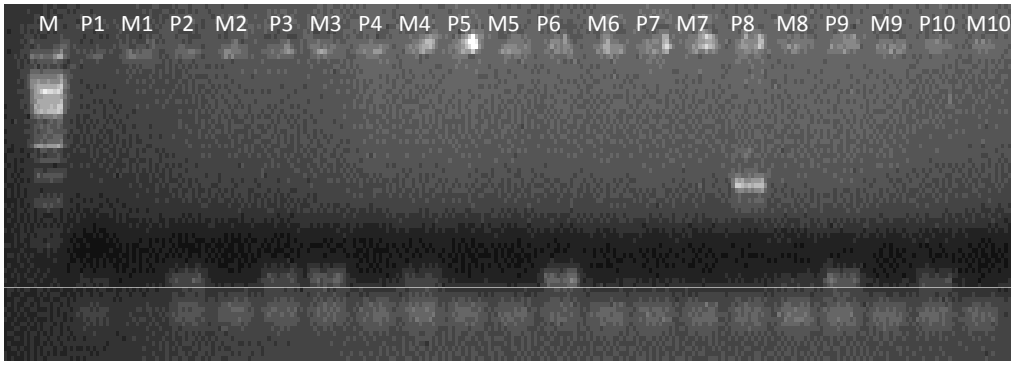
Plate 24

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



EST-SSR Pattern of apple mother trees and bud sports generated by primer E12
F: TTCCAACGACACCAACCTTT
R: GCGATGATGTATGGCACAGA

Plate 25



EST-SSR Pattern of apple mother trees and bud sports generated by primer E13
F: TCCGACAAATCAACATTGGA
R: CTGACGGACGCTCATAACAA

Plate 26

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2

4.3.2.3 EST-SSR pattern with primer E3

Primer E3 generated only two polymorphic bands to validate the differences between identified bud sports and their corresponding mother trees. Polymorphism was observed between Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 (Plate 21).

4.3.2.4 EST-SSR pattern with primer E5

Primer E5 amplified five bands which were polymorphic. E5 primer showed polymorphism in Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu and Kullu M ; Kullu 2 and Kullu M2 ; Shimla 1 and Shimla M1 (Plate 22).

4.3.2.5 EST-SSR pattern with primer E6

Primer E6 produced five polymorphic bands in five bud sports that were different from their respective mother trees. The polymorphism was found in Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kalpa 3 and Kalpa M3 ; Kullu and Kullu M ; Kullu 1 and Kullu M1 (Plate 23).

4.3.2.6 EST-SSR pattern with primer E11

Primer E11 amplified four bands which were polymorphic. Polymorphism was observed between four bud sports and their corresponding mother trees namely Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 ; Kullu 1 and Kullu M1 ; Shimla 1 and Shimla M1 (Plate 24).

4.3.2.7 EST-SSR pattern with primer E12

Primer E12 generated four polymorphic bands to validate the variation observed in bud sports as against their respective mother trees. Primer E12 showed polymorphism between Kalpa 3 and Kalpa M3 ; Kullu 1 and Kullu M1 ; Kullu 2 and Kullu M2 ; Shimla 1 and Shimla M1 (Plate 25).

4.3.2.8 EST-SSR pattern with primer E13

Primer E13 amplified six bands which were polymorphic for as many bud sports *vis-a-vis* their mother trees. The polymorphism was observed in Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 ; Kullu 1 and Kullu M1 ; Manikaran 5 and Manikaran M5 ; Shimla 1 and Shimla M1 ; Shimla 2 and Shimla M2 (Plate 26).

4.3.2.9 EST-SSR pattern with primer E18

Primer E18 amplified three bands which were polymorphic. E18 primer generated polymorphism between Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu and Kullu M (Plate 27) indicating differences between bud sports from their corresponding mother trees.

4.3.2.10 EST-SSR pattern with primer E23

As many as five bands were generated by primer E23 exhibiting polymorphism between bud sports and corresponding mother trees which are Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 3 and Kalpa M3 ; Shimla 1 and Shimla M1 ; Shimla 2 and Shimla M2 (Plate 28).

