MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF AMBRI APPLE IN DODA AND KISHTWAR DISTRICTS OF J&K

By

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(J-15-D-248-A)

Thesis submitted to Faculty of Postgraduate Studies

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE (FRUIT SCIENCE)

Division of Fruit Science

Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu,
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CERTIFICATE-I

This is to certify that the thesis entitled **"Morphological, Biochemical and Molecular Characterization of Ambri apple in Doda and Kishtwar Districts of J&K"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Horticulture (Fruit Science)** to the Faculty of Post-Graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, is original work and has similarities with published work not more than minor similarities as per UGC norms of 2018 adopted by the University. Further the level of minor similarities has been declared after checking the manuscript with **Urkund** software provided by the University.

The work has been carried out by **Ms. Koushalya Devi** under my supervision and guidance. No part of the thesis has been submitted for any other degree of diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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"Distance doesn't matter only first step is difficult"

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ABSTRACT

Title of the Thesis	:	**Morphological, Biochemical and Molecular Characterization of Ambri apple in Doda and Kishtwar Districts of J&K**
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ABSTRACT

The present investigation entitled "Morphological, Biochemical and Molecular Characterization of Ambri apple in Doda and Kishtwar Districts of J&K" was undertaken during 2016 -2017 and 2017 -2018 in *Ambri* apple growing areas of Doda and Kishtwar districts to assess the distribution and to record the range of genetic variability for different horticultural traits of the selected trees. Fifty seedling trees of *Ambri* apple were selected during the survey on the basis of variability for various morphological traits constituted the material study as per descriptor given by NBPGR New Delhi. The genotypes were analysed for various statistical parameters such as mean, standard deviation, range and coefficient of variation using various multivariate statistical tools.

The *Ambri* genotypes evaluated during study showed wide variation with respect to tree, leaf, flower and fruit characteristics**.** In the present study, out of fifty *Ambri* apple genotypes, none of the genotypes had extremely weak tree vigour, while 4 genotypes (8 per cent) showed weak tree vigour, 25 genotypes (50 per cent) had intermediate, 19 genotypes (38 per cent), had vigorous and rest 2 genotypes (4 per cent) exhibitted very vigorous tree vigour. In case of tree habit among the fifty *Ambri* apple genotypes 22 genotypes (44 per cent) had upright tree habit, while 23 genotypes (46 per cent) had spreading and rest 5 genotypes (10 per cent) genotypes had drooping tree habit. Among the selected genotypes tree height, trunk girth and tree spread varied from 5.23 m to 9.30 m,105. 28 cm to 340.31 cm and 3.20 m to 6.80 m respectively. Leaf size varied as small (8 per cent), medium (44 per cent) and large (48 per cent), leaf shape varied as oval (40 per cent), and broad elliptic (60 per cent). In case of flower characterstics, biennial flowering was observed in 35 genotypes (70 per cent), except 3 genotypes (6 per cent) which were found regular in flowering and 12 genotypes (24 per cent) showed irregularity in flowering. Self incompatibility was observed in all 50 genotypes (100 per cent). Majority of genotypes 38 (76 per cent) showed bearing on spurs and 12 genotypes (24 per cent) exhibited mixed bearing habit. The number of flower buds per inflorescence showed maximum six number of flower buds per inflorescence, followed by five and four number of flower buds per inflorescence. Flower stalk length showed wide variation from 10.7 mm to 25.15 mm. As date of start of flowering is

concerned, it varied from 1st to 3rd week of April. End of flowering varied 3rd to 4th week of April. Fruit shape varied from globose (38 per cent), globose conical (16 per cent), conical (20 per cent) and long conical (26 per cent) whereas, fruit base varied as narrow (34 per cent), intermediate (12 per cent) and broad (54 per cent). Out of fifty selected *Ambri* apple genotypes, 17 genotypes (34 per cent) had narrow base, 6 genotypes (12 per cent) had intermediate and rest 27 genotypes (54 per cent) had broad fruit base. Fruit base cavity depth showed that 18 genotypes (36 per cent) exhibited shallow, 18 genotypes (36 per cent) showed medium and 14 genotypes (28 per cent) possessed deep fruit base cavity depth. Fruit apex showed that 30 genotypes (60 per cent) possessed smooth apex and 20 genotypes (40 per cent) exhibitted grooved fruit apex. Wide variation was recorded in fruit weight (158.2g to 292.2 g), fruit length (4.35 cm to 6.37 cm) and fruit width (4.62 to 7.62 cm) among the selected genotypes. Days to fruit harvest ranged from 145 to 158 days. Wide variation was also observed in fruit ground colour, fruit over colour, pulp texture, pulp tatse, juiciness, fruit skin lenticels, productivity status and biotic stress susceptibility among the selected genotypes of *Ambri* apple. Cluster analysis of *Ambri* apple genotypes base on morphological data categorized them into three clusters. Cluster I contained 11 genotypes cluster II contained 25 genotypes and cluster III contained 14 genotypes which showed inter cluster variability which can be utilized for improvement purpose. Principle component analysis of morphological data explained the cumulative variance of 71. 89 per cent. According to loading plots, PC1 explained (14.84 per cent) while PC2 explained (11.92 per cent) of the total variance.

Considerable variation was recorded among the biochemical parameters. TSS (12.57 °Brix to 15.91 °Brix), total sugar (9.89 per cent to 13.15 per cent), pectin content (1.00 per cent 1.32 per cent), ascorbic acid (2.10 mg/100g to 4.81 mg/100g) and Phenolics (43.59 mg/100g to 64.05 mg/100g) varied among the selected genotypes. Principle component analysis of biochemical data explained total cumulative variance of 76.76 per cent. Loading plots showed (32.62 per cent) and (18.17 per cent) of the total variance.

Twenty nine SSR markers were used in molecular characterization of fifty *Ambri* apple genotypes. The PIC value varied from 0.02 (CH03c02) to 0.8 (CH05d11) primer. Polymorphic percentage was observed from 14.20 per cent to 62.50 per cent in primer CH04g10 and primer CH03d12 respectively. Similarity coefficient value ranged from 0.14 to 0.74. UPGMA based dendrogram was constructed based on SSR analysis which grouped fifty genotypes into 2 major clusters. Principal co-rdinate analysis differentiate the genotypes into two cor-dinates based on diversity exist among them.

Four genotypes namely, SKJAG-12, SKJABh-19, SKJABh-20 and SKJAM-45 were obtained with different similarity coefficient values suggest that a rich genetic variation exists between them and they can be used prospective parents in further breeding programme to get segregates from molecular analysis. Two genotypes SKJAD-29 and SKJAD-30 showed superiority over selected genotypes with respect to biochemical parameters. The existing biochemical diversity could be explored as pre indices for identification of biotic and abiotic stress tolerance ability of the studied *Ambri* apple genotypes.

Keywords : Genetic diversity, genotypes, *Ambri* apple, variability, SSR markers.

Signature of Major Advisor

Koushalya

Signature of the Student

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LIST OF ABBREVIATIONS

CD	critical difference
μg	micrograms
Bp	base pair
Cm	centimeter
CTAB	cetyl trimethyl ammonium bromide
dNTP	deoxy ribonucleotide triphosphate
ddH_2O	double distilled water
EDTA	ethylene diamine tetra acetic acid
Ha	hectare
G	gram
HCl	hydrochloride acid
Hr	hour
Kg	kilograms
L	liters
LC	liquid chromatography
M	meter
M	molar
Mg	miligrams
$MgCl_2$	magnesium chloride
Min.	minutes
Ml	milliliters
Mm	millimeter
MT	metric tone
N	normal

NaCl	sodium chloride
Nm	nanometer
pH	potential of hydrogen
ppm	parts per million
r.p.m	revolutions per minute
TBE Buffer	tris borate EDTA buffer
TE Buffer	tris EDTA
Tris	trisaminomethane
Tris-Cl	tris hydrochloride
V	volts
v/v	volume/volume
ºC	degree celsius
SSR	simple sequence repeats
DNA	deoxyribonucleic acid
%	per cent
/	per
+	plus
mg Kg^{-1}	miligram per kilogram

INTRODUCTION

CHAPTER-1

INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) is an important fruit of the temperate zones with a high diversity of commercial cultivars. Apple belongs to genus *Malus*, subfamily Pomoideae and family Rosaceae with a basic chromosome number of x = 17. Across the globe, more than 10,000 apple cultivars are known today, of which only 20 are known for their commercial demand (Janick *et al.,* 1996). The centre of origin of apple is in the southwestern Asia, in the Caucasus area near Gilan in Turkistan (Vavilov, 1951). Since then, it has been spread by man into almost all parts of the world. The genetic variability as well as its adaptability in different environments in the apple germplasm is the main reason behind its spread in all most all parts of the world (Juniper *et al.,* 2001). Commercial cultivation of apple in India is confined to J&K, H.P, Uttarakhand and in small scale in the North-Eastern states. In India about 33 apple varieties are commercially grown over an area of 314 thousand hectares with the production of 2503 thousand metric tonnes annually (Anonymous, 2019a). In the union territory of Jammu & Kashmir total area under apple is 1,64,742 hectares with a production of about 18,82,319 MT and productivity of 11.42 metric tonnes /hectare whereas in Jammu region, the area under apple is around 18,415 hectares with the production of about 30,595 MT (Anonymous, 2019b). The districts under apple cultivation in Jammu region are Doda, Kishtwar, Ramban, Rajouri and Poonch.

Fruit consumption is believed to have beneficial health effects, and its often well said about apple that "An apple a day keeps the doctor away."Apples form an important part of human diet as they are a rich source of sugars, minerals, dietary fibre and functional compounds such as ascorbic acid and phenolics (Bondonno *et al*., 2017; Wu *et al*., 2007).

According to Bamzai (1989) apples have been grown in Jammu and Kashmir Union Territory even in 2000 B.C. during the reign of Nara. Its cultivation got extended during the reign of Lalitaditya (1089 A.D.). The cultivation is presumed to have been of

indigenous varieties grown for the purpose of local consumption only (Farooqui *et al.*, 2004). Ancient records of *Rajatarangi* and *Alberuni* mentioned the existence of numerous varieties of fruit in Kashmir. Fruit trees growing all over the valley, were wild and thousands of acres skirting the foot of the hills were covered with apple in full bearing.

Ambri apple is said to be indigenous to Kashmir (Lawrence, 1895). According to Thaper (1960) it is of seedling origin and was extensively grown in Kashmir valley. It requires a cool climate as buds of apple exhibit long rest period and require more chilling than buds of other temperate fruit crops. It is an attractive apple with an extra ordinary keeping quality. The fruits are medium to large size, oblong conical in shape and slightly irregular. Distinctly flattened at the base, ribbed, flesh creamy whitish with excellent aroma. *Ambri* apple can be kept for about a year in a basement/underground cellars (Chadha, 1995). An *Ambri* apple left in a room keeps the room fragrant for many days. It is late season variety which matures in October and in some areas matures in early November.

Prospectus of selecting promising genotypes and bringing genetic improvement in temperate fruit crops largely depends upon the extent of genetic diversity present in the population. Greater the diversity, better are the chances of evolving promising and desired cultivars either by hybridization or by selection. The choice of parents for hybridization is of paramount importance because more diverse the parents, greater are the chances of obtaining higher amount of heterotic expression in F1's generation and broad spectrum of variability in subsequent generations (Watkins, 1974). In Jammu and Kashmir, profile of fruit industry has been dominated by Red Delicious variety of apple which occupies 60 per cent of the total area under apple. Presently *Ambri* germplasm existing in the form of different types is facing greatest genetic erosion due to natural mortality or replacement by highly improved exotic cultivars introduced from abroad in order to boost fruit production in the Union Territory of Jammu and Kashmir. These introductions have eliminated many locally adapted apple varieties. If this trend continues, it can result in elimination of native fruits from commercial and home garden production. Many local types of genetic value have almost completely disappeared and many others are likely to disappear. In spite of all this, *Ambri* plantations still exist on

some higher altitudes and within inaccessible areas. The plantation is old and has continued to yield without any application of package of practices. There is pressing need to diversify fruit industry which calls for extension of cultivation of other types and varieties of apple particularly our indigenous variety *Ambri.* This is possible only when the industry has an access to suitable varietal complex. One of the ways of achieving this is to capitalize on the diversity available indigenously. So far very little efforts have been made to tap the variability existing in the indigenous material particularly *Ambri* apple in the Union Territory. Historically, *Ambri* is considered a par excellent variety and still finds favour with the locals as well as the native Indians. The scattered plantations of *Ambri* apple can be found in Doda and Kishtwar districts of Jammu province. Due to the seedling origin and its highly cross pollinated nature, it has contributed towards the tremendous variability in size, shape and colour development which provide a platform for exploitation of vast gene pool of *Ambri* apple. Therefore, it has a special significance for fruit industry of the Union Territory despite of its long gestation period and biennial bearing habit. *Ambri* variety is one of the choicest parents used in Indian apple breeding programme for improving the shelf- life of Delicious apple. Introductions of early maturing exotic cultivars have eliminated many locally adapted apple varieties. Special emphasis on saving genetically important *Ambri* types from extinction is therefore to be given priority basis; this can prove useful in bringing genetic improvement in yield and quality of commercially grown apple varieties through known breeding methods and selection. The need to conserve this genetic resource is far greater in present time than ever before.

Characterization is the depth of genetic base that has direct bearing on the potential to identify different cultivars with improved performances. Accurate and rapid cultivar identification is especially important in vegetatively propagated plant species such as most fruit trees like apple, both for practical breeding purposes and for proprietary rights protection. Morphological characterization of these trees and fruits is the first and the most important step for the description, classification and characterization of germplasm collections (Verma *et al.,* 2006). Unfortunately, the traditional methods for characterization and assessment of genetic variability in perennial fruit crop species, based on morphological and biochemical studies are both time consuming and affected

by the environment. The long juvenile period of apple makes the task of genotype characterization for different traits more difficult. To overcome these limitations, molecular markers have been used to differentiate, characterize, and identify apple accessions. Among the DNA-based markers, microsatellites or SSRs (simple sequence repeats) allow a high level of resolution in genetic studies due to their high polymorphism, co-dominant inheritance, reproducibility, and easy detection by PCR (Gupta *et al*., 1996). SSR markers exhibit hyper variability and are highly informative in nature. These markers also have proven useful in the repository setting to examine potential redundancies and propagation errors within collections (Dangl *et al*., 2005).

The aim behind this study was to determine the degree of genetic diversity in collected genotypes from different areas of Jammu province and to understand the genetic relatedness and differentiation among them. The results emanating from this study will help us to provide scientific guidance about effective management, conservation and improvement of the existing *Ambri* apple resources more effectively and the best selections can form basis for the future breeding programme with *Ambri* as one of the parent. In light of above facts, the present investigation on "Morphological, Biochemical and Molecular Characterization of *Ambri* apple in Doda and Kishtwar Districts of J&K" was undertaken with the following objectives:

1. To study the genetic diversity in *Ambri* apple germplasm of seedling origin in Doda and Kishtwar districts of Jammu province through morphological, biochemical and molecular characterization.

