Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm

Aatifa Rasool

(2016-H-116-M)

Division of Fruit Science

Faculty of Horticulture

Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir

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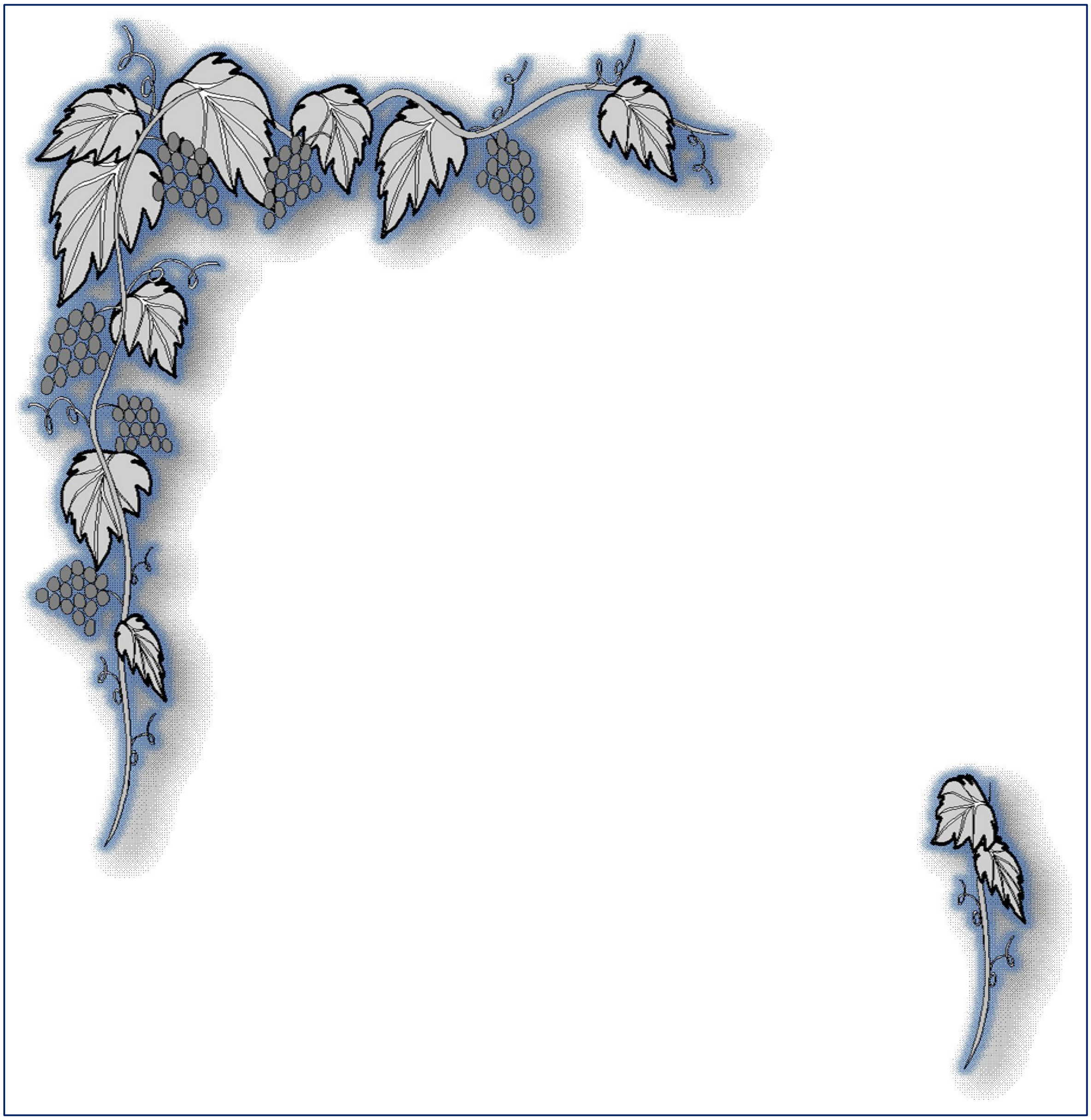

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Fruit Science

Certificate – I

This is to certify that the thesis entitled **“Morpho-Molecular characterization of apple (*Malus* × *domestica* borkh.) germplasm”** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in (Horticulture) Fruit Science to the Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Ms. Aatifa Rasool (Regd. No. 2016-H-116-M)** under my supervision and guidance. no part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or information received during the course of investigation have duly been acknowledged.

(Dr. K. M. Bhat)
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Endorsed

Prof. and Head,
Division of Fruit Science

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Fruit Science

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We, the members of the Advisory committee of **Ms. Aatifa Rasool (Regd. No. 2016-H-116-M)**, a candidate for the degree of **Master of Science in (Horticulture) Fruit Science**, have gone through the manuscript of the thesis entitled, **"Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the award of degree.

Advisory Committee

Chairman

Dr. K. M. Bhat
Professor,
Division of Fruit Science, SKUAST-Kashmir

Members

Dr. M. A. Mir
Associate Professor,
Division of Fruit Science, SKUAST-Kashmir

Dr. Zahoor Ah. Dar
Professor, G & PB,
SKUAST-Kashmir

Dr. Shakeel Ahmad Mir
Prof. & Head,
Division of Agri-Statistics, SKUAST-Kashmir

Dr. Asif Bashir Shikari,
Associate Professor,
Division of Plant Biotechnology, SKUAST-Kashmir

Dean's Nominee

Dr. Z. A. Qadri,
Professor,
Division of FLA, SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Fruit Science

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This is to certify that the thesis **“Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm”** submitted by **Ms. Aatifa Rasool (Regd. No. 2016-H-116-M)**, to the Faculty of Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, in partial fulfilment of the requirements for the award of the degree of **Master of Science in (Horticulture) Fruit Science** was examined and approved by his Advisory Committee and external examiner on ………………..

Chairman
Advisory Committee

External Examiner

Prof. and Head
Division of Fruit Science

Dean,
Faculty of Horticulture,
SKUAST-Kashmir

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Fruit Science

Name of the student	:	**Aatifa Rasool**
Registration No.	:	2016-H-116-M
Major subject	:	Fruit Science
Minor subjects	:	Plant Breeding and Bio-Technology
Major advisor	:	**Dr. K. M. Bhat** Professor, Division of Fruit Science, SKUAST-Kashmir
Title of the Thesis	:	**"Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm "**

ABSTRACT

The present investigation entitled "**Morpho-Molecular characterization of apple (*Malus x domestica* Borkh.) germplasm"** was carried out in order to document and characterize the apple germplasm comprising of forty apple cultivars to obtain information on the variability, genetic divergence and their allelic diversity with respect to various morphological and quality traits. Varieties differed significantly with respect to bloom dates and maturity. Benoni recorded earliest initial and full bloom whereas ASP-69 was the last to bloom. Irish peach was found to mature earliest (88 DAFB) while the last to mature was Maharaji (176 DAFB). Analysis of variance revealed significant differences among the selected varieties. Maximum range was observed for fruit weight (14.06-202.73g) followed by fruit length(28.1-85.26 mm).The genotypes under study were grouped into 3 clusters as per Mahalanobis D^2 analysis with maximum number of genotypes in cluster I (36 genotypes) followed by cluster II (3 genotypes) and cluster III was monogenic with 1 genotype. The maximum intra cluster distance was observed in cluster III (139.24) followed by cluster I (119.95) whereas the inter cluster distance was maximum between cluster I and III (1521.46) followed by cluster I and II (745.61). The per cent contribution towards the total genetic divergence revealed that fruit weight, acidity, fruit length and pedicel length were the main contributing characters towards total genetic divergence. The crosses between the genotypes of cluster I with III and I with II are likely to exhibit high heterosis and produce recombinants with desired traits in segregating generations. Besides, this principle component analysis (PCA) was performed to study correlation and to interpret relationship among genotypes. Results revealed that PC1 variation observed was 77.76% while from PC2 variation was observed as 6.9, PC3 accounted for 4.05% variation. Molecular characterization was performed for two fruit quality traits (scab resistance and fruit firmness). The molecular analysis of all the genotypes for scab resistance was done with one co-dominant primer (AL07) and one dominant primer AM19 and the results obtained with both of them confirmed the presence or absence of *Vf* gene. The co-dominant marker AL07 was very useful to discriminate homozygous from heterozygous plants for the *Vf* gene. Also the genotypes for ethylene biosynthesis potential were determined for the apple reference set using ACS1and ACO1 markers. Fuji Zhen Aztec, Gala Mast, Gala Redlum, Shalimar Apple-1, Red Velox and Oregon spur were found to have highly firm fruits some genotypes were soft while others were moderately firm.

Key words: Apple, *Malus*, Range, Heritability, Cluster, Scab, Firmness.

Signature of Student	**Signature of Major Advisor**
Dated : ___________	**Dated: ________________**

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Place : Shalimar, Srinagar *Aatifa Rasool*

Dated :

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Chapter-1

INTRODUCTION

The cultivated apple (*Malus* × *domestica* Borkh.) is an interspecific hybrid complex of allopolyploid origin (Korban and Skirvin, 1984). Apple belongs to the Rosaeceae and has been classified into the subfamily pomoideae and genus *Malus*. Apples have been cultivated since 4000 B.C and the primary centre of *Malus* is within the region from Asia Minor to the Western China. The Old Silk Road from central Asia to the Danube played an important role in the dispersal of the cultivated apple. Apple is economically the most important tree fruit crop in the temperate zones, presenting a high diversity of commercial cultivars. Apple is the most ubiquitous fruit in temperate regions and it has been cultivated throughout Europe and Asia since antiquity (Huxley, 1992; Janick *et al.* 1996). At global level, more than 10,000 cultivars of apple are known of which only 20 (0.2%) are said to be commercially important (Janick *et al.* 1996). It is the fourth most important fruit crop after citrus, grapes and banana. Apple was introduced into India by the British in the Kullu Valley of the Himalayan State of Himachal Pradesh as far back as 1865, while the coloured Delicious cultivars of apple were introduced to Shimla hills of the same State in 1917.

Apple is significant provider for human well being as a valuable source of phytonutrients, natural antioxidants and important minerals. Fruits of apple can be eaten in unlimited quantity who suffer from different illness (for instance obesity, diabetes, intestinal problems). Besides fresh consumption apple fruits are suitable for producing various processed products having different functional effects. The antioxidants in apple have disease preventing properties, thus truly justifying the adage, "an apple a day keeps doctor away". Apple fruits are used as a food crop and a source of pectin, which is used to thicken jams and culture media for laboratory uses (Brouk, 1975). In addition, apple fruits can be processed into sauces, slices, sweets, alcoholic beverages, vinegar or juice. They are also an excellent dentifrice, the mechanical action of eating a fruit serving to clean both teeth and gums (Grieve, 1984). In temperate regions, apples are very popular fruits as a result of their availability to consumers. Unlike other fruits, apples can be consumed off the tree or stored for months.

A number of apple cultivars are available all over the world, which have been originated as a result of open pollinated seedlings, controlled crosses in breeding programme and exploitation of naturally or induced somatic mutations. Apple germplasm and the maintenance of genetic diversity are important to future breeding because genetic diversity gives species the ability to adapt to changing environments and provide the raw material to breed new cultivars via hybridization (Doebley *et al.* 2006) or selection (Dzhangaliev, 2003). Pome fruit genetic resources collections constitute a highly valuable resource not only for fruit breeding but also for direct use by nurseries, growers and home gardeners. In order to use these resources efficiently and sustainably, reliable evaluation data on fruit and tree characteristics must be generated. Likewise, there is a growing interest in the characterization of local apple genotypes in order to preserve biodiversity and to search for agronomic and adaptive traits for production or breeding programs, such as better adaptation to local conditions and resistance to pathogens. Apple breeders have been eager to exploit the broad genetic and phenotypic diversity of this species in order to meet consumer demands for new and delicious apples thus detailed investigation on morpho-genetic characterization is required.

The application of morphological markers is the simplest method of evaluating crop diversity which has been used for many different fruit crops and it is the first step that should be done before molecular studies. Characters related to plants, flowers, fruits and leaves are used by several researchers to describe and characterize distinct apple varieties (Kiraly *et al.* 2012; Dar *et al.* 2015 and Kaya *et al.* 2015)using apple descriptors developed by the International Union for the Protection of New Varieties of Plants (UPOV, 2005) and the International Board for Plant Genetic Resources (IBPGR, 1982) and Distinctiveness, Uniformity and Stability (DUS,2012) guidelines described by Protection of Plant Varieties and Farmer's Rights Authority (PPV & FRA, Act 2001, MOA, Government of India, New Delhi. Fruit shape, size, colour, total soluble solids, acidity and firmness are the parameters most considered for defining apple quality standards. Research on existing cultivars suggests there is a strong consumer preference for apples with outstanding sweetness, crispness and juiciness (Hampson *et al.* 2000). These sensory fruit quality attributes present primary targets for consumer-focused plant breeding in apples. With regard to the sensory definition of freshness, the main attributes used by the consumer to judge apple freshness are crispness, juiciness and mealiness. The first two of these are considered to be positive factors, while the last one is negative. Crispness is an acoustic attribute, evaluated as the intensity and frequency of the sound produced by chewing. Juiciness is associated with a tactile sensation; it represents the juice amount released by the product during chewing (Ioannides *et al.* 2009). Mealiness is a qualitative defect, appearing as dry and "sandy" flesh which breaks down into fine particles as consequence of the weakening of intercellular bonding. In addition to these for the selection of varieties as genetic material for breeding purposes, other properties of the fruit: size, colour, fruit flesh firmness, total soluble solids, acidity of the fruit juice are significant. Old apple cultivars have enormous value from the point of view of biodiversity. The choice of fruit available in the market must be broadened if fruit consumption is to become more varied. This could be achieved not only by re-introducing old varieties into cultivation, but also by using them as crossing partners in the development of new cultivars.

As a complement to morphological characterization, molecular markers have been developed and are currently being used for germplasm genotyping alone or as a complement to morphological characterization (Federico *et al.* 2008). In case of apples, a high number of molecular markers have been described (Gianfranceschi *et al.* 1998; Liebhard *et al.* 2002; Dilworth *et al.* 2006). The trait specific markers can provide valuable genotypic information quickly and can be used in two areas: planning for new crosses and selection of parents in breeding populations. The allelic diversity of various genes involved in ethylene bio-synthesis and scab disease resistance have been reported by several workers. (Zhu and Barritt, 2008; Suprun and Tokmakov, 2013).

Being a climacteric fruit, loss of firmness in apple seems to be physiologically related to ethylene. Ethylene biosynthetic pathway is controlled by two large gene families coding for 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO). Retention of desirable firmness after prolonged storage is one of the key requirements for apple cultivars to provide year-round high-quality apples to consumers. Two allelic forms of Md-ACS1 are typically observed, i.e., Md-ACS1-1 and Md-ACS1-2. The three allelic combinations, ACS1-1/1, 1-1/2 and 1-2/2, generally confer high, medium and low ethylene production, respectively (Harada *et al.* 2000; Costa *et al.* 2005). Similarly, Md-ACO1 is primarily expressed in fruit tissues among the Md-ACO gene family members (Wakasa *et al.* 2006). MdACO1 has been demonstrated to have a relatively minor, but clear and independent role in ethylene biosynthesis In general, there is a close relationship among ethylene biosynthesis in genotype, ethylene production and fruit storability or shelf-life (Harada *et al.* 2000; Costa *et al.* 2005). The markers developed for ACS1 and ACO1 belong to the emerging category of molecular markers and termed as “functional” or “perfect markers”, which are derived from sequence variation of functionally analyzed genes. As their allele effects are known and they are developed “within or very close to” genes of interest, these markers are ideal for selection of desired genotypes.

Apple scab also known as black spot, caused by *Venturia inaequalis* (Cke.) Wint. is one of the most serious diseases of apple reported from almost all apple producing countries and causes huge economic losses (up to 70% reduction) The availability of the apple genotypes showing resistance to apple scab is highly relevant for the fruit industry through their use in integrated production and facilitating the production of organic fruit. Additionally, these cultivars could be incorporated into appropriate breeding programs, commencing with investigations on the genetic basis of their resistance. For the effective resistance breeding process, it is necessary to identify and evaluate valuable sources of resistance within genetic resources. Kashmir valley is known to host several hundred cultivars of apple, which constitute the bulk of diversity of apple germplasm in India and this germplasm is a potential source of important agronomic traits including scab resistance (Khajuria *et al.* 2014). The use of resistant apple cultivars is the best way of eliminating economic losses caused by fungal diseases.

In India apple is a prime commercial fruit crop of Jammu and Kashmir, Himachal Pradesh and Uttaranchal and some parts of north eastern states including Arunachal Pradesh, Sikkim, Nagaland, Meghalaya and Nilgiri hills of Tamil Nadu (Awasthi and Chauhan, 2001). The entire Himalayan region particularly Jammu and Kashmir State hosts a rich and remarkable genetic diversity of introduced, locally evolved and seedling based germplasm of apple. The ecozones of the Kashmir are very conducive for the optimum quality production of Apple and occupies an area of (67.4%) and production (77.26%) under fresh fruit cultivation in state (Anonymous, 2016). The cultivated apple in Kashmir is comprised of different groups of cultivars such as Delicious, Ambri, Trel and Maharaji etc. In each type one or few cultivars are only commercially successful e.g. Kashmiri Ambri, American Trel and Red Delicious etc. The rest of the cultivars in each group are sold in the market under the trade name of well-known cultivars. The apple cultivar '*Ambri*' is considered to be indigenous to Kashmir and has been grown long before Western introductions. The monoculture of cultivars like Red Delicious, Golden Delicious, American etc. from years are now associated with various problems like apple scab, alternaria, powdery mildew and have resulted in loss of diversity and depletion of indigenous apple germplasm and a number of apple cultivars are at the brim of extinction (Bhat *et al.* 2011). It is therefore important to characterize cultivars of each group so that well known cultivars are clearly distinguished from less known and commercially unsuccessful cultivars. The new cultivars with better characteristics could be identified and promoted to commercial level. The present investigation is a part of assessment of the apple germplasm present at Horticulture farm, Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology, of Kashmir, Shalimar, Jammu and Kashmir in order to generate passport data information, to identify elite cultivars for disease resistance and other fruit quality traits with the following objectives:

1. To assess the extent of variability in morpho-fruit quality characters in apple reference set.
2. To determine allelic diversity at loci for fruit quality traits using DNA markers.

Chapter-2

REVIEW OF LITERATURE

Improvement programmes involving the fruit crops are rendered difficult by the long juvenile phase of the plant and the limited ability to precisely identify the gene sources for particular characters. Selecting desirable genotypes from the existing varieties and to use the superior types in breeding programmes is an important tool to increase the productivity in any crop. The extent of genetic diversity determines the success of a crop improvement programme. The assessment of a viable diversity is basic to the development of improved cultivars. Characterization is the depth of the genetic base that has direct bearing on the potential to identify different cultivars with improved performances.

Frankel (1947) suggested that the characters of concern to a plant breeder must be observable or strongly expressed. These could be identified even in single plant (though very rarely) and must be expressed under growing conditions which are normal for cultivation. The characterization enables easy and quick discrimination between the phenotypes which are generally highly heritable, can be easily seen by the eye and are quickly expressed in all types of environment (Anonymous, 1984). According to Frankel (1986) characterization should provide a standardized record of readily assessable plant characters to identify an accession. The requirements of cultivar distinctness has scientific merits for a system that can ensure the release of genetically distinct elite cultivars, promote horticulture productivity and contribute to the conservation of genetic resources (Smith and Smith, 1989).

Apple (*Malus x domestica* Borkh.) being an economically important fruit crop is widely cultivated in most of temperate latitudes.The genetic base and genetic diversity of genus *Malus* was reviewed by Way *et al.* (1990) with major cultivars, place, date of origin and gave information on world apple production, fruit quality, nutritive value, yield and collected diverse genotypes of apple.

Noiton and Alspach (1996) used pedigrees of apple cultivars to study wide genetic diversity among clones used in modern apple breeding. The most frequent founding clones were Cox's Orange Pippin, Golden Delicious, Red Delicious, Jonathan and McIntosh.

Despite their wide distribution, the genetic basis of domesticated apple has been eroded and most of the world production relies on few varieties only (Hokanson *et al.* 2001). Kuden *et al.* (2001) reported diversity in apples, quince and pear etc., in Turkey a number of genotypes of apple and quince were collected at University of Cukurova to widen fruits genetic resources and to promote their conservation and utilization. The available diversity pool for fruit improvement could be expanded by including wide range of genotypes in breeding programmes.

The morphological characters which are specific to genotypes have been highlighted and used to classify the intra- specific genotypes in terms of tree morphology and to promote new agronomic practices. The inheritance of morphological traits depends upon the genotype and environment and their possible interactions (Costes *et al.* 2004).

Use of morphological markers has proved to be a strong base for characterization of apple cultivars however, the development of DNA technology has provided a number of methods, which may be employed for the much more authentic and reliable characterization of apple germplasm collections. The use of DNA-based molecular markers has been highly critical and valuable for pursuing studies to assess genetic diversity, determine genetic relatedness and identify genes of interest (Han and Korban 2010; Chen *et al.* 2010; Baraket *et al.* 2011). Markers such as simple sequence repeats (SSRs) are highly polymorphic, reproducible and are distributed throughout the genome, rendering these markers ideal for pursuing genetic diversity studies.Therefore, the use of DNA-based markers is a highly valuable and reliable tool for characterization of the germplasm (Gustavsson *et al.* 2008; Zhang *et al.* 2011.)

2.1 Flowering

De-Varies (1967) observed that time of bud break, beginning of bloom, full bloom and picking of apple cultivars are significantly related to each other. High correlations were obtained from data in the same or in different years on trees in the vegetative stage, leading to the conclusion that the above characteristics are caused by genetic differences between varieties.

Bellard *et al.* (1971) studied the average date of occurrence for flower bud break in apple at Washington State University Research and Extension Centre Prosser and they recorded the average date for green tip as 20/3, full pink 11/4, first bloom 18/4 and for full bloom 25/4. Studies on the performance of apple cultivars at fruit research station, Shalimar has been documented. It was found that flowering in cultivars Red Delicious, Ambri, Jonathan, Golden Delicious occurred during the 2nd week of April, whereas duration of blooming ends during last week of May (Anonymous, 1972).

Similar studies were conducted by Proebsting and Mills (1978) on six deciduous fruit species. Their results were in agreement with Bellard *et al.* (1971).

Ballard *et al.* (1978) studied that the average number of days from initial bloom to final bloom were seven days in Red Delicious, Golden Delicious, Winesap and Rome Beauty varieties of apple. Taburence (1983) found that late flowering in some cultivars may be due to their high chilling requirement.

Facteau *et al.* (1986) reported that flowering date and period may vary according to cultivar aptitude as well as ecological and cultural conditions. Tromp and Romer (1987) found that time of full bloom depends upon the regimes in Cox's Orange Pippin cultivar and it varies from 30-44 days.

Suresh and Sharma (1991) observed that the variation in dates of flowering may be attributed to the difference in agro-climatic conditions of a particular place. Blasse and Hofmann (1991) recorded dates of initial bloom, full bloom and harvest for 20 cultivars grown at Potsdam, Germany during 1970-1980. Cultivars were classified as early, mid early or mid late. The average flowering period was 11 days regardless of cultivar. The date of initial bloom was effected by air temperatures, in late March a maximum temperature of 30°C being critical. There was no relationship between the dates of initial bloom and harvest. The growth period of fruits being variable for each cultivar ranged between 126-172 days.

Kumar *et al.* (1997) evaluated six apple cultivars during 1994-1995 and found that flowering time varied from the last week of March to the first week of April. Sharma *et al.* (2005) carried out the flowering pattern of 13 cultivars (Arlet, Elstar, Exter cross, Fuji, Gala, Hardeman, Jonadel, Red Fuji, Royal Gala, Ruspippin, Spartan, Spur type Red Delicious and Summer Red) of apple and observed that all the cultivars started flowering from the second to the third week of March. Full bloom was recorded in the fourth week of April and the flowering period was 16-19 days. Sharma *et al.* (2006) reported that flowering duration in apple ranged from 10-17 days.

Baytekin (2006) reported that dates of bud break in Red chief/MM-106, Gala/M-9, Fuji/M-9 and Granny smith were observed as 12 March, 17 March, 18 March and 30 March respectively. Bodor and Toth (2007) reported that high spring temperature causes shorter blooming period and main bloom takes only few days for the whole cultivar assortment. The most effective method to consider the day to the beginning of blooming is when first flower opens and flowering continues in the following days (Prieto *et al.* 2009).

Bozbuga and Pirlak (2012) studied the phenological and pomological characteristics of some apple varieties (Galaxy, Mondial Gala, Red Chief, Super Chief, Oregon Spur, Scarlet Spur, Early Red One, Granny Smith and Fuji) grafted on dwarf and semi dwarf root stocks. In cultivars bud swell, bud break, beginning of blooming, full bloom and end of flowering were found 2 weeks later during 2007 than 2006 and such divergent results were attributed to the difference between the temperature dominance during early stages of vegetative development.

Pandit *et al.* (2017) studied floral phenology of various apple cultivars and the phenological studies indicated that varieties like Red Gravenstein, Ginger Gold, Braeburn were early bloomers, Gala Must, Royal Gala, Scarlet spur, Oregon spur, Golden Delicious and Red Gold as mid bloomers and Early Red One and Law Red Rome were found as late bloomers.

2.2 Fruit maturity and harvesting

Magness *et al.* (1926) suggested the indexes of maturity as ground colour, ease of separation of the fruit from the spur and pressure test. The number of days from full bloom to maturity was constant for each of the varieties used by them under different seasonal- conditions at a given locality and even under the widely varying climatic and soil conditions of widely separated localities. Hesse and Hitz (1939) suggested use of starch test for Jonathan and Grimes Gold apple as index of maturity.

Heller (1942) concluded that the number of days from full bloom to maturity was more reliable index of maturity than other factors studied. Performance studies on apple cultivars at Fruit Research Station, Shalimar revealed that harvesting of apple cultivars *viz.,* Cox's Orange Pippin, Golden Delicious, Jonathan and Red Delicious takes place during 2nd week of September to 4th week of October and 2nd to 4th week of October "in case of *Ambri* and Maharaji" (Anonymous, 1967-68, 1970-71,1971-72).

Blanpied (1979) observed that maturity dates for Delicious apples could be reliably predicted one week in advance by using average firmness, total soluble solids value for a geographic region when the firmness, total soluble solids are close to the maturity index line. Arumugam and Srinivasan (1983) studied the physico-chemical characters of some early, mid and late season apple varieties and concluded that the early varieties of apple are ready for harvest in 110 days, while mid and late season varieties take 130-150 days respectively from full bloom.

Tripathi (1984) reported that the number of days from full bloom to maturity was the most reliable index for determining maturity of Red Delicious. Further he observed that 124-129 days from full bloom to harvest was found most reliable index for determining maturity of Red Delicious apple cultivar.

Gupta (1986) observed that in Kashmir, the optimum picking time for cultivar Red Delicious occurred between first to third week of September or within 140-145 days after full bloom when physico-chemical and organoleptic parameters reached optimum level.

Farooqui (1992) observed apple cultivars Ambri, Red Delicious and Golden Delicious took 157, 151 and 148 days from full bloom to maturity respectively. Frecon (1997) studied Gala cultivars and observed that these cultivars are usually harvested from 115-130 days after full bloom Lydia's Gala are the exception and need to be picked about 108-120 days after full bloom.

Joshi *et al.* (1993) observed that picking of fruit depended upon optimum level reached by physico-chemical parameters and starch disappearance percentage. They suggested that every region should define its own criteria taking into account the climatic factors, varieties and dessert quality of the fruit. They added that most appropriate time for picking apple variety 'Benoni' was between 90-95 days after bloom stage.

Karacali (2004) reported that the most important criteria in apple cultivars are duration between full bloom and harvest date which depends upon cultivar, place, year, rootstock and ecological conditions. Wang *et al.* (2010) conducted Starch iodine test for determining maturation of apple cultivars "Gala", "Qinguan" and "Fuji" and concluded that the cultivars should be harvested for the long-term storage when the starch index reached 4.5, 6.0 and 7.7 for "Gala", "Qinguan" and "Fuji" apples respectively.

In a study on *Malus* germplasm diversity in south Serbia conducted by Mratinic and Aksic (2012), the characteristics including duration of flowering, blooming time, the period from the blooming time till the harvest time were studied. The results revealed that the duration of flowering time (DFT) of the accessions showed a narrow range (7 days), while the blooming time (BT) of examined genotypes showed a much wider (16 days).

2.3 Leaf characters

Costes (2003) reported that leaf area be considered as one of the most important components of the fruiting structures involved in the fruit size and quality because branches with a larger leaf area present a higher photosynthetic capacity, reducing the competition for photosyntates with other branches.

Wagner (2006) concluded that pubescence on leaves, long shoots and on flowers is an important characteristic to distinguish *Malus sylvestris* from *Malus domestica*. The shape and size of apple leaves can impact access to light, ultimately influencing flower number and fruit quality (Wünsche and Lakso, 2000).

Apple leaves are generally simple, with an elliptical-to-ovate shape. Previous studies in apple used linear measurements, such as length and width, to quantify leaf shape (Liebhard *et al*. 2003; Bassett *et al*. 2011).

Dar *et al*. (2015) studied leaf morphology of various apple cultivars and reported that leaf shape varied between ovate to oval, leaf apex ranged from acute to acuminate and incisions on leaf margins were found to vary between serrate type 1, crenate and bicrenate. Hassan *et al.* (2017) studied thirty three apple genotypes for various morphological and pomological characters. The genotypes showed variation with respect to various tree, leaf and fruit characters.Leaf blade length and width showed significant variations. Also leaf morphological studies revealed that the intensity of green color of leaf blade ranged from light green to dark green, different types of leaf margins were observed viz., bicrenate,serrate type 2, serrate type 2, biserrate. Pubescence on lower side of the leaves was found to be weak in majority of genotypes.

2.4 Fruit physical characters

The fruit characteristics provide the second important group of traits concerning the characterization of *Malus* species. The fruit appearance especially fruit shape represents an important trait for commercial purpose and the characteristic which defines fruit shape well and is dominantly influenced by genes is the length: width ratio. The shape of an apple fruit can be different under different environmental conditions. Lantz (1932) reported that each of the variety of apple carry factors for roundish, conic and oblate shape. He also reported that the primary forms namely conic, roundish, oblate and oblong appeared in most progenies but the proportion of each form in different progenies was far from constant.