4.3.2.11 EST-SSR pattern with primer E24

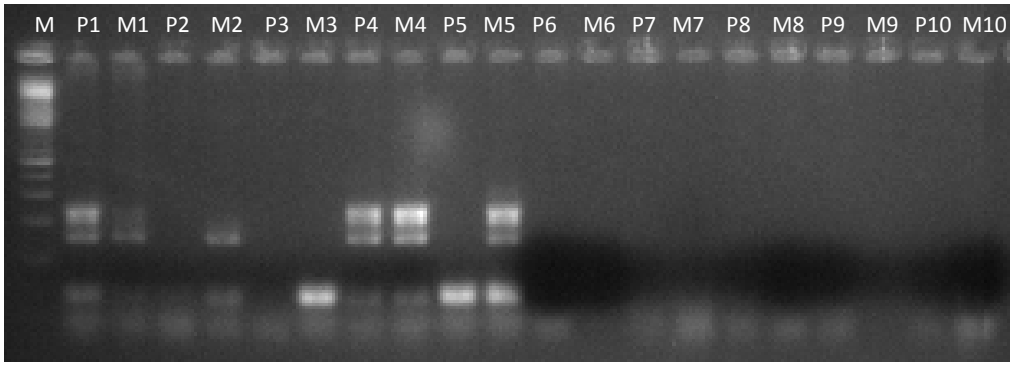
Primer E24 generated only one polymorphic band (Plate 29) to validate the variation observed in bud sport (Kullu M) from its mother tree (Kullu).

4.2.2.12 EST-SSR pattern with primer E25

As many as seven polymorphic bands were generated by primer E25 indicating differences between bud sports and their mother trees. The polymorphism was observed in Tabo 1 and Tabo M1 ; Kalpa 1 and Kalpa M1 ; Kalpa 2 and Kalpa M2 ; Kullu and Kullu M ; Kullu 2 and Kullu M2 ; Manikaran 5 and Manikaran M5 ; Shimla 2 and Shimla M2 (Plate 30).

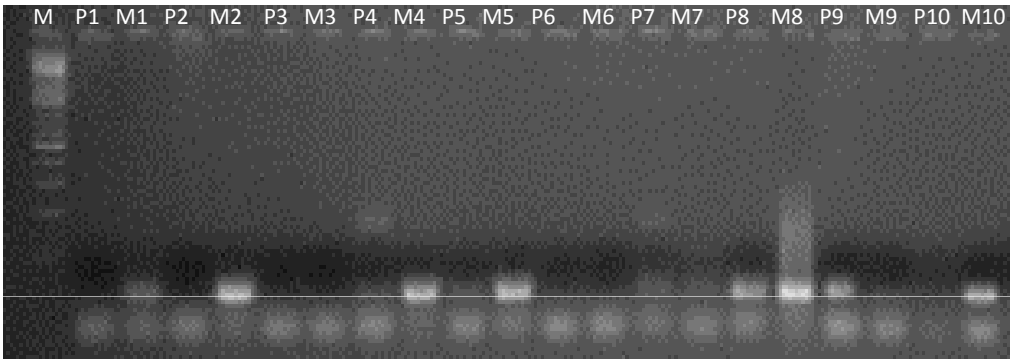
Aforesaid genomic SSR and EST-SSR pattern reveals that a considerable deal of polymorphism was observed in the present study as evident from number of informative primers/bands (Table 4.12) recorded. It is suggestive of some differences at DNA level between the identified bud sports and their mother trees. Overall, both the marker system used in the present study gave considerably high level of polymorphism (Table 4. 12) revealing genetic differences. Similar findings have been reported in apple earlier also (Korban *et al.*, 2005 ; Newcomb *et al.*, 2006) while carrying genetic diversity analysis. Wang (2009) reported one polymorphic band between bud sport and its progenitor. The above findings are preliminary in nature and warrant more detailed studies.

There is an absolute need to carry out further studies using more advanced and robust genetic marker tool to clearly distinguish and detect genetic variation between these bud sports and their parent trees.