2. To identify and select the most promising accession of *Ambri* apple for conservation and popularization.

REVIEW OF LITERATURE

CHAPTER-2

REVIEW OF LITERATURE

The knowledge of genetic diversity of a crop is fundamental to its improvement providing a basis for selection of superior parental combinations. The degree of genetic diversity could contribute to the efficient conservation, maintenance and utilization of germplasm resources. Review of information suggests traditional methods of cultivar characterization, which is based on agronomic and morphological parameters, are often used to distinguish cultivars of the same species (Cantini *et al.,* 1999; Barranco and Rallo, 2000). Selecting desirable genotypes from the existing varieties and to use superior types in breeding programmes is an important tool to increase productivity in any crop. The extent of genetic diversity determines, to a great extent the success of a crop improvement programme. The assessment of a viable diversity is basic to the development of improved cultivars. Genetic diversity in wild relatives is very important, as these contain genes resistant to biotic and abiotic stresses. Thus all unique selections need to be collected, characterized and preserved. According to Hallauer (2000) the two main factors needed for successful breeding programme are adequate genetic variation and effective selection. Evidences exist that the germplasm resources of fruit plants are threatened to extinction. Such reductions have serious implication for food security in long term. To balance the demand for future food security it is important to protect the genetic resources for their sustainable use. Assessment of the entire collection with molecular markers is generally accepted (Grenier *et al.,* 2000) as compared to morphological characters, which are considered quick and commonly used methods. Studies have established that apple molecular markers are the most appropriate for characterizing apple collections (Santesteban *et al.,* 2009). The morphological markers are considered necessary to complete germplasm descriptors (Romero *et al.,* 2014). Keeping these points in view, the research problem entitled "Morphological, Biochemical and Molecular Characterization of *Ambri* apple in Doda and Kishtwar Districts of J&K" has been planned. The literature pertaining to above said problem is reviewed under the following heads:

2.1.1 Morphological characterization

2.1.2 Biochemical characterization

2.1.3 Molecular characterization

2.1.1 Morphological characterization

Morphological characterization is an important element for the evaluation of distinctiveness in apple. Morphological characterization is considered as initial step for cultivar identification. Greater diversity more will be the chance for selection of superior types and broadening the genetic base of *Ambri* apple. Several tree, foliage, floral and fruit characters have been taken under the study for selecting better *Ambri* apple genotypes of seedling origin and cultivars are reviewed as follows.

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 flowering phenomenon is caused by genetic differences between varieties. Bellard *et al.* (1971) studied the average date of occurrence for flower bud break in apple at WSU 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.

Chadha and Sharma (1978) studied breeding in apple varieties and reported that the average time of full bloom in hybrid apple seedlings almost coincide with the Delicious parent but flowered earlier than the *Ambri* parents. Farooqui *et al.* (1986) while working on genetic upgrading of apple observed difference of 52 days from full bloom to maturity between the progenies of apple and their parents. Tromp and Romer (1987) studied temperature and fruit set in apples and reported that time the full bloom varies from 30-44 days, and he also recorded late flowering in some cultivars may be due to their high chilling requirement.

Chadha and Sharma (1978) while investigating the quality and yield parameters of different apple cultivars observed that the size of fruit varies with the load of the crop.

Alston (1981) studied the fruit size of apple and reported that 60-70 per cent derivates of Cox's Orange Pippin were small in size. He also reported that in most of the progenies over half the seedlings have too small fruit size. In general large fruit size is controlled recessive gene, in which progeny mean is being below mid- parent values**.** Sharma *et al.* (1986) studied breeding of apple varieties with *Ambri* apple and reported that crosses made with *Ambri* resulted in the release of few outstanding apple varieties viz., Ambred, Ambrich, Ambstarking and Ambroyal. These varieties possessed the characteristic aroma, shape, better storage qualities of *Ambri* and are popular among fruit growers of Himachal Pradesh. These varieties have also exhibited tolerance to scab disease.

Lombard *et al.* (1988) studied fruit and tree yield performance in nut crops and reported that flower bud density (number of flower buds per trunk cross sectional area) (TCSA) as an index to express the magnitude of flowering. Fruit set decreases as the flower density increases, because competition among the flowers or developing fruit is stronger (Dennis, 1986). Royo and Miranda (2002) found in apples that 21 cluster set was related to flower density per land area (number of flower buds per m^2). They also reported that apples cluster set was related to flower density per land area (number of flower buds per m^2).

Tromp (2000) studied fruit shape in 3-year-old potted trees of apple cv. Cox's Orange Pippin apple under various controlled environment conditions and reported that environmental factors had little or no influence on fruit shape. The number of seeds/fruit was also unaffected. He also reported that fruit shape is related to seed number, it is postulated that in the field, climatic factors influence fruit shape via their effects on seed formation during the flower and fruitlet stages, and that the environment has no further effect.

Blasse and Hofmann (1991) recorded great diversity in flowering (dates of onset of flowering, full bloom, end of flowering and harvest) in 20 apple cultivars. They classified cultivars as early, mid-early and mid-late. The average flowering period was recorded 11 days regardless of cultivar. They also reported that the date of initial bloom was affected by air temperatures and there was no relation between the dates of initial bloom and harvest. For each cultivar harvest dates varied within a range of 7-12 days.

Kumar and Sharma (1991) studied flowering and fruit set aspects in introductions of apple and observed variation in dates of apple flowering and this variation is attributed to difference in agro-climatic conditions of a particular place. Eccher and Noe (1993) studied influence of light on shape and quality of Golden Delicious apples and observed that apples grown in different light conditions showed slight but significant difference in fruit shape and skin russeting.

Janick *et al.* (1996) studied range of apple shapes and reported that marketers prefer fruits with tall shape and conical to ovate (oval) fruit profile. Irregular and oblate (flat) shapes are not acceptable in market. Noiten and Alspach (1996) conducted an experiment to determine genetic variability by using pedigrees of apple cultivar in modern apple breeding. And they obtained the frequent clones of Cox's Orange Pippin, Golden Delicious, Red Delicious, Jonathan and McIntosh.

Kumar *et al.* (1997) studied flowering behaviour of some scab resistant and susceptible apple cultivars and found that flowering time varied from the last week of March to the first week of April. They also added that percentage anthesis, dehiscence increased from 8.00 to 12.00 h, then it decreased slightly until 14.00 h and then declined rapidly.

Aziz *et al.* (1999) reported that fruit shape of apple was affected by the type of parent pollen. He further observed that 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.

Sumrah *et al*. (2000), studied the role of temperature in apple fruit growth and revealed that in some low chilling apple cultivars under sub mountainous climatic conditions observed that Anna fruits were significantly larger (7.1 cm in diameter), while Tropical Beauty. They further added that cultivar with highest number of fruits per tree were having smallest fruits (4.53 cm in diameter).

Dewit *et al.* (2000) studied tree architecture in terms of production efficiency and pruning aspects. They also investigated the genetic control and physiology of both growth habit and vigour. They observed tree architecture is important in terms of

production efficiency and pruning aspects. They also recorded that the natural tree habit is under genetic control, which important for selecting the suitable parents in a breeding programme.

Kuden *et al.* (2001) studied genetic resources of temperate zone fruits in Turkey. They reported diversity in apples, quince and pear. Number of genotypes of apple and quince were collected at University of Cukurova to promote their conservation, utilization and widen fruits genetic resources. Sandhu *et al.* (2001) studied evaluation of subtropical pear germplasm under Punjab conditions and found that earliest full bloom was 25th February in AR-90-17 but it was delayed to 3rd April in Strain No. 30. The duration of flowering ranged between 7 to 19 days in different strains.

Shibata *et al.* (2002) while studying the characteristics of Japanese pear cultivar Akemizu found yellowish red brown skin, and round shape of fruit, averaging 320 g. The fruit was slight acidity and fine, fresh in taste. They also observed that fruit quality can remain good for five days when the fruit is kept at 25^{o}C and 90 per cent humidity. Nath Rai (2002) studied cultivating pears in Netarhat area of Chota Nagpur plateau and reported that average fruit weight was higher (260.0 g) in Netarhat Local than that of Nakh pear (175.1 g).

Paganova (2003) reported in pear that leaf shape varies from broad oval to narrow lanceolate, orbicular-ovate to elliptic-serrated. Bist *et al.* (2003) studied performance of low chill pear cultivars under sub mountainous Tarai region. They reported higher leaf length (14.15 cm), leaf breadth (7.07 cm) and leaf area (14.59 cm2) in Gola as compared to Pathernakh in early group cultivar of apple.

Liebhard *et al.* (2003) found that later stages of fruit growth are largely associated with cell expansion. Genes that regulate cell production and cell expansion may therefore control final fruit size. They also reported that several quantitative traits loci (QTL) affecting fruit size in tomato and apple and two such genes, FW2.2 and FACIATED, have been isolated from tomato. FW2.2 negatively regulates fruit size through control of cell proliferation during early fruit development (Cong *et al.*, 2002). FACIATED encodes

a YABBY-like transcription factor that affects fruit size by regulating carpel number in tomato (Cong *et al.,* 2008).

Elshihy *et al.* (2004) reported in pear that tree vigour varied from small to intermediate, except one genotype which showed very vigorous tree vigour. The shoot length ranged between 18 cm (W.T1) and 40 cm (Abu-Satel), while the shoot length of the other genotypes ranged between 25- 35 cm.

Hwang *et al.* (2005) found in South Korea that cultivar of pear, Joseng whangkcum (Nitaka x Shinko) exhibited vigorous tree growth and upright spreading tree habit and faster spur formation as compared to Kosui. Singh *et al.* (2006) obtained a maximum mean trunk girth (88.75 cm) in strain X and minimum (57.13 cm) in strain VIII.

Singh (2006) reported fruit length of hard pear varied from 6.17 cm to 6.92 cm in Strain VII and Strain X respectively. He further recorded the pedicel length (2.06 cm) in Strain XI and (2.38 cm) in Strain V.

Iglesias (2008) observed the tree vigour of pear cultivars varied from extremely vigorous (Delbard Premiere), vigorous (Ercolini 6079), medium vigour (Turandot, Carmen and Norma) and extremely weak (Precoce di Fiorano).

Ahmed (2008) studied biodiversity in pears in Multan and reported that out of 60 selections in pear, 32 (53.3 per cent) had pyramidal growth habit, while 21 selections (35 per cent) had broad spreading type and rest of the selections (11.7 per cent) had upright growth habit. The selections with broad spreading growth habit had large sized, those with pyramidal were medium to large sized and those with upright growth habit were small to medium sized.

Singh (2008) studied characterization of pear varieties recommended for Punjab and found that Pathernakh cultivar of pear had maximum leaf length (10.57 cm) and breadth (7.33 cm) while Punjab soft pear had least length (8.43 cm) and breadth (4.51 cm). Reighard *et al.* (2008) observed the most vigorous cultivars found in the study were

the Chinese types ‘Ya Li’ and ‘Shin Li’ as well as the Japanese type ‘Hosui’ ‘Atago’ and ‘Shinko’ cultivar were found the smallest trees among the selected pear cultivar.

Raina *et al.* (2011) recorded the flowering in pear and reported that earliest flowering (15th to 20th February) was observed in strain I, strain II, strain V and strain VI of hard pear and 19th to 25th February in strains S20, S21, S22, S23, S24 and S25 of semi-soft pear. Cultivars Florida Home, Orient, Tenn, Ya Li, Kousi, T-Su-Li and Nijisseiki was observed flowering from 28th February, 1st March, 5th March, 7th March, 17th March 13th March and 10th March respectively. Further observations were also recorded on leaf characteristics and it was revealed that leaf shape was found orbicular, leaf margin was exhibited serrulate, leaf apex was observed acuminate and leaf base was found reinform in hard pear strains. The semi-soft pear strains retained ovate leaf shape, serrulate leaf margin, acute leaf apex and convex leaf base. Orbicular leaf shape was also found in Nijisseiki, Shinseiki, YaLi, Hosui, Kosui and T-Su-Liwere variants with serrate leafmargin, acute leaf apex and convex leaf base.

Kumar and Mir (2012) studied varietal evaluation and found that Red delicious recorded maximum fruit weight (182.63 g). Maximum TSS (16. 35 per cent) and total sugars (12.11 per cent) with least acidity (0.07 per cent) was recorded in *Ambri* apple. Cropping efficiency (61.06 per cent) was scored least heritability. Length (0.843) and breadth (0.854) of fruit was positively and significantly correlated with weight of the fruit. Acidity was negatively correlated with all other biochemical characters.

Kiraly *et al.* (2012) studied morphological and molecular (SSR) analysis of old apple cultivars and they evaluated five cultivars of apple in the Batul group and four cultivars of apple in the Sovari group and reported that within the Batul group very few differences were found for the flowers. The predominant colour of the flowers at the balloon stage was dark pink for all the cultivars, the arrangement of the petals was overlapping, and the stigmas and anthers were on the same level. They also found that cultivars had large and obloid or globose fruit, while those of Zold batul were regular very large and globose or slightly elongated globose in shape. During the characterisation of the leaves, clear differences were observed among the Beregi sovari Selection 1, Selection 2, Daru sovari and Nemes sovari in the incisions on the leaf margins. With

regard to the flower and fruit characteristics, Beregi sovari Selection 1 was clearly distinguished from Beregi sovari Selection 2.

Reim *et al*. (2012) recorded diversity in wild apple (*Malus sylvestris* L. Mill.) and reported that there was no pubescence on leaf surface (43 per cent) and only few hairs (37 per cent) was observed. Anthocyanin colouration also showed difference from base of petiole. Margins incisions of leaf blade varied around serrate type1, serrate type 2, biserrate, and bicrenate.

Bhat (2012) studied molecular characterization and hybridization in pear at Ludhiana reported that fruit weight varied from (7.27 to 162.63 g) with maximum value in Patharnakh (162.63 g) and minimum in Kainth (7.27 g). He further reported that pedicel length and diameter varied significantly among the different pear genotypes under study and reported that maximum pedicel length of(5.53 cm) in YaLi and minimum value in Patharnakh (2.32 cm), and pedicel diameter was maximum in Patharnakh (1.02 cm) and minimum (0.13 cm) in Shaira.

Rugienius *et al.* (2013) studied the genetic polymorphism of wild pear selections collected from Lithuania and found that intervals of variation for the leaf length and width (2.7- 5.9 cm), (2.9-8.6 cm) respectively. Based on characteristics of leaf blade 8, 73, 18 and 1% of selections had acute, right angled, obtuse and rounded shape of apex respectively, 36, 38, 24 and 2 per cent had right angled, obtuse, rounded and cordate shape of base respectively. The incision of margins were absent in 26 per cent of selections and varied from crenate (22 per cent) to blunty serrate (18 per cent and sharply serrate (34 per cent). The leaf shape was rounded (20 per cent), oblong (30 per cent) and egg- shaped (6 per cent) and reverse egg shaped (18 per cent) and elliptic (26 per cent) was also reported by the same workers.

Bozovic *et al*. (2015) found a great variation on blooming period and maturation time among apple varieties and classified them as very early, early, mid and late for blooming and early, middle, late and very late for maturation. Fruit weight range was recorded (40.76 g to 206.74 g) in Krupnaja and Krstovaca. Biggest fruits (191.83–206.74 g) was produced by Babovaca. Soluble solid content (SSC) (11.0 to 16.1 per cent)

varied among Borovaca, Aleksandrija, Krstovaca Dapsicanka, Bosnika, Rebraca and Babovaca recorded highest SSC values.