Crane and Lawrence (1938) and Klein (1958) observed that oblate shape is dominant over oblong. However, in their studies majority of the hybrids had conical to oblong fruits resembling their parents. According to Brown (1960) fruit shape of apple shows a quantitative pattern of inheritance. His studies revealed that the inheritance of fruit shape was under polygenic control and flat shape was, recessive to spherical shape. He further observed that the preference with flat, conical or angular fruit was very little.

Westwood (1962) observed the seasonal changes in shape of the apple fruits and stated that in Red Delicious, the weight, diameter and length/diameter ratio were 208 g, 7.5 cm and 0.87 respectively. Barden and Thompson (1962) found that average weight of Red Delicious fruit at harvest varied from 105 to 121 g in four years period. Workman (1963) studied the firmness in Golden Delicious apples harvested at weekly intervals and reported the pressure values to range between 12.6-16.1lbs. Westwood *et al.* (1967) reported that the fruit weight of small, medium, large and very large fruit of Golden Delicious apples were 134, 214, 294 and 385 g, respectively while overall average fruit weight was 257 g.

Russet usually starts early in life of the fruit, shortly after bloom, corresponding to the period of greatest tangential growth. Internal pressure created by the rapidly dividing and growing epidermal cells cracks the cuticle if it is unable to expand. Cells under cracks die and repair mechanism activates the formation of cork cambium which divides rapidly to create cork cells of which russett are composed. Russet usually starts in early stage of fruit development, shortly after bloom, corresponding to the period of tangential growth. Russet may also be genetic in nature and cultivar such as Cox's Orange Pippin can be heavily russeted by harvest time (Faust and Shaer, 1972).

Chadha and Sharma (1978), while investigating the quality and yield parameters of different apple cultivars observed that the size varies with the load of the crop. Alston (1981) described size as one of the most important selection criteria. In most of the progenies over half the seedlings have fruit which is too small. Cox's Orange Pippin is particularly poor parent; 60-70 per cent of derivates are too small. In general large fruit size is recessive, progeny mean being below mid- parent values. Fruit size is highly variable character because of the environmental factors as well as magnitude of the fruit set has a tremendous influence on it. Fruit size is a function of cell number, cell volume and cell density (Coombe, 1976; Scorza *et al*. 1991).

Looney (1993) suggested that large fruit size, attractive appearance and characteristic or distinctive flavour were amongst the most important fruit quality attributes. Fallahi *et al.* (1994) while evaluating 26 strains of Delicious apple reported that Ace', 'Imperial', 'Red King Oregon Spur', 'Rose Red', 'Starking' and 'Well spur' had heavier fruit, while fruit weight in 'August Red', 'Hardy spur' and 'Starkrimson' was lighter than that in most other strains.

Consumer surveys in peach and apple have shown that bigger is better and consumers are willing to pay more to make larger fruit more profitable (Parker *et al.*1991; Bruhn, 1995). Apple cortical firmness is an important criterion for assessing fruit maturity at harvest and fruit quality during and following storage. From a post-storage perspective, higher economic return to the grower is related to fruit firmness as higher firmness values are associated with better conditioned fruit having less incidence of rot, internal breakdown and other disorders (Stow, 1995).

Fruit skin colour is an important aspect of appearance. The overall colour of a fruit is reflected by the colour of the outer pericarp and the flesh colour. Pigments responsible for the colours are various modifications of anthocyanins, lycopenes and carotenoids. As the fruit matures they take on red blue, purple or yellow colour characteristic of the cultivar; at the same time most of the cultivars begin to loose their green under colour. Plotto *et al.* (1995) while studying the skin colour using the cultivars 'Gala', 'Fuji' and 'Braeburn' suggested that the observation of skin colour is a valuable method to monitor the ripening process. Lane *et al.* (1996) reported better red colouration in standard strains than in spur strains. Janick *et al.* (1996) stated that colour can be bright red, bright green, clear yellow or bicolour. Dull fruit colours were not acceptable although in some cases brighter coloured sports improved their marketability.

The inheritance of colour in apple is complex. Background or ground colour has two independent mechanisms: a yellow colour range and a green colour range. The yellow colour ranges from pale cream to yellow and is correlated to flesh colour (Janick *et al.* 1996). The green scale ranges from light green to green (due to chlorophyll).The over colour (anthocyanin production in the skin) can be nil to complete coverage. Combinations of over colour with background colour change the overall colour. An apple with a same red over colour is a bright pink when combined with a cream ground colour, a bright crimson with a yellow ground colour or a dark/ dull/red/brown on a green ground colour. As dull colours are unacceptable, ground colour is of primary importance in fruit with red colouring. The over colour can be patterned (stripped or blocked) and vary in intensity (intense to pale) and is highly sensitive to the environment. Stripe and blush traits are inherited independently.

Fruit size has a large genetic component thus selecting for larger fruit is relatively straight-forward (Janick and Moore, 1996). A wide range of fruit shapes are available but consumers/marketers prefer fruits with tall shape and conical to ovate (oval) profile (Janick *et al.* 1996) whereas irregular shapes are not acceptable nor are oblate (flat) shapes currently acceptable. Fruit size is polygenically inherited and studies have shown the progeny mean is 34 per cent lower than the mid-parent value (Janick *et al*. 1996), possibly due to dominance. They suggested a good strategy would be to select parents with oversize fruit, not commercially acceptable themselves, to ensure that the majority of offspring fall within a desirable size range. Like most of the traits important to agriculture, both fruit size and shape are quantitatively inherited (Grandillo and Tanksley, 1999).

Aziz *et al*. (1999) reported that fruit shape was influenced by the type of parent pollen. When cultivar Anna was crossed with cultivar Dorset-golden, the fruits obtained were round and flat but when Ein-Shemier was used as pollinizer round elongated fruits were obtained.

Currie *et al.* (2000) reported that fruit shape can be described by conicity (position of the widest point) and squareness. Fruit size together with shape and colour, are the most crucial fruit quality characteristics influencing consumers (Stanley *et al.* 2001). *Malus* species and hybrids have large differences in fruit size ranging from 4 cm or less in diameter for the wild progenitor *Malus sieversii* up to 10 cm for the modern cultivar Spokane Beauty (Harris *et al.* 2002).

Lukic (2005) reported that Breaburn had fruits of 184 g and 13.1% of soluble dry material. Takos *et al.* (2006) reported that in addition to the visual appeal of fruits, anthocyanins, the pigment that confer the red colouration of the skin as well as other organs of the plant, have been shown to be beneficial to human health due to their antioxidant activity.

Babojelic *et al*. (2007) studied chemical and sensory characteristics of three apple cultivars ‘Idared’, ‘Granny Smith’ and ‘Pink Lady’ and observed that cultivar Pink Lady had significantly highest firmness (7.3 kg cm-2), in comparison to ‘Granny Smith’ (6.4 kg cm-2) and ‘Idared’ (4.5 kg cm-2). Chauhan and Sharma (2008) observed that fruit firmness in Well Spur, Silver Spur, Oregon Spur and Vance Delicious were 7.5, 9.6, 7.5 and 7.7 kg/cm^2 respectively.

Kouassi *et al.* (2009) estimated genetic parameters for apple (*Malus* x *domestica*) fruit external traits (fruit size, ground colour, proportion of over colour and attractiveness) and sensory traits (firmness, crispness, texture, juiciness, flavour, sugar, acidity and global taste) using 2,207 pedigreed genotypes from breeding programmes in six European countries. The results revealed that heritability estimates ranged from medium to high for instrumental traits and genetic correlations between firmness and sugar were medium and low between firmness and acidity.

Nocker *et al.* (2012) studied the genetic diversity of red-fleshed apples (*Malus*) and reported that numerous genotypes of *Malus* are known to synthesize anthocyanin and these red-fleshed apple genotypes are an attractive starting point for development of novel diversities for consumption and nutraceutical use through traditional breeding and biotechnology.

Hofer *et al.* (2012) reported that the fruit height of the accessions as well as the fruit diameter varied up to three-times and the flat globose shape appeared in 65%, the flat shape in 14%, the globose shape in 11% and the broad globose conical shape in 4% of the accessions. They also reported that the ground colour showed a distribution over all scores, like yellow, whitish yellow, green yellow, green, green whitish.

Dar *et al.* (2015) studied 38 morphological characters on six apple cultivars with main emphasis on fruit and leaf morphology. The fruit characteristics such as shape, size, flesh and skin colour, pH, TSS and fruit firmness were evaluated. All the six cultivars exhibited distinct characteristics with respect to these traits.

Reig *et al.* (2015) studied agronomic, morphological and fruit quality traits for 80 accessions from an apple Germplasm Bank located in the central part of the Ebro Valley (Northeastern Spain, Zaragoza). Agronomic traits included bloom and harvest dates, while morphological traits included different fruit shape and size characteristics. Extensive variability was observed for most traits with significant correlations identified between many traits. Principal component analysis (PCA) revealed the main sources of variability in the set of accessions evaluated. Based on studies, the cluster analysis produced six different groups of accessions according to the Ward's minimum variance criterion. Eight groups of synonymies were founded, which represented 21% of the total accessions studied. On the contrary, some accessions with the same allelic profile exhibit phenotypic differences, suggesting the existence of clonal mutations not detected with molecular markers. This study provides useful information for agronomic, morphological and fruit quality traits from a wide range of accessions grown.

Kaya *et al*. (2015) studied some fruit quality characters and genetic variability of native apple germplasm resources from Van Lake Basin, eastern Turkey. With respect to fruit quality characters, apple genotypes had a range of 46.00–94.99 mm for fruit diameter, 43.04–310.99 g for fruit weight, 0.71–1.13 for fruit shape index, 3.99–14.05 kg cm–2 for fruit flesh firmness, 9.0%–14.4%. for soluble solid content and 0.15-1.75 % for titratable acidity.

Mushtaq *et al*. (2018) during an investigation on exotic cultivars found significant differences between the physical characteristics of fruits during the study. The maximum fruit weight was observed in variety Super Chief Sandidge (193.99 g) while the minimum in Fuji Zehn Aztec (157.62g).The variation in fruit weight may be due to varietal characters and presence of seeds in the fertilized fruits containing endosperm are the sites of giberrellic acid synthesis, where growth substances are produced.

2.5 Chemical characters

Coldwell (1922) studied the acidity in apples of different regions of North

America and reported that the quantity of acid varied with the variety, growing conditions and locations. In the eastern region of North America, an increase in the acidity of apples was observed from South to North wise. In Virginia acidity was low and ranged from 0.25 - 0.45%, in Pennsylvania the acidity was medium 0.30 - 0.55% while in Newascatia it ranged from 0.40- 0.55 percent. While working on the aroma of fruit, Wellington (1924) and Nybom (1958) have found that at optimal acidity the content of aromatic compounds become of primary importance for the appreciation of apple juice.

Pruthi *et al.* (1961) reported that the acidity in apple varieties ranged between 0.47 and 0.73 per cent. In Golden and Red Delicious varieties, the acidity contents were 0.73 and 0.58 per cent respectively. Nybom (1962) while evaluating the taste of apple grouped the samples in five classes (from very sweet to very acid), acid taste was positively associated with the acid content and negatively with the sugar content. Acidity and sweetness in progeny of apple crosses was found to be inherited independently, consequently apples with high percentage of sugar and low acid content were found sweet and insipid (Zieliniski *et al*. 1965 and Visser *et al.* 1968).

Fruit sugars, measured by refractive index and expressed as a percentage of soluble solids in the juice, is polygenically inherited (Brown and Harvey, 1971). A study by Brown and Harvey (1971) revealed that the concentrations of sugar and malic acid in ripe fruits accounted for the important variation among cultivars but they were relatively constant within a given cultivar. Jallel *et al.* (1973) reported that the level of total sugars in Maharaji, Golden Delicious, Kesri, Red Delicious and American Pippin cultivars were 8.45, 11.15, 10.31, 9.50 and 9.80 per cent, respectively and cultivars Maharaji, Golden Delicious, Kesri, Red Delicious and American had 0.49, 0.18, 0.49, 0.18 and 0.50 per cent acidity, respectively.

Reid *et al.* (1982) recommended a TSS content of 12 per cent as optimum for harvesting Granny Smith apples. During advance maturity period there is steady decline in acidity and increase in soluble solids in apple (Recasens *et al.,* 1989). Fallahai *et al.* (1994) found that 'Hardispur', 'Nured Royal', 'Silver spur' and 'Starkrimson' had high soluble solids concentrations (SSCs) at harvest and after storage. 'Apex', 'Classic Red', 'Improved Ryan Spur', 'Red King Oregon Spur', 'Silver spur' and 'Well spur' had satisfactory overall performance.

Yahia (1994) stated that successful cultivars are either medium acid (pH 3.2 -3.5) with medium (11-13%) or high (14-16%) sugar levels, or low acid (pH 3.5-3.7) with low (9-11%) sugar levels. Tu and De Baerdenaeker (1997) determined that a minimum content of 12° Brix for total soluble solids, a minimum acidity of 3g l^{-1}, as measured by the malate content and a minimum firmness of 45 N are the acceptable limits for the consumption of 'Golden Delicious' apples.

Zekri (2000) suggested TSS/acid ratio is a flavoring factor, the results depicted that with increase in the ratio there is a decrease in the acidity and with low TSS/acid ratio, quality of fruit is poor and taste of fruit becomes watery and insipid. Again the ratio is used to determine the fruit maturity standards, higher the Brix acid ratio the earlier is the fruit maturity.

At maturity TSS in different cultivars has been recorded in the ranges of 11 to 15°Brix (Anonymous, 2003).However, in general it has been reported to be 12 to 14°Brix in Delicious or spur type cultivars at harvest maturity.

Singh *et al.* (2006) studied ten apple cultivars (Ambrich, Granny Smith, Ambroyal, Rich-a-Red, Red Gold, Red Fuji, Red Delicious, Lord Lambourne, Royal Delicious and Golden Delicious) and observed that total soluble solids (TSS), moisture and acidity of these apples were 6.2-11.8° Brix, 84.6-95.0% and 0.134-0.804%, respectively.

Babojelic *et al*. (2007) while studying the chemical and sensory characteristics of three apple cultivars found that the total acids were highest in 'Granny Smith' (0.69 %, expressed as malic acid), somewhat lower in 'Pink

Lady' (0.54 %) and significantly lower in cultivar Idared (0.45 %). Values obtained for soluble solids (°Brix) ranged from 16.4 for Pink Lady, 15.4 for 'Idared' and 12.5 for cultivar 'Granny Smith'.

Drogoudi *et al.* (2008) reported a higher content of total acidity in pulp for "Granny Smith" apple variety, compared to the other varieties evaluated. Das *et al.* (2011) observed that the various apple cultivars were found to mature when the average starch iodine score reached 4.83 to 5.67. All the fruit physical parameters and TSS increased significantly from first to fifth sampling in all the cultivars. Accordingly, fruit acidity showed a declining trend from 2.53% in Gala Must to 0.38% in Royal Delicious.

Mratinic and Aksic (2012) in their studies on phenotypic diversity of apple germplasm in South Serbia reported that the soluble solid in ºBrix content varied from 12.55 to 19.24 and titratable acidity varied between 0.10 and 0.82%. Kumar and Mir (2012) assessed the agronomic performance and heritability estimates among different cultivars of apple in district Pulwama of Kashmir valley. Twenty cultivars were selected. Results revealed that Red Delicious apple recorded maximum TSS (16.35%) and total sugars (12.11%) and least acidity (0.07%) was recorded in Ambri apples. Bozovic *et al*. (2013) also reported almost similar results with TSS value ranging from 11.3 to 16% in some old apple varieties in central Montenegro

Mureşan *et al*. (2014) evaluated three varieties of apple (Jonathan, Golden Delicious and Starkrimson) to study the detailed chemical composition of apples. Jonathan variety showed an acidity of 0.29 % followed by Strakrimson variety with an acidity of 0.19 % and Golden Delicious with an acidity of 0.17 %. These values were below the maximum limit of 0.31%. The TSS values were between 19.25 and 23.25 °Brix.

Kotiyal *et al*. (2017) conducted a study with the objective to compare the physico-chemical properties of 10 apple cultivars grown in Uttarakhand hills of

India. Various parameters including total soluble solids, titratable acidity were measured in the apple cultivars Aurora, Brookfield, Braeburn, Galaxy, Azetec, Scarlet Gala, Marini Red, Jonagold, Royal Gala and Royal Delicious. The results showed great quantitative differences in the composition of the apple cultivars. Among all the cultivars the maximum T.S.S. noticed in *cv.* Scarlet Gala (14.27°Brix) and acidity in Marini Red (0.717%), while the minimum values of T.S.S. and acidity were observed in Marini Red (11.20°Brix) and Azetec (0.186%).

2.6 Sensory evaluation

Sensory evaluation can be a valuable addition to the breeder's selection criteria. It is an indicative of consumer responses to be useful in routine screening of breeding selections.Vickers and Bourne (1976) defined the crispness sensation as a characteristic sound of a range of frequencies emitted during biting. Gliha *et al.* (1981) concluded that consumers evaluate quality on the basis of their five senses: sight, hearing, smell, taste and touch. As a result scientists have attempted to quantify and evaluate quality using these same senses.

Consumer tests are impossible for routine screening of selections because of the limited availability of the fruit and other resources required for the large number of evaluations (70-200 consumers) that may be needed (Meilgaard *et al*. 1987). Spinnler *et al*. (1996) found texture and flavor combinations are the main preference indicator for apple, with United Kingdom consumers preferring either crisp, sweet apples or juicy, acidic apples.

Stainer *et al.* (1996) reported that in the course of an apple convention in Gorizia, Italy, consumers were asked to evaluate fruits of apple cultivars Fuji, Golden Delicious clone B, Red Chief, Gala, Florina, Braeburn and Granny Smith for appearance, flesh crispness and flavour. The respondents rated Gala and Braeburn as having most attractive fruits and Fuji and Braeburn as having best flavor and crisp fruit. Hampson *et al*. (2000) demonstrated that for good quality purpose the texture of flesh should be firm and crisp with plenty of juice. In apple it has been shown that among the textural traits, crispness accounts for 90% of texture appreciation and it has been largely recognised as the key attribute affecting consumer acceptability.

Kokai *et al.* (2001) examined sensory evaluation of 11 traditional and new apple cultivars (Elstar, Granny Smith, Idared, Jonagold, Jonathan, Jonica, Kovelit, Lyxgolden, Mutsu, Royal Gala and Red Spur Delicious). Apples were cut into unpeeled slices and samples were coded. The assessors were asked to taste them and rank them. Royal Gala and Mutsu ranked first and second respectively in terms of taste. Jonica was preferred mainly on the basis of its appearance, but ranked low in terms of taste. Jonathan ranked well in terms of both taste and appearance.

Fillion and Kilcast (2002), using a trained sensory panel and a consumer panel, defined the term ‘‘crunchy’’ as describing lower-pitched sounds that continue throughout chewing, whereas ‘‘crisp’’ described a higher-pitched sound resulting from the clean split of the first bite. Both crisp and crunchy designations, when applied to food, expess that the material breaks in the mouth rather than buckling or deforming.

Quality of fruit is made of its external and internal (morphological – physical, biochemical and organoleptic) factors. With its quality it has to correspond to wishes of many consumers so that it can satisfy most of their needs, preferences, tastes and habits (Harker *et al*. 2002). Abbott *et al.* (2004) compared the eating quality of a new apple cultivar, ‘GoldRush,’ with ‘Golden Delicious’ (one of its parents), ‘Fuji,’ and ‘Granny Smith’. Acceptability scores for the texture and flavor of ‘GoldRush,’ and of Fuji’ when included, were higher than those of ‘Granny Smith’ and ‘Golden Delicious.’

Shabeena *et al*. (2008) observed maximum organoleptic scoring in Gala Must followed by Florina, Red Delicious, Imperial Gala, Summer Red, Jonica, Red Chief and Red Fuji. Campeanu *et al.* (2009) reported that Organic acids are an important component of fruit flavour and, together with soluble sugars and aromas, contribute to the overall organoleptic quality of fresh apple fruits.

A trained sensory panel, as small as three experienced individuals, has been shown to be reliable in a postharvest study of fruit texture. The panel was able to discern greater separation among cultivars than was achieved with instrumental measures. (Brookfield *et al*. 2011). In a study, described by Evans *et al.* (2012), ‘‘crispness’’ refers to the intensity of the cracking noise of the first bite. ‘‘Firmness’’ is equivalent to ‘‘hardness’’ and determined while chewing. ‘‘Juiciness’’ is expressed juice on chewing. Schmitz *et al*. (2013) collected phenotypic data for the sensory texture traits of firmness, crispness and juiciness as well as for instrumental texture measures in the apple germplasm.‘GoldRush’ proved to be a promising new cultivar for fresh-cut apple slices.

Tanaka *et al*. (2015) compared flavors of “Fuji” apple cultivated with or without synthetic agrochemicals using quantitative descriptive analyses (QDA).Organic apples were weak in sweetness and floral characteristics and had enhanced green and sour flavors.

2.7 Molecular markers

Although morphological traits have been widely used to discriminate between varieties of the same species but these characteristics are affected by environmental conditions such as rainfall or solar radiation. DNA markers have been developed and are currently used for germplasm genotyping alone or as a complement to morphological characterization.

Nowadays, molecular marker techniques are widely applied in germplasm characterization in order to assist and complement phenotypic assessments (Bretting and Widrlechner 1995). Their main advantage is that variation can be measured directly at the DNA level, which makes these techniques insensitive to environmental influences. Microsatellites are generally considered one of the most reliable marker technologies in collaborative projects (Roder *et al.* 2002). Microsatellites have also been used to monitor variation in genes for important traits, such as resistance to apple scab (Vinatzer *et al.* 2004).Over the last decades, a large variety of different techniques have emerged (Spooner *et al.* 2005), including the use of microsatellites that are codominantly inherited and that are generally found to be highly informative and robust markers.

Shelf life determines the economic life time of mature apples. Good shelf life is highly associated with a slow decrease in fruit firmness which in turn depends on amount of ethylene produced. Studies on the cross Fuji x Braeburn revealed that Md-ACS1 and Md-ACO1 independently affect the internal ethylene concentration as well as the shelf life of apple, Md-ACS1 having the strongest effect. Descendants homozygous for Md-ACS1-2 and Md-ACO1-1 showed the lowest ethylene production as well as superior shelf life. These two genes are candidates to be included in marker assisted breeding (Costa *et al*. 2005).

A collection of 66 comercial apple cultivars was screened with 6 previously described SSR markers for molecular identification.In total, 55 polymorphic alleles were detected at the 6 SSR loci and the polymorphism information content averaged 0.72. Successful differentiation of all genotypes except for somatic mutants was accomplished by using only four SSR markers. (Galli *et al*. 2005)

Dilworth *et al.* (2006) reported that 86 reliable, highly polymorphic and well-scattered SSRs cover about 85% of apple genome. The molecular analysis conducted with the markers AL-07 and AM-19, detects the presence of the *Vf* gene, introgressed from *Malus floribunda* 821, which confers resistance to the pathogen, in the parental cultivars Liberty and Florina and in all the F1 hybrids belonging to these two genitors. Starkrimson and Golden Spur were susceptible scab parents with recessive homozygous genotype (*vfvf*), while Liberty and Florina were identified as heterozygous scab resistant parents with genotype (*Vfvf*). The results obtained with all of them confirmed the presence or absence of

Vf gene. The co-dominant marker AL-07 was very useful to discriminated homozygous from heterozygous plants for the *Vf* gene.

Zhu and Barritt (2008) genotyped 95 cultivars and selections for ethylene biosynthesis potential using two co-dominant functional markers (Md-ACS1 and Md-ACO1) and reported that these two functional markers can be efficiently utilized in an apple scion breeding program for selection of genotypes for low ethylene production, better storability and shelf life. Random amplified polymorphic DNA (RAPDs) and simple sequence repeats (SSR) were used to test the genetic variability and relatedness among 20 cultivars of apple. RAPD generated 107 polymorphic bands with 65.6% polymorphism while SSR generated 15 polymorphic alleles with 71.4% polymorphism.

The highest similarity was detected between 'Fuji' and 'Fuji Rekaki' (0.859) followed by 'Thanedar early flowering' and 'Fuji' by RAPD while between 'Galaxy' and 'Jonadel' (1.000) followed by 'Neomi' with 'Arlet', 'Fuji' and 'Royal Gala'; 'Fuji Rekaki' with 'Thanedar early flowering' and 'Reinette-Du-Canada' by SSR. The dendrogram generated from RAPD data grouped 'Fuji' and 'Fuji Rekaki' in one cluster and 'Jonadel' and 'Jonagold' in other cluster, while dendrogram derived from SSR data grouped 'Jonadel' and 'Jonagold' in one cluster and 'Arlet' and 'Royal Gala' in other cluster, which is generally in accordance with the recorded pedigree information (Pathania, 2010).

Patzak *et al*. (2011) studied the presence of genes for resistance to scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) using molecular markers in a sample of 279 apple cultivars from the Czech collection of apple genetic resources. The marker for the major scab resistance gene *Vf* was detected in all cultivars supposed to have *Vf*, except in Romus 1 and in the three small-fruited cultivars Malus Evereste, Golden Gem and Hilleri. The markers of the *Vr* and *Vh* scab resistance genes were detected in 22 cultivars in combination with the marker for *Vf,* in 56 reference world cultivars and in 82 old and local apple cultivars and reported that PCR molecular markers are useful tools for the identification of resistance genes within apple germplasm collections and can be used to increase the number of sources for disease resistance in breeding programmes.

Bilbao *et al*. (2012) used *Rvi6*-specific primers for evaluation of susceptibility of 92 apple cultivars to *V. inaequalis* and identified eight apple cultivars with low susceptibility to fire blight and resistance to apple scab, both to a mixed inoculum in the glasshouse and in the field.

Potts *et al*. (2012) used simple sequence repeats (SSRs) to assess genetic diversity and study genetic relatedness in a large collection of *Malus* germplasm. A total of 164 accessions from the *Malus* core collection, were genotyped using a single robust SSR marker from each of the 17 different linkage groups in *Malus*. Data were subjected to principal component analysis and a dendrogram was constructed to establish genetic relatedness. The diverse core collection showed high allelic diversity.

Suprun and Tokmakov (2013) studied the allelic diversity of the *Md-ACS1* and *Md-ACO1* genes involved in ethylene biosynthesis using a DNA-marker analysis of 48 apple varieties bred in Russia. Different allelic combinations of these genes were identified and found the prevalence of the allelic combinations associated with a long shelf life of apple fruits in the germplasm of Russian varieties which corresponds to the occurrence of these alleles in the worldwide gene pool.

Khajuria *et al*. (2014) screened eighty apple genotypes for presence of known genes conferring disease resistance using eight molecular markers. Different cultivars showed variable response to apple scab. Shireen and Firdous were found to harbor *Vf* (*Rvi6*) gene as a source of resistance.

Romero *et al.* (2015), characterized 29 apple accessions (*Malus x domestica* Borkh) belonging to 13 traditional cultivar denominations. For molecular characterization studies, 12 simple sequence repeat markers previously developed for apple species were used. Morphological characterization was performed using 33 fruit traits. A total of 115 alleles were amplified for the 12 loci, ranging from 7 to 13 alleles per locus, 41 alleles were unique to specific genotypes.

Chapter-3

MATERIALS AND METHODS

The present investigation entitled **"Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm"** was conducted in the Experimental fields of Horticulture farm, Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar, Jammu & Kashmir during the year 2017. The details of the materials used and the techniques followed during the course of investigation are described in this chapter.

3.1 Geographical features

District Srinagar, situated between 35º.5′-35º.7′ North latitude and 74º.8′-74º.9′ East longitude and at an altitude of 1500 meters above mean sea level, is surrounded by lofty Himalayan ranges on South-East and North-East sides. At the base of these ranges towards the North-East side lies the University campus, about 15 km from the main city of Srinagar.

3.2 Climate

The climate is temperate cum mediterranean and of continental type. Winter is severe extending over 100 days from the middle of December to March, during which the temperature often goes below the freezing point and the whole of Kashmir valley remains covered with snow. The valley is marked by extreme of temperature, ranging from a maximum of 35ºC in summer to a minimum of-10ºC in winter. The annual mean temperature is 15ºC. The climate is cold temperate. Rainfall is optimum well distributed about 80 cm per annum, mostly in the form of snow during winter and main rainfall is during March-April.

3.3 Soil

Soil is deep, well drained, fertile, loamy textured and suitable for horticultural purposes.

3.4 Experimental materials

The basic material for the present study comprised of 40 diverse genotypes of apple. On each tree four branches from four sides were marked, 20 fruits were selected randomly from the four marked branches. The phenological episodes, leaf and fruit characteristics for all the selected genotypes were recorded as per the guidelines for DUS test (Distinctiveness, Uniformity and Stability guidelines, 2012) on apple, under PPV & FRA, Act 2001 (Protection of Plant Varieties and Farmer's Rights Authority MOA, Government of India, New Delhi), International Union for the Protection of New Varieties of plants standard descriptor (UPOV, 2005) and RosBREED Apple Phenotyping Protocol (RosBREED phenotyping protocol www.rosbreed.org 2010) wherever feasible. The genotypes studied were:

Table 1: Plant material studied

Group	**Genotypes for the study**	**Total**
Released cultivars of SKUAST (K)	Lal Ambri, Sunhari, Firdous, Shireen, Akbar, Shalimar Apple-1 and Shalimar Apple-2.	7
Newly introduced cultivars of SKUAST (K)	Wiltons Star, Fuji Zhen Aztec, Super Chief, Gala Redlum, Red Velox, Silver Spur, Gala Mast, Mollies Delicious, Granny Smith, Braeburn, Oregon spur and Golden Delicious Reinders.	12
Old cultivars	Red Delicious, Chamure, Maharaji, Cox Orange Pippin, American Apirouge, June Eating, Lord Lambourne, Red Gold, Benoni, Golden Delicious, Starkrimson, Yellow Newton, Scarlet Siberian, Ambri and Irish Peach.	15
Scab resistant selections	ASP-1, ASP-3, ASP-4, ASP-10, ASP-12 and ASP-69.	6
Total		**40**

3.5 Experiment No. I: To determine the morphological and pomological diversity in apple reference set

3.5.1 Phenological stages (Reference Date-1st March)

3.5.1.1 Initial bloom (DARD)

This stage was observed visually when 10 per cent of flowers were open. The date of occurrence was recorded for each tree and then converted to days after reference date fixed arbitrarily as 1st March.

3.5.1.2 Full bloom (DARD)

This stage was observed visually when 80 per cent of flowers were open. The date of occurrence was recorded for each tree and then converted to days after reference date (1st March)

3.5.1.3 Maturity date (DAFB)

Maturity date was recorded when fruits had attained proper size and developed proper colouration with starch index rating of 3 on a 0-6 scale. The dates recorded were converted into days after full bloom.