EST-SSR Pattern of apple mother trees and bud sports generated by primer E18
F: TGGTGTGCCCTGAAGTGTTA
R: TATGGGAGCAGCAAATACCG

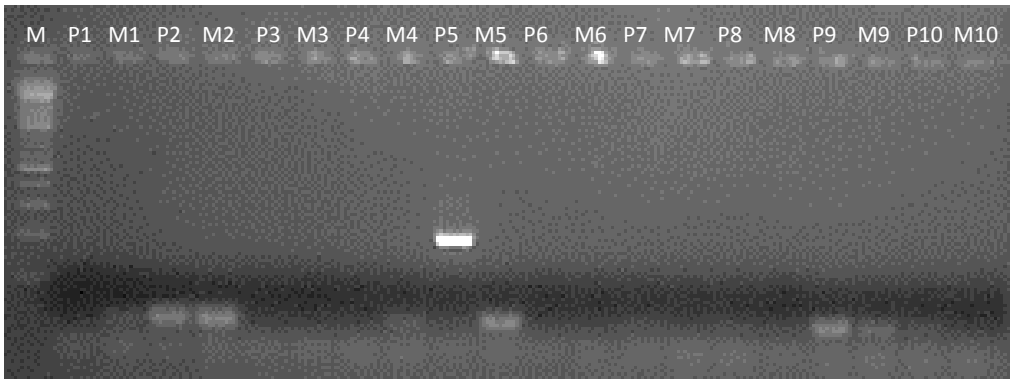
Plate 27



EST-SSR Pattern of apple mother trees and bud sports generated by primer E23
F: TGTTAGATCCGGACCCACTC
R: GATGGAAGGGGAGAAGAGC

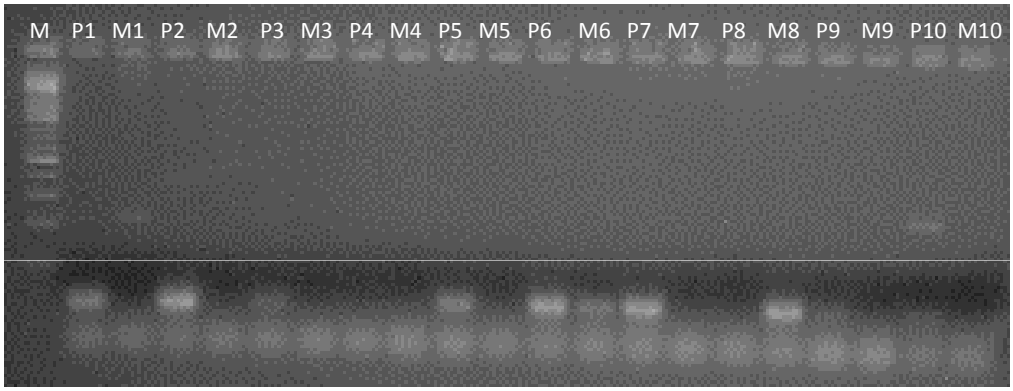
Plate 28

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2



EST-SSR Pattern of apple mother trees and bud sports generated by primer E24
F: TGAGGTGACCACAAGCAAAG
R: CAATTCTTTTGTGCGCGTAT

Plate 29



EST-SSR Pattern of apple mother trees and bud sports generated by primer E25
F: TTTTCCCCCTCAAGAACAAA
R: TGAAGCCGAAGGTTCATCAT

Plate 30

Code used for DNA studies	Description
P1	Tabo 1- mother tree (Cv. Starking Delicious) in Tabo
M1	Tabo M1- bud sport of Tabo 1
P2	Kalpa 1- mother tree (Cv. Starking Delicious) in Kalpa
M2	Kalpa M1- bud sport of Kalpa 1
P3	Kalpa 2- mother tree (Cv. Starking Delicious) in Kalpa
M3	Kalpa M2- bud sport of Kalpa 2
P4	Kalpa 3- mother tree (Cv. Red Delicious) in Kalpa
M4	Kalpa M3- bud sport of Kalpa 3
P5	Kullu- mother tree (Cv. Starking Delicious) in Kullu
M5	Kullu M- bud sport of Kullu
P6	Kullu 1- mother tree (Cv. Starking Delicious) in Kullu 1
M6	Kullu M1- bud sport of Kullu 1
P7	Kullu 2- mother tree (Cv. Starking Delicious) in Kullu2
M7	Kullu M2- bud sport of Kullu 2
P8	Manikaran 5- mother tree (Cv. Starking Delicious) in Manikaran 5
M8	Manikaran M5 - bud sport of Manikaran 5
P9	Shimla 1- mother tree (Cv. Red Delicious) in Shimla 1
M9	Shimla M1- bud sport of Shimla 1
P10	Shimla 2- mother tree (Cv. Vance Delicious) in Shimla 2
M10	Shimla M2- bud sport of Shimla 2