Duric *et al.* (2015) studied pomological assessment of ten European pear from Bosnia and Herzegovina and they recorded the highest average fruit weight (109.40 g), fruit length (67.50mm), fruit width (58.23 mm), stalk length 946.25 mm), fruit firmness (6.30 kg/cm^2) in Mioljnjaca cultivar and lowest fruit weight (31.10 g) in Zobnjaca, fruit length (67.50 mm) in Karamut, fruit width (38.34 mm) in Zobnjaca, stalk length (19.21 mm) in Poljakinja and fruit firmness (2.07 kg/cm^2) in Zujiceva zuta.

Sharma *et al.*(2016) studied the morphological characters of fruits in the collected variants of *Ambri* apple and reported that among different fruit shapes, conic type was dominant (44.12 per cent) followed by broad conic (20.59 per cent) and globose (17.65 per cent), while for other shapes such as globose conic, broad globose and ovoid, they ranged between 2.94-8.82 per cent. Majority of the variants (97.06 per cent) had creamy-white flesh colour. Closed locule aperture was observed in 41.18 per cent variants, while locule apertures were moderately closed and fully open each in 29.41 per cent variants.

Hassan *et al.* (2017) recorded great variation in leaf blade length (6.26 to 12.06 cm), leaf blade width (2.73 to 7.23 cm), petiole length (1.53 to 4.30 cm), fruit length (1.14 to 5.21 cm), fruit diameter (1.17 to 6.42 cm), length of fruit stalk (0.60 to 5.20 cm), fruit weight (1.06 to 81.34 g) and number of seeds (2.12 to 10.00) per fruit in apple. TSS varied from 6.70 to 16.30 ^{o}Brix and acidity was observed 0.06 to 0.79 per cent in apple.

Kumar *et al.* (2018) studied performance of apple cultivars under Bhaderwah climatic conditions of Jammu and Kashmir and reported that on the basis of plant growth and yield characteristics of fruits *cv.* Lal Ambri exhibited maximum plant height (480 cm), annual extension growth (60 cm), plant spread (N-S= 122.33 cm and E-W= 115.00 cm) and plant volume (23.70 m^3) during investigations.

Kumar *et al.* (2018) studied morphological diversity of fifteen plum varieties grafted on seedling rootstock at Central Institute for Temperate Horticulture, Srinagar, Jammu and Kashmir. The highest cross sectional area (177.37 cm-^{2}) was recorded in Santa rosa and canopy volume (25.42 m-^{3}) in Aurosa. The maximum fruit yield (57.91

Kg tree-^{1}) was recorded in Meriposa and productivity efficiency (0.785 kg cm-^{2}) was in Au-cherry. Highest fruit and pulp weight (58.59 g and 56.49 g) was recorded in Grand Duke and lowest seed weight (0.74 g) in Monarch. The highest fruit length width ratio (1.42) was recorded in Grand Duke and lowest (0.94) in Black Amber. The varieties Au-Cherry, Meriposa, Tarkol, Beauty and Monark recorded better yield whereas Grand Duke, Santa Rosa and Methley had better fruit quality.

2.1.2 Biochemical Characterization

Crop improvement programme, relies on diverse germplasm therefore, there is a need to collect diverse cultivated genotypes of *Ambri* apple as well as to evaluvate them at molecular and biochemical level. The outstanding variants are utilised for mass multiplication through vegetative propagation and in breeding programme for the development and evolution of new cultivars of better quality, which will improve the socio-economic condition of the farmers.

Sancez *et al*. (2003) evaluated the comparative study of Rosaceae family and studied six pear cultivars in term of Phenolics and Vitamin C contents and reported that total phenolics content ranged from 123.5 – 200.5 mg kg^{-1} in the peel and form 2.8 to 8.1 mg kg^{-1} in the flesh. Red D' Anjou pear cultivar had the highest total phenolic content in the peel (200.5 mg kg^{-1}) whereas lowest phenolics (123.5 mg was recorded in D' Anjous. six pear cultivars. Ascorbic acid was detected in peel, whereas only dehydro ascorbic was present in the flesh. Vitamin C content ranged from 116-228 mg kg^{-1} in the peel and 28 to 53 mg kg^{-1} in the flesh.

Davies *et al.* (1991) studied Vitamin C and its chemistry and biochemistry in apple and reported that Vitamin C, is the main class of vitamin in apple, which is present in two forms viz., apples-ascorbic acid and its oxidized form, dehydroascorbic acid. Mapson (1970) also reported that the total level of the two forms remain constant per unit weight during growth, although the ascorbic acid /dehydroascorbic acid ratio increases to at least 95/5 at fruit maturity. The variability of ascorbic acid levels between fruits of same cultivars and between years has been reported very high. (Lee and Kader, 2000).

Sugar content in apple fruits assumes importance both for obtaining high quality fruits for dessert (except diabetic dietary), as well as for industry (Lazar and Barbos, 2008; Magwaza and Opara, 2015). Sugar content in apple is reported to vary depending on the weather conditions, cultivars, culture technology, position and exposition of the fruits in the crown (Mitre *et al.,* 2009; Sestras *et al.,* 2010). Roth *et al.* (2007) reported that sucrose content of apples decline in during storage, while level of glucose and sorbitol content increased.

Nour *et al.* (2010) reported dry matter content in apple ranged between 12.49 per cent (Prima) and 20.09 per cent (Red Boskoop) and total sugar content varied between 9.5 per cent (Cadel) and 15.03 per cent (Red Boskoop). The highest titratable acidity (0.771 per cent) was found in Red Boskoop and minimum (0.101 per cent) was recorded in Starkrimson. Malic and citric acid was 522.2-1993.7 mg/100 g and 3.5-49.1 mg/100 g in all analysed samples respectively. The ascorbic acid on average was recorded 6.18 mg/100 g. Potassium content varied between 82.25 mg/100 g (Mutzu) and 160.85 mg/100 g (Florina). Calcium varied between 1.70 mg/100 g (Starkrimson) and 8.74 mg/100g (Prima) and iron content was observed 0.19 mg/100 g (Ionagold) and 0.40 mg/100 g (Cadel and Early Red).

Minnocci *et al.* (2010) analyzed the biochemical composition of two ancient, late-bearing apple varieties i.e, Gala and Panaia red. Who found that the total polyphenol content range between 56 and 221 mg GAE/100 g of wet weight in Gala and Panaia red cultivars, respectively.

Ahmed *et al.* (2011) observed appreciable variability in organoleptic rating, chemical composition and postharvest life in pear. It was found that fruits of all the genotypeswere determined as excellent for food quality.TSS and total sugar contents was recorded maximum in Frashishi genotypes (MZ32, SD49, BG25 and SD40) and minimum in genotype KT54 (Btangi) .

Mathur *et al.* (2011) studied pectin content as an index for screening different varieties of apple (*Malus*L.) of Kashmir (J&K) on the basis of antimicrobial activity. They recorded that the yield of pectin content was found to be maximum in Maharaj-ji

(20.60 per cent) followed by Delicious (14.40 per cent) and American (11.60 per cent). The pectin extracted was then evaluated for its in *vitro* antibacterial activity against different pathogenic bacterial cultures. The results investigated that pectin extracted from Delicious variety showed potent antibacterial activity against *Klebsiella pneumoniae* (MIC value: 0.8 mg/ml) followed by *Streptococcus* pyogenes (MIC value: 0.3 mg/ml), *E.coli* (MIC value: 0.7 mg/ml) and *Lactococcus* sp. (MIC value: 0.7 mg/ml).

Raina *et al.* (2011) studied genetic diversity in pear germplasm using morphological traits and DNA markers. Who found that in hard pear group, total soluble solids were highest (11.00 per cent) in strains IX, XI and XII and lowest (9.52 per cent) in strain IV. Among semi-soft group, maximum TSS was observed in strain S15, followed by strain S16 (15.08 per cent) and S8 (14.37 per cent) and ranged from 10.00 to 14.00 per cent in Asian soft pear group. On the other hand, in soft pear group, maximum acidity was observed in Packham's Triumph (0.40 per cent) and lowest in Nijisseiki (0.19 per cent).

Mratinic and Aksic (2012) studied the local apple germplasm in Serbia and found large variations for physico-chemical characteristics enabling the selection of some superior clones from the local apple types. Who recorded the highest coefficient of variation (80.42 per cent) for titratable acidity among different physico-chemical characters of apple.

Verma *et al.* (2014) found that total soluble solids (^{o}Brix) ranged from 12.90 to 17.80 in THP-3 and THB-1respectively, while fruit acidity varied from 0.11 to 0.40 per cent. Rana *et al.* (2015) found that for detecting variation in pear species it is important to know the ratio of fruit length to fruit diameter, persistency of calyx on mature fruits, fruit surface and pulp texture. Who further observed that wild species which were sub-acidic and medium sweet in taste they also showed high total soluble solids (TSS). Fruit over colour was observed yellow to yellow green for 34 selections.

Dar *et al.* (2015) observed diversity in six apple cultivars namely Kullu Delicious, Golden Delicious, Lal Ambri, Raj Ambri, American Trel and Ambri Cross. It was reported that all the six cultivars exhibited distinct attributes with respect to shape, size,

flesh and skin colour, pH, TSS and fruit firmness. Further observations were made on the basis of fruit quality parameters, and it was found that*cvs.* Kullu Delicious, Lal Ambri and American Trel are highly admissible in the Indian fruit market as compared to rest of cultivars.

Sharma *et al.* (2016) studied genetic diversity of *Ambri* apple (*Malus* × *domestica* Borkh.) in Jammu region. They observed variation in terms of TSS, titratable acidity and ascorbic acid in between (10.90 to 20.00 ºBrix), (0.31 to 1.78 per cent) and (2.10 to 6.80 (mg/ 100 g) respectively. They also recorded the highest coefficient of variation for titratable acidity (54.76 per cent) followed by (37.11 per cent) in ascorbic acid and (14.68 per cent) in TSS.

Mir *et al.* (2017) observed principal component analysis in apple and reported that colour traits L, b and Tint were positively correlated with TSS and fruit size. TSS was negatively correlatedwith 'a' values but was positively correlated with fruit firmness. L values ranged from 32.66 (Well Spur) to 76.48 (Supermore Gold). Orange Val (-5.17), Snow Drift (-1.67) and Greensleeves (-7.70) were found Negative 'a' value which showed that these cultivars are green in colour at the time of harvesting. The 'b' values was observed 6.86 (King Hacious) and 52.18 (Supermore Gold) which indicate that these cultivars are yellow in colour and none blue. Positive 'Tint' value (2.36) was observed in Greensleeves cultivar and remaining cultivars observed negative value. Fruit firmness was recorded 26.00 (Starkrimson Gold) and 90.85 RI (Top Red). TSS varied from 11.80 ^{o}Brix (Razakwari) to 20.03 ^{o}Brix (Salvapobedetalian). Principal component analysis showed that first three components with Eigen values were able to explain more than 78 per cent of total variation. PC1 explained 37 per cent, PC2 exhibited 28 per cent and PC3 reported 13 per cent variability. Cluster analysis divide all the cultivars into five distinct clusters and one cultivar Starkrimson Gold does not appearedin any cluster.

Kotiyal *et al*. (2017) studied physico-chemical evaluation of ten apple (*Malus* X *domestica* Borkh.) cultivars grown in Uttarakhand hills of India. They observed among all the cultivars the maximum fruit size (length x diameter) and weight were observed in *cv*. Royal Delicious the values being 50.66 mm× 74.73 mm and 170.12 g respectively, while the minimum values were measured in *cv*. Azetec (40.52 mm × 53.03 mm) and

Aurora (110.97 g), respectively. The highest volume of fruit registered as 196.79 ml (Royal Delicious), in comparison to the lowest value as 125.75 ml (Aurora). The maximum T.S.S. noticed in *cv*. Scarlet Gala (14.27 °Brix) and acidity in Marini Red (0.717 per cent), while the minimum values of TSS and acidity were observed in Marini Red (11.20 °Brix) and Azetec (0.186 per cent). The ascorbic acid varied from 6.07 mg/100gm (Royal Gala) to 9.86 mg/100g (Braeburn), whereas the total sugar ranged 11.36 per cent (Royal Gala) to 7.06 per cent (Jonagold).

2.1.3 Molecular Characterization

Microsatellite markers or SSRs have become the preferred technique for the molecular characterization of different plant species (Gupta and Varshney, 2000). Microsatellite markers are useful for genetic studies at varietal, species and genus level, due to high conservation of the flanking regions (Hamza *et al*., 2004). SSR analysis is an informative method to fingerprint apple and study genetic relationships in it.

Hokanson *et al*. (2001) discovered excessive levels of divergence with a mean of 26.4 alleles per locus. It was additionally reported that mean of direct count for heterozygosity across all eight loci is 0.623.

Galli *et al*. (2005) recorded fifty six polymorphic alleles at the six SSR loci (average 9.2 alleles per locus) and the polymorphism information content (PIC) averaged 0.72. Affluent distinction of all apple genotypes was experienced by using only four (CH03g07, CH04e03, CH05d11, and CH05e03) SSR markers except for somatic mutants.

Patocchi *et al.* (2009) studied development and test of 21 multiplex PCRs composed of SSRs spanning most of the apple genome. They developed a series of twenty one multiplex (MP) polymerase chain reactions containing simple sequence repeat (SSR) markers spanning most of the apple genome. They selected eighty-eight SSR markers, well distributed over all seventeen linkage groups (LGs). Eighty-four of them were included in twenty one different MPs while four could not be included in any MPs.

Treuren *et al.* (2010) studied microstatellite genotyping of apple (*Malus*× *domestica* Borkh.) genetic resources in Netherlands. They reported that among the total sample, four hundred seventy five different genotypes were distinguished based on multi-locus microstatellite variation, which revealed a potential redundancy within the total sample of 32 per cent.

Miranda *et al.* (2010) recorded 9 to 15 alleles per locus in eight simple sequence repeat (SSR) with heterozygosity of 0.65 to 0.89. They identified that all the selections except for 16 had at least one of the 48 rare alleles (frequency < 0.05), while seven unique alleles were found in six selections. It was further reported that 75 per cent germplasm had genetic separatenessas compare to the main pear cultivars.

Farrokhi *et al.* (2011) revealed that 45 alleles were generated at sixteen SSR loci with polymorphism information content (PIC) content ranged between 0.18 to 0.76 in apple. Mean polymorphism information content (0.49) was recorded for all loci. Jaccard's similarity coefficient (0.19 to 0.79) was observed in apple cultivars.

Dequigiovanni *et al.* (2012) identified SSR markers, developed for pear and other fruit species that are effective in characterizing fruit germplasm and in demonstrating their use in providing support for genetic breeding programs. They investigated that out of total 62 SSRmarkers, 23 were yielding reproducible and polymorphic patterns which were used to 42 pear selections of the Brazilian Pear Germplasm Bank (PGB).