3.5.2 Leaf characters

3.5.2.1 Leaf blade length (mm)

The leaf blade length was measured with the help of a scale and recorded in millimeters

3.5.2.2 Leaf blade width (mm)

The leaf blade width was measured with the help of a scale and recorded in millimeters.

3.5.2.3 Petiole length (mm)

The petiole length was measured with the help of a scale and recorded in millimeters.

3.5.2.4 Leaf shape

Leaf shape was recorded as:

i) Oval

ii) Ovate

3.5.2.5 Leaf blade: Intensity of green colour (DUS, 2012)

Intensity of green colour of the leaf blade was observed visually and recorded as:

I. Light green (1)

II. Green (2)

III. Dark green (3)

3.5.2.6 Leaf blade: Pubescence on lower side (DUS, 2012)

Pubescence on lower side of the leaf was observed with the help of a magnifying glass and recorded as:

I. Less/Weak (1)

II. Moderate (3)

III. High (5)

3.5.2.7 Leaf blade: Serrations (DUS, 2012)

Leaf margins of mature leaves were classified into following categories

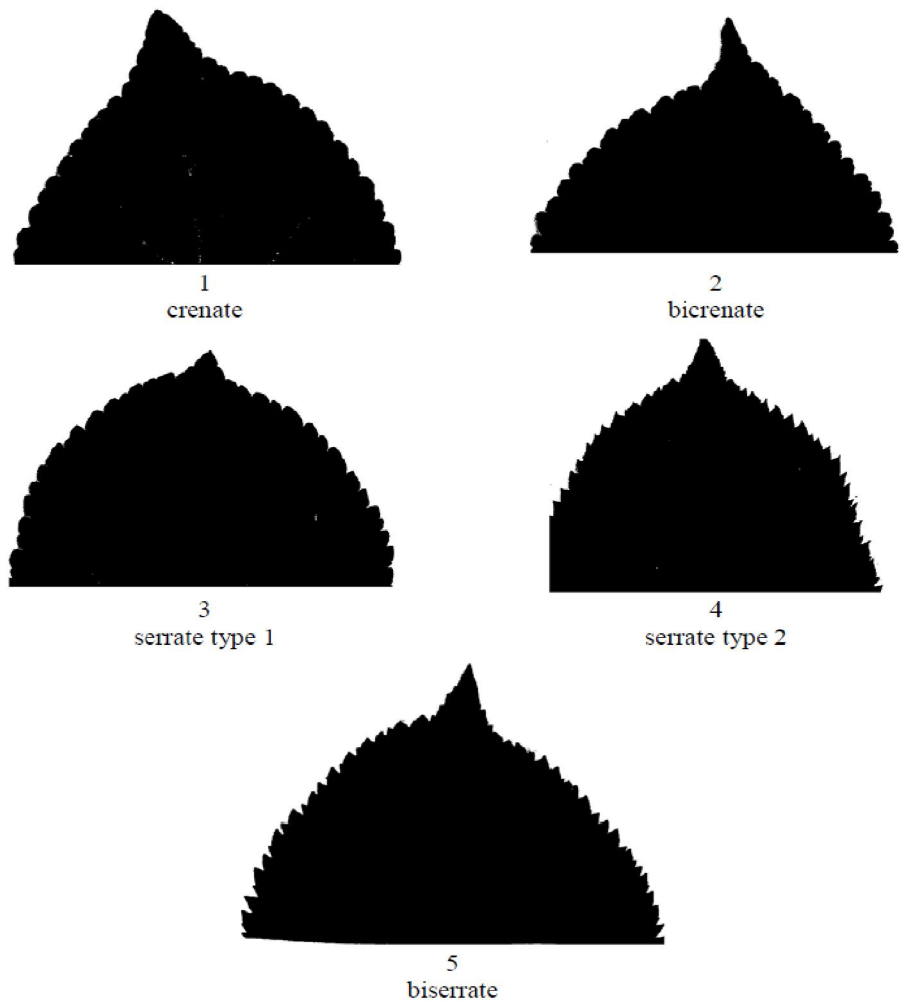
I. Crenate (1)

II. Bicrenate (2)

III. Serrate type 1 (3)

IV. Serrate type 2 (4)

V. Biserrate (5)



3.5.2.8 Leaf tip

Leaf tip was recorded as:

I. Acute

II. Acuminate

3.5.3 Fruit characters

3.5.3.1 Fruit length (mm)

The length of ten randomly selected fruits from each replication was measured from shoulders to apex with the help of a vernier caliper, averaged and recorded in millimeters.

3.5.3.2 Fruit diameter (mm)

The diameter of ten randomly selected fruits from each replication was measured along the cheek at maximum point with the help of a vernier calliper, averaged and recorded in millimeters.

3.5.3.3 Fruit weight (g)

Ten fruits from each replication were weighed individually on a sensitive monopan balance and average weight was recorded in grams.

3.5.3.4 Pedicel length (mm)

Length of the fruit pedicel was measured with the help of a scale and recorded in millimeters.

3.5.3.5 Fruit firmness (kg/cm^2)

Fruit firmness was measured with the help of Effegi penetrometer model, Ft-3-27 having 7/16 diameter of the head with a penetration of 5/6. In each replication, fruits were punched at two different places on its surface after removing about one square inch of peel and firmness was recorded as kg/cm^2.

3.5.3.6 Total soluble solids (°Brix)

A digital hand refractrometer ranging from 0-33 (^{o}Brix Erma make Japan) was used to determine the total soluble content of fresh fruit samples.

3.5.3.7 Acidity (% Malic acid)

Acidity was determined by taking a known weight of fruit sample (10 g) which was crushed and added to 100 ml distilled water, then filtered through Whatman's No. 1 filter paper. 10 ml of aliquot was titrated against N/10 NaOH using phenolphthalein indicator and end point was determined by pink colouration. The total titrable acidity was calculated in terms of malic acid on the basis of 1ml of 0.1N NaOH solution equivalent to 0.0067 and expressed in terms of per cent acidity (A.O.A.C., 1990). Acidity was calculated as malic acid by using the following formula:

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{normality of alkali} \times \text{volume made up} \times 67}{\text{Weight of sample} \times \text{volume of aliquot taken} \times 1000} \times 100$$

3.5.3.8 TSS-Acid ratio

Total soluble solids/acid ratio was determined using the formula:

$$\text{TSS/Acid ratio} = \frac{\text{Total Soluble Solids (\%)}}{\text{Acidity (\%)}}$$

3.5.3.9 Fruit shape (DUS, 2012)

The shape from the frontal view was observed and compared with the shapes provided in the descriptor and coded as under:

I. Conical (1)
II. Cylindrical (2)
III. Cylindrical waisted (3)
IV. Ellipsoid (4)
V. Globose (5)
VI. Obloid (6)
VII. Ovoid (7)

3.5.3.10 Ground colour (UPOV, 2005)

Ground colour of fruit was observed visually and recorded as:

I. Not visible (1)
II. Whittish yellow (2)
III. Yellow (3)
IV. Whitish green (4)

V. Yellow green (5)

VI. Green (6)

3.5.3.11 Fruit over colour (UPOV, 2005)

Fruit over colour was observed visually and recorded as:

I. Orange red (1)

II. Pink Red (2)

III. Red (3)

IV. Purple Red (4)

V. Brown red (5)

3.5.3.12 Pattern of over colour (DUS, 2012)

Pattern of over colour was observed visually and recorded as:

I. Only solid flush (1)

II. Solid flush with weakly defined stripes (2)

III. Solid flush with strongly defined stripes (3)

IV. Weakly defined flush with strongly defined stripes (4)

V. Flushed and mottled (5)

VI. Flushed striped and mottled (6)

3.5.3.13 Fruit russet amount (RosBREED, 2010)

Fruit area was recorded as:

I. No russet (rare but can occur) (1)

II. Stem cavity (2)

III. Slight (3)

IV. Severe (4)

3.5.3.14 Fruit lenticels (DUS, 2012)

Fruit Lenticels were counted per cm^2 and depending on the their number were recorded as:

I. Few (<4) (1)

II. Medium (4-7) (3)

III. Many (>7) (5)

3.5.3.15 Prominence of lobes at calyx end (DUS, 2012)

Prominence of lobes were recorded as:

I. Absent (1)

II. Weak (3)

III. Moderate (5)

IV. Strong (7)

3.5.3.16 Colour of flesh (DUS, 2012)

Flesh of the freshly cut fruits was recorded as:

I. Whitish (1)

II. Creamy (2)

III. Pinkish (3)

IV. Greenish (4)

V. Yellowish (5)

3.5.3.17 Calyx opening (RosBREED, 2010)

Calyx opening was recorded as:

I. All closed (1)

II. Mixed (2)

III. All open (3)

3.5.3.18 Pedicel end of the fruit (DUS, 2012)

Based on the length of the stalk as measured with the scale pedicel end of the fruit was recorded as:

I. Shallow (<10 mm) (1)
II. Medium (10-20 mm) (3)
III. Deep (>20 mm) (5)

3.5.3.19 Fruit greasiness of skin (RosBREED, 2010)

Fruit greasiness of skin was detected by sliding the thumb or finger firmly across the surface of the fruit and was recorded as:

I. None (1)
II. Slight (2)
III. Medium (3)
IV. Very greasy (4)

3.5.3.20 Core opening (RosBREED, 2010)

Core opening was observed visually and recorded as:

I. Open (0)
II. Closed (1)

3.5.3.21 Water core (RosBREED, 2010)

Water core was observed at harvest and was recorded as:

I. None (1)
II. Slight (2)
III. Medium (3)
IV. Severe (4)

3.5.3.22 Sensory evaluation (5 point scale)

To assess the consumer acceptability (physicals appearance, colour, taste, flavour, crispness, texture, firmness and overall quality), the fruits were rated on a scale of 1-5 by a panel of five experts as:

1	=	Poor
2	=	Fair
3	=	Good
4	=	Very Good
5	=	Excellent

3.5.4 Statistical and biometrical analysis

The data recorded during the present investigation was subjected to following statistical and biometrical analysis.

- Analysis of variance and estimation of the components of variability
- Estimation of heritability and expected genetic gain,
- Estimation of genotypic correlation coefficient,
- Divergence analysis.

3.5.4.1 Analysis of variance and estimation of the components of variance

3.5.4.1.1 Analysis of variance

Analysis of variance for all the characters was carried out for testing variation among the genotypes.

The Analysis of variance table was set up as under:

Source of variation	**d.f**	**SS**	**MSS**	**Fcal**
Replication	r-1	SSQr	MSR= SSQr/r-1	
Treatments (Adjusted)	K^2-1	SSQt	MST=SSQt/K^2-1	MST/MSE
Blockswithin replication (adjusted for treatments)	r (K-1)	SSQb	MSB= SSQb/r (K-1)	MSB/MSE
Intra-block error	(k-1) (rk-k-1)	SSQe	MSE= SSQe/ (k-1) (rk-k-1)	
Total	rK^2-1			

Where,

R	=	number of replications
K^2	=	number of treatments
K	=	number of blocks
SS	=	Sum of squares
MSS	=	Mean sum of squares
SSQr	=	sum of squares due to replication
MSR	=	Mean sum of squares due to replication
SSQb	=	sum of squares due to blocks with in replication, adjusted for treatments
MSB	=	Mean sum of squares due to blocks with in replication, adjusted for treatments.
SSQe	=	sum of squares due to intra-block error
MSE	=	Mean sum of squares due to intra-block error
SSQt	=	sum of squares due to treatments (adjusted)
MST	=	Mean sum of squares due to treatments (adjusted)

The significance of varietal differences was tested by F-test comparing calculated F-value at 5 and 1 per cent level of significance at treatment (t-1) and error (t-1) (r-1) degrees of freedom. If the F-value was found to be significant, comparison was further extended to compare each treatment mean with every other treatment utilizing Duncan's Multiple Range Test (DMRT).

3.5.4.1.2 Genotypic variance

Genotypic variance was calculated using the method suggested by Johnson *et al.* (1955).

$$\hat{\sigma}^2_g = \frac{MSG - MSE}{r}$$

Where,

$\hat{\sigma}^2_g$	=	Genotypic variance,
MSG	=	mean sum of squares due to genotypes,
MSE	=	mean sum of squares due to error and
R	=	number of replications

3.5.4.1.3 Phenotypic variance

Phenotypic variance was calculated as per the procedure given by Johnson *et al.* (1955).

$$\hat{\sigma}^2 p = \hat{\sigma}^2_g + \hat{\sigma}^2_e$$

Where,

$\hat{\sigma}^2 p$	=	Phenotypic variance
$\hat{\sigma}^2_g$	=	genotypic variance and
$\hat{\sigma}^2 e$	=	error variance

3.5.4.1.4 Phenotypic and genotypic co-efficient of variation

The magnitude of phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) existing in a trait was worked out by the formula given by Burton (1952):

$$PCV = \frac{\sqrt{\hat{\sigma}^2 p}}{\overline{x}} \times 100$$

Where,

$\hat{\sigma}^2 p$ = Phenotypic variance and

$\overline{x}$ = mean of the trait studied

$$GCV = \frac{\sqrt{\hat{\sigma}^2 g}}{\overline{x}} \times 100$$

Where,

$\hat{\sigma}^2 g$ = Genotypic variance and

$\overline{x}$ = mean of the trait studied

3.5.4.2 Estimation of heritability genetic advance and expected genetic gain

3.5.4.2.1 Heritability (broad sense)

It was estimated as per the procedure presented by Burton and De vane (1953), Johnson *et al.* (1955) and Hanson *et al.* (1956).

$$h^2 = \frac{\sigma^2 g}{\sigma^2 p}$$

Where,

h^2 = Estimate of heritability in broad sense,

$\sigma^2 g$ = Genotypic variance and

$\sigma^2 p$ = Phenotypic variance

3.5.4.2.2 Genetic advance

Genetic advance at 5 per cent selection intensity was worked out by using the formula given by Lush (1949) and Johnson *et al.* (1955).

$$GA = \frac{\sigma^2 g}{\sigma^2 p} \times (\sigma^2 p) \text{ x } K$$

Where,

GA	=	Genetic advance of the trait,
$\sigma^2 g$	=	genotypic variance of the trait,
$\sigma^2 p$	=	phenotypic variance of the trait and
K	=	selection differential (K= 2.06 at 5 per cent selection intensity)

3.5.4.2.3 Expected genetic gain (genetic advance as per cent of mean)

It was estimated as per the method suggested by Johnson *et al.* (1955).

$$\text{Genetic gain} = \frac{GA}{\overline{X}} \times 100$$

Where,

G.A.	=	Genetic advance of the trait and
$\overline{X}$	=	mean of the trait

3.5.4.3 Estimation of genotypic covariances and correlation coefficients

Covariance analysis followed the same pattern as the variance analysis. The genotypic and phenotypic covariances between two characters were obtained in the same fashion as corresponding variances. Estimate of genotypic and phenotypic variances and covariances were substituted in the following formula suggested by Panse and Sukatme (1985) to calculate correlation co-efficient between all possible pairs of characters.

Genotypic correlation co-efficient

$$r_{xy}\ (g) = \frac{\hat{\sigma}^2{}_{xy}\ (g)}{\sqrt{\hat{\sigma}^2{}_x\,(g)\ \hat{\sigma}^2{}_y\,(g)}}$$

Phenotypic correlation coefficient

$$r_{xy}\ (p) = \frac{\hat{\sigma}^2{}_{xy}\ (p)}{\sqrt{\hat{\sigma}^2{}_x\,(p)\ \hat{\sigma}^2{}_y\,(p)}}$$

Where,

$r_{xy}\ (g), r_{xy}\ (p)$	=	Genotypic and phenotypic correlation coefficients, respectively, between a pair of characters x and y
$\hat{\sigma}^2 xy(g), \hat{\sigma}^2 xy(p)$	=	Genotypic and phenotypic covariances, respectively, for a pair of characters x and y
$\hat{\sigma}^2 x(g), \hat{\sigma}^2 y(g)$	=	Genotypic variance for characters x and y, respectively and
$\hat{\sigma}^2 x(p), \hat{\sigma}^2 y(p)$	=	Phenotypic variance for character x and y, respectively.

Test of significance

The significance of a correlation co-efficient was tested by the following formula:

$$t = \frac{r\ (n - 2)^{0.5}}{(1 - r^2)^{0.5}}$$

Where,

R = Correlation coefficient and

N = number of observations

Any value (±) exceeding the table value of t at n-2 d.f is significant.

3.5.4.4 Estimates of genetic divergence

The genetic divergence was computed using the procedure. The details of analysis are described under the following heads as described by Rao (1952) and Singh and Choudhary (1985):

1. Computation of D^2 values,
2. Relative contribution of individual characters towards total divergence and
3. Group constellation

3.5.4.4.1 Computation of D^2 values

For each pair-wise combination of the varieties the differences in transformed values for various characters were computed and D^2-values were calculated according to the following formula:

$$D^2 = \sum_{i-1}^{p} (\overline{Y}_{ij} - Y_{ik})^2$$

Where,

P	=	number of characters studied and
Y_{ij} and Y_{ik}	=	are two transformed variables of the i^{th} character for two genotypes

3.5.4.4.2 Relative contribution of individual characters towards total divergences

The ranking of differences in uncorrelated means between all the characters for all pair-wise combinations of varieties was carried out, with first rank being assigned to the highest differences. Finally relative contribution of a character towards total divergence was estimated by calculating the percentage of first rank in that character.

3.5.4.4.3 Group constellation

Tocher's method was used for assigning various varieties to different clusters.The two varieties having smallest distance from each other were considered first to which a third variety having smallest average D^2 value from the first two varieties was added. Next come the nearest fourth variety and the process continued till the average D^2 value increased. The remaining varieties were then considered for the next cluster and the process was continued till all varieties were included in various clusters.

The spatial distances between clusters were arrived at by taking square root of average intra and inter cluster D^2 values.

For each combination (pair of genotypes) the mean deviation (d^2i) i.e. Y_1-Y_1 with I = 1, 2, 3 ……….. p was computed and D^2 values were calculated as sum of these deviations i.e. (y_i^1-y_i^2), where, y_i is the transformed variable from the original variable xi. Accordingly D^2 values for all combinations were calculated. The D^2 values so obtained for each pair of population were treated as x^2 and were tested against the tabulated values of λ^2 for p degrees of freedom, where p is the number of traits considered.

In all combinations each character was ranked on the basis of d_i = y_{ij}-y_{ik} values. Rank I was given to the highest mean difference and rank p to the lowest mean difference, where p is the total number of characters. In this manner contribution of each character to the total divergence was computed.

Tocher method for grouping of varieties into various clusters was adopted. This method is detailed in a simplified way by Rao (1952) and Singh and Choudhary (1985).

All the above computations were carried out using the software Indostat at Bio-informatics Laboratory of the Division of Genetics and Plant Breeding SKUAST-Kashmir, Shalimar.

3.6 Experiment II: Assessment of allelic diversity among apple genotypes for fruit quality characteristics using gene specific markers

3.6.1 DNA Extraction

DNA extraction involves two steps *viz*., lysis of cell walls and membranes to release DNA into solution and purification of DNA by precipitating protiens and polysaccharides. The standard Cetyltrimethyl ammonium bromide (CTAB) method gives a good quality of relatively pure DNA. In our experiment DNA was extracted from fresh and young leaves of 40 apple genotypes using CTAB method (Doyle and Doyle, 1990 with some minor modifications).

Procedure

- About 100 mg of leaf tissue was crushed in pre chilled mortar and pestle in presence of liquid nitrogen. Thorough crushing of leaves was done before adding extraction buffer but not ground into a very fine powder as it results in shearing of DNA.20 mg of PVP (Polyvinylpyrollidene) was added to each sample during grinding step.
- The powder was transferred to 2 ml eppendrof tubes and 1 ml of pre warmed (65^{0}C) CTAB uffer (Cetyl Trimethyl Ammonium Bromide buffer 2% w/v-CTAB, 1.4M NaCl, 20Mm EDTA, 100 mM Tris-HCL, 0.2% Mercaptoethanol) was added.
- The contents were mixed well by vigorous shaking and tubes were incubated at 65^{0}C for one hour in water bath. Occasional mixing was performed during this period and then the tubes were cooled at room temperature.
- 1ml of chloroform: isoamyl alcohol (24:1) (v/v) was added and contents were mixed by inverting the tubes for 5 minutes.
- Samples were centrifuged for 15 minutes at 10,000 rpm at room temperature so as to separate the phases.

- Supernatant (aqueous phase) was carefully pipetted out without disturbing the interface to other fresh 1.5 ml eppendrof tube.
- Chilled isopropanol (350 µl) was added to the supernatant (500 µl) and it was kept in the refrigerator overnight so as to precipitate the DNA.
- Tubes were then centrifuged at 10,000 rpm for 5 minutes so as to precipitate DNA at the bottom of tube. The supernatant was discarded.
- The pellet was washed twice with 70% ethanol.
- Ethanol was drained and the pellet was allowed to dry at room temperature.
- The pellet was dissolved in 100 µl of TE buffer (pH 8.0). and stored at-20º C for further purification.

3.6.2 Purification

- After melting of DNA by thawing, 200 µl of RNase A (10 µl/ml) was added to each sample mixed properly and incubated at 37ºC for 1 hour in water bath.
- Mixture of phenol:chloroform (1:1) was added in equal amounts. The samples were gently mixed by inverting for 10 minutes and centrifuged at 13,000 rpm for 10 minutes
- Supernatant was collected and equal volume of chloroform and isoamyl alcohol (24:1) was added and centrifuged at 13,000 rpm for 10 minutes.
- Supernatant was collected in fresh 2.0 ml centrifuge tubes. 0.1 volume of 3M sodium acetate (pH 8.0) and double volume of chilled ethanol was added.The tubes were centrifuged at 7,000 rpm for 5 minutes to get the DNA pellet.
- Supernatant was discarded and pellet was washed with 1ml of 70% ethanol for 10 minutes and centrifuged at 1,000 rpm for 5 minutes.

- Ethanol was discarded and pellet was dried at room temperature for 1 hour.
- The pellet containing DNA was dissolved in 50 µl of TE buffer and stored at 4º C.

3.6.3 DNA quantification

- The concentration and purity of DNA was checked by Agrose Gel electrophrosis. Various steps involved are given here:
- 0.8 g agrose was dissolved in 100 ml of 1X TAE electrophoresis buffer
- The mixture was heated till the agrose dissolved completely i.e., when the solution became transparent and clear. It was cooled down to 65^{0}C with constant stirring. Ethidium bromide was added to a final concentration of 0.5µg/ml of buffer.
- Agrose solution was then poured into an already prepared gel mould with combs and left for 20-30 minutes for solidification.
- DNA samples for loading were prepared by adding 2 µl loading dye
- DNA samples were loaded into wells with the help of micropipette.
- Gel was run for about half an hour at voltage of 5 V/cm and visualized under UV transilluminator.
- DNA samples were photographed using photo gel documentation system
- Quality of DNA samples was judged based on whether DNA formed a single high molecular weight band (good quality) or a smear (degraded or poor quality).

3.6.4 PCR standardization and amplification

A mixture of 20 µl of various PCR reagents, based on the stock and final concentration of different components was prepared as under:

Table 2: Stock and final concentration of different components used in PCR

S.No.	Component	Stock concentration	Final concentration	Volume (µl)
1.	Water	-		10.2
2.	PCR buffer	10X*	1X	2.0
3.	MgCl2	25 mM	1.5 mM	1.2
4.	dNTPs	100 mM	2 mM	0.4
5.	Primer Forward	100 µM	10 µM	2
6.	Primer Reverse	100 µM	10 µM	2
7.	*Taq* Polymerase	5U/5 µl	1 Unit	0.2
8	DNA template	50 ng/µl	100 ng/µl	2
9.				**20**

*10X PCR buffer: 200 mM Tris-HCl (pH 8.3), 500 mM KCl

Table 3: Temperature profile in PCR

S. No.	Step	Temperature (°C)	Time (minutes)	No. of cycles
1.	Initial Denaturation	94.0	5	1
2.	Denaturation	94.0	1	35
3.	Annealing	58-63	1	
4.	Elongation (Extension)	72.0	2	
5.	Final Extension	72.0	7	
6.	Hold	4.0		

3.6.5 Marker based genotyping of apple germplasm for fruit quality traits

Genotyping of the apple varieties was carried out using gene specific markers which are given below:

Table 4: Markers for detection of scab and fruit quality traits

S. No.	Trait	Markers	References
1	Fruit storability	Md-ACS1, Md-ACO1	Zhu and Barritt (2008)
2	Scab	AL-07, AM-19	Tartarini *et al.* (1999)

Chapter - 4

EXPERIMENTAL FINDINGS

The results obtained during the present investigation entitled, **"Morpho-Molecular characterization of apple (*Malus x domestica* Borkh.) germplasm"** are presented in the following sections of this chapter under two experiments.

4.1 Experiment No. I: To determine the morphological and pomological diversity in apple reference set

4.1.1 Phenological stages

4.1.1.1 Initial bloom

Initial bloom dates for each genotype was recorded and compared taking Red Delicious as the standard genotype (Table 5). It is evident from the recorded observations that the initial bloom ranged from 7 to 16th April. Benoni was the earliest to reach the initial bloom stage (7th April) 4 days prior to Red Delicious as against ASP-69 which was the last to reach the initial bloom stage (16th of April) 5 days after Red Delicious.

4.1.1.2 Full bloom

Full bloom dates for each genotype was recorded and compared taking Red Delicious as the standard genotype (Table 5). From the Table it is evident that the full bloom dates ranged from 14th to 26th April. Benoni retained the bloom till 14thApril 5 days prior to Red Delicious while the genotypes ASP-69 and ASP-12 retained the bloom till 26th April 7 days after Red Delicious.

4.1.1.3 Maturity date

Perusal of the data related to maturity date cited in Table 5 indicates that the maturity date in apple genotypes studied ranged from 3rd week of July to 3rd week of October. It is evident from the table that Irish peach was the earliest to mature (3rd week of July) while Maharaji was the last genotype to mature (3rd week of October). The maturity dates were estimated based on number of days

Table 5: Phenological stages of apple genotypes

S. No.	Genotypes	Initial bloom (10%)	Initial bloom w.r.t Red Delicious	Full bloom (80%)	Full bloom w.r.t Red Delicious	Maturity date (DAFB)	Maturity date (DAFB) w.r.t. Red Delicious
1	Wiltons Star	12 April	+1	21 April	+2	12 September (144)	-6
2	Cox's Orange Pippin	12 April	+1	21 April	+2	8 September (140)	-8
3	Shireen	11 April	0	20 April	+1	20 August (122)	-27
4	Sunhari	13 April	+2	20 April	+1	28 September (161)	+12
5	Shalimar Apple-2	11April	0	20 April	+1	12 September (145)	-4
6	Shalimar Apple-1	9 April	-2	17 April	-2	25 August (130)	-22
7	Maharaji	13 April	+2	22 April	+3	15 October (176)	+29
8	American Apirouge	13 April	+2	23 April	+4	21 September (151)	+5
9	Scarlet Siberean	8 April	-3	16 April	-3	4 October (171)	+18
10	Irish Peach	9 April	-2	18 April	-1	15 July (88)	-63
11	June Eating	10 April	-1	17 April	-2	19 July (93)	-59
12	Lord Lambourne	9 April	-2	17 April	-2	26 August (131)	-21
13	Oregon Spur	11 April	0	18 April	-1	30 August (134)	-17
14	Braeburn	10 April	-1	19April	0	1 October (165)	+15
15	Granny Smith	12 April	+1	20April	+1	10 October (173)	+24
16	Mollies Delicious	11 April	0	19April	0	7 August (110)	-40
17	Gala Mast	11 April	0	20April	+1	16 August (118)	-31
18	Red Delicious	11 April	0	19April	0	16 September (150)	0
19	Benoni	7 April	-4	14April	-5	19 July (96)	-59
20	Chamure	11 April	0	19April	0	2 October (166)	+16
21	Red Gold	11 April	0	18 April	-1	9 September (144)	-7

Contd..

Table 5: Contd..

22	Golden Delicious	12 April	+1	20 April	+1	26 September (159)	+10
23	Starkrimson	10 April	-1	17 April	-2	22 August (127)	-25
24	Yellow Newton	11 April	0	19 April	0	29 September (163)	+13
25	Golden Delicious Reinders	12 April	+1	20 April	+1	21 September (154)	+5
26	Silver Spur	12 April	+1	19 April	0	7 September (141)	-9
27	Red Velox	13 April	+2	21 April	+2	3 September (135)	-13
28	Fuji Zhen Aztec	13 April	+2	21 April	+2	4 October (166)	+18
29	Gala Redlum	10 April	-1	17 April	-2	24 August (129)	-23
30	Super Chief	11 April	0	18 April	-1	12 September (147)	-4
31	Lal Ambri	12 April	+1	20 April	+1	1 October (164)	+15
32	Firdous	11 April	0	19 April	0	26 August (129)	-21
33	Akbar	12 April	+1	20 April	+1	26 September (159)	+10
34	ASP-3	11 April	0	21 April	+2	4 September (136)	-12
35	ASP-4	11 April	0	21 April	+2	21 September (153)	+5
36	ASP-69	16 April	+5	26 April	+7	31 August (127)	-16
37	ASP-1	14 April	+3	24 April	+5	30 August (128)	-17
38	ASP-10	14 April	+3	24 April	+5	24 July (91)	-54
39	ASP-12	15 April	+4	26 April	+7	29 August (125)	-18
40	Ambri	13 April	+2	21 April	+2	5 October (167)	+19

from full bloom to harvest and also with respect to Red Delicious. Irish Peach matured 88 days after full bloom and 63 days before Red Delicious while as Maharaji matured 176 days after full bloom and 29 days after Red Delicious.

4.1.2 Leaf characters

4.1.2.1 Leaf blade length (mm)

The data pertaining to the length of leaf blade of various apple genotypes is presented in Table 6. It reveals that average leaf blade length varied from 58.66 to 101.00 mm. Among the genotypes under study, the maximum average leaf blade length of 101.00 mm was observed in ASP-4 and ASP-10 followed by 99.3 mm in Shalimar Apple-1. Minimum leaf blade length (58.66 mm) was recorded in Scarlet Siberean.

4.1.2.2 Leaf blade width (mm)

The observation related to the width of leaf blade of apple genotypes under study is recorded in Table 6 which showed a significant difference among the genotypes. It is evident from the data that the variety Lal Ambri recorded maximum average width of leaf blade of about 67.66 mm. Maharaji recorded an average leaf blade width of 67.00 mm. Minimum leaf blade width of 36.00 mm was recorded in Scarlet Siberean.