Table 4.12: Summary of polymorphism using genomic SSRs and EST-SSRs

S. No.	**Polymorphic Genomic Primer**	**No. of Polymorphic Bands**
1	CH01f02	7
2	CH01d08	4
3	CH01h01	7
4	CH02a04	7
5	CH01e12	5
6	CH01a07b	6
7	CH01f03b	8
8	CH05e03	7
	Total	**51**
	Polymorphic EST-SSRs Primer	**No. of Polymorphic Bands**
1	E1	5
2	E2	6
3	E3	2
4	E5	5
5	E6	5
6	E11	4
7	E12	4
8	E13	7
9	E18	3
10	E23	5
11	E24	1
12	E25	7
	Total	**54**

4.4 CLONAL PROPAGATION

All the 10 identified bud sports of apple resulting from present investigation were vegetatively propagated (Plate 31) to raise the plants for the further studies in future. Bud wood was collected from the marked trees in 2017 and unsuccessful grafts was again collected in 2019 and vegetatively propagated. This work done in accordance with the contentions of previous workers with respect to determination of stable nature of altered character and its perpetuation (Brown, 1975 ; Lacey and Compbell, 1987).

Kullu, Kullu 1, Kullu 2

Kullu, Manikaran 5

Kalpa 1, Kalpa 2

Kalpa 3, Tabo 1

Plate 31 : Clonal propagation of some identified apple bud sports

Chapter-5
SUMMARY AND CONCLUSION

The present investigation **"Studies on identification of bud sports in apple (*Malus* x *domestica* Borkh.)"** were carried out during the period 2016-18. The various findings obtained in the study are summarized as under :-

1. Extensive field surveys were conducted to screen existing apple plantations in as many as 40 orchards located in the districts of Shimla, Kullu, Kinnaur and Lahaul & Spiti of Himachal Pradesh during 2016-17.
2. A total of 7154 apple trees were preliminary screened for identification of bud sports based on pre-selection criteria of variations in bearing habit, time and pattern of fruit skin colour development, time of maturation and other horticulturally desirable characters.
3. As many as 10 bud sports were identified namely Shimla 1, Shimla 2, Kullu, Kullu 1, Kullu 2, Manikaran 5, Tabo 1, Kalpa 1, Kalpa 2 and Kalpa 3. Out of these 10, two were early colouring (Shimla 1, Shimla 2), five solid coloured (Kullu, Kullu 1, Kullu 2, Manikaran 5, Kalpa 2), two spur-type (Kalpa 3, Tabo 1) and one late colouring (Kalpa 1).
4. All the identified bud sports were evaluated alongwith their mother trees for various phenological, flower and fruit characters. Biochemical and organoleptic analysis was also carried out. Early colour development was observed some six weeks earlier in Shimla 1 and some four weeks earlier in Shimla 2 as compared to their mother trees. Their fruits also matured earlier. In five solid coloured bud sports, fruit skin colour covered the entire surface (Kullu, Kullu 1, Kullu 2, Manikaran 5 and Kalpa 2) whereas fruits on their respective mother trees exhibited striped pattern till harvesting time. On the contrary, Kalpa 1 was late in colour maturation as compared to its mother tree. Other two limb sports Tabo 1and Kalpa 3 were spur bearing on standard trees of cv. Starking Delicious and cv. Red Delicious in districts Lahaul & Spiti and Kinnaur, respectively. Considerable differences were observed for several characters like time and pattern of fruit skin colour development, time of fruit maturation, fruit shape, fruit firmness, TSS content etc.

5. Genomic SSR (10) and genic SSR (20)-EST derived were used to carry out molecular marker analysis of bud sports and their mother trees which generated considerable polymorphism revealing genetic differences.
6. Bud wood of all the identified bud sports was collected and propagated successfully for further studies in future.