Cao *et al.* (2012) studied genetic diversity of cultivated and wild Ussurian pear (*Pyrus ussuriensis* Maxim.) in China. They evaluated cultivated and wild pear with M13-tailed eight genomic SSR markers and found that the M13-tailed method was effective in discriminating all the 32 wild selections.

Fan *et al.* (2013) developed a set of 120 simple sequence repeats (SSRs) from the newly assembled pear sequence and evaluated them for polymorphisms in seven genotypes of pear from different genetic backgrounds. They revealed that 67 (55.8 per cent) primer pairs produced polymorphic amplifications and together, the 67 SSRs detected 277 alleles with an average of 4.13 per locus.

Darvishzadeh *et al.* (2014) scored 44 alleles at SSR loci and 2 to 5 alleles per locus with average of 3.14. Effective number of alleles (2.3), expected heterozygosity (0.56), and observed heterozygosity (0.36) was recorded in apple rootstocks. The polymorphism information content (PIC) varied between 0.5 to 0.86.

Liu *et al.* (2014) studied identification of apple cultivars on the basis of simple sequence repeat markers. They examined 60 apple cultivars and varieties from descendants (Fuji x Telamon). They observed that out of the twenty pairs of SSR primers screened, eight pairs gave reproducible, polymorphic DNA amplification patterns which were used to construct a CID map. Each cultivar in this study was distinguished from the others completely, indicating that this method can be used for efficient cultivar identification.

Moazedi *et al.* (2014) studied the genetic relationships of cultivars of Asian pears (*Pyrus pyrifolia* Nakai) and native pears of Northern Iran. They used eleven SSR markers and found that UPGMA cluster analysis located the 20 genotypes into two main groups. The first main group had two subgroups including seven native genotypes and commercial Asian pears and the second group also had two subgroups involving the inter-specific hybrids of Asian and European pears, and Bartlett pear.

Muzher *et al.* (2014) evaluated the genetic relationships among seven pear genotypes (3 wild type genotypes of *Pyrus syriaca* Boiss and three local Syrian pears cultivars, in addition to Egyptian Licontei cultivar) by using 32 RAPD primers and 10 AFLP primer combinations. The level of polymorphism was achieved for all genotypes as revealed by RAPD and AFLP was 81.47 per cent, and 92.5 per cent, respectively. RAPD and AFLP revealed different genetic similarity (according to Jaccard coefficient) among the seven pear genotypes.

Romero *et al*. (2014) analyzed molecular and morphological characterization of local apple cultivars in Southern Spain. A total of 115 alleles were amplified for the 12 loci, ranging from 7 (CH01h01, CH01h10, and GD 12) to 13 alleles per locus (CH02c11). Forty-one alleles were unique to specific genotypes. The locus with the highest number of detected unique alleles was CH01f03b with 6 alleles. Expected

heterozygosity ranged from 0.74 for CH01h10 to 0.88 for CH02c11, with an average of 0.82. Observed heterozygosity varied from 0.45 for CH01h01 to 1.0 for CH02d08, with an average of 0.86.

Wolko *et al.* (2015) studied genetic diversity and population structure of wild pear (*Pyrus pyraster* (L.) and reported 17 SSR loci in six populations (192 selections) and 19.5 alleles per locus with a mean of 0.806. High Heterozygosity (Ho = 0.751) and low Fis (0.007) showed high level of diversity. Number of migrants per generation (Nm = 6.996) indicated a high gene flow and weak inter-population differentiation.

Dhyani *et al.* (2015) studied inter simple sequence repeat markers based genetic characterization of selected Delicious group of apple cultivars. They revealed that out of the 45 screened ISSR primers, 14 produced 129 clear and reproducible fragments ranging from 220 to 1400bp in size with an average of 7.92 fragments per primer. The ISSR markers revealed 8.26 per cent to 52.89 per cent polymorphism (Pp). Expected heterozygosity was in range of 0.035 to 0.186. Analysis of molecular variance revealed 27 per cent to 47 per cent variance among cultivars and 53 to 73 per cent within cultivars.

Hassan *et al.* (2017) evaluated the genetic diversity of *Malus* sp. in Kashmir. Fruit weight (1.06 g)in Genotype 3 and (81.34 g) in Genotype 20 followed by TSS (6.70 °Brix) in Genotype 10 and (16.30 °Brix) in Genotype 4. Mahalanobis D2 analysis categorized genotypes into 8 clusters. Maximum (17 genotypes) was grouped in cluster I followed by (8 genotypes) in cluster III, 3 genotypes in (cluster II) and rest were monogenotypic. The highest intra cluster distance was observed in cluster III (139.24) followed by cluster I (67.77) where as maximum (5213.52) inter cluster distance was observed between cluster II and V followed by cluster II and IV (4895.20). Cluster means in fruit weight (1.34 g in cluster IV to 79.19 g in cluster II) followed by TSS (6.70 °Brix in cluster IV to 16.30 °Brix in cluster V) was observed in genotypes. Besides this, principle component analysis (PCA) results revealed that PC1 variation observed was 88.19 per cent while from PC2 variation was observed as 8.84 per cent.

Ganopoulos *et al*. (2018) detected medium extent of polymorphism and 38 typical alleles (5.4 alleles per primer pair) were identified. Apple cultivars were group to their type by multivariate and distance clustering approach.

Omasheva *et al.* (2018) an experiment was conducted on genetic diversity of apple cultivars growing in Kazakhstan. A set of six SSR markers was used for identification of 30 varieties of Kazakhstani, 40 foreign varieties, and 16 Dzhangaliev's apple clones in wild apple populations. Values of expected (*H*e) and observed (*H*o) heterozygosity in groups of all analyzed varieties were high from 0.735 to 0.812 and from 0.661 to 0.721 respectively.

Dar *et al.* (2019) obtained 218 polymorphic fragments in 19 apple cultivars. 3 and 14 alleles per primer were recorded with an average of 7.51 alleles per SRR.

Mazeikiene *et al.* (2019) identified 81 polymorphic alleles. Out of these, 5 alleles were unique, found only in four individual genotypes. The most homozygous (44.7 per cent) and heterozygous (90.0 per cent) was observed in COL locus and CH02c11 locus respectively in referenced cultivars. CH04e05 locus was the most heterozygous (84.4 per cent) between the analysed genotypes.

Gitta *et al*. (2020) studied genetic diversity and similarity of pear (*Pyrus communi* L.) cultivars in Central Europe by SSR markers. Eighty-eight cultivars were analysed employing eight SSR primers resulting in a total of 216 alleles. Seventy-seven cultivars were thoroughly analysed. Among the samples 29 were considered to be diploids and 59 triploids. A genetic diversity analysis was computed based on a Neighbour-Joining algorithm and combined with a PCA indicating close genetic relationship and an overall high amount of genetic diversity among the samples tested.

MATERIALS AND METHODS

CHAPTER-3

MATERIALS AND METHODS

The present investigation entitled "Morphological, Biochemical and Molecular Characterization of *Ambri* apple in Doda and Kishtwar districts of J&K." was carried out during the year 2016 - 2017 and 2017 - 2018 in different selected apple growing areas of Jammu province.The morphological and biochemical analysis was carried out in the Division of Fruit Science, and molecular marker analysis was carried out in the Plant Genomics Laboratory, School of Biotechnology, Sher-e-Kashmir University of Agricultural Science and Technology of Jammu. Observations were recorded on selected genotype for different morphological characters at various growth and development stages.

3.1 Geographic features of site of study

The site of study is situated in Northern Western Himalayan region of Jammu and Kashmir. The study areas fall in Doda and Kishtwar districts. Doda is a district situated in eastern part of Jammu region. The district has mostly a hilly terrain. Doda is situated at 33.08^0 north latitude and 75.32^0 east longitude at an altitude of 1107 meters above mean sea level. The climate of the district is almost dry due to scanty of rainfall. The area receives rainfall during monsoon from July to September. The average annual rainfall is 926 mm and snowfall of about 135 mm. Kishtwar district lies in the north–eastern corner of Jammu region at 33^o 19'12''N and 75^o 46' 12"E coordinates. It is situated on the banks of the Chenab river. Kishtwar district is situated at height of 1,640 m above msl and is about 232 Kms from Jammu. The area is mainly hilly and mountainous with valleys and stretches of plains. Dachhan, Marwah, Paddar, Chatroo are major valleys of Kishtwar. General climate of Kishtwar is sub-tropical to temperate. Kishtwar district summer highest day temperature is in between 6°C to 27°C. Average temperature of January is -1°C , February is 1 °C , March is 6 °C , April is 9 °C , May is 13 °C . The average rainfall for Kishtwar has been recorded as 865 mm. The snow lasts for quite some time on the mountain tops, whereas in lower valley the snow cover

lasts only for short duration. The maximum precipitation is in the form of snowfall although the monsoon rains are also quite good. Kishtwar is bounded on the north by Kashmir and Zanskar Valleys, on the south by Badherwah and Doda, on the east by Himachal Pradesh and on the west by Anantnag and Ramban Districts. The farmers name,collection site, geographical coordinates and altitude for selected areas are presented in Table 1.

3.2 Technical programme

Survey was done during the year 2016 - 2017 and 2017 - 2018 at different apple growing areas of district Doda and Kishtwar of Union territory of Jammu and Kashmir, to select promising accession among the diverse *Ambri* apple genotypes and assess variability in their morphological characters. During the survey to get first hand information department of Horticulture and local inhabitants were consulted to identify the hotspots of *Ambri* and also on the basis of personal contact with local growers, one hundred fifty *Ambri* apple trees were surveyed, marked and evaluated based upon the superiority in terms of fruit size, quality and colour. The location was selected with respect to the availability of diversity in *Ambri* apple genotypes. Initially a total of 150 naturally growing seedling trees were marked and the data on various tree, leaf, flowering and fruit morphological parameters were recorded to select the elite genotype. The sample of 100 trees were rejected and finally, trees of fifty (50) *Ambri* apple genotypes (more than fifteen year old) with divergent characters were selected at fruit maturity stage on the basis of size, shape, weight, colour and quality characteristics.Codes were assigned to each genotype on the basis of their spot (even as SKJAB stands for SKUAST-Jammu *Ambri* Bhaderwah, SKJAT stands for SKUAST-Jammu *Ambri* Thathri, SKJAG stands for SKUAST-Jammu *Ambri*Gandoh, SKJABh stands for SKUAST-Jammu *Ambri* Bhagwah, SKJAK stands for SKUAST-Jammu *Ambri* Kishtwar, SKJAD stands for SKUAST-Jammu *Ambri* Dool and SKJAN stands for SKUAST-Jammu *Ambri* Nagsani, SKJAM stands for SKUAST-Jammu *Ambri* Mughalmadain and SKJAC stands for SKUAST-Jammu *Ambri* Chatroo number were allotted for identification) and geo tagging was done for selected genotypes. Regular visits were made during the period of flowering, fruit setting, fruit maturity and ripening stages during the years of study.A short description of all *Ambri* apple genotypes/ accessions was recorded in the form of

Table 1: Name of the farmer and geographical location of site of fifty Ambri apple (*Malus* × *domestica* Borkh.) genotypes

S. No.	Name with parentage	Address	Total number of plants selected	Genotypes	Geographical location of selected plant		
					Latitude	Altitude	Longitude
1)	Sh. Ashok Kumar S/O Sh. Sham Lal Kotwal	Bhadarwah Village:Paneja	**5**	SKJAB 01	N $32^0$58.270′	1751m	N $32^0$58.270′ E $075^0$42.983′
2)	Sh. Ashok Kumar S/O Sh. Sham Lal Kotwal	Bhadarwah Village:Paneja		SKJAB 02	N $32^0$58$^{0.}$ 266′	1753m	N $32^0$58$^{0.}$ 266′ E $075^0$42. 979′
3)	Sh. Ashok Kumar S/O Sh. Sham Lal Kotwal	Bhadarwah Village:Paneja		SKJAB 03	N $32^0$58.246′	1754m	N $32^0$58.246′ E $075^0$42.984′
4)	Sh. Ashok Kumar S/O Sh. Sham Lal Kotwal	Bhadarwah Village:Paneja		SKJAB 04	N $32^0$58.246′	1754m	N $32^0$58.246′ E $075^0$42.984′
5)	Sh. Ashok Kumar S/O Sh. Sham Lal Kotwal	Bhadarwah Village:Paneja		SKJAB 05	N $32^0$58.261′	1753m	N $32^0$58.261′ E $075^0$42.963′
6)	Sh. Abdul Latif Wani S/O Sh. Deen MohamadWani	Jangalwar, Thatri	**4**	SKJAT 06	N= $33^0$11.079′	1567m	N= $33^0$11.079′ E=$075^0$28.575′
7)	Sh. Abdul Latif Wani S/O Sh. Deen MohamadWani	Jangalwar,Thatri		SKJAT 07	N =$33^0$11.060′	1593m	N =$33^0$11.060′ E=075^0.28.580′
8)	Sh. Abdul Latif Wani S/O Sh. Deen MohamadWani	Jangalwar, Thatri		SKJAT 08	N= $33^0$10.106′	1594m	N= $33^0$10.106′ E= $075^0$28.576′
9)	Sh. Abdul Latif Wani S/O Sh. Deen MohamadWani	Jangalwar, Thatri		SKJAT 09	N= 3311.097′	1593m	N= 3311.097′ E=07528.580′
10)	Sh. Punjab Singh S/O Sh. Puran Singh	Gundoh, , near court of Munsiff, Judicial Magistrate 1st class	**8**	SKJAG 10	N= $33^0$01.934′	1672m	N= $33^0$01.934′ E=075^0 54.556′
11)	Sh. Punjab Singh S/O Sh. Puran Singh	Gundoh, , near court of Munsiff, Judicial Magistrate 1st class		SKJAG 11	N= $33^0$08.872′	1653m	N= $33^0$08.872′ E=075^0 46.559′