4.1.2.3 Petiole length (mm)

Perusal of data of petiole length of the 40 apple genotypes as depicted in Table 6 showed a significant difference among all genotypes under study. The petiole length varied from 17.66 to 40.33 mm with maximum petiole length (40.33 mm) recorded in Akbar while minimum petiole length (17.66 mm) was recorded in American Apirouge

4.1.2.4 Leaf shape

The shape of the leaf for various apple genotypes was categorized as oval and ovate. Among the selected genotypes oval shape of the leaf was noticed in 29

(72.5%) genotypes while ovate shape was noticed in 11 (27.5%) genotypes (Table 8).

4.1.2.5 Leaf blade: Intensity of green colour

The data related to the intensity of green colour of the leaf blade is given in Table 8.The intensity of green colour ranged from green to dark green. Dark green colour was recorded in maximum number of genotypes i.e 28 (70%) while as green colour of the leaf blade was observed in 12 (30%) genotypes.

4.1.2.6 Leaf blade: Pubescence on lower side

The observation related to the pubescence on the lower side of leaf blade is inscribed in Table 8. The pubescence on the lower side of leaf blade was studied as less/weak, moderate and high based on the descriptor. Out of the 40 selected genotypes, less/weak pubescence was recorded in 16 (40%) genotypes, high amount of pubescence on the lower side of the leaf was recorded in 13 (32.5%) genotypes while moderate amount of pubescence was observed in the remaining in 11 (27.5%) genotypes.

4.1.2.7 Leaf blade: Serrations

Incisions of the leaf margins were studied as serrate type 1, serrate type 2, biserrate, crenate and bicrenate. Among the 40 selected apple genotypes biserrate type of leaf margins were observed in 17 (42.5%) genotypes, serrate type-2 in 11 (27.5%), crenate in 5 (12.5%), bicrenate in 4 (10%) genotypes and serrate type-1 in the remaining 3 (7.5%) genotypes (Table 8).

4.1.2.8 Leaf tip

The observations recorded with respect to leaf tip are presented in Table 8.From the table it is evident that leaf tip was recorded as acuminate and acute. Acuminate tip was observed in 38 (95%) genotypes while as acute tip was observed only in 2 (5%) genotypes

Table 6: Mean values of leaf characteristics of apple genotypes

S. No.	Genotypes	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)
1.	Wiltons Star	$92.00^{defghij}$	47.00^{mnop}	38.66^{ab}
2.	Cox's Orange Pippin	75.66^{rs}	57.33^{cdefgh}	21.00^{qrs}
3.	Shireen	83.00^{mnopq}	58.33^{cdef}	32.66^{cdefgh}
4.	Sunhari	$91.00^{defghijk}$	$54.66^{efghijk}$	32.66^{cdefg}
5.	Shalimar Apple-2	$86.00^{jklmnop}$	57.00^{cdefgh}	26.00^{lmnop}
6.	Shalimar Apple-1	99.33^{ab}	53.66^{ghijk}	27.00^{klmno}
7.	Maharaji	$88.33^{eghijklm}$	67.00^{a}	$28.33^{hijklmn}$
8.	American Apirouge	73.33^{s}	51.00^{jklmn}	17.66^{s}
9.	Scarlet Siberean	58.66^{u}	36.00^{r}	18.66^{rs}
10.	Irish Peach	$89.33^{efghijkl}$	58.66^{cdef}	$32.00^{defghij}$
11.	June Eating	94.00^{bcdef}	$54.66^{efghijk}$	24.00^{klmno}
12.	Lord Lambourne	83.66^{lmnopq}	53.66^{ghijkl}	$30.33^{efghijk}$
13.	Oregon Spur	75.00^{s}	47.66^{mnop}	26.33^{klmnop}
14.	Braeburn	$87.00^{ijklmno}$	56.00^{defghi}	$32.33^{defghij}$
15.	Granny Smith	$92.00^{defghij}$	59.66^{bcd}	$29.66^{efghijklm}$
16.	Mollies Delicious	$93.33^{defghij}$	47.66^{mnop}	36.00^{bcd}
17.	Gala Mast	$87.66^{hijklmn}$	48.66^{mnop}	26.00^{lmnop}
18.	Red Delicious	74.66^{s}	43.00^{s}	27.00^{klmno}
19.	Benoni	81.00^{pqr}	51.66^{jklm}	28.33^{jklmn}

Contd..

Table 6: contd..

20.	Chamure	$87.33^{hjklmno}$	61.00^{bc}	26.00^{lmnop}
21.	Red Gold	83.33^{lmnopq}	54.66^{hijkl}	28.33^{jklmn}
22.	Golden Delicious	94.33^{bcde}	58.00^{cdefg}	23.33^{opqr}
23.	Starkrimson	94.00^{bcdefg}	47.66^{mnop}	36.66^{bc}
24.	Yellow Newton	78.00^{qrs}	54.00^{fghijk}	23.00^{opqr}
25.	Golden Delicious Reinders	95.33^{abcd}	52.00^{ijklm}	$29.66^{fghijklm}$
26.	Silver Spur	82.00^{nopq}	45.66^{op}	33.33^{cdef}
27.	Red Velox	65.33^{t}	40.33^{qr}	26.33^{klmno}
28.	Fuji Zhen Aztec	81.66^{opqr}	$55.33^{defghij}$	25.66^{mnop}
29.	Gala Redlum	$87.33^{hijklmno}$	50.00^{klmno}	22.33^{pqr}
30.	Super Chief	$88.33^{efghijklm}$	45.00^{pq}	28.66^{gijklm}
31.	Lal Ambri	$92.66^{cdefghi}$	67.66^{a}	$29.66^{efghijklm}$
32.	Firdous	85.33^{klmnop}	49.00^{lmnop}	20.66^{qrs}
33.	Akbar	98.33^{abc}	53.66^{ghijk}	40.33^{ab}
34.	ASP-3	85.66^{klmnop}	54.00^{fghijk}	33.66^{cde}
35.	ASP-4	101.00^{a}	63.00^{ab}	31.66^{efghij}
36.	ASP -69	83.00^{mnopq}	57.33^{cdefgh}	$30.00^{efghijkl}$
37.	ASP -1	83.00^{mnopq}	$54.66^{efghijk}$	$29.33^{fghijklm}$
38.	ASP –10	101.00^{a}	56.00^{defghi}	26.00^{lmnop}
39.	ASP –12	$88.00^{fghijklm}$	58.66^{cde}	$29.33^{fghijklm}$
40.	Ambri	$88.66^{eghijkl}$	46.66^{nop}	$32.33^{cdefghi}$

Table 7: Leaf characteristics of apple genotypes

S. No.	Genotypes	Leaf shape	Leaf blade: intensity of green colour	Leaf blade: pubescence on lower side	Leaf blade: serrations	Leaf tip
1.	Wiltons Star	Oval	Dark green (3)	Moderate (3)	Serrate type 1 (3)	Acuminate
2.	Cox's Orange Pippin	Ovate	Green (2)	High (5)	Biserrate (5)	Acuminate
3.	Shireen	Oval	Dark Green (3)	High (5)	Biserrate (5)	Acuminate
4.	Sunhari	Oval	Dark Green (3)	Moderate (3)	Serrate type 2 (4)	Acuminate
5.	Shalimar Apple-2	Oval	Dark Green (3)	Moderate (3)	Biserrate (5)	Acuminate
6.	Shalimar Apple-1	Ovate	Dark Green (3)	Weak (1)	Crenate (1)	Acuminate
7.	Maharaji	Ovate	Dark Green (3)	Weak (1)	Biserrate (5)	Acuminate
8.	American Apirouge	Oval	Dark Green (3)	Moderate (3)	Bicrennate (2)	Acuminate
9.	Scarlet Siberean	Oval	Green (2)	Weak (1)	Serrate type 2 (4)	Acuminate
10.	Irish Peach	Ovate	Dark Green (3)	Moderate (3)	Serrate type 1 (3)	Acuminate
11.	June Eating	Oval	Dark Green (3)	Weak (1)	Biserrate (5)	Acuminate
12.	Lord Lambourne	Oval	Green (2)	Weak (1)	Biserrate (5)	Acuminate
13.	Oregon Spur	Ovate	Dark Green (3)	High (5)	Biserrate (5)	Acuminate
14.	Braeburn	Oval	Dark Green (3)	High (5)	Serrate type 2 (4)	Acute
15.	Granny Smith	Oval	Dark Green (3)	High (5)	Serrate type 1 (3)	Acuminate
16.	Mollies Delicious	Oval	Green (2)	High (5)	Bicrennate (2)	Acuminate
17.	Gala Mast	Oval	Dark Green (3)	Moderate (3)	Biserrate (5)	Acuminate
18.	Red Delicious	Ovate	Dark Green (3)	High (5)	Crennate (1)	Acuminate
19.	Benoni	Oval	Green (2)	Weak (1)	Serrate type 2 (4)	Acuminate

Contd..

Table 7 contd..

20.	Chamure	Oval	Dark Green (3)	High (5)	Biserrate (5)	Acuminate
21.	Red Gold	Ovate	Dark Green (3)	High (5)	Bicrennate (2)	Acuminate
22.	Golden Delicious	Oval	Dark Green (3)	High (5)	Serrate type 2 (4)	Acuminate
23.	Starkrimson	Oval	Dark Green (3)	Moderate (3)	Serrate type 2 (4)	Acuminate
24.	Yellow Newton	Ovate	Green (2)	Weak (1)	Biserrate (5)	Acuminate
25.	Golden Delicious Reinders	Oval	Dark Green (3)	Moderate (3)	Biserrate (5)	Acuminate
26.	Silver Spur	Oval	Dark Green (3)	High (5)	Serrate type 2 (4)	Acuminate
27.	Red Velox	Ovate	Green (2)	High (5)	Bicrennate (2)	Acuminate
28.	Fuji Zhen Aztec	Oval	Dark Green (3)	Moderate (3)	Serrate type 2 (4)	Acuminate
29.	Gala Redlum	Oval	Dark Green (3)	Moderate (3)	Serrate type 2 (4)	acuminate
30.	Super Chief	Oval	Dark Green (3)	Moderate (3)	Serrate type 2 (4)	Acuminate
31.	Lal Ambri	Ovate	Dark Green (3)	Weak (1)	Biserrate (5)	Acuminate
32.	Firdous	Oval	Green (2)	High (5)	Biserrate (5)	Acuminate
33.	Akbar	Oval	Dark Green (3)	Weak (1)	Crenate (1)	Acuminate
34.	ASP-3	Oval	Green (2)	Weak (1)	Biserrate (5)	Acuminate
35.	ASP-4	Oval	Green (2)	Weak (1)	Biserrate (5)	Acuminate
36.	ASP-69	Oval	Dark Green (3)	Weak (1)	Serrate type 2 (4)	Acuminate
37.	ASP-1	Oval	Green (2)	Weak (1)	Biserrate (5)	Acuminate
38.	ASP -10	Ovate	Dark Green (3)	Weak (1)	Crenate (1)	Acute
39.	ASP -12	Oval	Dark Green (3)	Weak (1)	Biserrate (5)	Acuminate
40.	Ambri	Oval	Green (2)	Weak (1)	Crenate (1)	Acuminate

* Values within parenthesis are scores as per descriptor

Table 8: Summary of frequency of leaf characters of apple genotypes

Leaf character	Category	Number of genotypes	Percentage (%)
Leaf shape	Oval	29	72.5
	Ovate	11	27.5
Leaf blade intensity of green colour	Light Green (1)	0	0
	Green (2)	12	30
	Dark Green (3)	28	70
Leaf blade pubescence on lower side	Less/weak (1)	16	40
	Moderate (3)	11	27.5
	High (5)	13	32.5
Leaf blade serrations	Crenate (1)	5	12.5
	Bicrenate (2)	4	10
	Serrate type 1 (3)	3	7.5
	Serrate type 2 (4)	11	27.5
	Biserrate (5)	17	42.5
Leaf tip	Acute	2	5
	Acuminate	38	95

* Values within parenthesis are scores as per descriptor

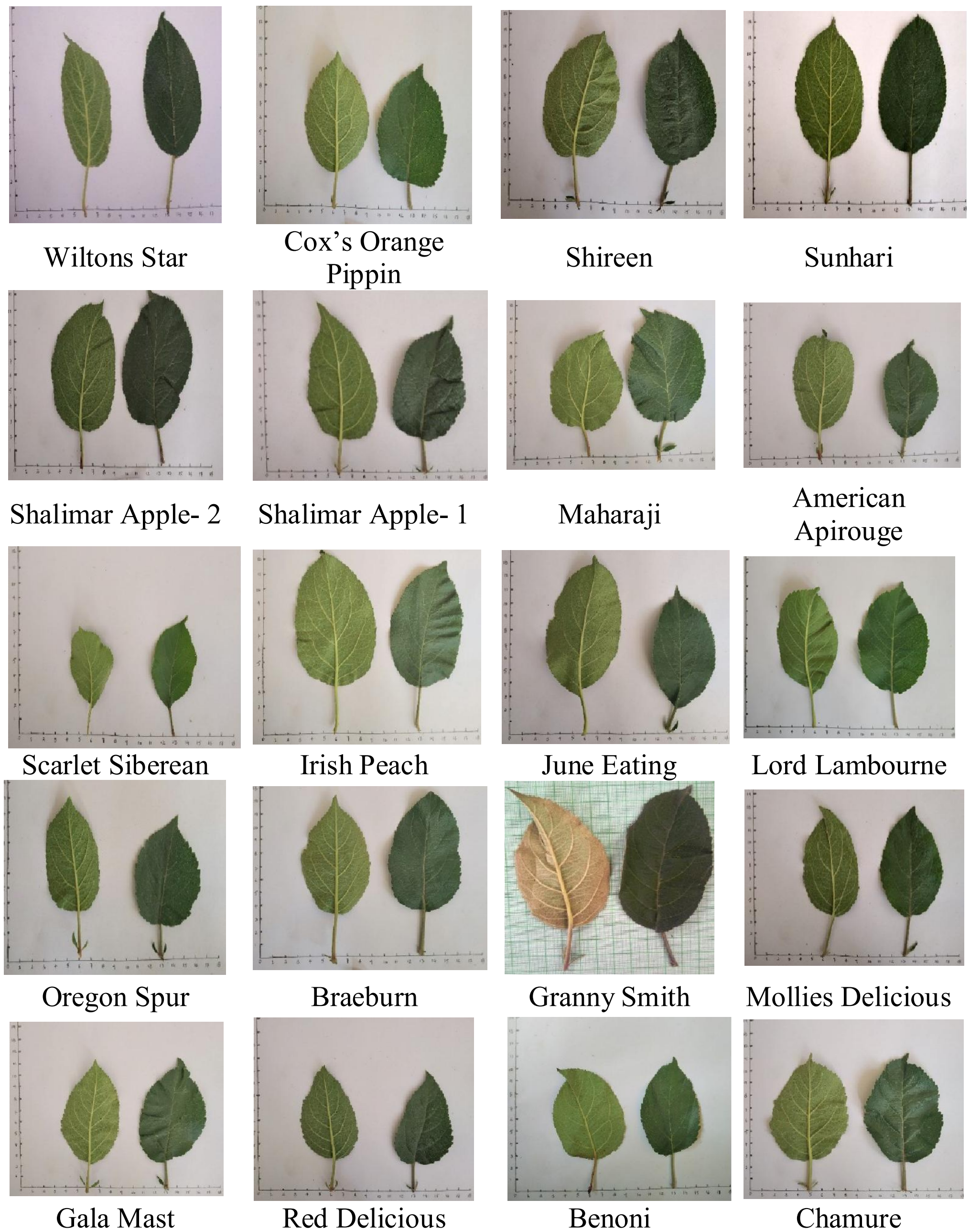
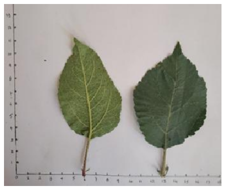
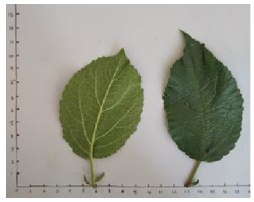


Plate 1: Leaf morphological characteristics of apple genotypes

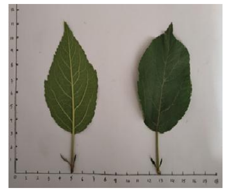
Plate 1 contd…



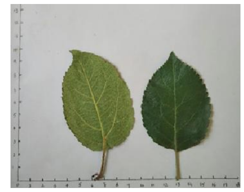
Red Gold



Golden Delicious



Starkrimson



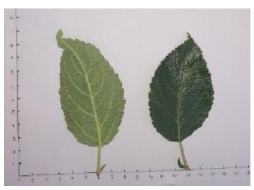
Yellow Newton

Golden Delicious Reinders

Silver Spur

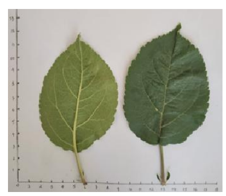
Red Velox

Fuji Zhen Aztec

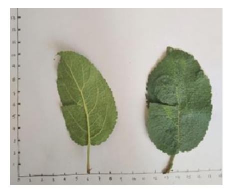


Gala Redlum

Super Chief



Lal Ambri



Firdous

Akbar

ASP-3

ASP-4

ASP-69

ASP-1

ASP-10

ASP-12

Ambri

4.1.3 Fruit characters

4.1.3.1 Fruit length (mm)

Table 9 reveals significant difference with respect to fruit length among the genotypes under study. Average length of fruits varied from 28.1 to 85.2 mm. Among the genotypes under study, the maximum average fruit length of 85.2 mm was observed in Lal Ambri followed by 81.1 mm in ASP-69 and minimum length of fruits (28.1mm) was noticed in Scarlet Siberean.

4.1.3.2 Fruit diameter (mm)

The observation related to the diameter of fruits of apple genotypes under study is recorded in Table 9 which showed a significant difference among the genotypes. It is evident from the data that variety Chamura recorded maximum average fruit diameter of 85 mm followed by 82.9 mm in ASP-69. Minimum fruit diameter of 30.03 mm was noticed in Scarlet Siberian.

4.1.3.3 Fruit weight (g)

The data related to the weight of fruit documented in Table 9, reveals a significant difference in the average fruit weight of apple genotypes under study. The maximum average fruit weight of 202.73 g was recorded in Lal Ambri which was followed by 184g in ASP-69. Minimum average fruit weight of 14.06g was recorded in Scarlet Siberean.

4.1.3.4 Pedicel length (mm)

The analysis of the data on the pedicel length (mm) of the fruit of apple genotypes under study as displayed in Table 9 showed that the genotypes differed significantly with maximum pedicel length equal to 38 mm recorded in ASP-69 followed by 34 mm in Red Gold.The minimum pedicel length of 15 mm was recorded in Benoni.

4.1.3.5 Fruit firmness (kg/cm^2)

Data on fruit firmness as depicted in the Table 9 shows a significant difference among the genotypes under study. The fruit flesh firmness varied from 5.35 to 9.43 kg/cm^2 between the genotypes under study. It is obvious from the noted observations that Scarlet Siberean recorded maximum fruit flesh firmness of 9.43 kg/cm^2 followed by 9.06 kg/cm^2 in Ambri. The minimum fruit flesh firmness of 5.35 kg/cm^2 was observed in June Eating.

4.1.3.6 Total soluble solids (°Brix)

The observations related to total soluble solids of genotypes under study shown in Table 9 reveal that highest total soluble solids (14.93 °Brix) were recorded in Shalimar Apple-1 followed by 14.4 °Brix in Shalimar Apple-2. Minimum TSS (10.76 °Brix) was noticed in June Eating

4.1.3.7 Acidity of fruit juice (% Malic acid)

Perusal of data of acidity as malic acid as depicted in the Table 9 showed a significant difference among all genotypes when compared with each other. The acidity of fruits varies from 0.12-0.33 per cent between the genotypes under study during the period of investigation. It is pertinent from the noted observations that variety Braeburn recorded maximum acidity of 0.33 per cent followed by 0.31 per cent in Granny Smith and ASP-12. The minimum acidity of 0.12 per cent was observed in Ambri and ASP-69.

4.1.3.8 TSS-acid ratio

Perusal of the information with respect to the TSS/acid given in Table 9 reveals that TSS/acid ratio varied from 39.1-116.7 in the genotypes under study. There was a significant difference between these genotypes. Ambri recorded the highest TSS/acid ratio of 116.7 which was followed by 113.5 in ASP-69. The minimum TSS/acid ratio of 39.1 was recorded in Braeburn.

4.1.3.9 Fruit shape

The fruits of apple genotypes studied during the programme were found to have different shapes *viz.*, globose, conical, cylindrical waisted, cylindrical, globose and obloid. Globose shape was observed in 14 (35%) genotypes, 11 (27.5%) genotypes were conical in shape, cylindrical waisted and cylindrical shape was observed in 6 (15%) and 5 (12.5%) genotypes respectively, while the remaining 4 (10%) had obloid shape. (Table 11).

4.1.3.10 Ground colour

The ground colour of fruits in apple genotypes studied varied from green, yellow green, yellow to whittish yellow. Green ground colour was observed in 20 genotypes (50%), 13 (32.5%) genotypes had yellow ground colour while yellow green ground colour was observed in 5 (12.5%) genotypes, only 2 (5%) genotypes i.e. Super Chief and Red Gold were found to have whittish yellow ground colour. (Table 11).

4.1.3.11 Fruit over colour

Fruit over colour of the apple genotypes was studied as orange red, pink red, red, purple red and brown red. Perusal of Table 11 revealed that maximum genotypes i.e. 17 (42.5%) genotypes had orange red over colour, 7 (17.5%) genotypes had red over colour, pink red and purple red over colour were found respectively in 6 (15%) and 5 (12.5%) genotypes, only 2 (5%) genotypes i.e. Wiltons Star and Yellow Newton had brown red over colour. Genotypes Granny Smith, Golden Delicious and Golden Delicious Reinders did not show any particular over colour with reference to the descriptor.

4.1.3.12 Pattern of over colour

Among the genotypes studied 17(42.5%) genotypes were found to have only solid flush**,** 12(30%) genotypes had solid flush with weakly defined stripes, 7(17.5%) genotypes had a solid flush with stripped and mottled fruits while only

4(10%) genotypes had a solid flush with strongly defined stripes (Table 11).

4.1.3.13 Fruit russet

The russet amount on fruits was observed visually and was recorded as per the descriptor. 23 (57.5%) genotypes were found to have no russet, russet at stem cavity was observed in 11 (27.5%) genotypes, slight russet was observed in 5 (12.5%) genotypes. ASP-1 was the only genotype that had severe amount of russet on fruits (Table 11).

4.1.3.14 Fruit lenticels

Maximum number of genotypes i.e. 20 (50%) were found to have many lenticels, while medium number of lenticels were found in 12 (30%) genotypes and only 8 (20%) genotypes were observed with few lenticels on their fruit surface (Table 11).

4.1.3.15 Prominence of lobes at calyx end

The data related to the prominence of lobes in fruits is given in (Table 11). It is clear that 24 (60%) genotypes did not possess prominent lobes and had a more or less flat surface at the calyx end, lobes were weakly prominent in 11 (27.5%) genotypes, strong lobes were observed in only 3 genotypes while as moderately prominent lobes were observed in 2 (5%) genotypes.

4.1.3.16 Colour of flesh

Freshly cut fruits were analyzed for flesh colour. The flesh colour in 40 apple genotypes studied showed variations with creamy flesh in 12 (30%), genotypes, whittish flesh colour in 10 (25%), yellowish flesh colour in 10 (25%) and greenish in 8 (20%) genotypes (Table 13).

4.1.3.17 Calyx opening

Calyx end of the fruits was observed visually and the observation was recorded in Table 13. From the table it is evident that among the genotypes under study 18 (45%) genotypes had open calyx end with all the sepals open,15 (37.5%)

genotypes had closed calyx end having tightly closed sepals while 7 (17.5%) genotypes had mixed calyx ends with some sepals open while others closed.

4.1.3.18 Pedicel end of fruit

Pedicel length was measured with the help of a scale and was coded according to the standard descriptor as shallow, medium and deep in Table 13. Consequently among the 40 genotypes under study 37 (92%) genotypes had deep pedicel, 3 (7.5%) had medium pedicel length while none of the genotypes showed shallow pedicel end.

4.1.3.19 Fruit greasiness of skin

Greasiness of skin was recorded by sliding the thumb firmly across the surface of the fruit. From the observation recorded in Table 13 it is clear that fruit surface greasiness was absent in 23 (57.5%) genotypes, 15 (37.5%) genotypes were slightly greasy, 2 (5%) genotypes had medium greasiness and none was found to be very greasy.

4.1.3.20 Core opening

On cutting the fruit in two equal halves core was found open in 29 (72.5%) genotypes while 11 (27.5%) genotypes had closed core opening (Table 13).

4.1.3.21 Water core

Water core was observed in freshly cut fruit. The observations related to water core are given in Table 13. 21 (52.5%) genotypes did not show any sign of water core, slight water core was present in 11 (27.5%) genotypes while water core was medium in 8 (20%) genotypes.

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Table 9: Mean values of fruit characteristics of apple genotypes

S. No	Genotypes	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Pedicel length(mm)	Fruit firmness (kg/cm^2)	TSS (^{o}Brix)	Acidity (%)	TSS/Acid
1	Wiltons Star	58.44^{qr}	73.13^{jklmn}	148.40^{bcd}	30.00^{abcdefghi}	7.60^{bcdef}	14.06^{abcd}	0.22bc^{defghijkl}	63.93^{defgh}
2	Cox's Orange Pippin	51.83^{s}	59.40^{r}	114.50^{cde}	18.33^{klm}	8.43^{abcdef}	13.06^{abcdef}	0.23ab^{cdefghijk}	55.33^{fgh}
3	Shireen	67.10^{hij}	63.13^{q}	160.50^{bc}	24.33^{cefghijkl}	7.96^{abcdefg}	13.66^{abcd}	0.18^{fghijkl}	76.00^{bcdefgh}
4	Sunhari	75.20^{c}	71.30^{klmno}	165.96^{bc}	31.00^{abcdefg}	7.80^{abcdefgh}	13.63^{abcd}	0.21^{cdefghijkl}	64.93^{defgh}
5	Shalimar Apple-2	58.50^{qar}	66.40^{pq}	153.60^{bc}	24.00^{cefghijklm}	8.10^{abcdef}	14.40^{ab}	0.18^{fghijkl}	77.40^{abcdefg}
6	Shalimar Apple-1	68.70^{ghi}	70.10^{no}	167.16^{bc}	30.00^{abcdefghij}	7.40^{bcdefgh}	14.93^{a}	0.14^{ijkl}	102.56^{abc}
7	Maharaji	62.10^{nop}	76.20^{efghij}	164.66^{bc}	24.00^{cefghijklm}	8.66^{abcdef}	13.13^{abcdef}	0.29^{abcd}	44.30^{gh}
8	American Apirouge	53.60^{s}	65.40^{pq}	123.33^{bcde}	25.00^{bcdefghijkl}	7.70^{bcdefgh}	13.60^{abcd}	0.19^{efghijkl}	71.63^{cdefgh}
9	Scarlet Siberean	28.10^{v}	30.03^{t}	14.06^{f}	31.00^{abcdefg}	9.43^{a}	14.23^{abc}	0.21^{cdefghijkl}	66.86^{defgh}
10	Irish Peach	53.70^{s}	65.33^{pq}	71.06^{def}	22.00^{ijklm}	6.06^{hi}	11.16^{efg}	0.25^{abcdefg}	43.63^{gh}
11	June Eating	37.73^{u}	47.83^{s}	49.96^{ef}	22.33^{hijklm}	5.33^{i}	10.76^{fg}	0.26^{abcdef}	41.73^{gh}
12	Lord Lambourne	57.70^{r}	68.16^{op}	135.26^{bcd}	24.66^{cdefghijkl}	7.36^{cdefgh}	12.80^{abcdef}	0.25^{abcdefg}	50.63^{gh}

Contd…

Table 9 contd..

13	Oregon Spur	71.33^{def}	74.70^{hijk}	180.00^{bc}	$30.00^{abcdefg}$	8.56^{abcdef}	12.00^{def}	$0.20^{defghijkl}$	59.10^{efgh}
14	Braeburn	62.43^{lmno}	65.10^{q}	141.20^{bcd}	$27.00^{bcdefghijk}$	8.93^{abcd}	12.90^{abcdef}	0.33^{a}	39.10^{gh}
15	Granny Smith	76.86^{c}	81.33^{bcd}	180.20^{bc}	$25.00^{cdefghijkl}$	8.63^{abcdef}	12.70^{bcdef}	0.31^{ab}	40.46^{gh}
16	Mollies Delicious	63.03^{mno}	71.29^{lmno}	143.26^{bcd}	$28.00^{bcdefghij}$	8.83^{abcde}	13.73^{abcd}	$0.25^{abcdefgh}$	54.96^{fgh}
17	Gala Mast	65.46^{jkl}	64.60^{q}	119.60^{cde}	33.00^{abcd}	$7.76^{abcdefgh}$	12.90^{abcdef}	$0.23^{bcdefghijk}$	55.56^{fgh}
18	Red Delicious	71.76^{def}	77.90^{cde}	161.33^{bc}	$31.00^{abcdefghi}$	8.03^{abcdef}	12.03^{cdef}	$0.20^{defghijkl}$	59.26^{efgh}
19	Benoni	42.56^{t}	45.50^{s}	50.66^{ef}	15.00^{m}	6.16^{ghi}	10.96^{g}	$0.24^{abcdefghij}$	42.30^{h}
20	Chamure	70.16^{defg}	85.06^{a}	135.66^{bcd}	17.33^{lm}	8.66^{abcde}	13.40^{abcd}	$0.25^{abcdefg}$	53.00^{gh}
21	Red Gold	65.50^{jkl}	74.20^{ijklm}	124.56^{bcde}	34.00^{ab}	$7.90^{abcdefg}$	13.63^{abcd}	$0.19^{defghijkl}$	69.46^{cdefgh}
22	Golden Delicious	74.16^{c}	76.53^{efghi}	162.86^{bc}	31.33^{abcdef}	$7.43^{bcdefgh}$	13.30^{abcde}	$0.21^{defghijkl}$	63.33^{defgh}
23	Starkrimson	64.33^{klmn}	63.26^{q}	161.00^{bc}	33.33^{abc}	$7.66^{bcdefgh}$	11.93^{def}	$0.20^{defghijkl}$	59.70^{efgh}
24	Yellow Newton	70.53^{defg}	77.76^{efgh}	158.66^{bc}	21.66^{jklm}	8.23^{abcdef}	13.16^{abcdef}	$0.25^{abcdefg}$	52.03^{gh}
25	Golden Delicious Reinders	72.40^{d}	78.13^{def}	181.00^{bc}	32.33^{abcde}	6.86^{fghi}	12.10^{bcdef}	0.27^{abcde}	44.33^{gh}
26	Silver Spur	77.40^{c}	82.26^{abc}	180.90^{bc}	$31.00^{abcdefgh}$	8.63^{abcdef}	12.36^{bcdef}	$0.19^{fghijkl}$	65.20^{defgh}
27	Red Velox	69.36^{efgh}	75.03^{ghij}	143.30^{bcd}	23.00^{gijklm}	$7.73^{abcdefgh}$	12.90^{abcdef}	$0.22^{bcdefghijk}$	57.83^{efgh}

Contd…

Table 9 contd..