From the findings of the present study, it may be concluded that the identified bud sports comprise a valuable genepool having variations over their parents. These variations have been validated to some extent by molecular marker studies yet warrant further testing. Systematic field trials of these bud sports be laid out for determining their commercial acceptance.

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Department of Fruit Science
Dr Yashwant Singh Parmar University of Horticulture & Forestry
(Nauni) Solan (HP) - 173 230 India

Title of the Thesis	:	**Studies on identification of bud sports in Apple (*Malus* x *domestica* Borkh.)**
Name of the Student	:	Heerendra Prasad
Admission Number	:	H-2014-10-D
Major Discipline	:	Fruit Science
Minor Discipline	:	Genetics and Plant Breeding
Degree Awarded	:	Ph.D Fruit Science
Year of Award of Degree	:	2020
No. of Pages in Thesis	:	76
Major Advisor	:	Dr Krishan Kumar

ABSTRACT

The present investigation was undertaken during 2016-18 to identify bud sports in apple. Field surveys of existing apple plantation in as many as 40 orchards/sites located in districts of Shimla, Kullu, Kinnaur and Lahaul & Spiti in Himachal Pradesh were carried out during 2016-17. Based upon pre-selection criteria such as bearing habit, time of colour development and time of fruit maturation, screening of 7154 bearing apple trees was done. Feedback information was also collected from the orchardists/grower and the identified trees were marked for systematic evaluation studies. In all, 10 bud sports were identified. Two of them were early colouring (Shimla 1 in cv. Red Delicious and Shimla 2 in cv. Vance Delicious) in which colour development started 4-6 weeks earlier than in their mother trees. As many as five bud sports were identified for developing solid colour pattern (washed out) over entire fruit surface as against streaked pattern of colour in the fruits of their mother trees. There were namely Kullu, Kullu 1, Kullu 2, Manikaran 5 and Kalpa 2. Interestingly all the five solid coloured bud sports were discovered in respective mother trees of single cv. Starking Delicious at different locations in the districts of Kullu and Kinnaur. One bud sport Kalpa 1 discovered was unique being late in fruit skin development as compared to fully red coloured fruits on its mother tree. Red streaked fruits over yellow ground colour on the bud sports did not mature even at harvesting. Other two bud sports (Tabo 1 and Kalpa 3) were spur-type as against standard mother trees of cv. Starking Delicious and cv. Red Delicious in districts of Lahaul & Spiti and Kinnaur, respectively. Apart from the distinguishing character for which the bud sports were identified, they also differed in fruit shape and size, fruit firmness, fruit maturity, TSS, acidity and starch-iodine test stained area. The distinct morpho-physico-chemical variation observed in bud sports were validated by molecular marker studies. Both genomic SSR and genic SSR markers generated considerable polymorphism to detect differences between bud sports and their mother trees. The identified bud sports have been vegetatively propagated for further studies in future.

Signature of the Student
Name: Heerendra Prasad
Date:

Signature of Major Advisor
Name: Dr Krishan Kumar
Date:

Head of the Department

BRIEF BIO-DATA OF THE STUDENT

Name : Heerendra Prasad

Father's Name : Sh. Girvar Prasad Sagar

Mother's Name : Smt. Ramidhakeli

Date of Birth : 07-06-1989

Permanent Address : Sagar Clinic, Station Road Nadbai, Bharatpur, Rajasthan 321602

Educational Qualifications :

Certificate/ degree	Class/ grade	University/ Board	Year
Matriculation	First	RBSE, Ajmer	2003
10+2	Second	RBSE, Ajmer	2005
Graduation	First	Marathwada Agriculture University Parbhani (MS)	2010
Post Graduation	First	Dr Y S Parmar University of Horticulture and Forestry, Nauni, Solan (HP)	2013

Title of Thesis in M Sc **:** Effect of foliar spray 2,4-D, Urea and Zinc Sulphate on Kinnow Mandarin

Fellowship/ Scholarship/ Gold Medals Award any other Distinction received during the study period **:** UGC (RGNF) Rajiv Gandhi National Fellowship New Delhi

Publication **:** 12

Scientific Popular Articles **:** 3

Place : Nauni, Solan
Date : **(Heerendra Prasad)**