12)	Sh. Brij Lal S/O Sh. Ravi Dass	Gundoh , Hospital (Primary Health Centre)		SKJAG 12	N= $33^0$08.871/	1654m	N= $33^0$08.871/ E=$075^0$46.558/
13)	Sh. Brij Lal S/O Sh. Ravi Dass	Gundoh , Hospital (Primary Health Centre)		SKJAG 13	N= $33^0$08.869/	1654m	N= $33^0$08.869/ E=$075^0$46.554/
14)	Sh. Brij Lal S/O Sh. Ravi Dass	Gundoh , Hospital (Primary Health Centre)		SKJAG 14	N= $33^0$08.851/	1666m	N= $33^0$08.851/ E=$075^0$46.574/
15)	Sh. Brij Lal S/O Sh. Ravi Dass	Gundoh , Hospital (Primary Health Centre)		SKJAG 15	N= $33^0$08.855/	1666m	N= $33^0$08.855/ E=$075^0$46.574/
16)	Sh. Brij Lal S/O Sh. Ravi Dass	Gundoh , Hospital (Primary Health Centre)		SKJAG 16	N= $33^0$08.854/	1667m	N= $33^0$08.854/ E=$075^0$46.574/
17)	Sh. Punjab Singh S/O Sh. Puran Singh	Gundoh, near court of Munsiff, Judicial Magistrate 1st class		SKJAG 17	N= $33^0$08.843/	1669m	N= $33^0$08.843/ E=$075^0$46.583/
18)	Sh.Pyare Lal S/O Sh. Jodh Ram	Bhagwa	**3**	SKJABh 18	N= $33^0$08.851/	1672m	N= $33^0$08.851/ E=$075^0$46.580/
19)	Sh.Pyare Lal S/O Sh. Jodh Ram	Bhagwa		SKJABh 19	N= $33^0$11.956/	1577m	N= $33^0$11.956/ E=$075^0$28.528/
20)	Sh.Pyare Lal S/O Sh. Jodh Ram	Bhagwa		SKJABh 20	N= $33^0$11.981/	1580m	N= $33^0$11.981/ E=$075^0$28.511/
21)	Sh. Shokat Ali Minto S/O Sh. Moh. Akbar Mintoo	Samina Colony, Kishtwar	**1**	SKJAK 21	N= $28^0$08.751/	1711m	N= $28^0$08.751/ E=$075^0$46.580/
22)	Sh. Jaffar Ali S/O Sh. Moh. Akbar	Filter plant , Kishtwar	**2**	SKJAK 22	N= $28^0$08.751/	1728m	N= $28^0$08.751/ E=$075^0$46.580/
23)	Sh. Jaffar Ali S/O Sh. Moh. Akbar	Filter plant , Kishtwar		SKJAK 23	N= $28^0$08.751/	1726m	N= $28^0$08.751/ E=$075^0$46.580/
24)	Sh. Ishtaq Dar S/O Sh. Gulam Rasool Dar	Bhagwan Mohallah	**4**	SKJAK 24	N= $28^0$08.751/	1736m	N= $28^0$08.751/ E=$075^0$46.580/
25)	Sh. Ishtaq Dar S/O Sh. Gulam Rasool Dar	Bhagwan Mohallah		SKJAK 25	N= $28^0$08.751/	1733m	N= $28^0$08.751/ E=075^0 46.580/

26)	Sh. Ishtaq Dar S/O Sh. Gulam Rasool Dar	Bhagwan Mohallah		SKJAK 26	N= 28^{0}08.751$^{/}$	1735m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
27)	Sh. Ishtaq Dar S/O Sh. Gulam Rasool Dar	Bha-gwan Mohallah		SKJAK 27	N= 28^{0}08.751$^{/}$	1739m	N= 28^{0}08.751$^{/}$ E=075^{0}46.580$^{/}$
28)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool	**9**	SKJAD 28	N= 28^{0}08.751$^{/}$	1553m	N= 28^{0}08.751 E=075^{0} 46.580$^{/}$
29)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 29	N= 2808.751$^{/}$	1643m	N= 2808.751$^{/}$ E=075^{0} 46.580$^{/}$
30)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 30	N= 28^{0}08.751$^{/}$	1642m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
31)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 31	N= 28^{0}08.751$^{/}$	1644m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
32)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 32	N= 28^{0}08.751$^{/}$	1647m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
33)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 33	N= 28^{0}08.751$^{/}$	1648m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
34)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 34	N= 28^{0}08.751$^{/}$	1649m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
35)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 35	N= 28^{0}08.751$^{/}$	1650m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
36)	Zubair Ahmad S/O Gulam Hussain	Lonepora Dool		SKJAD 36	N= 28^{0}08.751$^{/}$	1652m	N= 28^{0}08.751$^{/}$ E=075 46.580$^{/}$
37)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni	**6**	SKJAN 37	N= 28^{0}08.751$^{/}$	1570m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
38)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni		SKJAN 38	N= 28^{0}08.751$^{/}$	1500m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
39)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni		SKJAN 39	N= 28^{0}08.751$^{/}$	1555m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
40)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni		SKJAN 40	N= 28^{0}08.751$^{/}$	1550m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
41)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni		SKJAN 41	N= 28^{0}08.751$^{/}$	1560m	N= 28^{0}08.751$^{/}$ E=075^{0} 46.580$^{/}$
42)	Sh. Abdul Latif Lone S/O Abdul Rashid Lone	Near Tehsildar Office Nagseni		SKJAN 42	N= 28^{0}08.751$^{/}$	1561m	N= 28^{0}08.751$^{/}$ E=075 ^{0}46.580$^{/}$

43)	Shri Altaf Hussain Meer S/O Shri Gulam Rasool Meer	Mugalmaidan	**5**	SKJAM 43	N= $28^{0}08.751^{/}$	1270m	N= $28^{0}08.751^{/}$ E=075 46.580$^{/}$
44)	Shri Altaf Hussain Meer S/O Shri Gulam Rasool Meer	Mugalmaidan		SKJAM 44	N= $28^{0}08.751^{/}$	1273m	N= $28^{0}08.751^{/}$ E=075^{0} 46.580$^{/}$
45)	Shri Altaf Hussain Meer S/O Shri Gulam Rasool Meer	Mugalmaidan		SKJAM 45	N= $28^{0}08.751^{/}$	1270m	N= $28^{0}08.751^{/}$ E=075 46.580$^{/}$
46)	Shri Altaf Hussain Meer S/O Shri Gulam Rasool Meer	Mugalmaidan		SKJAM 46	N= $28^{0}08.751^{/}$	1270m	N= $28^{0}08.751^{/}$ E=075 46.580$^{/}$
47)	Shri Altaf Hussain Meer S/O Shri Gulam Rasool Meer	Mugalmaidan		SKJAM 47	N= $28^{0}08.751^{/}$	1272m	N= $28^{0}08.751^{/}$ E=075 46.580$^{/}$
48)	Shri Gh. Mohd S/o Shri Fathu Kholi	Chatroo	**3**	SKJAC 48	N= $28^{0}08.753^{/}$	1799m	N= $28^{0}08.753^{/}$ E=075 46.580$^{/}$
49)	Shri Gh. Mohd S/o Shri Fathu Kholi	Chatroo		SKJAC 49	N= $28^{0}08.753^{/}$	1799m	N= $28^{0}08.753^{/}$ E=075 46.580$^{/}$
50)	Shri Gh. Mohd S/o Shri Fathu Kholi	Chatroo		SKJAC 50	N= $28^{0}08.753^{/}$	1798m	N= $28^{0}08.753^{/}$ E=075 46.580$^{/}$

passport data for different morpho-physiological characters with the help of Apple descriptor NBPGR- 2002.For molecular analysis young leaves were collected for DNA extraction by CTAB method (Doyle and Doyle, 1990).The morphological and biochemical data were analyzed for various statistical parameters such as mean, range, coefficient of variation using multivariate statistical tools.Molecular characterization was done using twenty nine polymorphic SSR markers and the allelic data was analyzed using DARwin software.

3.2.1 Morphological diversity analysis

The characterization of the seedling *Ambri* apple genotypes for diversity analysis at morphological level was carried out as per the NBPGR (National Bureau of Plant Genetics and Resources) descriptor guidelines (NBPGR-2002).

3.2.1.1 Tree characteristics

All the six tree characteristics *viz.*, type of planting material, tree height, trunk girth, tree spread, tree habit and tree vigour were scored as per the descriptor NBPGR-(2002).

3.2.1.1.1 Type of planting material

Only healthy and bearing *Ambri* apple trees of seedling origin were selected and subjected for further evaluation.

Seedling = 1
Cutting = 2
Layered = 3
Grafted = 4
Budded = 5

3.2.1.1.2 Tree height (m)

Tree height was measured with the help of marked bamboo stick from the ground level to the maximum height attained by the tree during dormancy and expressed in meters (m).

3.2.1.1.3 Trunk girth (cm)

Trunk girth of seedling *Ambri* apple genotypes were measured at 25 cm above ground level during dormancy with the help of measuring tape during dormancy and expressed in centimeters (cm).

3.2.1.1.4 Tree spread(m)

Tree spread of seedling *Ambri* apple genotypes were measured by putting the marked bamboo stick horizontally to the tree from east-west and north-south direction and mean spread was worked as in meters (m) during active growth period.

3.2.1.1.5 Tree habit

Tree habit was observed in the month of May-June during active growth period and rated as per descriptor as mentioned below:

Upright = 3

Spreading = 5

Drooping = 7

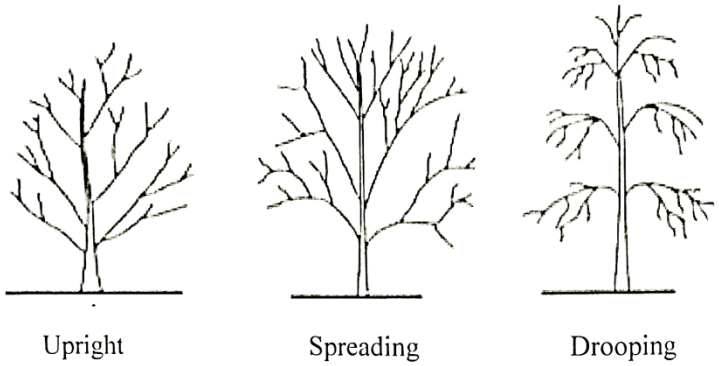


Fig. 1: Tree growth habit

3.2.1.1.6 Tree vigour

Tree vigour was observed as the overall abundance of branches during the active growth period and rated as per descriptor as mentioned below:

Extremely weak = 1

Weak = 3

Intermediate = 5

Vigorous = 7

Very vigorous = 9

3.2.1.2 Leaf characteristics

All the observations on the leaf were made on fully developed leaves and were scored as per the descriptor NBPGR-(2002).

3.2.1.2.1 Leaf size

Leaf size was recorded on mature leaf and rated as per descriptor as mentioned below:

Small = 3

Medium = 5

Large = 7

3.2.1.2.2 Leaf shape

Leaf shape was recorded on mature leaf and rated as per descriptor as mentioned below:

Oval = 1

Ovate = 2

Broad elliptic = 3

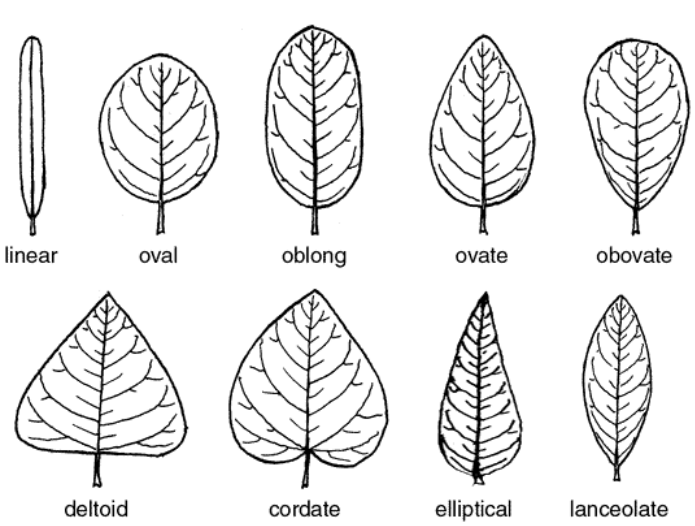


Fig. 2: Different leaf shapes of apple

3.2.1.3Flower characteristics

Four shoots in each of the four directions (East, West, North and South) were tagged and these shoots were studied for the flowering pattern.

3.2.1.3.1 Number of flower buds per inflorescence

Number of flower buds per inflorescence was recorded as average of 10 random inflorescences on different shoots in four directions.

3.2.1.3.2 Flower stalk (pedicel) length (mm)

Flower stalk length was recorded as average of 10 random stalks.

3.2.1.3.3 Date of initiation of flowering (dd/mm/yy)

Date of initiation of flowering was recorded when 5 to 10 per cent per cent buds had opened and expressed as Day/Month/Year.

3.2.1.3.4 Date of end of flowering (dd/mm/yy)

Date of end of flowering was recorded when 85-90 per cent buds have opened and expressed as Day/Month/Year.

3.2.1.3.5 Regularity of flowering

Regularity of flowering was recorded on the basis of flowering flush in the current year and rated as per descriptor as mentioned below:

Regular = 1

Biennial = 2

Irregular = 3

3.2.1.3.6 Self incompatibility

Self incompatibility was recorded during the flowering flush and rated as per descriptor as mentioned below:

Incompatible = 1

Partially compatible = 2

Compatible = 3

3.2.1.3.7 Bearing habit

Bearing habit was recorded during flowering stage by the location and types of buds which produce flower and fruit and rated as per descriptor as mentioned below:

On spurs = 1

On shoot tips = 2

On old shoots = 3

Mixed = 4

3.2.1.3.8 Age of first bearing

Age of first bearing (years) was recorded as number of years to attain first fruiting.

3.2.1.4 Fruit characteristics

All observation on the fruit were recorded on physiologically ripe fruits immediately after harvest.

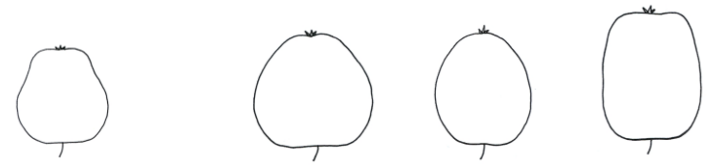
3.2.1.4.1 Days to fruit harvest

Days to fruit harvest were recorded as number of days from date of end of flowering to date of harvest.

3.2.1.4.2 Fruit shape

Fruit shape was recorded on mature fruit and rated as per descriptor as mentioned below:

Globose	=	1
Globose- conical	=	2
Short-globose conical	=	3
Flat	=	4
Flat-globose (oblate)	=	5
Conical	=	6
Long-conical	=	7
Intermediate-conical	=	8
Ellipsoid	=	9
Ellipsoid-conical (Ovate)	=	10
Oblong	=	11
Oblong -conical	=	12
Oblong-waisted	=	13
Other	=	9



Cylindrical waisted conic ovoid Cylindrical

Ellipsoid globose Obloid

Fig. 3: Different fruit shapes of apple.

3.2.1.4.3 Fruit base

Fruit base was recorded on mature fruit and rated as per descriptor as mentioned below:

Narrow = 3

Intermediate = 5

Broad = 7

3.2.1.4.4 Fruit base cavity depth

Fruit base cavity depth was recorded on mature fruit and rated as per descriptor as mentioned below:

Shallow = 3

Medium = 5

Deep = 7

3.2.1.4.5 Fruit apex

Fruit apex was recorded on mature fruit and rated as per descriptor as mentioned below:

Smooth = 1

Wrinkled = 2

Grooved = 3

Others = 99

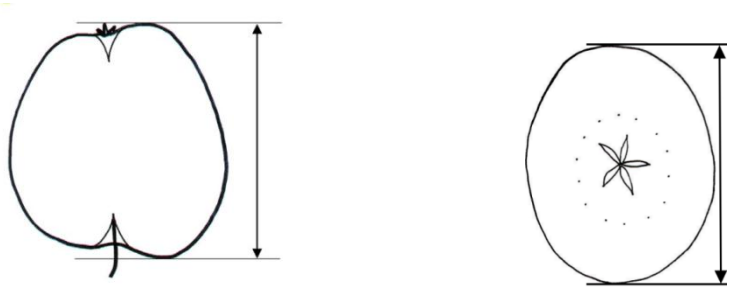
3.2.1.4.6 Fruit length

Fruit length was recorded with help of Digital Vernier Calipers as average of 10 random mature fruits and expressed in centimetres (cm).