28	Fuji Zhen Aztec	69.16^{fgh}	74.60^{ijkl}	171.90^{bc}	24.00^{defghijk}	8.96^{abc}	12.23^{bcdef}	0.22^{bcdefghijkl}	55.60^{fgh}
29	Gala Redlum	64.96^{klm}	70.10^{no}	144.80^{bcd}	23.00^{fgijklm}	8.40^{abcdef}	13.96^{abcd}	0.25^{abcdefghi}	55.90^{fgh}
30	Super Chief	72.03^{d}	74.83^{ghij}	173.56^{bc}	27.00^{bcdefghij}	7.20^{defgh}	13.20^{abcdef}	0.19^{defghijkl}	67.10^{cdefgh}
31	Lal Ambri	85.26^{a}	81.53^{bc}	202.73^{ab}	30.00^{abcdefghij}	8.96^{abc}	13.80^{abcd}	0.15^{hjkl}	92.03^{abcde}
32	Firdous	66.30^{ijk}	71.20^{mno}	161.56^{bc}	23.66^{efghijklm}	7.86^{abcdefgh}	11.96^{def}	0.22^{bcdefghijk}	52.90^{gh}
33	Akbar	71.13^{defg}	75.86^{fghij}	169.90^{bc}	26.00^{bcdefghijkl}	8.10^{abcdef}	12.80^{abcdef}	0.20^{defghijkl}	63.10^{defgh}
34	ASP-3	71.36^{de}	79.36^{efg}	180.06^{bc}	24.66^{cefghijk}	6.13^{ghi}	13.93^{abcd}	0.14^{kl}	97.00^{abcd}
35	ASP-4	60.50^{opq}	66.00^{pq}	135.16^{bcd}	26.00^{bcdefghijkl}	7.00^{efghi}	14.03^{abcd}	0.16^{ghijkl}	90.00^{abcdef}
36	ASP-69	81.10^{b}	82.96^{ab}	184.00^{bc}	38.00^{a}	7.20^{defghi}	13.56^{abcd}	0.12^{l}	113.56^{ab}
37	ASP–1	60.23^{pqr}	63.96^{q}	134.56^{bcd}	31.00^{abcdefghi}	7.70^{abcdefgh}	12.00^{def}	0.25^{abcdefg}	46.70^{gh}
38	ASP–10	60.00^{pqr}	66.06^{pq}	126.93^{bcde}	22.66^{gijklm}	7.40^{cdefgh}	12.63^{bcdef}	0.23^{bcdefghijk}	54.20^{gh}
39	ASP–12	64.23^{klmn}	70.46^{no}	157.43^{bc}	27.00^{bcdefghijkl}	8.43^{abcdef}	13.26^{abcdef}	0.31^{abc}	42.50^{gh}
40	Ambri	75.10^{c}	71.43^{klmno}	169.93^{bc}	24.33^{cefghijklm}	9.06^{ab}	14.06^{abcd}	0.12^{l}	116.70^{a}

Table 10: **Fruit characteristics of apple genotypes**

S. No.	Genotypes	Fruit shape	Fruit ground colour	Fruit over colour	Pattern of over colour	Fruit russet amount	Fruit lenticels	Prominence of lobes at calyx end
1.	Wiltons Star	Conical (1)	Green (6)	Brown Red (5)	1	No russet (1)	Many (5)	Absent (1)
2.	Cox's Orange Pippin	Globose (5)	Yellow (3)	Orange Red (1)	6	Stem cavity (2)	Medium (3)	Absent (1)
3.	Shireen	Conical (1)	Yellow Green	Pink red (2)	2	Slight (3)	Medium (3)	Absent (1)
4.	Sunhari	Cylindrical (2)	Green (6)	Orange red (1)	1	No russet (1)	Many (5)	Absent (1)
5.	Shalimar Apple-2	Globose (5)	Yellow (3)	Red (3)	6	Stem cavity (2)	Many (5)	Absent (1)
6.	Shalimar Apple-1	Cylindrical (2)	Green (6)	Pink Red (2)	2	No russet (1)	Many (5)	Weak (3)
7.	Maharaji	Globose (5)	Yellow (3)	Orange Red (1)	6	No russet (1)	Medium (3)	Absent (1)
8.	American Apirouge	Obloid (6)	Yellow green (5)	Orange Red (1)	1	Stem cavity (2)	Few (1)	Absent (1)
9.	Scarlet Siberean	Obloid (6)	Yellow (3)	Orange Red (1)	1	No russet (1)	Few (1)	Absent (1)
10.	Irish Peach	Obloid (6)	Yellow (3)	Pink Red (2)	2	No russet (1)	Few (1)	Weak (3)
11.	June Eating	Obloid (6)	Green (6)	Orange Red (1)	1	Stem cavity (2)	Few (1)	Absent (1)
12.	Lord Lambourne	Globose (5)	Green (6)	Red (3)	6	Stem cavity (2)	Medium (3)	Absent (1)

Contd..

Table 10 contd..

13.	Oregon Spur	Cylinderical waisted (3)	Yellow (5) Green	Orange Red (1)	2	No russet (1)	Many (5)	Moderate (5)
14.	Braeburn	Cylinderical (2)	Yellow (3)	Orange Red (1)	2	No russet (1)	Medium (3)	Absent (1)
15.	Granny Smith	Conical (1)	Green (6)	Green	1	No russet (1)	Many (5)	Weak (3)
16.	Mollies Delicious	Conical (1)	Green (6)	Red (3)	2	No russet (1)	Medium (3)	Weak (3)
17.	Gala Mast	Conical (1)	Yellow (3)	Red (3)	1	No russet (1)	Many (5)	Absent (1)
18.	Red Delicious	Cylinderical waisted (3)	Yellow (3)	Red (3)	6	No russet (1)	Medium (3)	Strong (7)
19,	Benoni	Globose (5)	Yellow (3)	Pink Red (2)	3	Stem cavity (2)	Few (1)	Absent (1)
20.	Chamure	Conical (1)	Green (6)	Orange red (1)	1	Slight (3)	Few (1)	Absent (1)
21.	Red Gold	Globose (5)	Whittish yellow (2)	Purple Red (4)	1	Stem cavity (2)	Many (5)	Absent (1)
22.	Golden Delicious	Conical (1)	Yellow Green (5)	Yellow	1	Stem cavity (2)	Many (5)	Absent (1)
23.	Starkrimson	Cylinderical waisted (3)	Green (6)	Purple red (4)	1	No russet (1)	Many (5)	Weak (3)
24.	Yellow Newton	Globose (5)	Yellow (3)	Brown Red (5)	1	Slight (3)	Many (5)	Weak (3)
25.	Golden Delicious Reinders	Globose (5)	Green (6)	Yellow	1	Stem cavity (2)	Many (5)	Absent (1)
26.	Silver Spur	Globose (5)	Green (6)	Purple Red (4)	2	No russet (1)	Many (5)	Strong (7)
27.	Red Velox	Globose (5)	Green (6)	Pink Red (2)	1	No russet (1)	Many (5)	Weak (3)
28.	Fuji Zhen Aztec	Globose (5)	Yellow (3)	Orange Red (1)	2	No russet (1)	Many (5)	Absent (1)

Contd..

Table 10 contd..

29.	Gala Redlum	Conical (1)	Green (6)	Purple Red (4)	1	No russet (1)	Many (5)	Absent (1)
30.	Super Chief	Cylinderical waisted (3)	Whittish Yellow (2)	Purple Red (4)	1	No russet (1)	Many (5)	Strong (7)
31.	Lal Ambri	Cylinderical waisted (3)	Green (6)	Orange Red (1)	6	No russet (1)	Medium (3)	Moderate (5)
32.	Firdous	Conical (1)	Green (6)	Pink Red (2)	1	No russet (1)	Many (5)	Weak (3)
33.	Akbar	Cylinderical (2)	Yellow (3)	Orange Red (1)	3	No russet (1)	Medium (3)	Weak (3)
34.	ASP-3	Cylinderical waisted (3)	Green (6)	Orange red (1)	2	Slight (3)	Many (5)	Weak (3)
35.	ASP-4	Globose (5)	Green (6)	Orange Red (1)	2	Slight (3)	Medium (3)	Absent (1)
36.	ASP-69	Conical (1)	Yellow Green (5)	Orange Red (1)	2	Stem cavity (2)	Few (1)	Absent (1)
37.	ASP-1	Cylinderical (2)	Green (6)	Red (3)	6	Severe (4)	Medium (3)	Weak (3)
38.	ASP-10	Globose (5)	Green (6)	Red (3)	3	No russet (1)	Many (5)	Absent (1)
39.	ASP-12	Conical (1)	Green (6)	Orange Red (1)	2	Stem cavity (2)	Medium (3)	Absent (1)
40.	Ambri	Globose (5)	Yellow (3)	Orange Red (1)	3	No russet (1)	Few (1)	Absent (1)

* Values within parenthesis are scores as per descriptor

Table 11: Summary of frequency of fruit characters of apple genotypes

Fruit character	Category	Number of genotypes	Percentage (%)
Fruit shape	Conical (1)	6	15
	Cylindrical (2)	11	27.5
	Cylindrical waisted (3)	0	0
	Ellipsoid (4)	5	12.5
	Globose (5)	0	0
	Obloid (6)	14	35
	Ovoid (7)	4	10
Fruit ground colour	Not visible (1)	0	0
	Whitish yellow (2)	2	5
	Yellow (3)	13	32.5
	Whitish green (4)	0	0
	Yellow green (5)	5	12.5
	Green (6)	20	50
Fruit over colour	Orange red (1)	17	42.5
	Pink red (2)	6	15
	Red (3)	7	17.5
	Purple red (4)	5	12.5
	Brown red (5)	2	5
Pattern of over colour	Only solid flush (1)	17	42.5
	Solid flush with weakly defined stripes (2)	12	30
	Solid flush with strongly defined stripes (3)	4	10
	Weakly defined flush with strongly defined stripes (4)	0	0
	Flushed and mottled (5)	0	0
	Flushed striped and mottled (6)	7	17.5
Fruit russet amount	No russet (1)	23	57.5
	Stem cavity (2)	11	27.5
	Slight russet (3)	5	12.5
	Severe (4)	1	2.5
Fruit lenticels	Few (1)	8	20
	Medium (3)	12	30
	Many (5)	20	50
Prominence of lobes at calyx end	Absent (1)	24	60
	Weak (3)	11	27.5
	Moderate (5)	2	5
	Strong (7)	3	7.5

* Values within parenthesis are scores as per descriptor

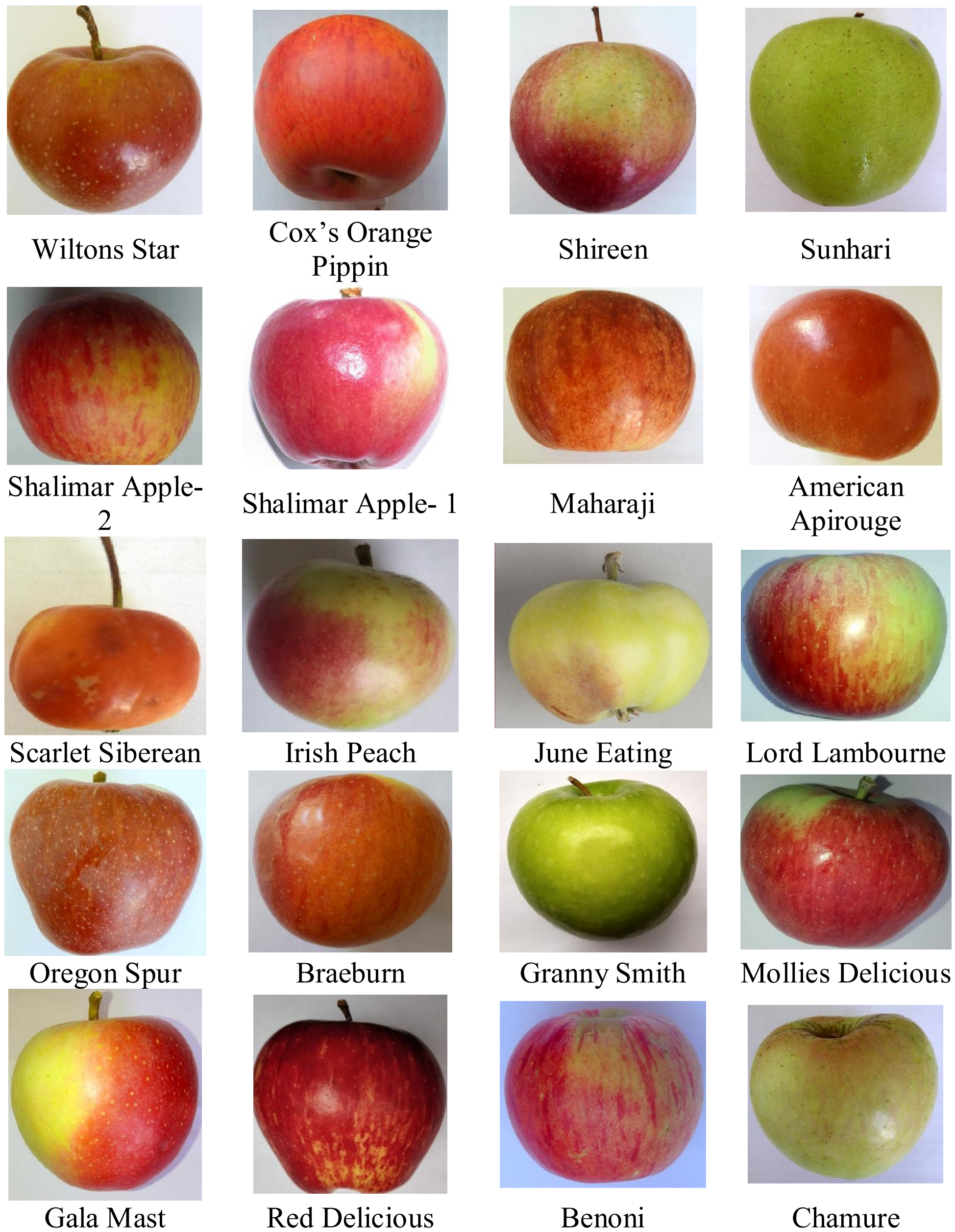
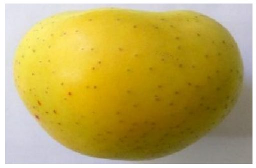


Plate 2: Fruit shape of apple genotypes

Plate 2 contd…


Red Gold


Golden Delicious


Starkrimson


Yellow Newton


Golden Delicious Reinders


Silver Spur


Red Velox


Fuji Zhen Aztec


Gala Redlum

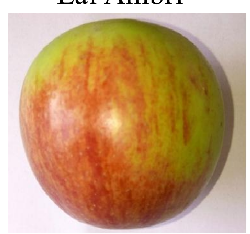

Super Chief


Lal Ambri


Firdous

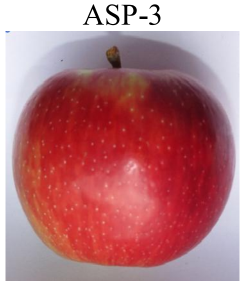

Akbar


ASP-3


ASP-4


ASP-69


ASP-1


ASP-10


ASP-12


Ambri

Table 12: Fruit morphological characteristics of apple genotypes

S. No.	Genotypes	Colour of flesh	Calyx opening	Pedicel end of the fruit	Fruit greasiness of skin	Core opening	Water core
1	Wiltons Star	Greenish (4)	All closed (1)	Deep (5)	None (1)	Open (0)	Slight (2)
2	Cox's Orange Pippin	Yellowish (5)	All open (3)	Medium (3)	Slight (2)	Closed (1)	Medium (3)
3	Shireen	Creamy (2)	All open (3)	Deep (5)	Slight (2)	Open (0)	Slight (2)
4	Sunhari	Whitish (1)	Mixed (2)	Deep (5)	Slight (2)	Open (0)	None (1)
5	Shalimar Apple-2	Greenish (4)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)
6	Shalimar Apple-1	Greenish (4)	All closed (1)	Deep (5)	None (1)	Open (0)	None (1)
7	Maharaji	Creamy (2)	All open (3)	Deep (5)	None (1)	Closed (1)	Slight (2)
8	American Apirouge	Creamy (2)	All open (3)	Deep (5)	None (1)	Open (0)	Medium (3)
9	Scarlet Siberean	Yellowish (5)	All open (3)	Deep (5)	Slight (2)	Closed (1)	Slight (2)
10	Irish Peach	Creamy (2)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)
11	June Eating	Greenish (4)	All closed (1)	Deep (5)	None (1)	Closed (1)	None (1)
12	Lord Lambourne	Whitish (1)	All closed (1)	Deep (5)	Slight (2)	Closed (1)	Medium (3)
13	Oregon Spur	Whitish (1)	All closed (1)	Deep (5)	None (1)	Open (0)	Slight (2)
14	Braeburn	Yellowish (5)	All open (3)	Deep (5)	Slight (2)	Open (0)	None (1)
15	Granny Smith	Greenish (4)	All closed (1)	Deep (5)	None (1)	Closed (1)	Medium (3)
16	Mollies Delicious	Whitish (1)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)
17	Gala Mast	Whitish (1)	All closed (1)	Deep (5)	None (1)	Open (0)	None (1)
18	Red Delicious	Yellowish (5)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)

Contd..

Table 12 contd..

19	Benoni	Creamy (2)	All open (3)	Medium (3)	Slight (2)	Closed (1)	None (1)
20	Chamure	Whitish (1)	All open (3)	Medium (3)	Medium (3)	Open (0)	Slight (2)
21	Red Gold	Creamy (2)	Mixed (2)	Deep (5)	Slight (2)	Open (0)	Medium (3)
22	Golden Delicious	Creamy (2)	All closed (1)	Deep (5)	Slight (2)	Open (0)	None (1)
23	Starkrimson	Greenish (4)	Mixed (2)	Deep (5)	None (1)	Open (0)	None (1)
24	Yellow Newton	Whittish (1)	All closed (1)	Deep (5)	Medium (3)	Closed (1)	Slight (2)
25	Golden Delicious Reinders	Yellowish (5)	Mixed (2)	Deep (5)	Slight (2)	Open (0)	None (1)
26	Silver Spur	Greenish (4)	All open (3)	Deep (5)	Slight (2)	Open (0)	Medium (3)
27	Red Velox	Creamy (2)	All closed (1)	Deep (5)	None (1)	Open (0)	None (1)
28	Fuji Zhen Aztec	Yellowish (5)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)
29	Gala Redlum	Whitish (1)	All open (3)	Deep (5)	None (1)	Open (0)	None (1)
30	Super Chief	Creamy (2)	Mixed (2)	Deep (5)	None (1)	Open (0)	None (1)
31	Lal Ambri	Yellowish (5)	All open (3)	Deep (5)	None (1)	Closed (1)	None (1)
32	Firdous	Whitish (1)	All closed (1)	Deep (5)	None (1)	Open (0)	Medium (3)
33	Akbar	Creamy (2)	Mixed (2)	Deep (5)	None (1)	Open (0)	None (1)
34	ASP-3	Yellowish (5)	Mixed (2)	Deep (5)	None (1)	Open (0)	Slight (2)
35	ASP-4	Yellowish (5)	All open (3)	Deep (5)	Slight (2)	Open (0)	None (1)
36	ASP-69	Creamy (2)	All closed (1)	Deep (5)	None (1)	Open (0)	Slight (2)
37	ASP-1	Greenish (4)	All closed (1)	Deep (5)	Slight (2)	Open (0)	Medium (3)
38	ASP-10	Yellowish (5)	All closed (1)	Deep (5)	None (1)	Closed (1)	Slight (2)
39	ASP-12	Creamy (2)	All open (3)	Deep (5)	Slight (2)	Open (0)	Slight (2)
40	Ambri	Whittish (1)	All closed (1)	Deep (5)	Slight (2)	Closed (1)	None (1)

*Values within parenthesis are scores as per descriptor

Table 13: Summary of frequency of fruit characters of various apple genotypes

Fruit character	Category	Number of genotypes	Percentage (%)
Colour of the flesh	Whittish (1)	10	25
	Creamy (2)	12	30
	Pinkish (3)	0	0
	Greenish (4)	8	20
	Yellowish (5)	10	25
Calyx opening	All closed (1)	15	37.5
	Mixed (2)	7	17.5
	All open (3)	18	45
Pedicel end of the fruit	Shallow (1)	0	0
	Medium (3)	4	10
	Deep (5)	36	90
Fruit greasiness of skin	None (1)	23	57.5
	Slight (2)	15	37.5
	Medium (3)	2	5
	Severe (4)	0	0
Core opening	Open (0)	29	72.5
	Closed (1)	11	27.5
Water core	None (1)	21	52.5
	Slight (2)	11	27.5
	Medium (3)	8	20
	Severe (4)	0	0

* Values within parenthesis are scores as per descriptor

4.1.3.22 Sensory evaluation

The forty genotypes were evaluated by a panel of five judges and the score were given for their colour, taste, flavor, texture, firmness, sweetness and overall acceptability. The genotype which performed the best in all the parameters was Fuji Zhen Aztec and with an overall score of 4.16 out of 5 followed by Gala Redlum with an overall score of 4.11. Varieties Irish peach and Benoni were statistically at par with overall scores of 2.55 and 2.65 out of 5 respectively (Table 14).

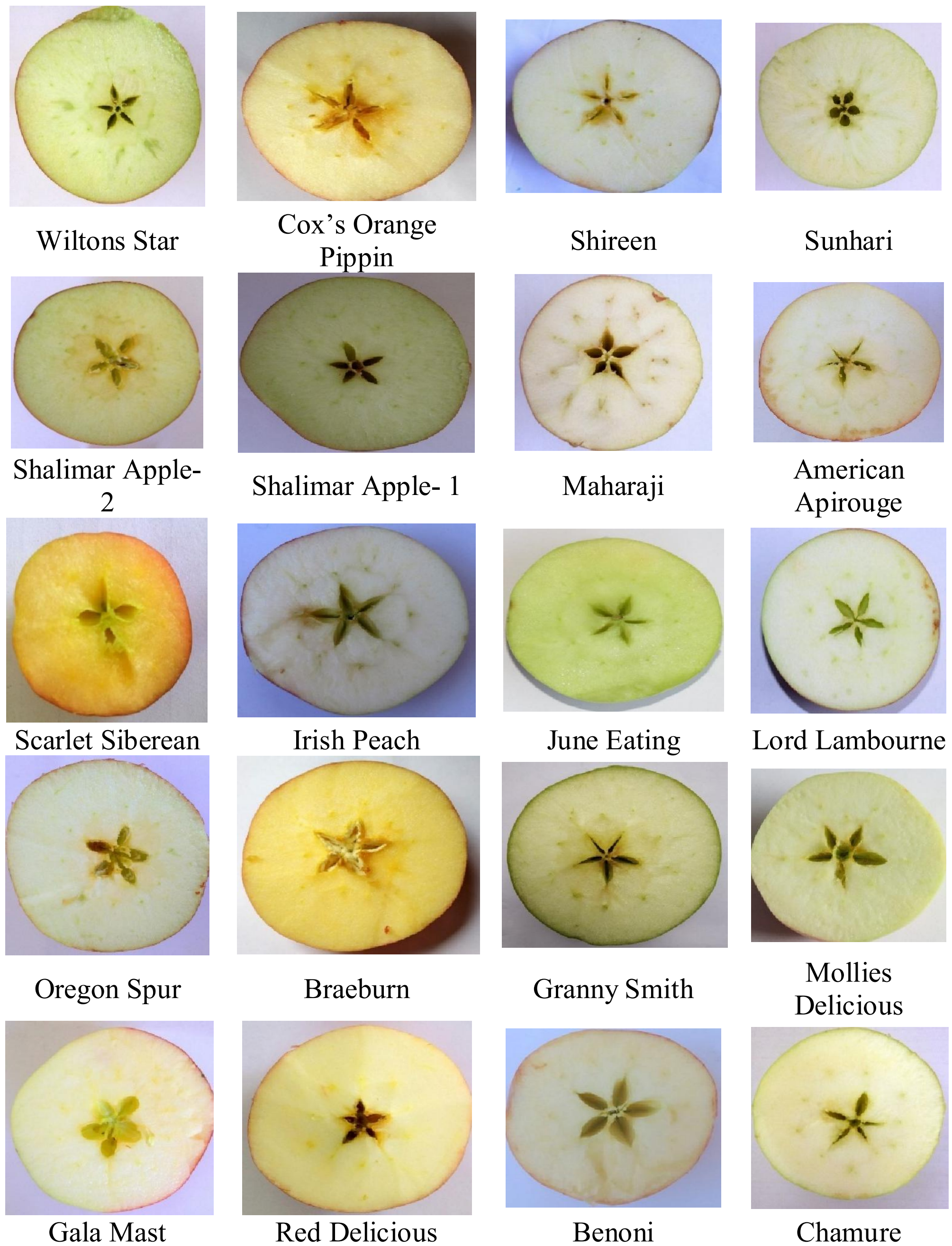
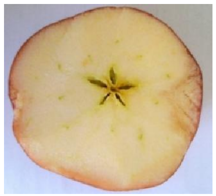
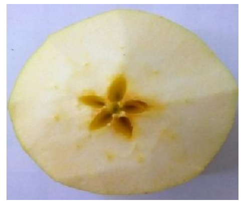


Plate 3: Core of the fruits

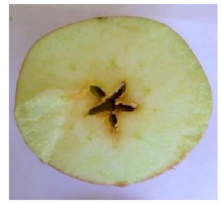
Plate 3 contd…



Red Gold



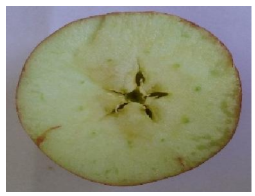
Golden Delicious



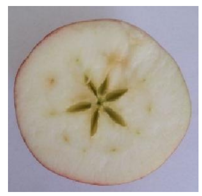
Starkrimson

Yellow Newton

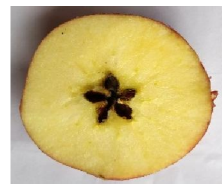
Golden Delicious Reinders



Silver Spur

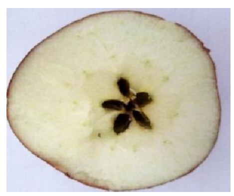


Red Velox

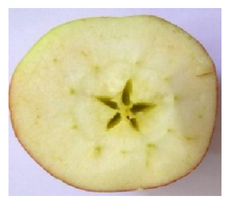


Fuji Zhen Aztec

Gala Redlum

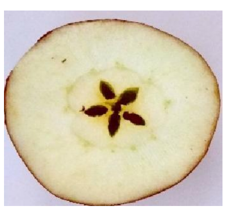


Super Chief



Lal Ambri

Firdous



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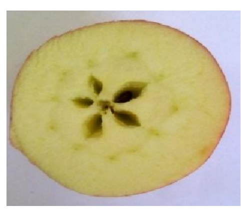
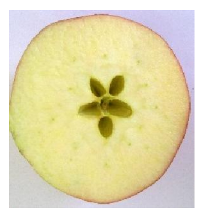
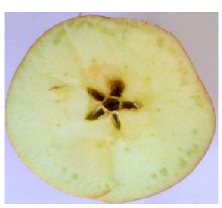


ASP-3

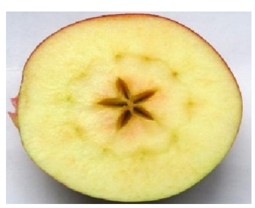


ASP-4

ASP-69



ASP-1



ASP-10

ASP-12

Ambri

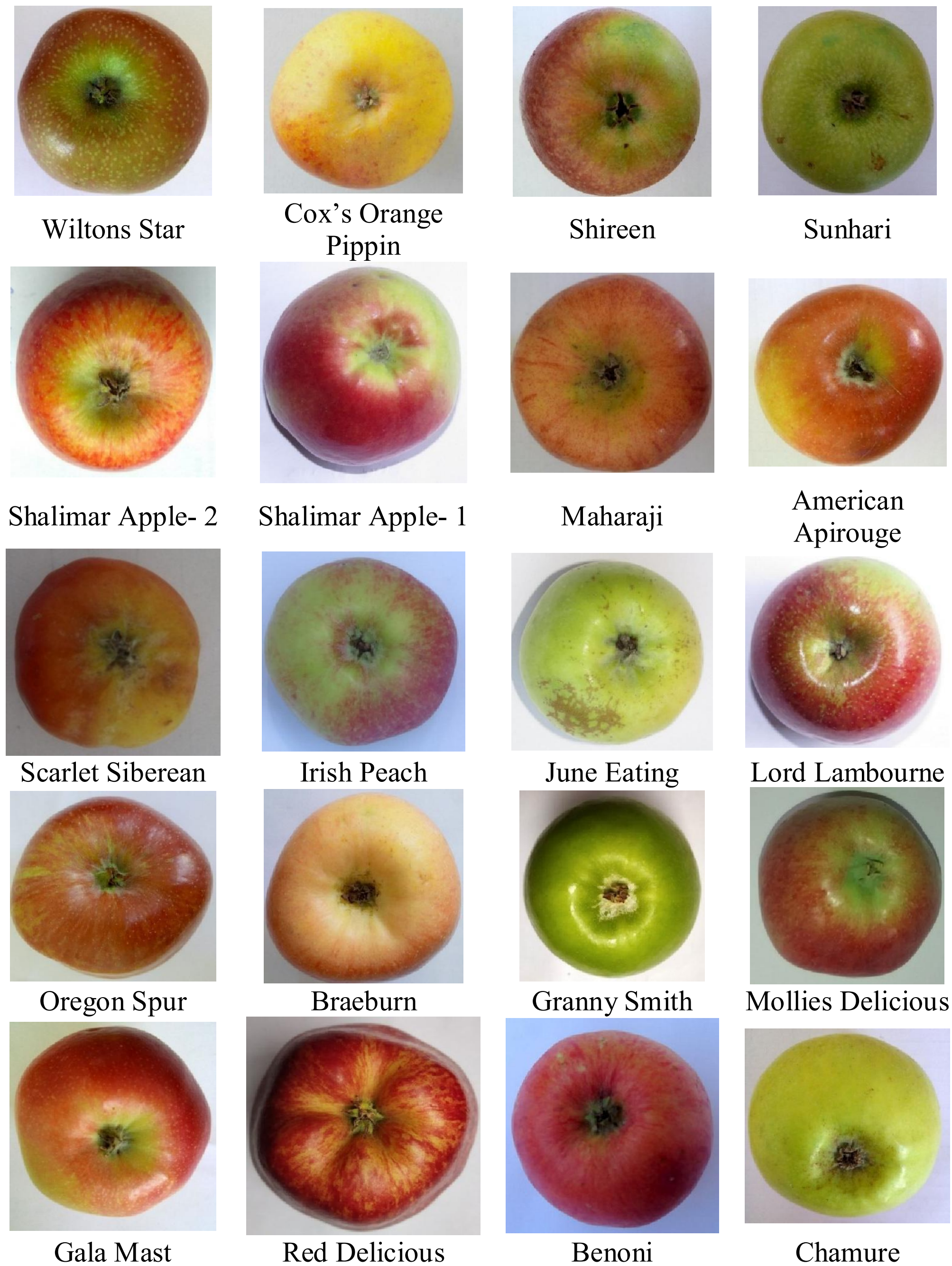
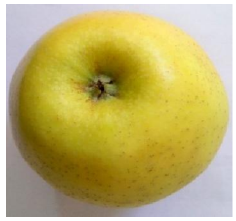


Plate 4: Calyx end of the fruits

Plate 4 contd…

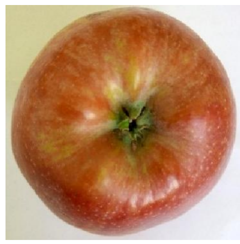

Red Gold


Golden Delicious


Starkrimson


Yellow Newton


Golden Delicious Reinders


Silver Spur


Red Velox

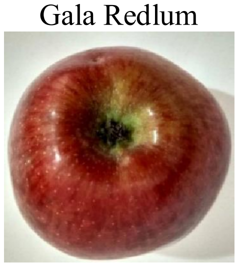

Fuji Zhen Aztec

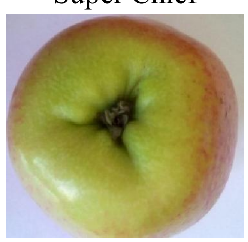

Gala Redlum

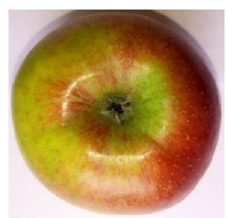

Super Chief


Lal Ambri

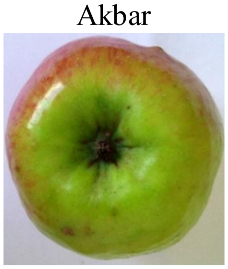

Firdous

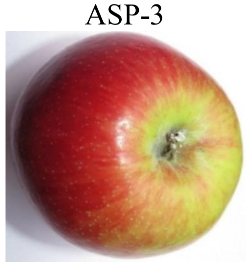

Akbar


ASP-3


ASP-4

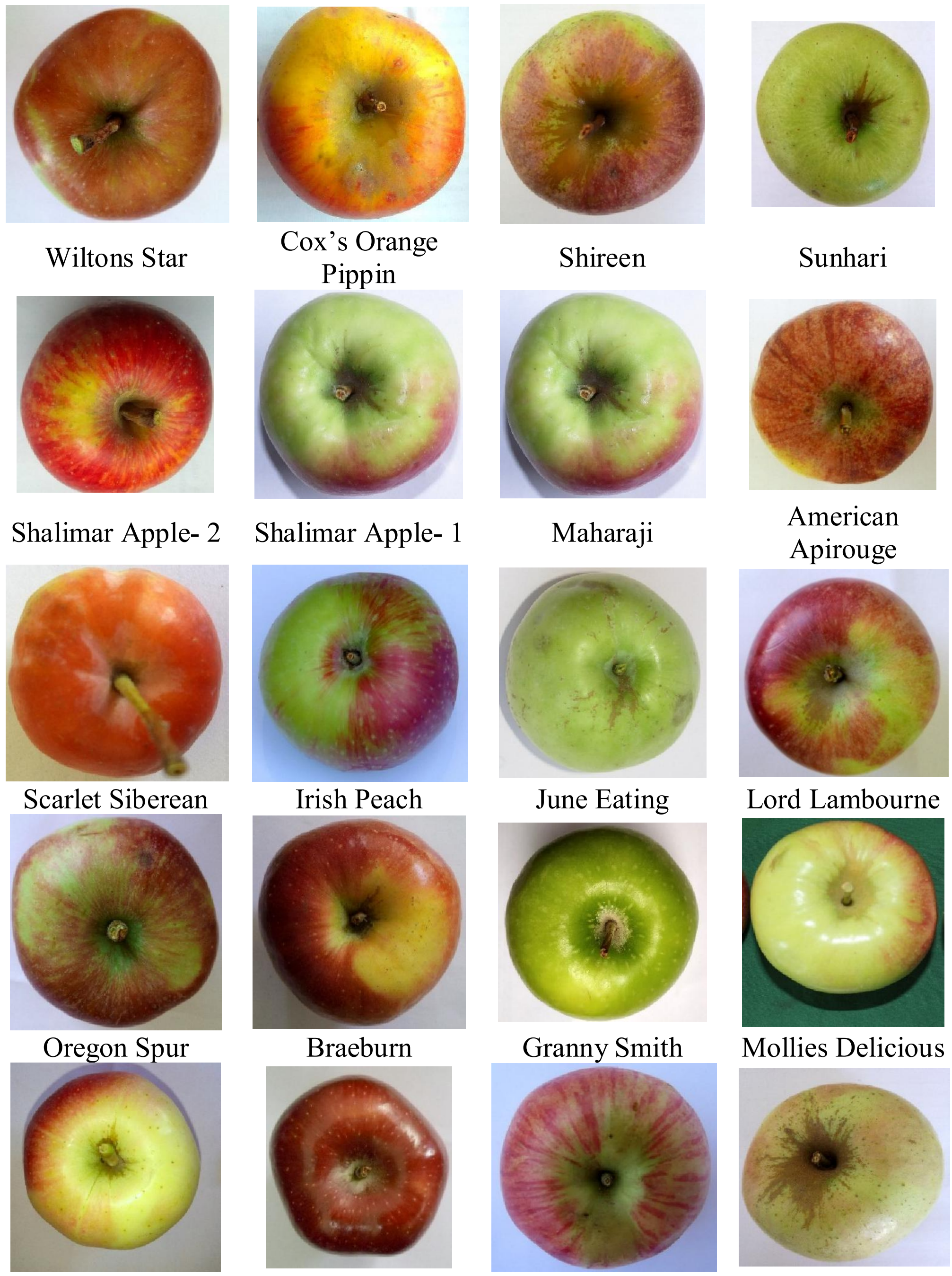

ASP-69


ASP-1


ASP-10


ASP-12


Ambri



Wiltons Star | Cox's Orange Pippin | Shireen | Sunhari

Shalimar Apple- 2 | Shalimar Apple- 1 | Maharaji | American Apirouge

Scarlet Siberean | Irish Peach | June Eating | Lord Lambourne

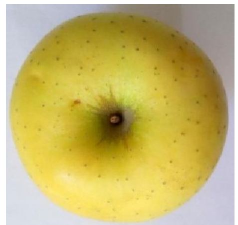
Oregon Spur | Braeburn | Granny Smith | Mollies Delicious

Gala Mast | Red Delicious | Benoni | Chamure

Plate 5: Pedicel End of the Fruits

Plate 5 contd…

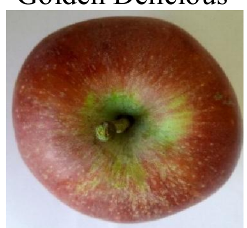

Red Gold


Golden Delicious

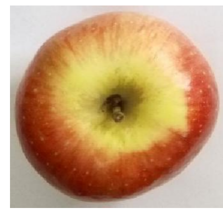

Starkrimson

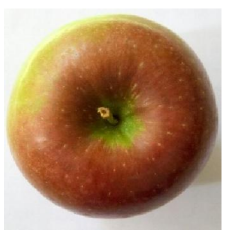

Yellow Newton


Golden Delicious Reinders


Silver Spur


Red Velox


Fuji Zhen Aztec


Gala Redlum


Super Chief

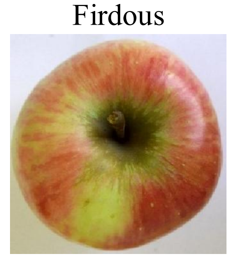

Lal Ambri


Firdous


Akbar

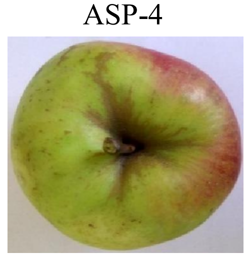

ASP-3


ASP-4


ASP-69


ASP-1


ASP-10


ASP-12

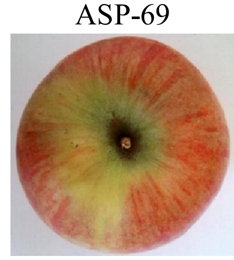

Ambri

Table 14: Sensory evaluation of apple genotypes

S. No.	Genotypes	Colour (1-5)	Taste (1-5)	Flavor (1-5)	Texture (1-5)	Firmness (1-5)	Sweetness (1-5)	Overall acceptability (1-5)
1	Wiltons Star	3.3	3.4	3.4	3.5	3.7	3.5	3.46
2	Cox's Orange Pippin	3.3	3.6	3.7	3.1	3.1	3.7	3.41
3	Shireen	3.4	4	3.9	3.	3.4	3.6	3.55
4	Sunhari	3.2	3.1	3.1	3.2	3.1	3.9	3.31
5	Shalimr Apple-2	3.5	3.6	3.7	3.8	3.7	3.7	3.66
6	Shalimar Apple-1	4	4.1	3.9	3.5	3.4	3.5	3.75
7	Maharaji	3.5	3.4	3.7	3.9	4	3.1	3.6
8	American Apirouge	4	3.9	4.1	4.1	3.9	4.1	4.01
9	Scarlet Siberean	3.8	3.5	3.6	3.9	3.1	3.1	3.5
10	Irish Peach	2.6	2.4	2.7	2.7	2.3	2.6	2.55
11	June Eating	2.6	2.8	2.7	2.6	2.7	2.5	2.65
12	Lord Lambourne	3.1	3.1	3.1	2.9	2.7	2.9	2.96
13	Oregon spur	4.1	3.8	3.8	3.7	3.5	3.5	3.73
14	Braeburn	3.5	4.1	3.9	4.1	4.1	3.9	3.93
15	Granny Smith	3.2	3.2	3.3	3.7	4.2	3.8	3.56
16	Mollies Delicious	3.4	3.4	3	3.1	2.8	3.5	3.2
17	Gala Mast	3.5	3.8	3.5	3.8	3.7	4.1	3.73
18	Red Delicious	4.1	3.9	3.5	3.7	3.7	4.	3.81
19	Benoni	2.8	2.6	2.9	2.6	2.1	2.9	2.65
20	Chamure	3.1	3.6	4.1	3.9	3.8	3.8	3.71
21	Red Gold	3.5	3.6	3.8	3.2	3.1	3.9	3.51

Contd..

Table 14 contd…

22	Golden Delicious	3.3	3.8	3.9	3.9	3.8	3.8	3.75
23	Starkrimson	3.7	3.5	3.4	3.3	3.2	3.2	3.38
24	Yellow Newton	3	3.1	3.3	3.4	3.7	3.5	3.33
25	Golden Delicious Reinders	3.4	4	4.1	4.1	3.8	3.9	3.88
26	Silver Spur	4.1	3.9	3.9	3.8	3.8	4.1	3.93
27	Red Velox	4	3.5	3.4	3.6	3.4	3.4	3.55
28	Fuji Zhen Aztec	3.8	4.3	4.1	4.3	4.2	4.3	4.16
29	Gala Redlum	4.1	4.2	4.2	4.1	4	4.1	4.11
30	Super Chief	4.3	3.9	3.8	3.7	3.6	3.6	3.81
31	Lal Ambri	4.2	3.9	3.9	3.7	4.1	3.9	3.95
32	Firdous	3.5	3.4	3.1	3.7	3.6	3.4	3.45
33	Akbar	3.7	3.3	3.4	3.6	3.4	3.7	3.51
34	ASP-3	3.8	3.1	3.1	2.9	2.9	3.1	3.15
35	ASP-4	2.9	3.1	2.9	3.2	3.3	3	3.06
36	ASP-69	3.7	3.2	3.1	3.2	3	3.1	3.21
37	ASP-1	3.6	3.1	2.9	3.1	3.1	2.9	3.11
38	ASP-10	3.3	3.2	3	2.9	2.9	3.1	3.06
39	ASP-12	2.9	2.9	2.8	2.8	3.2	3.1	2.95
40	Ambri	3.6	4	3.8	3.9	4.2	4.1	3.9

4.1.4 Analysis of variance

Analysis of variance for various traits under study is presented in Table 15. The data revealed significant variation among all the cultivars for all the traits studied during the programme. Mean sum of squares due to cultivars for all the traits was found highly significant.

4.1.5 Variability and genetic components of variation

The magnitude of phenotypic and genotypic variance and phenotypic and genotypic coefficient of variation in various traits is presented in Table 16. Perusal of Table 16 revealed that the estimates of phenotypic variance were higher than the corresponding estimates of genotypic variance. The magnitude of phenotypic and genotypic coefficient of variation was low (<10 per cent) only for TSS whereas it was moderate (10-30 per cent) for fruit length, fruit diameter, fruit weight, fruit firmness acidity, leaf blade length, leaf blade width, petiole length and pedicel length.

4.1.5.1 Fruit length (mm)

Fruit length of the 40 apple genotypes studied ranged from 28.1-85.2 mm with an overall mean of 64.7 mm. The phenotypic variance (PV) and genotypic variance (GV) was found to be 126.91 and 125.03 respectively. Phenotypic coefficient of variation (17.38%) and genotypic coefficient of variation (17.26%) also were observed for this trait. The heritability (broad sense) and genetic gain (as per cent of mean) was found to be 0.98 and 35.29% respectively (Table 16).

4.1.5.2 Fruit diameter (mm)

The fruit diameter of all genotypes studied showed a range of 30.03-85.06 mm with an overall mean of 69.93 mm. The phenotypic and genotypic variances were observed as 114.73 and 111.85 respectively. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were found to be 15.31% and 15.12% respectively where as the heritability and genetic advance (as per cent of mean) were found to be 0.97and 30.75% respectively (Table 16).

4.1.5.3 Fruit weight (g)

Fruit weight is a variable character and ranged from 14.06-202.73 with an overall mean of 145.13g. The results showed high phenotypic variance and genotypic variance which were 1562.73 and 1555.20 respectively. The phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance (as per cent of mean) were found to be 27.23%, 27.17%, 0.99 and 55.84% respectively (Table 16).

4.1.5.4 Fruit firmness (kg/cm^2)

The Fruit firmness of all genotypes studied ranged from 5.33-9.43 kg/cm^2 with an overall mean of 7.85 kg/cm^2. The phenotypic and genotypic variances were observed as 0.86 and 0.78 respectively. Also the phenotypic coefficient of variation and genotypic coefficient of variation for this trait were observed as 11.86% and 11.27% respectively. The heritability (broad sense) and genetic advance (as per cent of mean) were found to be 0.90 and 22.0% respectively (Table 16)

4.1.5.5 Leaf blade length (mm)

Leaf blade length of the 40 genotypes studied ranged from 58.66-101.00 mm with an overall mean of 86.21. The phenotypic variance (PV) and genotypic variance (GV) was found to be 87.22 and 77.65 respectively. The phenotypic coefficient of variation of 10.83% and genotypic coefficient of variation of 10.22% was observed for this trait. The heritability (broad sense) and genetic gain (as per cent of mean) was found to be 0.89 and 19.86% respectively (Table 16).

4.1.5.6 Leaf blade width (mm)

Leaf blade width of the 40 apple genotypes studied showed a range of 36.00-67.66 mm with an overall mean of 53.19 mm. The phenotypic variance (PV) and genotypic variance (GV) was found to be 47.55 and 41.85 respectively. The phenotypic coefficient of variation of 12.96% and genotypic coefficient of variation of 12.16% was observed for this trait. The heritability (broad sense) and genetic gain (as per cent of mean) was found to be 0.88 and 23.50% respectively (Table 16).

4.1.5.7 Petiole length (mm)

Petiole length of the 40 apple genotypes studied ranged from 17.66-40.33 mm with an overall mean of 28.52 mm. The phenotypic variance (PV) and genotypic variance (GV) was found to be 29.32 and 24.09 respectively. The phenotypic coefficient of variation of 18.98% and genotypic coefficient of variation of 17.20% was observed for this trait. The heritability (broad sense) and genetic gain (as per cent of mean) was found to be 0.82 and 32.13% respectively (Table 16).

4.1.5.8 Pedicel length (mm)

The stalk length of all genotypes studied ranged from 15.00-38.00 mm with an overall mean of 26.67 mm. The phenotypic and genotypic variances were observed as 24.63 and 22.98 respectively. Also the phenotypic coefficient of variation and genotypic coefficient of variation for this trait were observed as 18.60 and 17.97 respectively. The heritability (broad sense) and genetic advance (as per cent of mean) were found to be 0.93 and 35.76 % respectively (Table 16).

4.1.5.9 Total Soluble Solids (ºBrix)

The TSS of all the genotypes studied during the programme ranged from 10.7-14.9 ºBrix with an overall mean of 13.02ºBrix. The phenotypic variance and genotypic variance were found to be 1.04 and 0.84% respectively. The phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance (as per cent of mean) were found to be 7.84, 7.07, 0.81 and 13.13% respectively (Table 16).

4.1.5.10 Acidity (%)

The acidity of all the genotypes studied during the programme ranged from 0.12-0.33% with an overall mean of 0.22%. The phenotypic variance and genotypic variance were found to be 0.003 and 0.002 respectively. The phenotypic coefficient of variation, genotypic coefficient of variation, heritability (broad sense) and genetic advance (as per cent of mean) were found to be 23.31%, 21.98%, 0.88 and 42.69% respectively (Table 16).

4.1.6 Heritability and Genetic Gain

Heritability estimates (broad sense) and expected genetic gain were calculated for the quantitative traits (Table 16). Estimates of heritability were classified into three distinct classes with value >60.0 per cent as high heritability, >30.0 ≤ 60.0 as medium and < 30.0 per cent as low. Estimates were high for all the traits. Highest heritability estimate (99.5 per cent) was found for fruit weight followed by (98.5 per cent) for fruit length. Genetic advance was estimated at 5 per cent of selection intensity and converted into expected genetic gain (per cent of mean). Estimates of genetic gain were classified into three distinct classes with value >30.0 per cent as high genetic gain, >10≤30 per cent as medium and < 10.0 per cent as low genetic gain. The result revealed that the expected genetic gain was high for fruit weight (55.84 per cent), acidity (42.69 per cent), pedicel length (35.76 per cent), fruit length (35.29 per cent), petiole length (32.13 per cent),fruit diameter (30.75 per cent) and medium for leaf blade width(23.50 per cent),fruit firmness (22.09 per cent), leaf blade length (19.86 per cent) and TSS (13.13 per cent)

Table 15: Analysis of Variance (ANOVA) for various traits in various apple genotypes

S. No.	Source of variation	d.f	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Fruit firmness (kg/cm^2)	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)	Pedicel length (mm)	TSS (^{o}Brix)	Acidity (%)
1.	Replications	2	1.40**	0.69**	1.43**	0.03**	7.75**	14.23**	8.40**	1.97**	0.31**	0.00036**
2.	Genotypes	39	376.98**	338.43**	4673.15**	2.44**	242.53**	131.26**	77.51**	70.60**	2.73**	0.0073**
3.	Error	78	1.87	2.87	7.52	0.083	9.56	5.70	5.22	1.65	0.194	0.000294

*Significant at 0.05 probability level

**Significant at 0.01 probability level

Table 16: Estimates of mean, range, phenotypic variance, genotypic variance, phenotypic and genotypic. Coefficient of variation, heritability (bs) and genetic advance (as % of mean) for different quantitative traits in apple genotypes

S. No.	Parameters	Mean	Range	Phenotypic variance σ^2p	Genotypic variance σ^2g	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)	Heritability (h^2 bs)	Genetic advance (as% of mean)
1.	Fruit length (mm)	64.78	28.1-85.26	126.91	125.03	17.38	17.26	0.98	35.29
2.	Fruit diameter (mm)	69.93	30.03-85.06	114.73	111.85	15.31	15.12	0.97	30.75
3.	Fruit weight (g)	145.13	14.06-202.73	1562.73	1555.2	27.23	27.17	0.99	55.84
4.	Fruit firmness (kg/cm^2)	7.85	5.33-9.43	0.86	0.78	11.86	11.27	0.90	22.09
5.	Leaf blade length (mm)	86.21	58.66-101.00	87.22	77.65	10.83	10.22	0.89	19.86
6.	Leaf blade width (mm)	53.19	36.00-67.66	47.55	41.85	12.96	12.16	0.88	23.50
7.	Petiole length (mm)	28.52	17.66-40.33	29.32	24.09	18.98	17.20	0.82	32.13
8.	Pedicel length (mm)	26.67	15.00-38.00	24.63	22.98	18.60	17.97	0.93	35.76
9	TSS (^{o}Brix)	13.02	10.76-14.93	1.04	0.84	7.84	7.07	0.81	13.13
10.	Acidity (%)	0.22	0.12-0.33	0.003	0.002	23.31	21.98	0.88	42.69

4.1.7 Correlation coefficient

Correlation coefficient for various traits was estimated and presented in Table 17. The results reveal that fruit length had a significantly positive correlation with fruit diameter, fruit weight, fruit firmness, pedicel length, leaf length, leaf width, petiole length, TSS and negative correlation of fruit length was observed with acidity. Similarly fruit diameter had positive correlation with fruit length, fruit weight, fruit firmness, pedicel length, leaf blade length, leaf blade width, petiole length, TSS and negative correlation with acidity. Fruit weight showed positive correlation with fruit length, fruit diameter, fruit firmness, pedicel length, leaf blade length, leaf blade width, petiole length, TSS and negative correlation with acidity. Regarding the fruit firmness it was observed that fruit firmness was positively correlated with fruit length, fruit diameter, fruit weight, pedicel length, TSS, acidity and negatively correlated with leaf blade length, leaf blade width and petiole length, pedicel length was positively correlated with TSS and other parameters excluding acidity with which it was negatively correlated. Similarly petiole length was positively correlated with pedicel length and negatively correlated with TSS and acidity. Leaf blade length and width were positively correlated. TSS and acidity were negatively correlated.

Based upon the performance of cultivars, they were grouped in to three clusters (Table 18) as per Mahalanobis D^2 analysis employing Tocher's method (Rao, 1952). The cluster diagram and dendrogram (Fig. 1 and 2) indicated that maximum number of genotypes fall in cluster I (36) followed by cluster II (3) and cluster III (1).Cluster I consisted of Maharaji, ASP-12,Fuji Zhen Aztec, Firdous, Yellow Newton, Gala Redlum, Akbar, Mollies Delicious, Red Delicious, Super Chief, Sunhari, Golden Delicious, Oregon Spur, Shireen, Golden Delicious Reinders, Shalimar Apple-2,Wiltons Star, Starkrimson, Red Velox, Braeburn, Lord Lambourne, ASP-1, Shalimar Apple-1, Silver Spur, Ambri, ASP-3, Granny Smith, Red Gold, ASP-4, ASP-10, Gala Mast, Chamure, Amercian Apirouge, Cox's Orange Pippin, ASP-69 and Lal Ambri, cluster II consisted of June Eating, Benoni and Irish Peach. Cluster III consisted of only one genotype i.e. Scarlet Siberean.In addition to grouping of selections into different clusters, non-hierarchical analysis was also performed to identify the diverse and desirable selections in terms of inter cluster distance and mean performance of clusters for various characters respectively. The average intra and inter cluster distance (D^2) values revealed that the cluster I had the highest intra cluster distance value of 119.95 followed by cluster II (83.89) (Table 19). The inter cluster distance was highest between cluster I and cluster III (1521.46) followed by inter cluster distance between cluster I and cluster II (745.61). Therefore cluster I and III were most divergent with maximum inter cluster distance.

The cluster means for different characters are given in Table 20. Cluster I had maximum mean values for fruit length (67.48 mm), fruit diameter (72.47 mm), fruit weight (156.10 g) and petiole length (28.83 mm). Cluster II is characterized by highest leaf length (88.11 mm), leaf width (55.00 mm), acidity (0.26%) and lowest fruit firmness (5.86 kg/cm^2), pedicel length (19.78 mm) and TSS (10.97 ºBrix). Cluster III had maximum mean values for fruit firmness (9.43 kg/cm^2), pedicel length (31.00 mm), TSS (14.23 ºBrix) and lowest mean value for fruit length (28.10 mm), fruit diameter (30.03 mm), fruit weight (14.07 g), leaf length (58.67 mm), leaf width (36.00 mm), petiole length (18.67 mm) and fruit acidity (0.21%) (Table 20).

The per cent contribution of the traits towards total divergence (Table 21) revealed that fruit weight was the main factor contributing towards divergence (54.10%) followed by acidity (12.30%), fruit length (10.23%) and pedicel length (7.82%). The minimum contribution towards divergence was from TSS (0.26%).

Table 17: Estimates of genotypic (above diagonal) correlation coefficients among different traits in various apple genotypes

S. No.	Parameters	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Fruit firmness (kg/cm^2)	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)	Pedicel length (mm)	TSS (^{o}Brix)	Acidity (%)
1.	Fruit length (mm)	-	0.9093**	0.9217**	0.2505*	0.2946*	0.2235*	0.3472**	0.3584**	0.2193*	-0.3060**
2.	Fruit diameter (mm)		-	0.8641**	0.1794	0.2866*	0.2923*	0.3094**	0.2017*	0.1707	-0.1403
3.	Fruit weight (g)			-	0.2966*	0.3171**	0.2307*	0.3638**	0.3346**	0.2901*	-0.2692*
4.	Fruit firmness (kg/cm^2)				-	-0.2996*	-0.0877	-0.0877	0.1160	0.4149**	0.0907
5.	Leaf blade length (mm)					-	0.5242**	0.5009**	0.0766	0.0317	0.0011
6.	Leaf blade width (mm)						-	0.1056	-0.2145*	0.0738	0.1539
7.	Petiole length (mm)							-	0.2564*	-0.0038	-0.1177
8.	Pedicel length (mm)								-	0.2059*	-0.3223**
9.	TSS (^{o}Brix)									-	-0.4867**
10.	Acidity (%)										-

*, ** = Significant at 5% and 1% respectively

Table 18: Distribution of apple genotypes into clusters based on D^2 Statistics

S. No.	Cluster	No. of genotypes	Name of the genotypes
1.	I	36	Maharaji, ASP-12, Fuji Zhen Aztec, Firdous, Yellow Newton, Gala Redlum, Akbar, Mollies Delicious, Red Delicious, Super Chief, Sunhari, Golden Delicious, Oregon Spur, Shireen, Golden Delicious Reinders, Shalimar Apple-2,Wiltons Star, Starkrimson, Red Velox, Braeburn, Lord Lambourne, ASP-1, Shalimar Apple-1, Silver Spur, Ambri, ASP-3, Granny Smith, Red Gold,ASP-4,ASP-10,Gala Mast, Chamure, Amercian Apirouge, Cox's Orange Pippin, ASP-69, Lal Ambri,
2.	II	3	June Eating, Benoni and Irish Peach
3.	III	1	Scarlet Siberean

Table 19: Average intra cluster (Diagonal) and inter cluster (Above Diagonal) Distance Values in various apple genotypes

S. No.	Cluster	I	II	III
1.	I	**119.95**	745.61	1521.46
2.	II	-	**83.89**	394.36
3.	III	-	-	**0.00**

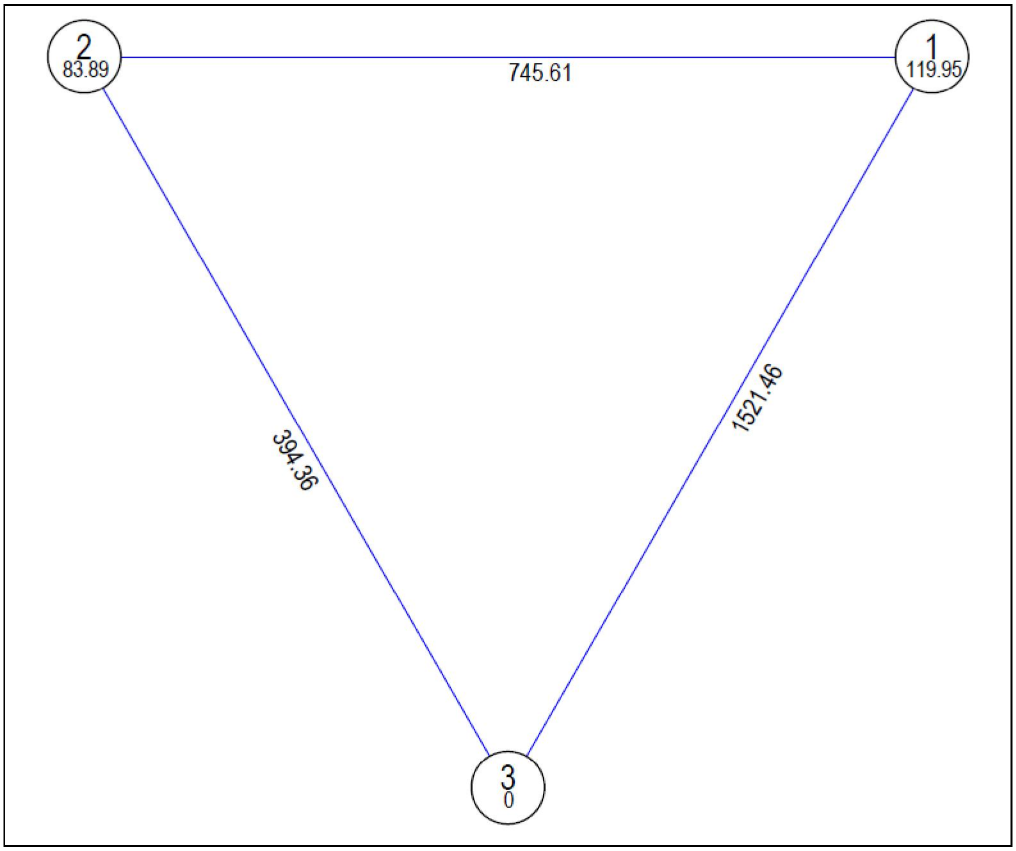


Fig. 1: Mahalnobis Euclidean Distance (Not to the Scale)