3.2.1.4.7 Fruit width

A random sample of 10 healthy fruits was selected and fruit width was recorded with the help of Digital Vernier Calipers as average of 10 random mature fruits and expressed in centimetres (cm).

Length Width



Vertical orientation Horizontal orientation

Fig. 4: Fruit length and Fruit width

3.2.1.4.8 Fruit weight

Fruit weight was analysed with electronic weighing balance as average of 10 random mature fruits and expressed in grams (g).

3.2.1.4.9 Fruit ground colour

Fruit ground colour was recorded as skincolour of fully mature fruit and rated as per descriptor as mentioned below:

Cream white = 1

Yellow = 2

Green yellow = 3

Green = 4

Orange = 5

Red = 6

Others = 99

3.2.1.4.10 Fruit over colour

Fruit over colour was recorded as skincolour of fully mature fruit and rated as per descriptor as mentioned below:

Yellow (golden) = 1

Pink = 2

Green = 3

Orange = 4

Red = 5

Dark red = 6

Brown = 7

Purple = 8

Dark brown = 9

Others = 99

3.2.1.4.11 Fruit skin lenticels

Fruit skin lenticels was recorded on mature fruit and rated as per descriptor as mentioned below:

Absent = 0

Low = 3

Medium = 5

High = 7

3.2.1.4.12 Pulp texture

Pulp texture was recorded on mature fruit and rated as per descriptor as mentioned below:

Soft = 3

Intermediate = 5

Firm = 7

3.2.1.4.13 Pulp taste

Pulp taste was recorded on mature fruit and rated as per descriptor as mentioned below:

Acidic = 1

Sub acidic = 2

Medium sweet = 3

Sweet = 4

3.2.1.4.14 Juiciness

The juice content was recorded on mature fruit and estimated by using the following formula:

$$\text{Juice content (per cent)} = \frac{\text{Total volume of juice (ml)} \times 100}{\text{Weight of fruit (g)}}$$

Juice content was coded under different categories as given below:

Less = 3

Medium = 5

High = 7

3.2.1.4.15 Productivity status

Productivity status was recorded at the time of harvest as the production per unit area and rated as per descriptor as mentioned below:

Low = 3

Medium = 5

High = 7

3.2.1.4.16 Biotic Stress Susceptibility

Infestation was recorded during the fruit growth and development and rated as per descriptor as mentioned below:

Very low or no visible sign of susceptibility = 1

Low = 2

Intermediate = 5

High = 7

Very high = 9

The data recorded on morphological parameters during the investigation was assigned to statistical analysis. The data on fifty *Ambri* apple genotypes were examined with the XLSTAT software (version 2014.5.03) for reducing the dimensionality and retaining the variability of the data at same time.

3.2.2 Biochemical diversity analysis

For assessment of chemical composition, fruit samples were selected and analyzed. The following chemical compositions were studied.

3.2.2.1Total Soluble Solids (^{o}Brix)

The total soluble solids (TSS) of the fruit juice was recorded with the help of Erma hand refractometer (0-32^{0}B) according to standard procedure as given in AOAC (1994) in terms of degree Brix (^{0}B) at room temperature. A temperature correction chart was applied when the readings were taken at a temperature other than 20^{0}C. The refractometer was calibrated with distilled water before use.

3.2.2.2Titratable acidity (per cent)

The per cent titrable acidity (as per cent maleic acid) was analyzed by titrating known volume of juice against 0.1 N NaOH solution, using phenolphthalein as an indicator (A.O.A.C., 2000).

25 grams of fruit pulp was mixed with distilled water. The volume was build to 250 ml, and then filtered through Whatman No. 1 filter paper. 10 ml sample of solution was titrated against N/10 NaOH using phenophthalein as an indicator. The total titrable acidity was calculated in terms of malic acid on the basis of 1 ml N/10 NaOH being equivalent to 0.0067g anhydrous malic acid. The results were expressed as per cent of fresh weight of fruit pulp.

$$\text{Titrable acidity (per cent)} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made up} \times \text{eq. weight of acid}}{\text{Aliquot} \times \text{Weight of sample} \times 1000} \times 100$$

3.2.2.3 Ascorbic acid (mg/ 100g)

The ascorbic acid was estimated by the method of AOAC (1994).

Reagents

Indophenol dye (0.04%): Weighed 40 mg sodium 2, 6-dichlorophenol indophenol. Added 150 ml hot distilled water. Then added 42 ml sodium bicarbonate. Cooled the contents made volume to 200 ml with distilled water and kept in refrigerator.

Metaphosphoric acid (3%): Dissolved 30g metaphosphoric acid in water and made volume to 1000 ml.

Standard ascorbic acid (0.1%): Dissolved 100 mg ascorbic acid in 100 ml of oxalic acid. Diluted 10 ml to 100 ml with metaphosphoric acid (1 ml=0.1 mg ascorbic acid).

Standardization of dye: Taken 5 ml standard ascorbic acid and added 5 ml HPO_3. Fill a microburette with dye. Titrate against dye solution to a light pink colour and determine dye equivalent.

$$\text{Dye equivalent} = 0.5/\text{Titre}$$

Procedure

Ascorbic acid was extracted from the pulp by macerating 10 g of sample with 3 % metaphosphoric acid. The extract was filtered and volume made to 100 ml. 10 ml of the aliquot was titrated against standardized dye (2,6 dicholorophenol indophenol) till the light pink colour appeared at the end point. Results were expressed as mg/100 g of fruit weight.

Calculation

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Titre x dye equivalent. x dilution}}{\text{Weight of sample}} \text{ x } 100$$

3.2.2.4 Total Sugars (per cent)

Twenty-five grams of fruit pulp thoroughly mixed with distilled water ina beaker and 25 ml of lead acetate was added to extract the juice from filtrate. To this 25 ml of potassium oxalate was also added for removing excess lead and volume was made up to 250 ml by adding distilled water. The solution was filtered through Whatsman filter paper No. 1. From the above aliquot 100 ml of solution was brought and hydrolysed by pouring 10 ml of concentrated hydrochloric acid and left for overnight. The excess of HCl was neutralized by saturated NAOH solution. Total sugars were estimated by titrating a solution against mixture containing 5 ml each of Fehling's solution A and B using methylene blue as indicator. The titration was continued till brick red colour appeared. Total sugars were estimated by Lane and Eynon's Method reported by Ranganna (1995) with the formulas given below:

$$\text{Total sugars (per cent)} = \frac{\text{mg of invert sugar} \times \text{dilution} \times 10}{\text{Titre (after inversion)} \times \text{weight of sample (g)}}$$

3.2.2.5 Reducing Sugar (per cent)

Reducing sugars were determined by Fehling's solution method using methylene blue as an indicator

$$\text{Reducing sugars (per cent)} = \frac{\text{mg of invert sugar} \times \text{dilution} \times 100}{\text{Titre (after inversion)} \times \text{weight of sample (g)} \times 1000}$$

3.2.2.6 Non reducing sugar (per cent)

The non reducing sugar was analyzed by deducting reducing sugar from total sugar and multiplying the difference by 0.95. Results were showed in terms of percentage of juice.

3.2.2.7 Pectin content (per cent)

Pectin content was extracted by the method of Ranganna (1995).

Reagents

1N Acetic acid: 30 ml of glacial acetic acid was diluted to 500 ml with distilled water.

1N Calcium chloride: 55 g anhydrous $CaCl_2$ was dissolved in distilled water and diluted to 1000 ml.

Silver nitrate (1 per cent): Dissolved 5 g $AgNO_3$ in glass distilled water and diluted to 500 ml. It was dissolved by keeping it in hot water bath for 10 minutes.

Procedure

25 g of fruit sample was taken in one litre beaker with 400 ml of 0.05 N HCl. It was boiled for 1 hour at 80-90 ^{0}C. The evaporated water was replaced by addition of distilled water. After Cooling, transfer it to 500 ml volumetric flask and volume was made with distilled water and then filtered through what man filter paper No.4 100 ml of filtrate was taken in two beakers and 300 ml distilled water was added to each beaker**.** Then 10 ml of 1 N NaOH solution was added and kept overnight. 50 ml of 1 N acetic acid was added and kept for 5 minutes. $CaCl_2$ solution was added and kept for one hour.

Therefore it was boiled for one minute. Whatsman filter paper no. 4 were taken and washed wit distilled water, dried in an oven at 100 ^{0}C for two hours and then these filter papers weighed. The solution was filtered through Whatsman filter paper No. 4. It was washed with distilled to make it free from chloride ions. A few drops of silver nitrate solution were added. The white precipitates (one filter paper in a petridish) was put in an oven, dried and weighed again.

Calculation

$$\text{Pectin (per cent)} = \frac{\text{Weight of calcium pectate}}{\text{Weight of fruit sample}} \times 100$$

3.2.2.8 Phenolics (mg/100g)

The total phenols of *Ambri* apple was measured using the Folin-Ciocalteu's colorimetric assay (Singleton *et al.* 1999). In reaction mixture, methanolic solution, extract, distilled water; sodium carbonate and Folin- Ciocaltue reagent were used.Certain amounts of these reagents were mixture, and allowed to stand for 2 hr in the dark. The sample was measured at 750nm absorbance (Spectrophotometer). The total phenolic contents were determined as a chlorogenic acid equivalents against the fresh weight of the samples.

3.2.2.9 TSS/ Acid ratio

TSS acid ratio was determined after dividing TSS by titrable acidity.

3.2.3.1 Genetic diversity using SSR molecular markers

3.2.3.1.1 Collection of leaf samples

In the present investigation, fifty genotypes of *Ambri* apple comprised of young, fresh, disease and insect free leaves were selected from different areas (Table 1). Leaf samples were collected in butter papers and were placed in ice containers while transferring from field to the laboratory. In laboratory, the leaves were stored in deep freezer at -20^{o}C for DNA isolation and SSR marker studies at subject to further analysis at Plant Genomics Laboratory, School of Biotechnology, SKUAST-Jammu.

3.2.3.1.2 Methods

1. **Genomic DNA Isolation**: The reagents required for isolation and purification of DNA were prepared as follows:

(A) Preparation of stock solutions (500ml) for DNA extraction

a) 1M Tris HCL pH 8.0

For this, 24.22 g of 1M Trizma base having molecular weight 121.14 was dissolved in the 150 ml of distilled water and pH was adjusted to 8.0 with 1N NaOH. The volume of the stock was finally made up to 200 ml with distilled water. Then solution was autoclaved and stored at room temperature.

b) 0.5 M EDTA

To prepare this stock, 37.22 g of ethyl diamine tetra acetic acid (EDTA) having molecular weight 372.24 was dissolved in 150 ml of distilled water, pH was adjusted to 8.0 and volume was made up to 200 ml. The 0.5 M EDTA was autoclaved and stored at room temperature.

c) 5 M NaCl

For preparing this stock, 29.22 g of NaCl (molecular weight 58.44) was dissolved in 100 ml of distilled water. The solution was autoclaved and stored at room temperature.

d) TE Buffer pH 8.0

The buffer was prepared with the following constituents:

Tris - HCl pH 8.0 = 2.0 ml

EDTA pH 8.0 = 0.4 ml

They were dissolved properly and the volume was made upto 200 ml by adding distilled water.

e) DNA extraction buffer

The extraction buffer was prepared with the following constituents:

1 M Tris	=	15.0 ml
0.5 M EDTA	=	6.0 ml
5 M NaCl	=	42.0 ml
CTAB	=	2.0 g

They were mixed and dissolved properly and then the volume was made up to 150 ml by adding distilled water. ß-mercaptoethanol 0.2 per cent in 100 ml of extraction buffer was added freshly and extraction buffer was pre warmed before use.

f) TBE Buffer (10X)

It was prepared with the following constituents:

Tris Base	=	108.0 g
Boric acid	=	55.0 g
0.5 M EDTA	=	40.0 ml

They were dissolved properly and the volume was made up to 100 ml by distilled water with final concentration 10X.

g) Chloroform: Isoamyl alcohol (C:I)

For preparing 100 ml of C:I stock solution, 96 ml of chloroform and 4 ml of isoamyl alcohol were taken and mixed well.

h) RNase

10 mg was dissolved in 1 ml of 10mMTris- HCl and 15mM NaCl and heated to 100°C for 15 min. It was then cooled slowly to room temperature and stored at -20°C.

i) Working stocks of Primers

The primers were supplied in the lyophilized form. Stocks were prepared by adding the double distilled Mili-Q water and from the stocks working concentration of 10 picomole of each primer set was prepared.

3.2.3.1.3 Isolation of genomic DNA

Genomic DNA was isolated following Doyle and Doyle (1990) method, with slight modifications. About 6-8 cm, young and actively growing fresh leaves were harvested for genomic DNA extraction. About 5 gm of the plant tissue was taken for each genotype and grinded in liquid nitrogen by using pestle and mortar to obtain fine powder. It was followed by putting this fine powder into 2 ml eppendorf tube which contained 800 µl of extraction buffer (CTAB buffer). The eppendorf tubes were incubated in a water bath at 65 ˚C for 60 min. and contents of the tubes were mixed by intermittently inverting them after every 10 min. An equal volume of Chloroform: Isoamyl alcohol (24:1) was added in the tube and slowly mixed by inverting the tubes for 5 min. The samples were then transferred to centrifuge tubes and centrifuged at 8,000 rpm for 10 min. The supernatant (upper phase) was transferred into fresh tubes and again treated with Chloroform: Isoamyl alcohol (24:1), mixed slowly for 10 min and centrifugation was done at 10,000 rpm for 10 min. To precipitate the DNA, 0.66 volume of chilled Isopropanol was added to the supernatant and stored at 4 ^{o}C for 1-2hrs. Centrifugation was done at 10,000 rpm for 15 min at 4 ^{0}C. The supernatant was discarded and the pellets were washed with 70 per cent ethanol, centrifuged at 10,000 rpm for 5 min. and the pellets air dried. Then, 200µl of 1x TE buffer was added to dissolve the pellet. The DNA was spooled out, air dried and resuspended in 0.5-1.0 ml TE or deionized distilled water and stored at 4^{0}C.

3.2.3.1.4 DNA purification

RNase treatment was given to sample by adding 2µl of RNase (10mg/ml) to the samples (1ml T.E/DNA mixture) and incubated at 37 ^{o}C for 1hr. in water bath. An equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and gently mixed for 10 min and centrifuged at 13,000 rpm for 10 min. The supernatant was collected in another tube and again added equal volume of C: I (Chloroform: Isoamyl alcohol) (24:1) was added. The tubes containing supernatant were centrifuged for 10 min at 13,000 rpm and then 0.6 volume of ice chilled pure ethanol was added and it was kept in refrigerator for 10 min. It was centrifuged at 7000 rpm for 5 min for pelleting the DNA. The DNA

Table 2: List of selected SSR primers along with their primer sequence.

S. No.	Primers	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing Temperature (°C)	Expected amplicon size (bp)
1	CH03g07	AAT AAG CAT TCA AAG CAA TCC G	TTT TTC CAA ATC GAG TTT CGT T	55	119-171
2	CH04e03	TTG AAG ATG TTT GGC TGT GC	TGC ATG TCT GTC TCC TCC AT	56	179-222
3	CH04g10	CAA AGA TGT GGT GTG AAG AGG A	GGA GGC AAA AAG AGT GAA CCT	55	127-168
4	CH05c02	TTA AAC TGT CAC CAA ATC CAC A	GCG AAG CTT TAG AGA GAC ATC C	55	168-200
5	CH05d11	CAC AAC CTG ATA TCC GGG AC	GAG AAG GTC GTA CAT TCC TCA A	55	171-211
6	CH05e03	CGA ATA TTT TCA CTC TGA CTG GG	CAA GTT GTT GTA CTG CTC CGA C	55	158-190
7	CH02h11a	CTGTTTGAACCGCTTCCTTC	CGTGGCATGCTTATCATTTG	50	104-132
8	CH03d12	ATTGCTCCATGCATAAAGGG	GCCCAGAAGCAATAAGTAAACC	50	108-154
9	CH03e03	AAAACCCACAAATAGCGCC	GCACATTCTGCCTTATCTTGG	53	106-216
10	CH03g12z	CAAGGATGCGCATGTATTTG	GCGCTGAAAAAGGTCAGTTT	50	127-155
11	CH04a12	ATCCATGGTCCCATAAACCA	CAGCCTGCAACTGCACTTAT	50	158-196
12	CH05d04	TCCG GGTATGCTTCGATT	ACTTGTGAGCCGTGAGAGGT	50	174-214
13	CH05e03	CAAGTTGTTGTACTGCTCCGAC	AAGTGCACCCACACCCTTAC	51	158-190
14	Hi01d06y	GGAGAGTTCCTGGGTTCCAC	GTTTAAGTTCGCCAACATCGTCTC	53	129-155
15	Hi02d04	TGCTGAGTTGGCTAGAAGAGC	GTTTGTTGCTGTTGGATTATGCC	51	224-250
16	Hi03a03	ACACTTCCGGATTTCTGCTC	GTTTAACAGCGGGAGATCAAGAAC	51	160-228
17	Hi03e03	ACGGGTGAGACTCCTTGTTG	GTGCAGAGTCTTTGACAAGGC	53	186-200
18	CH03c02	TCACTATTTACGGGATCAAGCA	TGTCTCAAGAACACAGCTATCACC	51	116-136
19	Hi02b10	GTTTCTTGGAGGCAGTAGTGCAG	TGCTGAGTTGGCTAGAAGAGC	60	200-254
20	Hi02d04	GTTTAAGTTCGCCAACATCGTCTC	TGATGCATTAGGGCTTGTACTT	60	224-250
21	CH05c07	GGGATGCATTGCTAAATAGGAT	TTGTGGACGTTCTGTGTTGG	59	111-149
22	CH03a02	TTGTGGACGTTCTGTGTTGG	CAAGTTCAACAGCTCAAGATGA	60.6	124-184
23	CH02b12	GGCAGGCTTTACGATTATGC	CCCACTAAAAGTTCACAGGC	59.7	101-143
24	Hi03e03	ACGGGTGAGACTCCTTGTTG	GTTTAACAGCGGGAGATCAAGAAC	60	186-200
25	CN444636	CACCACTTGAGTAATCGTAAGAGC	GTTTAACAGCGGGAGATCAAGAAC	60	239-243
26	CH05g07	CCCAAGCAATATAGTGAATCTCAA	TTCATCTCCTGCTGCAAATAAC	60	149-197
27	CH02c02	CTTCAAGTTCAGCATCAAGACAA	TAGGGCACACTTGCTGGTC	60	128-150
28	Hi03a03	ACACTTCCGGATTTCTGCTC	GTTTGTTGCTGTTGGATTATGCC	60	160-228
29	CN444542	ATAAGCCAGGCCACCAAATC	GTTTGCAGTGGATTGATGTTCC	60	110-156

pellet was washed with 70 per cent ethanol, centrifuged at 7000 rpm for 5 min, air dried, dissolved in 100µl of 1xTE (Tris-cl, EDTA) buffer and stored at 4^0C for further use.

3.2.3.1.5 DNA quantification

Quality and quantity of genomic DNA was estimated by using Agarose gel electrophoresis and Nanodrop method. Quality of DNA was checked by spectrophotometer by measuring absorbance from 230 nm to 320 nm to detect other possible contaminants.

3.2.3.1.5.1 Agarose gel electrophoresis

Before proceeding for gel electrophoresis, stock solutions used for electrophoresis were prepared as follows:

a) DNA loading dye (6X) (for 10ml)

Following ingredients were used to prepare loading dye:

Bromophenol blue (0.25 % w/v)	=	0.025 g
Glycerol (40 %)	=	4.0 ml

They were dissolved properly and the volume was made up to 10ml by 1X TAE and stored at 4^0C.

b) Electrophoresis buffer (TBE 50X) (For 100ml)

Tris base	=	24.2 g
Boric acid	=	5.7 ml
0.5M EDTA	=	10.0 ml

They were combined and volume was made upto 100 ml, autoclaved and stored at room temperature.

c) Ethidium bromide (10mg/ml)

Ethidium bromide	=	10.0 mg
Distilled water	=	1.0 ml

They were dissolved properly and stored at 4°C.

They were dissolved and stored at 4ºC. For resolving the DNA fragments on the gel, the DNA samples were loaded on to the wells of 0.8 per cent agarose gel. For this, 3µl of DNA of each genotype was mixed with 2µl of loading dye (0.25 per cent w/v bromophenol blue, 50 per cent glycerol in sterile water). For preparation of agarose gel, 0.8 g of agarose was weighed and mixed in 100 ml TBE (Tris, Borate EDTA, 1x) buffer and heated in a microwave for 3 minutes for dissolving agarose. It was then cooled for few minutes, followed by addition of 6 µl of ethidium bromide for visualization of DNA bands and stirred for some time. The gel was poured into the casting tray with combs in it and allowed to polymerize at room temperature for 20-25 minutes. Marker DNA (ladder) of known band size was also loaded for precise quantitative estimation of DNA bands in gels. The electrophoresis was carried out at 80V for 1 hour. DNA samples were observed under photo gel documentation system (DNR Bioimaging system, Israel). The intensity of fluorescence of each sample was compared with that of a standard marker and then DNA concentration of each sample was ascertained. The quality of DNA samples were judged based on whether DNA formed a single high molecular weight band (good quality) or a smear (degraded/poor quality).

3.2.3.1.6 SSR Assay

3.2.3.1.6.1 Primer used for DNA Amplification

A set of twenty nine (29) arbitrary highly polymorphic random primers (Table 2) were selected based on earlier studies for use in amplification of genomic DNA (Farrokhi*et al.*,1986 and Galli *et al.*, 2005)

3.2.3.1.6.2 Components used for PCR Reactions

For performing Polymerase Chain Reaction following reagents were used

Table 3: List of reagents used for performing Polymerase Chain Reaction

S.No.	Reagents	Final Concentration in PCR reaction	Quantity
1.	Template DNA	50 ng/ µl	1.0 µl
2.	DNTPs	2.5 mM/ µl	0.3 µl
3.	Primer	10 pmole	1.0 µl
4.	PCR Buffer with $MgCl_2$	10 X buffer &15mM ($Mgcl_2$)	2.2 µl
5.	*Taq*polymerase	5 U	0.2µl
6.	Sterile water		5.3 µl
	Total		**10 µl**

3.2.3.1.6.3 PCR amplification

DNA amplification was carried out in Polymerase Chain Reaction (PCR) tubes containing 10 µl reaction mixture. The reaction mixture contained 1 µl of template DNA (50ng/µl), 2.5 m M/ µl of each dNTP (dTTPs, dGTPs, dCTPs, dATPs), 0.5 µl of each forward and reverse primers, 5 U of Taq polymerase (D1806- Sigma Aldrich, USA), 2.2 µL of 10X PCR buffer with $MgCl_2$, The quantity of these components used in a reaction for the SSR presented in Table 3.

3.2.3.1.6.4 Resolution of PCR products

Amplified DNA fragments were resolved in horizontal electrophoresis using 3 per cent agarose gels and visualized by staining with ethidium bromide (10 mg/ml). Gel casting plate was washed with distilled water and dried. Plate was wipped with ethanol, air-dried and ends were sealed with tape, 3 per cent agarose gel solution was prepared by heating 3 g agarose in 100 ml 1 X TBE Buffer in oven. The solution was cooled to about 50 ^{o}C by keeping it at room temperature for 5-10 minutes. Ethidium bromide was added in the gel at a concentration of 2 µg/ml and mixed. It intercalates between the nitrogenous base pairs in the double helix. Agarose gel was then poured into the gel casting plate inserted with an appropriate comb with required well number and size to get 0.5 cm thick gel. Gel plate was then placed in the sealing tapes were removed from both the ends. Gel plate was then placed in the electrophoresis chamber and submerged using 1 X TBE (Tris Borate EDTA) buffer. The comb was removed gently. DNA samples were prepared by

Table 4: Thermal profiles used for DNA amplification

Step	**Temperature (ºC)**	**Time**	**No. of cycles**
Initial denaturation	95	5 minutes	1
Denaturation	94	1 minutes	35
Annealing	53-60	30 seconds	
Extension	72	40 seconds	
Final Extension	72	5minutes	1

adding loading dye solution (1µl/ml). The samples were loaded in the wells using micropipette. Cover was placed on electrophoresis unit and proper connections were made using power supply. Electrophoresis was carried out a constant voltage (3 V/cm of gel) until the dye had moved to 3/4 length in the gel. PCR amplification products were viewed by UV light (high UV wavelength 350 nm) under gel documentation system.

3.2.3.1.6.5 Scoring of bands

The molecular data was scored as per 'allelic format' as required for diversity analysis using DARwin5 software (Perrier and Collet, 2006). The DNA bands were scored on the basis of relative mobility in gel, the allele difference was determined according to their fragment size (bp) corresponding to the 100 bp standard marker (Sigma Aldrich, U.S.A). The banding patterns of all genotypes against every primer were compared.

3.2.3.1.6.6 Data analysis

In order to check the informartiveness and discriminatory power of SSR markers used in this study, certain parameters like polymorphism information content and alleles per locus were calculated.

a) Number of alleles per locus

The variation in the number of alleles across multiple loci within a population can be expressed as the average number of alleles per locus:

$$\text{Number of alleles per locus} = \frac{\Sigma \text{ number of alleles}}{\text{Number of loci}}$$

Distance based approach which is based on calculating pair wise distance matrix was computed by calculating a dissimilarity matrix using a shared allele index with DARwin software (Perrier and Collet, 2006). An unweightedneighbour joining tree was constructed using the calculated dissimilarity index. The genetic distance between accessions was estimated using Nei's coefficient (Nei, 1973) with bootstrap procedure of resampling (1000) across markers and individuals from allele frequencies.

b) Polymorphism information content (PIC)

Average PIC indicates the ability of utilized markers to differentiate the genotypes. It was calculated according to the following formula given by (Anderson *et al.*, 1993).

$$PIC = 1 - \sum_{i=1}^{n} (P_{ij})^2$$

Where P_{ij} in the frequency of j^{th} allele in i^{th} marker, and summation over on alleles PIC value exceeding 0.5 indicated presence of polymorphism for the alleles.

c) Percent Polymorphism (PP)

The proportion of Polymorphic loci is a measure used to quantify genetic diversity in a population. The ratio was calculated by determining the number of polymorphic loci and dividing by the total number of loci examined.

$$\text{Percent Polymorphism} = \frac{\text{number of polymorphic loci}}{\text{total number of loci examined}} \times 100$$

d) Principle co-ordinate analysis

The principle co-ordinate analysis of fifty *Ambri* apple genotypes was performed by using PAST - PAlaeontological Statistics (Oyvind Hammer *et al*., 2001).

3.3.1 Comparison of best selections with other commercial variety apple

The comparison of two best selections with other standard commercial variety Red Delicious was done for morphological, biochemical and molecular parameters. Comparison for morphological parameters was based on NBPGR-2002 descriptor and observations were made on two best seedling *Ambri* apple trees growing at their respective locations and commercial varieties of apple i.e., Red Delicious growing in the orchard of apple. For comparison of biochemical the values available in secondary literature were referred.

Collaboration with other Division

Requisite collaboration was sought from Plant Genomics Laboratory, School of Biotechnology, SKUAST-Jammu FOA, Chatha.

RESULTS

CHAPTER-4

RESULTS

The present investigation "Morphological, Biochemical and Molecular Characterization of *Ambri* apple (*Malus* × *domestica* Borkh.) in Doda and Kishtwar districts of J&K." was carried out at Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu during 20016-2017 and 2017-2018. The study comprised of survey, selection and characterization of *Ambri* apple genotypes of seedling origin *Ambri* apple growing at various location in Doda and Kishtwar districts of Union Terriority of Jammu and Kashmir. The study was carried out on fifty *Ambri* apple genotypes to assess the extend of diversity by morphological, biochemical and molecular characterization using NPBGR descriptor (NPBGR-2002), and 29 SSR markers. The data collected was subjected to appropriate statistical analysis and the results emanate from the present studies are presented in this chapter under the following headings:

4.1Morphological characterization

4.1.1 Tree characters

4.1.2 Leaf characters

4.1.3 Flower characters

4.1.4 Fruit characters

4.1.5 Cluster analysis

4.1.6 Principle Component Analysis

4.2 Biochemical characterization

4.3 Coefficient of variation

4.4 Cluster Analysis

4.5 Principle Component Analysis

4.6 Molecular characterization

4.7 Comparison of best genotypes with Red Delicious apple

4.1 Morphological characterization

4.1.1 Tree characters

The data pertaining to tree characters of the genotypes in the present study w.r.t. type of planting material, tree height, trunk girth, tree spread, tree habit and tree vigour is presented in the Table 5, 6, Fig. 1and Plate 1(A), 1(B), 1(C), 1(D) and 1 (E).

4.1.1.1 Type of planting material

The genotypes selected during the survey were seedling in origin and the details of the studied genotypes are mentioned in Table 5.

4.1.1.2 Tree height

Tree height in all the selected genotypes studied are presented in the Table 5. Out of fifty selected genotypes maximum height was observed in genotype SKJAK-28 (9.301m) whereas minimum tree height was observed in SKJAAT-08 (5.23 m).

4.1.1.3 Trunk girth

Trunk girth of each genotype studied are given in Table 5. Among the fifty *Ambri* apple genotypes maximum trunk girth was observed in genotype SKJAG-15 (340.31 cm) and minimum was observed in SKJAT-08 (105.28 cm).

4.1.1.4 Tree spread

Tree spread of all the fifty selected genotypes studied are presented in the Table 5, which shows that genotype SKJAD -28 (6.80 m) exhibited maximum tree spread while SKJAT -08(3.20 m) possessed minimum tree spread.

4.1.1.5 Tree habit

The tree habit in the genotypes studied was categorized to upright, spreading and dropping. The descriptive data presented in the Table 6 shows that 22 genotypes (44 per cent) exhibited upright tree growth habit, 23 genotypes (46 per cent) showed spreading tree growth habit and 5 genotypes (10 per cent) were dropping in tree growth habit.