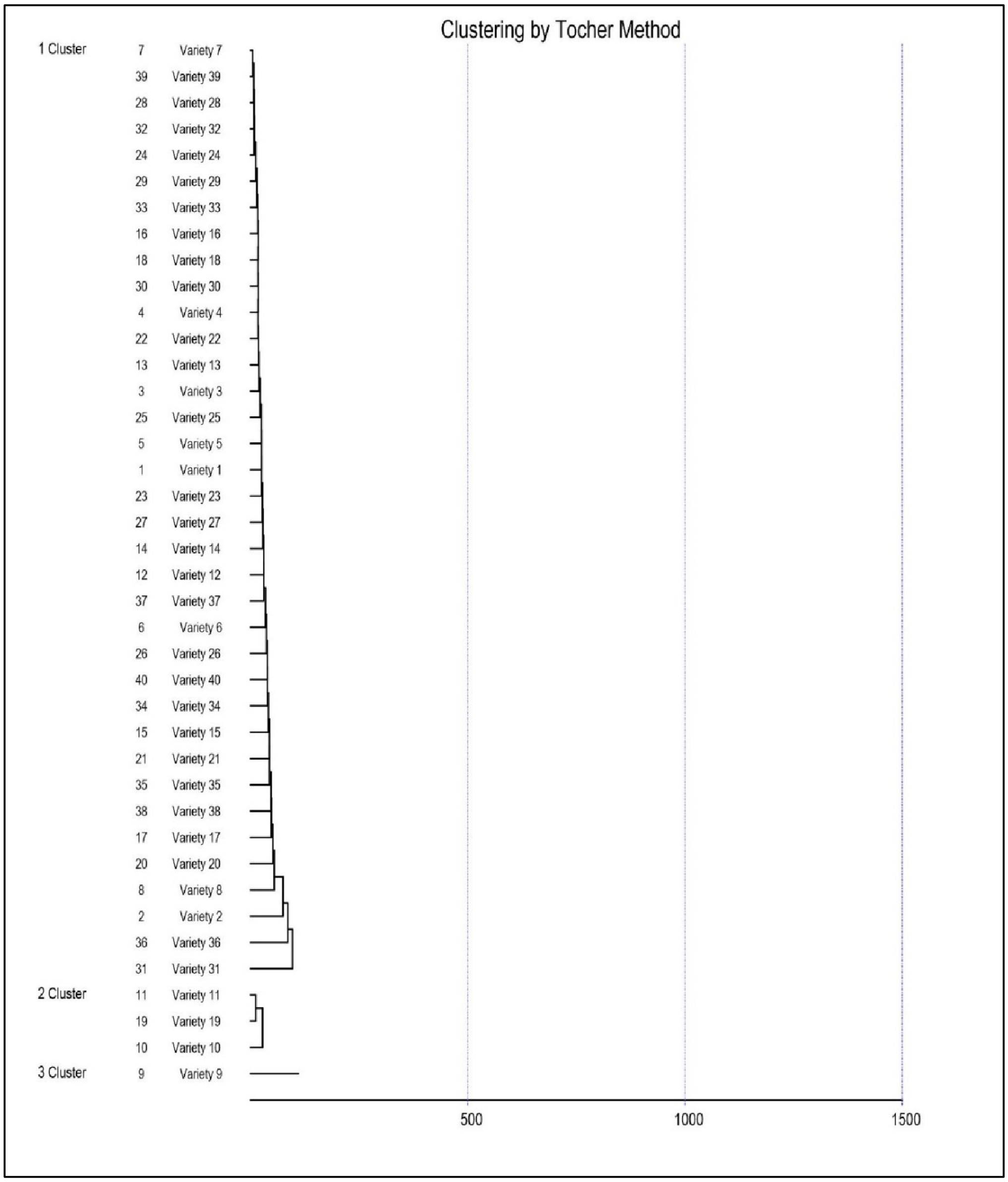


Fig. 2: Dendrogram showing clustering of apple genotypes by Tocher method

Table 20: Cluster means for various characters in different clusters of various apple genotypes

S. No.	Cluster	Fruit length (mm)	Fruit diameter (mm)	Fruit weight (g)	Fruit firmness (kg/cm^2)	Leaf blade length (mm)	Leaf blade width (mm)	Petiole length (mm)	Pedicel length (mm)	TSS (^oBrix)	Acidity (%)
1.	I	67.48	72.47	156.10	7.98	86.82	53.52	28.83	27.13	13.16	0.22
2.	II	44.67	52.89	57.23	5.86	88.11	55.00	28.11	19.78	10.97	0.26
3.	III	28.10	30.03	14.07	9.43	58.67	36.00	18.67	31.00	14.23	0.21

Table 21: Per cent contribution of individual traits towards total genetic divergence

S. No.	Traits	Times ranked 1st	Contribution (%)
1.	Fruit length (mm)	72	10.23
2.	Fruit diameter (mm)	43	6.51
3.	Fruit weight (g)	422	54.1
4.	Fruit firmness (kg/cm^2)	15	1.92
5.	Leaf blade length (mm)	31	4.17
6.	Leaf blade width (mm)	12	1.54
7.	Petiole length (mm)	9	1.15
8.	Pedicel length (mm)	60	7.82
9.	TSS (^{o}Brix)	2	0.26
10.	Acidity (%)	96	12.30

4.1.8 Principal Component Analysis

Three components of PCA viz., PC1, PC2 and PC3 were studied to explain the trait variation. PC1 accounted for 77.76% variation, the PC2 accounted for 6.95% and PC3 accounted for 4.05% variation. In particular the PC1 was positively and strongly associated with fruit weight (0.773) and negatively associated with leaf blade length. Highest PC2 scores were for acidity (0.663) PC3 score was found highest for fruit diameter (0.370). The most desirable trait which showed variation was fruit weight (0.773) (Table 22).

Table 22: The three principal components (PC) and contributions to the total variation (%) in various apple genotypes

S. No.	Variable	Component loadings		
		PC1	PC2	PC3
1	Fruit length (mm)	0.440	0.024	0.341
2	Fruit diameter (mm)	0.365	0.313	0.370
3	Fruit weight (g)	0.773	-0.069	-0.338
4	Fruit firmness (kg/cm^2)	0.080	-0.007	-0.537
5	Leaf blade length (mm)	-0.052	0.027	0.091
6	Leaf blade width (mm)	0.018	0.214	0.300
7	Petiole length (mm)	0.0123	-0.047	-0.088
8	Pedicel length (mm)	0.033	-0.575	-0.165
9	TSS (^{o}Brix)	0.095	-0.179	.0013
10	Acidity (%)	0.017	0.66	-0.436
Eigen value		4127.25	369.06	215.05
% Var.		77.76	6.95	4.05

4.2 Experiment II: Assessment of allelic diversity among apple genotypes for fruit quality characteristics using gene specific markers

4.2.1 Molecular screening of apple genotypes for scab resistance using *Vf* gene specific markers

Forty genotypes of apple were screened for the presence of *Vf* gene conferring resistance against apple scab disease. To confirm the presence of *Vf* gene, two gene specific markers were used. The marker AL07 (*Vf*) amplified at 820 bp and 570 bp alleles, the 570 bp fragment was observed in resistant genotypes Shalimar Apple-1, ASP-1, ASP-69, Firdous, ASP-3, Shireen, ASP-12, ASP-10, Golden Delicious, Maharaji, Red Gold, Benoni and was absent in susceptible genotypes, while the 820 amplicon was observed in both resistant and susceptible genotypes. The markers AM19 led to the amplification of 520 bp fragment in genotypes Shalimar Apple-1, ASP-1, ASP-69, Firdous, ASP-3 Shireen, ASP-12, ASP-10, Maharaji (Fig.3).

4.2.2 Marker analysis for fruit firmness

The markers Md-ACS1 and Md-ACO1 were used for screening of 40 apple genotypes. Two allelic forms of both Md-ACS1 and Md-ACO1 were typically amplified as Md-ACS1-1, Md-ACS1-2 and Md-ACO1-1, Md-ACO1-2. Out of the 40 genotypes evaluated, 3 were found homozygous ACS1-2/2 *viz.,* Gala Mast, Gala Redlum and Fuji Zhen Aztech, 17 were heterozygous ACS1-1/2 *viz.,* Super Chief, Braeburn, Shalimar Apple-1, Red Velox, Granny Smith, Golden Delicious Reinders, Mollies Delicious, ASP-69, Firdous, Starkrimson, Ambri, American Apirouge, ASP-12, ASP-10, Silver Spur, Irish Peach and Wiltons Star and 9 were homozygous ACS1-1/1 *viz.,* ASP-1, Lal Ambri, Sunhari, Shireen, ASP-3, Akbar, Cox's Orange Pippin, June eating and Yellow newton.. Amplification was not observed in the remaining genotypes (Fig. 4).

For Md-ACO1, 3 genotypes were found homozygous Md-ACO1-1/1 *viz.,* Shalimar Apple-1, Red Velox, Oregon Spur, 34 were heterozygous Md-ACO1-1/2 *viz.,* Super Chief, Braeburn, ASP-1, Lal Ambri, Granny Smith, Golden Delicious Reinders, Sunhari, Gala Redlum, Mollies Delicious, ASP-69, Gala Mast, Firdous, Starkrimson, Fuji Zhen Aztec, Ambri, American Apirouge, Shireen, ASP-10, ASP-4, ASP-3, Akbar, Shalimar-Apple 2, Silver Spur, Golden Delicious, Red delicious, Cox's Orange Pippin, June eating, Lord Lambourne, Red Gold, Benoni, Yellow Newton, Scarlet Siberean, Irish Peach and Wiltons Star and none was homozygous Md-ACO1-2/2 (Fig. 4).

Table 23: Marker based genotyping of apple germplasm for fruit quality traits

S. No.	Genotypes	ALO7	AM19	S. No.	Genotypes	ACO1	ACS1
1.	Super chief	rr	-	1.	Super chief	1/2	1/2
2.	Braeburn	rr	-	2.	Braeburn	1/2	1/2
3.	Shalimar Apple-1	Rr	R	3.	Shalimar Apple-1	1/1	1/2
4.	Red Velox	rr	-	4.	Red Velox	1/1	1/2
5.	ASP-1	Rr	R	5.	ASP-1	1/2	1/1
6.	Lal Ambri	rr	-	6.	Lal Ambri	1/2	1/1
7.	Granny Smith	rr	-	7.	Granny Smith	1/2	1/2
8.	Golden Delicious Reinders	rr	-	8.	Golden Delicious Reinders	1/2	1/2
9.	Sunhari	rr	-	9.	Sunhari	1/2	1/1
10.	Gala Redlum	-	-	10.	Gala Redlum	1/2	2/2
11.	Mollies delicious	rr	-	11.	Mollies delicious	1/2	1/2
12.	ASP-69	Rr	R	12.	ASP-69	1/2	1/2
13.	Gala Mast	-	-	13.	Gala Mast	1/2	2/2
14.	Firdous	Rr	R	14.	Firdous	1/2	1/2
15.	Starkrimson	rr	-	15.	Starkrimson	1/2	1/2
16.	ASP-3	Rr	R	16.	Fuji Zhen Aztec	1/2	2/2
17.	Ambri	rr	-	17.	Ambri	1/2	1/2
18.	American Apirouge	rr	-	18.	American Apirouge	1/2	1/2
19.	Shireen	Rr	R	19.	Shireen	1/2	1/1

Contd…

Table 23 contd…

20.	ASP-12	Rr	R	20.	ASP-12	-	1/2
21.	ASP-10	Rr	R	21.	ASP-10	1/2	1/2
22.	ASP-4	rr	-	22.	ASP-4	1/2	-
23	Fuji Zhen Aztec	rr	-	23	ASP-3	1/2	1/1
24.	Akbar	rr	-	24.	Akbar	1/2	1/1
25.	Shalimar Apple-2	rr	-	25.	Shalimar Apple-2	1/2	-
26.	Silver Spur	rr	-	26.	Silver Spur	1/2	1/2
27.	Oregon Spur	-	-	27.	Oregon Spur	1/1	-
28.	Golden Delicious	Rr	-	28.	Golden Delicious	1/2	-
29.	Red Delicious	-	-	29.	Red Delicious	1/2	-
30.	Chamure	-	-	30.	Chamure	-	-
31.	Maharaji	Rr	R	31.	Maharaji	-	-
32.	Cox's Orange Pippin	-	-	32.	Cox's Orange Pippin	1/2	1/1
33.	June Eating	-	-	33.	June Eating	1/2	1/1
34.	Lord Lambourne	rr	-	34.	Lord Lambourne	1/2	-
35.	Red Gold	RR	-	35.	Red Gold	1/2	-
36.	Benoni	RR	-	36.	Benoni	1/2	-
37.	Yellow Newton	-	-	37.	Yellow Newton	1/2	1/1
38.	Scarlet Siberean	-	-	38.	Scarlet Siberean	1/2	-
39.	Irish Peach	rr	-	39.	Irish Peach	1/2	1/2
40.	Wiltons Star	rr	-	40.	Wiltons Star	1/2	1/2

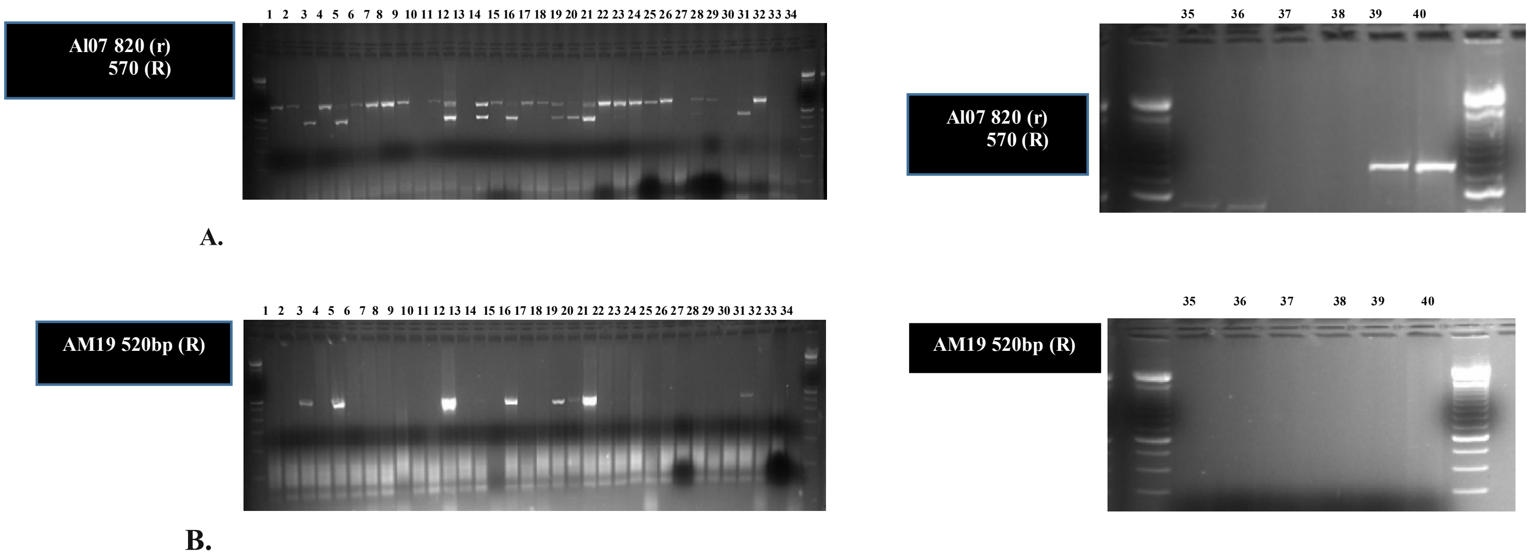


Fig. 3: Gel electrophoresis profile for markers AL07 and AM19

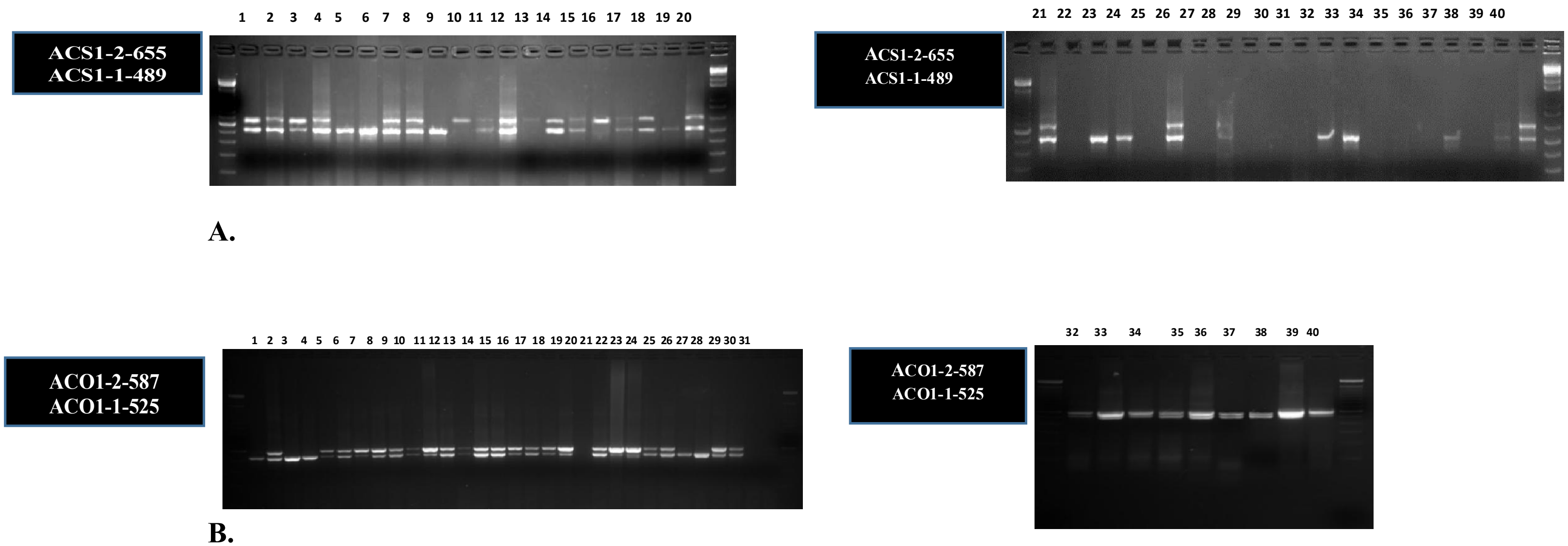


Fig. 4: Gel electrophoresis profile of markers Md-ACS1 and Md-ACO1

Chapter-5

DISCUSSION

For the success of any breeding programme and for the effective selection of cultivars, genetic variability must be present in the breeding material. It is therefore, necessary to assess the relative magnitude of variability in order to use such information, together with other selection parameters for the improvement of yield and quality of any fruit crop through adoption of effective breeding methods (William, 1964; Briggs and Knowles, 1967). Knowledge of extent of genetic variation and diversity for fruit phenology, maturity and quality component traits in locally available apple genotypes and subsequent identification of adapted superior genotypes as potential donors for yield and quality improvement is therefore essential. The genotypes observed in this study could serve as an outstanding basis and source of germplasm for apple breeding aimed at developing new cultivars with desired characters of superior quality and resistance to diseases. The apple being highly cross pollinated crop, each seedling raised plant is therefore a distinct genotype due to its heterozygous nature. Thus tremendous genetic variability is created which on the outer play of environmental conditions produces some excellent genotypes (possessing many desirable traits in a single plant). Keeping in view the importance of variability/diversity of the apple genotypes, it is important to characterize these populations and identify superior genotypes for their use in the future breeding programme. The study was accordingly taken up to characterize the apple germplasm and identify some promising genotypes for future improvement of apple crop species. In the present study 40 apple genotypes were evaluated for various morphological and qualitative traits, also allelic diversity for fruit quality traits was studied and the results obtained are discussed in this chapter.

5.1 Experiment No. I: To determine the morphological and pomological diversity in apple reference set

5.1.1 Flowering

Remarkable variations were observed in the phenological aspects of different genotypes from the date of initial bloom to full bloom stage. Initial bloom was first noticed at 37 days after reference date (DARD) in the genotype Benoni and last of all (46 DARD) in the genotype 'ASP-69'.The differences in the phenological stages may be due to their genetic differences or the differential chilling requirements of these varieties may be the reason for such variations. Further these varieties may be different in their photo sensitivity and response to temperature resulting in such variations. These results are in agreement with the results of Gasser (1994) who has reported that beginning of blooming depends highly on the site of cultivation. Beginning of bloom can be observed with highest accuracy which indicates the genetic differences among varieties the best. Soltesz (2002) investigated the stability of the blooming order of varieties in the case of for 20 years and pointed out that beginning of blooming never shows same trend even under similar circumstances. According to him 10 years of observations can be informative but cannot define the place of a variety in the blooming order.

Date of full bloom stage, was observed first in 'Benoni' (44 DARD) and very late (56 DARD) in 'ASP-69' and 'ASP-12'. These differential results may be due to the different requirements of temperature, cultural practices followed and ecological conditions of the cultivars, as the average temperature during bloom period may affect the flowering duration. These results are in conformity with the findings of Facteau *et al.* (1986) who reported that beginning of flowering, full bloom and end of flowering were two weeks later during 2007 than 2006 year. Such divergent results might be due to the difference between the temperatures during early stage of vegetative development. In these two year during this study the average temperature in March and April which is normal flowering period of apple in research area was 7.3 and 11.9°C respectively. Flowering date and period varies according to cultivar aptitude as well as ecological and cultural conditions (Facteau *et al.* 1986). According to Kronenberg (1985) an eight day difference existed in the bloom time of apple at 119 locations across Europe. Soltesz (1992) supports the concept that bloom time can greatly vary over years at the same location and the length of bloom period was affected more by number of rainy days than the amount of rain. The relative bloom time of the cultivar is the time a specific cultivar blooms compared to the chosen or standard cultivar. Soltesz (1992) reported that during the 20 years period neither relative time of bloom nor the order of full bloom occurred at the same time in any two years. In the current study difference between initial and full bloom varied between 7-10 days. Our results are also in consonance with those of Blasse and Hofmann (1991) who recorded dates of initial bloom, full bloom and harvest for 20 cultivars grown at Potsdam, Germany during 1970-1980 and reported that the average flowering period was 11 days regardless of cultivar. Bodor and Toth (2007) reported that high spring temperature causes shorter blooming period and main bloom takes only few days for the whole cultivar assortment. According to Sharma *et al.* (2005) and Sharma *et al.* (2006) flowering duration in apple ranged from 10-17 days in Himachal Pradesh. Our results are in consonance with Bozbuga and Pirlak (2012) who recorded the beginning of flowering, full flowering and end of flowering in Turkish apple cultivars on 14-19th April, 19-26th April and 25th April-2nd May respectively.

The maturity date of apple genotypes was also recorded and ranged from 88-176 (DAFB). The variation among genotypes in the date of maturity may be due to the difference in their genetic makeup and inherent parental characters of these varieties. Ingle and D'souza (1986) reported similar harvest time for Red Delicious cultivars (130-144 days). These results are further supported by the findings of Tripathi (1984) who reported that 124-129 days from full bloom to maturity was found most reliable index for determining maturity of Red Delicious apple cultivar.Our results are in consonance with the findings of Magness *et al.* (1926) who suggested the indexes of maturity as ground colour, ease of separation of the fruit from the spur and pressure test and the number of days from full bloom to maturity was constant for each of the varieties used by them under different seasonal conditions at a given locality and even under the widely varying climatic conditions and soil conditions of widely separated localities. According to Karacali (2004), harvest date depends on cultivar, place, year, rootstock and ecological conditions. Our results are in agreement with the results of Das *et al.* (2011) who found that cultivars like Vance Delicious, Starkrimson, Oregon Spur, Red Chief, Gala Must, Royal Delicious, Red Delicious and Golden Delicious took 110, 117, 117.5, 120, 125, 120,132 and 145.5 days respectively to attain maturity. This variation in the harvest dates may be due to difference in bloom dates, prevailing temperature, sunshine durations, overcast days and rainfall conditions during fruit growth period. Apple maturity gets delayed if cooler temperature prevails during summer. The variation among cultivars in the date of maturity may also be due to the difference in their genetic makeup and inherent parental characters of these varieties.

5.1.2 Leaf characters

Leaves play an important role in growth and development of plants as they serve as a source of food to plants. Various types of leaf characters were studied. The results obtained in this study are in close confirmation with the findings of Dar *et al*. (2015) who reported that leaf shape in various apple cultivars varied between ovate to oval, leaf apex ranged from acute to acuminate and incisions on leaf margins were found to vary between serrate type 1, crenate and bicrenate. Our results are in agreement with Wohner *et al.* (2014) who reported that the leaves in *Malus* species could reach a length of 8-11 cm and the intensity of green colour ranged from light, medium to dark. Leaf pubescence on lower side was observed as weak/absent, medium to strong. Incisions on margins of leaf blade varied between serrate type1, serrate type 2, biserrate, bicrenate. Linden and Iwarson

(2014) observed thick and doubly serrate leaf margins in crab apples. Hassan *et al.* (2017) observed similar results with respect to incision of leaf margins and pubescence on lower side of the leaf blade.

5.1.3 Fruit physical characters

The data recorded in the present investigation reflects significant difference between the physical characteristics of fruits. The study of fruit characters is important for diversity studies. Various types of fruit characters of different apple genotypes were recorded during the present study. Fruit characters *viz.,* length, diameter, weight, shape and pedicel length were recorded. Fruit size is an important parameter for selection of superior genotypes through breeding programmes (Westwood and Blaney, 1963).Fruit size is an important marketing parameter determining economical value in horticultural crops especially for pear and apple (Gillaspy *et al.* 1993). Fruit size in apple has been greatly increased by rigorous selections over the years from small wild types to the size of the present day commercial apple. It has been reported that the larger the fruit of the parents the greater will be the proportion of the seedlings producing fruits of acceptable size. Among all other physical parameters fruit size is the most important selection character. Fruits below 65mm in diameter have little value and fruit size of 65-70 mm is preferable. (Alston, 1981 and Knight, 1966, 1969).

All the apple genotypes in this study showed great variation as far as the fruit size is concerned. The fruit length and diameter showed variation and ranged from 28.1-85.2 mm and 30.03-85.06 mm respectively. The variation in size of the fruits have been reported by several workers and is attributed to several factors like variety, soil, age and vigor of plant and orchard management practices being followed. Our results are in line with the findings of Dar *et al. (*2015) who observed similar results with respect to fruit length and diameter of apple genotypes under Kashmir conditions. Atashi *et al*. (2015) observed among two cultivar, Golden Delicious (77.74mm and 82.24mm) was higher than Red Delicious (69.16mm and 78.29mm) in fruit length and diameter, respectively.

According to Weber (2001), fruit size is closely related to number of fruits per tree and tree volume. Our results are in agreement with the findings of Farooqui and Dalal, 1999 who reported that cultivars like Cox's Orange Pippin, American, Irish peach are reported to carry genes for small fruits. Other cultivars such as Ambri, Sunhari, Lal Ambri carry genes for large fruit size. The results are in agreement with Warrington *et al.* (1990), Fallahi *et al.* (1994) Chauhan and Sharma (2008) who observed similar results in relation to fruit size variation in spur and standard cultivars.

Another character observed was fruit weight which showed a huge variation and ranged between 14.06-202.73 g. The variation in fruit weight may be due to varietal characters and presence of seeds in the fertilized fruits as the seeds with endosperm are the sites of giberrellic acid synthesis, where growth substances are produced. It is a well-known fact that the genetics, environment and cultural practices all interact to determine fruit weight. Producing bigger fruits might be the inherent ability of genotype to utilize resources efficiently to achieve a certain fruit size (Stanley *et al.* 2000). The results of this study are close to results of Mratinic and Aksic (2011) who reported fruit weight of 70g to 193.33g in some Turkish *Malus* species. Variation in fruit size and weight might be under control of genetic factors involving their phylogenic behavior (Cowman *et al.*, 2001 and Harada *et al.* 2005). The variation of fruit weight in different apple cultivars is attributed to the inter-varietal differences associated with the genetic makeup of the cultivars and governed by the cell size and inter-cellular spaces of the fruit flesh. The results on the fruit weight and volume are supported from the observations made by Westwood *et al*. (1967), who also registered the fruit weight of small, medium and large fruits of Golden Delicious apples as134.00 g, 214.00 g, 294.00 g and 386.00 g, respectively. Sharma *et al*. (1997) in their experiment with quality evaluation of apple fruits at different elevation ranges registered the respective values of fruit weight in cultivars Golden Delicious and Red Gold as; 97.20 g and 94.60 g (mid hills), 107.83 g and 127.80 g (high hills) and 175.30 g and 116.25 g (dry temperate). In another study, the mean fruit weight for eight apple cultivars namely; Ruby Gala, Buckeye, Royal Beaut, Obrogala, Brookfield, Schniga, Galaxy and Mondial Gala was recorded as 198.00, 223.60, 215.20, 188.90, 214.60, 198.20, 194.90 and 187.00 g, respectively (Iglesias *et al*. 2008).

The fruits shapes under the present investigations have been recorded as per the apple descriptor (PPV, FRA 2012).The apple fruit shape is a very important component of visual quality, especially in regions of the world where certain shapes of apple are not acceptable for consumption. Fruit shape in this study varied between globose, conical, cylindrical, cylindrical waisted and obloid. However majority of genotypes showed fruits with globose shape. Janick *et al.* (1996) studied wide range of fruit shapes from conical to ovate which are preferred by consumers. Fruit shape though is influenced by the type of pollen parent (Aziz *et al.* 1999). Hofer *et al.* (2012) also studied fruit shape and reported flat globose in 65%, flat shape in 14%, globose shape in 11% and broad globose conical shape in 4% of the accessions. Dar *et al.,* 2015 reported that apple fruit shape is highly variable and is likely to depend on the genetic makeup of the cultivar. Pedicel length of the genotypes under study varied between 16-36 mm. Our results are in line with those of Verma *et al*. (2018) who observed similar results with respect to fruit shape and pedicel length in different apple cultivars.

Fruit skin colour is a very important character for assessing the quality and maturity of fruits. Skin colour consists of two components- background colour and over colour. The background colour in all apple genotypes under study varied from yellow to green, with an orange red to purple red over colour although the intensity of over colour varied from one genotype to another and from one fruit to other within a cultivar. Pattern of over colour also varied to a great extent where some genotypes showed either a strong or a weak solid flush pattern while others had strongly or weakly defined stripes over the solid flush, in some genotypes mottling along with solid flush was also observed. Colour of flesh varied from whittish, creamy, yellowish to greenish. Fruit colour is an important parameter to evaluate fruit characters which directly correlate with environmental conditions in prevailing localities. Fruit colour is significantly influenced by temperature, location of plant, light penetration and growth habit of tree. Sunlight is main factor responsible for synthesis of anthocyanin in fruit skin (Erez and Flore, 1986) and thus fruit colour (Marini *et al.* 1991). Janick *et al.* (1996) reported that inheritance of colour in apple is complex and can be bright red, bright green, clear yellow or bicolour free from russet or totally russeted. Mratinic and Aksic (2012) reported the background colour from cream white (38.89%), over yellow (16.67%) to green yellow (44.44%), while over colour ranged from red (50.0%) to dark red or purple (5.56%) in some *Malus* species in South Serbia.

Fruit russet amount did not show much variation and majority of genotypes had absent/small russet amount, only 11 genotypes had russet at stem cavity, 5 genotypes were covered with russet over the shoulders while only 1 genotype had large amount of russet. Russet usually develops in early stage of fruit development shortly after bloom, corresponding to the period of tangential growth and can also be genetic in nature such as in Cox's orange Pippin (Faust and Shaer, 1972). Russet formed in response to the damage of epidermal cells may result from application of crop protection agents, particularly those having a high degree of oil solubility as these can penetrate the cuticle directly (Goffinet and Pearson, 1991) and russeting has been shown to be controlled by a major dominant gene (*Ru*) with minor modifying and polygenic effects (Brown, 1992).

Fruit firmness is an important character that determines the quality and shelf life of apple fruit (Stow *et al.* 2000) and loss of fruit firmness is a serious problem resulting in quality losses. The firmness of the fruit depends on transpiration and respiration rates, resulting in loss of solutes and water (Gavalheiro *et al.* 2003; Erturk *et al.* 2003; Ghafir *et al,* 2009) and the pectin composition. Billy *et al.* (2008) reported that the firmness of the apple fruit significantly decreased with increasing storage duration. Fruit firmness of the apple genotypes under study ranged between 5.35 - 9.43 $kgcm^{-2}$ with maximum firmness observed in Scarlet Siberean and minimum in June Eating. The variations in fruit firmness may be due to varietal differences as reported by Magness *et al.* (1928). Willey and Thompson (1960) reported pressure values for Stayman, Golden Delicious, York imperial and Rome Beauty as 12.88, 12.98, 17.51 and 14.91 $lbs/inch^2$ respectively, Shabeena (2003) also reported maximum fruit firmness of (19.60 $lbs/inch^2$) in cultivar Gala Mast. Our results are in agreement with the findings of Damyar *et al.* (2007) who reported that fruit firmness varied greatly and was classified in 6 groups ranging from very soft (4%) to very firm (>7%). Our results are also in line with Kaya *et al.* (2015) who on their studies on fruit quality characters and genetic variability of apple germplasm in Turkey reported that the fruit flesh firmness had a range of 3.99-14.05 kg/cm^2. The significant difference between the cultivars in the fruit flesh firmness may be due to difference in fruit size specific to each variety, number of fruits, maturity of cultivars. These findings are supported by Fallahi *et al.* (1994) who observed marked differences in the physical quality parameters of cultivars due to varietal characteristics.

5.1.4 Chemical properties

Chemical aspects of fruits such as total soluble solids, acidity provide important information to the consumers in terms of recognizing a more nutritious fruit (Drogoudi *et al.* 2008).). The variability in fruit characteristics especially in fruit composition is not only genetic factor but also influenced by climatic factors (Wang, 1982).TSS is influenced by environmental factors such as temperature, light (duration and intensity), rainfall/supply of water and locations (Ahmed, 2008). In the present study the total soluble solids (TSS) showed a range of 8.96 - 14.93 (^{o}Brix) and the titratable acidity showed a range of 0.12-0.33%. The appreciable differences with respect to TSS and titrable acidity in different apple cultivars may be explained on the basis of their genetic differences, which subsequently affect the synthesis of photosynthates and their further breakdown into simple metabolites. Our results are similar to Mratinic and Aksic (2012) who in their studies on phenotypic diversity of apple germplasm in South Serbia reported that the total soluble solids in ºBrix content varied from 12.55 to 19.24 and titratable acidity varied between 0.10 and 0.82%. At maturity TSS in different cultivars has been recorded in the ranges of 11 to 15°Brix (Anonymous, 2003). However, in general it has been reported to be 12 to 14°Brix TSS in Delicious or spur type cultivars at harvest maturity. Singh *et al.*(2006) studied ten apple cultivars (Ambrich, Granny Smith, Ambroyal, Rich-a-Red, Red Gold, Red Fuji, Red Delicious, Lord Lambourne, Royal Delicious and Golden Delicious) and observed that total soluble solids (TSS), moisture and acidity of these apples were 6.2-11.8° Brix, 84.6-95.0% and 0.134-0.804%, respectively. Our results are in line with Kaya *et al. (*2015) who in their studies on fruit quality characters and genetic variability of apple germplasm in Turkey reported that the range for total soluble solids was 9.0-14.4^{0}Brix and 0.15-1.75% for titratable acidity. Our results are also in accordance with Kumar and Mir (2012) who reported maximum TSS (16.35^{0}Brix) with least acidity (0.07%) in Ambri apples. Bozovic *et al. (*2013) also reported almost similar results with TSS value ranging from 11.3 to 16% in some old apple varieties in central Montenegro.

The consumer acceptability is closely related to acid content which varies with the cultivar and forms an important standard of maturity (Wills *et al.* 1980). The intervarietal differences in the acidity level of fruits are attributed to the presence of varying amount of organic acids in them. The extent of variation in sugars in different apple cultivars is obviously due to fruit leaf ratio, abundance of photosynthates and variable amount of starch in young fruits, which in turn gets converted into sugar at maturity. Adhikari *et al*. (1985) studied on apple variety Fanny and estimated the ranging amount of titratable acidity from 0.80 to 0.26% (1978) and 1.06 to 0.44% (1979) during the 2nd week of June to 1st week of August. Kumar (2002) while comparing the standard and spur type of apple cultivars found Tydeman's Early Worcester having maximum acidity (0.45%), whereas minimum acidity was found in Top Red (0.21%). In close agreement with our results Tripathi *et al*. (2002) reported the acidity of two strains of Ambri apple, which varies between 0.10 to 0.12 per cent in the hilly region of Uttarakhand. While studying three apple cultivars namely; Red Boskoop, Lobo and Jonagold, Adamczyk *et al*. (2009) reported the acidity of 0.71 g 100g-1 (Red Boskoop), 0.41 g 100g-1 (Lobo) and 0.42 g 100g-1 (Jonagold), whereas it was 0.24, 0.35 and 0.17 per cent for Mutzu, Jonathan and Delicious, respectively. Similarly, Yosef and Belal (2014) reported that among two cultivar, Golden Delicious (0.39%) was higher than Royal Starking (0.26%) in total acidity. Our results are in agreement with the findings of Kotyal *et al*. (2017) who studied physico-chemical properties of ten apple (*malus domestica* borkh.) cultivars and recorded maximum T.S.S in *cv*. Scarlet Gala (14.27°Brix) and acidity in Marini Red (0.717%), while the minimum values of T.S.S. and acidity were observed in Marini Red (11.20°Brix) and Azetec (0.186%).

Considering the fact that the balance between sugars and acids has an important role in consumer acceptance and that apple cultivars with very low TSS/Acid ratio are appropriate for processing and cider production, while others with TSS/Acid ratio between 40-80. TSS/Acid ratio is used to determine the taste, texture and particular flavour of the fruits. In the present study this ratio was found to be highest in Ambri (117.7) and lowest in Braeburn (39.1). The variation among genotypes may be varietal differences due to environmental factors and difference in the sugar content of varieties. Our results for TSS, acidity and TSS/Acid ratio are in conformity with the findings of Kumar *et al.* (2006) who evaluated various standard, spur type and colour mutant varieties of apple for various chemical characteristics. They noticed that TSS of spur types was in the range of 11.34 to 13.50 °B and that of colour mutants was in the range of 10.25 to 12.31°Brix. Acidity was in the range of 0.22 to 0.39 per cent, TSS: acid ratio ranged from 29.08 to 67.63.

5.1.5 Sensory evaluation

The methods of sensory evaluation have provided reproducible quantitative data on quality of genotypes. Large number of genotypes can be screened with minimal resources. In the present case to evaluate varieties on the basis of sensory evaluation, a panel of five judges was selected and the scores were given for their colour, taste, flavor, texture, firmness, sweetness and overall acceptability. The genotype which performed the best in all the parameters was Fuji Zhen Aztec which scored 4.16 out of 5 followed by Gala Redlum which scored 4.11 out of 5 and the genotype which performed the lowest in the varieties under study was Irish Peach with an overall score of 2.55 out of 5 followed by June Eating with a score of 2.65 out of 5. The in-house panel results for colour, taste, firmness, sweetness were similar to the instrumental data. Our results are in agreement with Abbott *et al.* (2004) who compared the eating quality of a new apple cultivar, 'GoldRush,' with 'Golden Delicious' (one of its parents), 'Fuji,' and 'Granny Smith'. Acceptability scores for the texture and flavor of 'GoldRush,' and of Fuji' when included, were higher than those of 'Granny Smith' and 'Golden Delicious.'Shabeena *et al*. (2008) observed maximum organoleptic scoring in Gala Must followed by Florina, Red Delicious, Imperial Gala, Summer Red, Jonica, Red Chief and Red Fuji.

5.1.6 Analysis of variance

Analysis of variance for the various traits under study is presented in Table 15. The data revealed significant differences at genotypic level among the genotypes than the effect of environment on the traits and thus indicating that the material selected was genetically diverse for all the traits studied. Similar results were reported by Sarkar *et al.* (1991) while estimating the genetic variability in litchi and the results were also in agreement with Mir (2015) in wild apple. The magnitude of variability present in various traits under study revealed existence of wide range of variability for all the traits and thus the knowledge of extent of genetic variation and diversity and subsequent identification of adapted superior genotypes as potential donors for yield and quality improvement.

5.1.7 Genetic variability, heritability and genetic advance (as per cent of mean)

The range in the values reflect the amount of phenotypic variability which is not very reliable since it includes genotypic, environmental and genotypic × environmental interaction and does not reveal as to which character is showing higher degree of variability. The phenotype of crop further is influenced by additive gene effect (heritable), dominance (non-heritable) and epitasis (no allelic interaction). Hence variability is to be necessarily splitted into phenotypic coefficient of variation and genotypic coefficient of variation which ultimately indicates the variability extent for various traits.

The estimates of phenotypic coefficient of variation and genotypic coefficient of variation for all the characters studied are presented in Table 16. Generally phenotypic coefficient of variation agreed closely with the values of genotypic coefficient of variation however, the value of the PCV is slightly greater than that of the GCV for all the characters which indicates environmental effect in the expression of traits under observation. The results obtained in the present study follow the same trend and was in agreement with the study of Bandale *et al.* (2006) and Chattopadhay *et al.* (2011). From Table 16 moderately high phenotypic coefficient of variation and genotypic coefficient of variation was observed in fruit weight (27.238, 27.172%) followed by acidity (23.3, 21.9%), petiole length (18.9,17.2%), pedicel length (18.6,17.9%), fruit length (17.3,17.2%) and fruit diameter(15.3,15.1%) thus indicating that the genotypes had broad genetic base for these characters. The values of both phenotypic coefficient of variation and genotypic coefficient of variation indicate greater improvement to be expected for these characters. The results were supported by Bhat and Dhillon (2015) who reported high phenotypic and genotypic coefficient of variation for fruit yield, fruit length, fruit weight and pedicel length in pear and by Bisati (2012) who reported low magnitude of phenotypic and genotypic coefficient of variation (<10 per cent) for TSS whereas the values were moderate

(10-30 per cent) for other traits in apple cultivar Ambri.

The phenotypic coefficient of variation and genotypic coefficient of variation does not give a true picture of the inheritance of a character. Therefore the heritability is important as it enables the breeder to decide the extent of selection pressure to be applied under a particular environment which separates out the influence of environment from the total variability. Heritability estimation plays an important role in determining the effectiveness of selection provided it is considered in combination with the predicted genetic advance (Panse and Sukhatme, 1957 and Johnson *et al.*(1955).According to them the estimated heritability associated with genetic advance is more reliable than heritability alone for prognosticating the impact of selection. Heritability magnitude indicates the reliability with which the genotype will be recognized by its phenotypic expression and measure the proportion of variability of a trait which gets transmitted to the off spring.

Heritability (broad sense) estimates are more informative as they indicate the relative importance of genotype and environmental contribution to the variability exhibited. The estimate of heritability ranged from 0.81 to 0.96 for different traits in the present study and high heritability accompanied by greater genetic advance was observed in fruit weight, acidity, fruit length, fruit diameter, petiole length and pedicel length which revealed that these characters had additive gene effect and therefore have more role in proficient selection. High values of heritability for the traits clarified that they were least affected by environmental modification and selection based on phenotypic performance would be reliable. The results were supported by Srivastava *et al*. (2014), Moghaddama *et al.* (2013), Sharma *et al.* (2004) and Sharma and Sharma (2007) who reported high heritability with high genetic gain for different parameters in sweet cherry and apple. Hajnajari *et al.* (2012) in their study on heritability of morphological traits in apple early-ripening full-sib and half-sib offspring showed that the heritability varied among the traits, ranging from moderate to high values and a high level of heritability was found in leaf chlorophyll concentration (LCC) and seedling heights in both half-sib and full-sib families. High heritability with high genetic advance as per cent mean were recorded in papaya for characters seeds per fruit, pulp to seed ratio, yield per plant, fruit length, distance of first fruiting node from ground level, fruits per plant, fruit cavity, fruit diameter and average fruit weight by Jhambhale *et al.* (2014) indicating that these traits are predominantly governed by additive gene action.

5.1.8 Correlation Coefficient

Correlations are important in plant breeding as they measure the degree of association between two or more traits and give an indication of the traits that could be used to identify more important ones for a particular selection programme. Genotypic correlation is the heritable association between two variables which may be either due to pleiotropy or linkage. The correlation due to pleiotropy is useful in genetic improvement whereas the correlation due to linkage hinders the genetic improvement because it changes in segregating population (Singh and Narayanam, 2009). Mode and Robinson (1959) have studied the implication of phenotypic and genotypic correlation on breeding programmes. The pleotropic nature of genes could be the reason for correlation among traits. Genotypic correlation between two or more traits may result from pleotropic effect of genes or linkages of genes governing inheritance of two or more traits (Stebbins, 1950; Adams, 1967). The existence of interrelations among the characters is of utmost significance which otherwise are very difficult and in some crops impossible to identify during the segregation In present study the correlations among different traits like fruit diameter, fruit length and fruit weight showed a positive correlation among themselves indicating that weight of fruits depend on the fruit length and fruit diameter. Pedicel length was positively correlated with TSS and other parameters excluding acidity with which it was negatively correlated. Similarly petiole length was positively correlated with pedicel length and negatively correlated with TSS and acidity. Leaf blade length and width were positively correlated. TSS and acidity were negatively correlated. Similar correlation results between fruit length and diameter were recorded by Kumar and Srivastava (1983).

A number of other workers on apple and other crops have found both positive and negative correlations. The significant positive correlation between different characters could be useful for genetic improvement of various traits. Shin *et al.* (1986) observed positive association with leaf characters i.e. leaf area, weight and dry leaf weight. Klossowski (1976) between shoot length and yield, Shin *et al.* (1986) for leaf area with fruit length, Lauri *et al.* (1996) for fruit set and leaf area.

Verma *et al.* (2002) observed significant positive correlations for characters like tree height, tree spread, fruit set, fruit weight and fruit size. Bisati (2012) observed that highest direct positive effect was shown by fruit length breadth ratio and highest indirect negative was shown by fruit size through fruit length breadth ratio on yield per tree. Islam *et al.* (2010) reported strong positive correlation between yield per plant and individual fruit weight, stone weight, fruit length, fruit breadth and pulp-stone ratio in ber. Apart from these Zhang *et al.* (1994) obtained negative correlation between fruit firmness and TSS content. The varied positive and negative correlations obtained in the present study may be attributed to the genetic make up of the studied cultivars, varied agro-climates condition, site; orchard management practices individually or collectively may have influenced the performance of cultivar and the resultant correlations

Multivariate technique using D^2 statistics (Mahalanobis, 1928) is a very powerful and multivariate statistical tool in quantifying the degree of divergence among the genotypes (Murty and Arunachalam, 1966). In order to identify genetically diverse parents for hybridization, Mahalanobis D^2 statistics has been used in almost all crop species. Use of Mahalanobis D^2 statistics to estimate or evaluate the net/total divergence in breeding for crop improvement has been indicated by number of workers in different fruit crops (Saran *et al.* 2007). The use of genetically divergent parents in hybridization under transgressive breeding programme depends on the categorization of breeding material on the basis of appropriate criteria (Santos *et al*. 2011). Apart from providing requisite assistance or help in selection of divergent parents in hybridization, D^2 statistics also adequately assists in the measurement of diversification and the contribution of the relative proportion of each component trait towards the total genetic divergence or variation. This estimation of genetic divergence helps in reducing the large data of genotypes to manageable proportions. It is assumed that the parents showing wide genetic divergence are best suited for being used in the hybridization programmes. The utility of multivariate analysis in quantifying the degree of divergence between populations so as to understand the trend of their evolutionary pattern and assess the relative contribution of different components to the total divergence together with the nature of forces operating at intra and inter cluster levels had greatly been emphasized (Mishra *et al.* 1994).

In the present investigation 40 apple genotypes were evaluated to estimate the diversity as per Mahalanobis D^2 statistics. The genetic variation can be effectively employed in intra-specific crosses with hope that would lead to transmission of higher genetic gain for desirable traits. Classification of the genotypes led to the formation of 3 clusters. Cluster I comprised of maximum genotypes (36) followed by cluster II (3 genotypes) and only 1 genotype in cluster III. The studies of Sharma *et al.* (2013) on genetic divergence of apple revealed 4 clusters with maximum intra cluster distance between II and I cluster (30.331). The results were supported by Sharma *et al.* (2015) who also studied D^2 statistics for apple germplasm including 42 apple genotypes.

The clustering of genotypes from different eco-geographic locations into one cluster could be attributed to exchange of breeding materials from one place to another and may be due to unidirectional selection practiced for a particular character at several places produced similar phenotypes which were aggregated in one cluster irrespective of their distant geographic origin (Singh and Bains, 1968).

Another case is where many genotypes originating from one place were scattered over different clusters and such diversity among genotypes of common geographic origin could be attributed to factors like heterogeneity, genetic architecture of the populations, past history of selection, developmental traits and degree of general combining ability (Murty and Arunachalam, 1966).

The average intra and inter cluster distance (D^2) values revealed that the cluster I had the highest intra cluster distance value of 119.95 followed by cluster II (83.89) (Table 19) whereas the inter cluster distance was highest between cluster I and cluster III (1521.46) followed by inter cluster distance between cluster I and cluster II (745.61). Therefore cluster I and III were most divergent with maximum inter cluster distance.The results clearly indicate that hybridization between the cultivars from cluster I and cluster III can be utilized for getting the superior recombinants in segregating generations. Cluster means for various morpho-taxonomic and quality related characters revealed that substantial variability existed for all the traits and identified the traits to be chosen for hybridization. The results were in conformity with the Pereira *et al.* (2003), Saran *et al.* (2007), Sharma *et al.* (2015) and Bhat and Dhillon (2015).

The per cent contribution of the traits towards total divergence (Table-21) revealed that fruit weight was the main factor contributing towards divergence (54.10 per cent) followed by acidity (12.30 per cent), fruit length (10.23 per cent) and pedicel length (7.82 per cent). The minimum contribution towards divergence was from TSS (0.26 per cent each).The traits contributing maximum towards the divergence should be given great emphasis for deciding the clusters to be chosen for hybridization and the subsequent selection of the parents from the clusters be based on their performance (De *et al.* 1988).

5.1.9 Principal Component Analysis

Principal component analysis (PCA) one of the multivariate statistical procedures, has been used to study correlations among fruit traits to establish genetic relationship among genotypes within sets of apple genotypes. Associations between traits obtained from PCA may correspond to genetic linkage between loci controlling traits or a pleiotropic effect (Oraguzie *et al.* 2001). Principal component analysis has been studied previously to evaluate apple germplasm (Currie *et al.* 2000; Echeverria *et al.* 2005). In the present study PCA was used to explain the variation among different apple genotypes and to study the correlation among different traits of these 40 apple genotypes. Three components of PCA viz., PC1, PC2 and PC3 were studied to explain the trait variation. PC1 accounted for 77.76% variation, the PC2 accounted for 6.95% and PC3 accounted for 4.05% variation. In particular the PC1 was positively and strongly associated with fruit weight (0.773) and negatively associated with leaf blade length. Highest PC2 scores were for acidity (0.663). PC3 score was found highest for fruit diameter (0.370). The most desirable trait which showed variation was fruit weight (0.773) (Table 22). The results were supported by Mratinic and Aksic (2011) who reported that PC1, PC2 and PC3 accounted for 30.825, 22.562 and 13.709% respectively of total variation.

5.2 Experiment No.II: Assessment of allelic diversity among apple genotypes for fruit quality characteristics using gene specific markers.

Kashmir valley represents the richest repository of apple germplasm in India. However during the recent years many of the low yielding yet prized cultivars have been sacrificed on the altar of commercialization. This has led to widespread cultivation of few commercial cultivars many of which are susceptible to apple scab. On the contrary most of the wild relatives are resistant to scab due to the presence of resistance gene. Many of these resistance genes have been identified and characterized in depth with the use of these molecular markers. Of these *Vf* (Rvi6) has been widely characterized and evaluated.

Molecular screening was performed in all forty genotypes for the presence of *Vf* gene conferring resistance against apple scab disease using *Vf* gene specific primers, AL07 and AM19. Primer for the marker AL07 amplified two fragments 820 bp and 570 bp, the former 820 bp band represents the recessive *vf* allele and the latter the dominant allele *Vf.* Hence, this marker can be used for the identification of homozygous and heterozygous genotypes. Interestingly, the 820 amplicon was observed in both resistant and susceptible genotypes, while the fragment of 570 bp amplicon was amplified only in resistant genotypes. Primers for the marker AM19 being dominant in nature, led to the amplification of one band in resistant genotypes Shalimar Apple-1, ASP-1, ASP-69, Firdous, ASP-3 Shireen, ASP-12, ASP-10 and Maharaji. The molecular marker AM19 proved to be highly useful because of its ability to distinguish resistant and susceptible genotypes on the basis of presence or absence of single band on gel. Using this marker only one band of 520 bp was amplified in resistant nine cultivars. Therefore this marker seems to be highly specific in detecting presence of *Vf* (Rvi6) gene in apple genotype.s However, due to the dominant nature of this marker, it cannot distinguish homozygous and heterozygous genotypes containing *Vf* (Rvi6) gene. Our results are in agreement with the findings of Patrascu *et al*. (2006). Similar results were reported by Khajuria *et al*. (2014) by screening apple germplasm of north western Himalayas.

Ethylene regulates several physiological processes related to fruit ripening including changes in skin colour, flesh texture and synthesis of aromatic flavor compounds (Giovannoni 2004). Ethylene biosynthesis during apple fruit ripening is generally regarded as a primary factor leading to softening. Apple is a climacteric fruit and ripening is characterized by an ethylene burst accompanied by an increase in respiration. The role of ethylene in apple ripening has been studied using ethylene action inhibitors and transgenic approaches. Fruit ethylene production genotypes for Md-ACS1 and Md-ACO1 were determined for 40 apple genotypes. ACS1 had a much greater influence on fruit firmness than ACO1. The association between ACS1 and ACO1 allelotypes and observed firmness phenotypes at harvest supports the practical utilization of both ACS1 and ACO1 functional markers for selecting the progeny at the seedling stage with low ethylene production, firm fruit and long storage potential. Two alleles for each gene are commonly found in cultivated apple. Earlier studies showed that cultivars homozygous for the ACS1-2 allele produce less ethylene and have firmer fruit than ACS1-1/2 and ACS1-1/1 genotypes. ACO1 plays a minor role compared to ACS1, with homozygous ACO1-1 having lower ethylene production**.** Our results reveal low ethylene production genotypes for both Md-ACS1 and Md-ACO-1 in cultivars Fuji Zhen Aztec, Gala Mast, Gala Redlum and Shalimar Apple-1, Oregon Spur and Red Velox. Most of the genotypes under study had intermediate firmness with heterozygous genotypes for both Md-ACS1and Md-ACO-1 i.e. Md-ACS1-1/2 and Md-ACO1-1/2. The preponderance of heterozygotes is common for many apple traits (King *et al*. 2000). Zhu and Barritt (2008) reported similar results for Md-ACS1and Md-ACO1 in 60 apple cultivars. The data reported in the present study is important for marker assisted selection of progeny for breeding low ethylene producing apple cultivars for better storability and improved consumer acceptance.

Chapter-6

SUMMARY AND CONCLUSION

The results obtained for the study entitled **"Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm"** are summarized in this chapter as under :

6.1 Experiment No. I: To determine the morphological and pomological diversity in apple reference set

- Among the genotypes under study ASP-69 took maximum number of days after reference date (46 DARD) to reach the initial bloom stage while as Benoni took minimum days (37 DARD) to attain this stage.
- ASP-69 and ASP-12 took maximum number of days (56 DARD) to attain full bloom stage compared to Benoni which reached to this stage at 44 DARD.
- Maharaji took maximum number of days to reach maturity after the full bloom date (176 DAFB) while Irish Peach was the earliest to mature (88 DAFB).
- Leaf shape was mostly oval however ovate shape was also noticed in some genotypes. Similarly leaf tip was found to be acuminate in most of the genotypes (95%).
- The shape of apple fruits varied greatly among the genotypes, however most of the genotypes had globose shape. Ground colour was mostly green while over colour and pattern of over colour showed wide range of variation with maximum number of genotypes having orange red over colour and solid flush pattern.
- The phenotypic variance was higher than genotypic variance for each trait.

The phenotypic coefficient of variation and genotypic coefficient of variation was highest for fruit weight followed by acidity and lowest for TSS. In general the phenotypic coefficients of variation were slightly higher than genotypic coefficients of variation, which indicates the minor role of environment in the expression of traits under observation.

- High heritability accompanied by greater genetic advance was observed in fruit weight, acidity, fruit length, fruit diameter, petiole length and pedicel length which revealed that these characters had additive gene effect and therefore have more role in proficient selection.
- Correlation coefficient for various traits was estimated and the results revealed that fruit length and fruit weight showed a positive correlation among themselves indicating that weight of fruits depend on the fruit length and fruit diameter. Pedicel length was positively correlated with TSS and other parameters excluding acidity with which it was negatively correlated. Similarly petiole length was positively correlated with pedicel length and negatively correlated with TSS and acidity. Leaf blade length and width were positively correlated. TSS and acidity were negatively correlated.
- Estimates of divergence among forty apple genotypes revealed that significant divergence existed among them. The genotypes under study were grouped into 3 clusters as per Mahalanobis D^2 (1928) analysis with maximum number of genotypes in cluster I (36 genotypes) followed by cluster II (3 genotypes), cluster III (1 genotype).Cluster I had the highest intra cluster distance value of 119.95 followed by cluster II (83.89) whereas the inter cluster distance was highest between cluster I and cluster III (1521.46) followed by inter cluster distance between cluster I and cluster II (745.61). Therefore cluster I and III were most divergent with maximum inter cluster distance.

- The per cent contribution of the traits towards total divergence revealed that fruit weight was the main factor contributing towards divergence (54.1%) followed by acidity (12.31%), fruit length (10.23%) and pedicel length (7.82%).

- Principal component analysis of apple genotypes studied revealed that PC1 accounted for 77.76% variation, the PC2 accounted for 6.95% and PC3 accounted for 4.05% variation.

6.2 Experiment No.II: Assessment of allelic diversity among apple genotypes for fruit quality characteristics using gene specific markers.

- Molecular screening was carried out in all forty genotypes for the presence of *Vf* gene conferring resistance against apple scab disease using *Vf* gene specific primers. The results obtained provide sufficient evidence of resistance phenomenon carried by apple genotypes like Firdous, ASP-3 Shireen, Shalimar Apple-1, ASP-1, ASP-69, ASP-12, ASP-10 and Maharaji.

- Furthermore fruit ethylene production genotypes for Md-ACS1 and Md-ACO1 were determined for forty apple genotypes. Out of 40 genotypes evaluated 3 were homozygous ACS1-2/2 with highly firm fruits *viz.,* Gala Mast, Gala Redlum and Fuji Zhen Aztech. ACO1 plays a minor role compared to ACS1, with homozygous ACO1-1 having lower ethylene production. Our results reveal low ethylene production genotypes for Md-ACO-1 in genotypes *viz.,* Shalimar-Apple 1, Oregon Spur and Red Velox. Most of the genotypes under study had intermediate firmness with heterozygous genotypes for both Md-ACS1and Md-ACO-1 i.e. Md-ACS1-1/2 and Md-ACO1-1/2.

On the basis of investigations conducted in the present study, the following broad line conclusions are drawn:

Conclusions:

I. Estimates of variance and coefficient of variation (genotypic and phenotypic) indicate that there is appreciable diversity in the apple germplasm under study.

II. Considering high magnitude of genetic advance and heritability (b. s.) in various traits of horticultural interest, the existing biodiversity can be gainfully exploited either by direct selection of individual genotypes or as prospective parents.

III. On the basis of comparative evaluation ASP-10 was found to be the best with respect to various fruit quality among early maturing genotypes. Gala Redlum and Fuji Zhen Aztec were found to be the best among mid-season and late maturing varieties respectively.

IV. The results obtained from this study show that the gene specific primers identified in present study can be directly used for screening large apple germplasm in short period of time for developing marker assisted resistant varieties against apple scab.

V. Our results support the practical use of ACS1 and ACO1 markers in apple breeding in marker-assisted selection for firm apple fruit with improved storability and shelf life.

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*Original not seen

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Faculty of Horticulture, Division of Fruit Science

CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner Dr. Javid Iqbal Mir, Senior Scientist, ICAR-CITH, Srinagar during Viva-Voce examination held on 29-10-2018 have been incorporated in the manuscript entitled **"Morpho-Molecular characterization of apple (*Malus* × *domestica* Borkh.) germplasm"** submitted by **Ms. Aatifa Rasool (Regd. No. 2016-H-116-M).**

(Dr. K. M. Bhat)
Chairman
Advisory Committee