Table 5: Tree and leaf characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Type of planting Material	Tree habit	Tree vigour	Tree height (m)	Trunk girth (cm)	Tree spread (m)	Leaf size	Leaf shape
1.	SKJAB -01	1	5	7	5.82	122.00	3.57	5	3
2.	SKJAB -02	1	5	7	6.77	177.37	4.85	5	3
3.	SKJAB -03	1	3	7	6.57	165.00	4.73	7	3
4.	SKJAB -04	1	3	5	8.45	259.11	5.57	7	1
5.	SKJAB -05	1	5	7	7.77	225.80	5.27	7	3
6.	SKJAT -06	1	5	5	6.53	152.85	3.65	3	3
7.	SKJAT -07	1	3	5	5.31	115.80	3.45	3	3
8.	SKJAT -08	1	5	5	5.23	105.28	3.20	5	3
9.	SKJAT -09	1	7	5	6.10	155.11	3.87	5	3
10.	SKJAG -10	1	3	5	5.43	120.59	3.48	5	3
11.	SKJAG -11	1	3	5	6.41	165.71	3.97	7	3
12.	SKJAG -12	1	7	7	5.75	139.20	3.79	5	3
13.	SKJAG -13	1	5	7	5.59	119.28	3.78	5	3
14.	SKJAG -14	1	7	9	6.35	160.59	3.83	5	3
15.	SKJAG -15	1	5	9	8.41	340.31	5.67	7	1
16.	SKJAG -16	1	7	5	6.71	179.35	3.75	5	1
17.	SKJAG -17	1	3	7	5.58	157.09	3.78	5	3
18.	SKJABh -18	1	3	5	6.15	219.35	4.49	3	3
19.	SKJABh -19	1	3	5	5.55	113.54	3.32	3	3
20.	SKJABh -20	1	3	5	7.00	192.76	5.19	5	3

Legend

Planting material	Note	Tree habit	Note	Tree vigour	Note	Leaf size	Note	Leaf shape	Note
Seedling	1	Upright	3	Extremely weak	1	Small	3	Oval	1
		Spreading	5	Weak	3	Medium	5	Broad elliptic	3
		Drooping	7	Intermediate	5	Large	7	Others	99
				Vigrous	7				
				Very vigorous	9				

Cont…

Table 5: Tree and leaf characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Type of planting Material	Tree habit	Tree vigour	Tree height (m)	Trunk girth (cm)	Tree spread (m)	Leaf size	Leaf shape
21.	SKJAK -21	1	5	5	7.00	210.94	5.77	7	1
22.	SKJAK -22	1	3	5	8.00	244.06	5.84	7	3
23.	SKJAK -23	1	5	5	8.30	251.76	6.05	7	1
24.	SKJAK -24	1	3	5	9.20	287.09	6.59	5	1
25.	SKJAK -25	1	3	5	9.10	250.28	6.61	5	1
26.	SKJAK -26	1	5	5	9.30	267.21	6.77	7	1
27.	SKJAK -27	1	3	5	8.30	248.39	6.50	7	1
28.	SKJAD -28	1	3	5	9.31	269.89	6.80	7	1
29.	SKJAD -29	1	3	7	7.74	191.29	5.46	7	3
30.	SKJAD -30	1	5	7	7.62	160.45	5.60	7	3
31.	SKJAD -31	1	5	7	8.21	172.14	3.50	5	1
32.	SKJAD -32	1	5	7	8.61	204.89	3.41	7	1
33.	SKJAD -33	1	5	5	8.35	181.59	3.80	5	1
34.	SKJAD -34	1	5	5	7.00	155.80	4.78	7	3
35.	SKJAD -35	1	3	7	9.11	258.71	4.75	5	1
36.	SKJAD -36	1	3	7	8.63	129.73	3.67	7	1
37.	SKJAN -37	1	5	7	8.71	137.99	3.79	7	1
38.	SKJAN -38	1	5	7	9.10	205.11	4.53	7	3
39.	SKJAN -39	1	5	7	8.90	222.58	4.77	7	3
40.	SKJAN -40	1	3	7	9.14	263.79	5.70	7	3

Legend

Planting material	Note	Tree habit	Note	Tree vigour	Note	Leaf size	Note	Leaf shape	Note
Seedling	1	Upright	3	Extremely weak	1	Small	3	Oval	1
		Spreading	5	Weak	3	Medium	5	Broad elliptic	3
		Drooping	7	Intermediate	5	Large	7	Others	99
				Vigrous	7				
				Very vigorous	9				

Cont…

Table 5: Tree and leaf characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Type of planting Material	Tree habit	Tree vigour	Tree height (m)	Trunk girth (cm)	Tree spread (m)	Leaf size	Leaf shape
41.	SKJAN -41	1	3	7	8.21	233.87	5.35	5	3
42.	SKJAN -42	1	7	7	7.79	189.78	3.34	7	1
43.	SKJAM-43	1	3	5	9.51	277.78	5.70	5	1
44.	SKJAM-44	1	5	5	8.70	229.56	5.40	7	3
45.	SKJAM-45	1	3	5	8.11	205.77	5.13	5	3
46.	SKJAM -46	1	5	5	6.45	139.72	3.59	5	1
47.	SKJAM -47	1	5	3	6.20	113.17	3.10	7	1
48.	SKJAC -48	1	5	3	9.00	271.10	5.72	5	3
49.	SKJAC -49	1	5	3	8.15	127.31	4.56	7	3
50.	SKJAC -50	1	3	3	8.79	149.12	3.29	5	3
General mean					7.56	192.73	4.66		
SD					1.31	57.00	1.10		
CV					17.32	29.57	24.66		

Legend

Planting material	Note	Tree habit	Note	Tree vigour	Note	Leaf size	Note	Leaf shape	Note
Seedling	1	Upright	3	Extremely weak	1	Small	3	Oval	1
		Spreading	5	Weak	3	Medium	5	Broad elliptic	3
		Drooping	7	Intermediate	5	Large	7	Others	99
				Vigrous	7				
				Very vigorous	9				

Table 6: Summary of frequency of tree and leaf characters of Ambri apple (*Malus × domestica* Borkh.) genotypes.

Trait	Category	No. of Selections	Percentage
Type of planting material	Seedling -1	50	100
Tree habit	Upright - 3	22	44
	Spreading- 5	23	46
	Drooping -7	5	10
Tree vigour	Extremely weak-1	-	-
	Weak - 3	4	8
	Intermediate - 5	25	50
	Vigrous - 7	19	38
	Very vigorous - 9	2	4
Tree height (m)	Below 6	8	16
	6-9	34	68
	Above 9	8	16
Trunk girth (cm)	Below 150	13	26
	150-200	15	30
	Above 200	22	44
Tree spread (m)	Below 4	24	48
	4-6	20	40
	Above 6	6	12
Leaf size	Small - 3	4	8
	Medium - 5	22	44
	Large – 7	24	48
Leaf shape	Oval - 1	20	40
	Broad elliptic - 3	30	60
	Others – 99	-	-

Genotype 1

Genotype 2

Genotype 3

Genotype 4

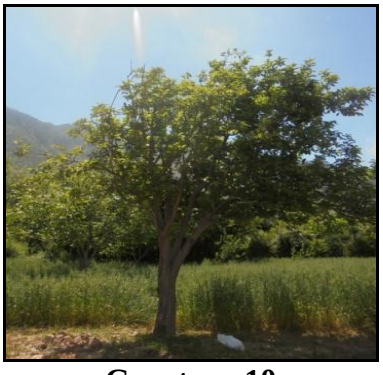
Genotype 5

Genotype 6

Genotype 7

Genotype 8

Genotype 9



Genotype 10

Plate 1 (A): Varaiability in tree characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 11

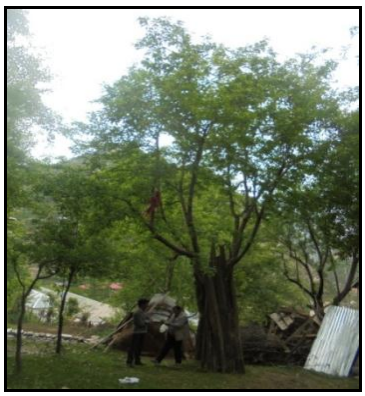
Genotype 12

Genotype 13

Genotype 14

Genotype 15

Genotype 16



Genotype 17

Genotype 18

Genotype 19

Genotype 20

Plate 1 (B): Varaiability in tree characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 21

Genotype 22

Genotype 23

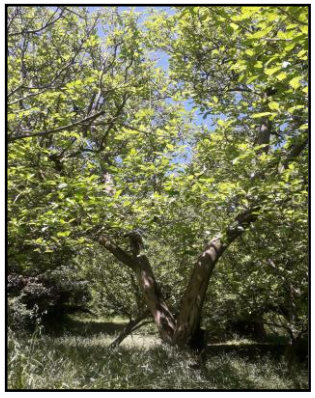
Genotype 24

Genotype 25

Genotype 26

Genotype 27

Genotype 28



Genotype 29

Genotype 30

Plate 1 (C): Varaiability in tree characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 31

Genotype 32

Genotype 33

Genotype 34

Genotype 35

Genotype 36

Genotype 37

Genotype 38

Genotype 39

Genotype 40

Plate 1 (D): Varaiability in tree characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 41

Genotype 42

Genotype 43

Genotype 44

Genotype 45

Genotype 46

Genotype 47

Genotype 48

Genotype 49

Genotype 50

Plate 1 (E): Varaiability in tree characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

4.1.1.6 Tree vigour

The tree vigour in the selected genotypes studied was found to vary from extremely weak, weak, intermediate, vigorous and very vigorous. The descriptive data of tree vigour showed in Table 6 showed that that among the fifty genotypes, 4 genotypes (8 per cent) had very vigorous, 19 genotypes (38 per cent) showed vigorous, 25 genotypes (50 per cent) possessed intermediate and 4 genotypes (8 per cent) exhibited weak tree vigour.

4.1.2 Leaf characters

The leaf description of the fifty selected genotypes in the present study with respect to two characters i.e., leaf size and leaf shape are presented in Table 5, 6 , Fig. 2 and Plate 2(A), 2(B), 2(C) and 2(D).

4.1.2.1 Leaf size

The leaf size of all the genotypes studied was found to vary from small, medium to large. The descriptive data of leaf size presented in the Table 6 showed that among the fifty genotypes, 4 genotypes (8 per cent) had small, 22 genotypes (44 per cent) medium and 24 genotypes (44 per cent) exhibited large leaf size.

4.1.2.2 Leaf shape

Leaf shapes was categorized as per descriptor as oval, ovate and broad elliptic. The data showed that out of fifty genotypes, the leaf shape of 20 genotypes (40 per cent) was oval in shape and 30 genotypes (60 per cent) were broad elliptic in leaf shape.

4.1.3 Flower characters

The mean descriptive data of various floral characteristics *viz.*, number of flower buds per inflorescence, flower stalk (pedicel) length, date of start of flowering , date of end of flowering, regularity of flowering and self incompatibility are presented in the Table 7, 8 and 9.

4.1.3.1 Number of flower buds per inflorescence

The no of flower buds per inflorescence showed variation among all the selected genotypes. The maximum six number of flower buds per inflorescence was recorded in

genotypes SKAB -04, SKJAAT -07, SKJAT -08, SKJAG -16, SKJAG -17, SKJAK-27, SKJAK -28, SKJAK -29, SKJAD-35 SKJAM-45 and SKJAM-46 followed by five number of flower buds per inflorescence in genotypes SKJAB -05, SKJAT-09, SKJAAG-10, SKJAG-11, SKJABh -18, SKJABh -19, SKJABh -20, SKJAK -24, SKJAK -25, SKJAK -26, SKJAD -30, SKJAD -31, SKJAD -32, SKJAD -34, SKJAD -36, SKJAN -39, SKJAN -42, SKJAM -43, SKJAM -44, SKJAC -49 and SKJAC -50 and followed by four number of flower buds per inflorescence were observed in genotypes SKJAB -01, SKJAB -02, SKJAB -03 , SKJAT-06, SKJAG -12, SKJAG -13, SKJAG -14, SKJAG -15 , SKJAK -21, SKJAK -22, SKJAK -23, SKJAD -33, SKJAN-37, SKJAN -38, SKJAN -40, SKJAN -41, SKJAM -47 and SKJAC -48.

4.1.3.2 Flower stalk (pedicel) length (mm)

Flower stalk length showed wide variation from 10.7 mm to 25.15 mm. The maximum (25.1mm) stalk length was observed in genotype SKJAC -48 followed by stalk length (25.0 mm) in SKJAK -27. While the minimum flower stalk length (10.7 mm and 10.9 mm) was observed in SKJABh -20 and SKJAB -03 genotypes respectively.

4.1.3.3 Date of start of flowering

As far as date of start of flowering is concerned, it varied from 1st to 3rd week of April. It is evident from the Table 7 that the out of fifty selected genotypes only 16 genotypes (32.00 per cent) started flowering from 7thApril to 10thApril (early bloomers), 26 genotypes (52.00 per cent) initiated flowering from 11th to 15thApril (mid bloomers) while 8 genotypes (16.00 per cent) initiated flowering from 16thto 17thApril (late bloomers). Hence it is evident from the data that genotype SKJAG-15 was earliest to initiate blooming and genotype SKJAC-50 showed late blooming among all the selected genotypes.

4.1.3.4 Date of end of flowering

The trees were observed during complete blooming period. It was evident from Table 7 that the end of flowering (85-90 per cent of flowering) varied from third to fourth week of April. The data pertaining the end of flowering analysis of flower characters given in Table 7 showed that out of fifty *Ambri* apple genotypes the end of flowering

Table 7: Flower characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S. No.	Genotype number	No of flower buds per inflorescence	Flower stalk (pedicel) length (mm)	Date of start of flowering (dd/mm/yy)	Date of end of flowering (dd/mm/yy)
1.	SKJAB -01	4	19.3	10th April, 2017	17th April, 2017
2.	SKJAB -02	4	17.2	9th April, 2017	18th April, 2017
3.	SKJAB -03	4	10.9	11th April, 2017	16th April, 2017
4.	SKJAB -04	6	17.9	10th April, 2017	15th April, 2017
5.	SKJAB -05	5	19.1	9th April, 2017	15th April, 2017
6.	SKJAT -06	4	23.4	12th April, 2017	18th April, 2017
7.	SKJAT -07	6	22.0	15th April, 2017	20th April, 2017
8.	SKJAT -08	6	21.0	11th April, 2017	16th April, 2017
9.	SKJAT -09	5	12.8	11th April, 2017	17th April, 2017
10.	SKJAG -10	5	13.5	9th April, 2017	15th April, 2017
11.	SKJAG -11	5	15.7	8th April, 2017	16th April, 2017
12.	SKJAG -12	4	14.4	9th April, 2017	15th April, 2017
13.	SKJAG -13	4	18.3	8th April, 2017	18th April, 2017
14.	SKJAG -14	4	23.1	8th April, 2017	15th April, 2017
15.	SKJAG -15	4	21.2	7th April, 2017	15th April, 2017
16.	SKJAG -16	6	20.1	10th April, 2017	19th April, 2017
17.	SKJAG -17	6	18.5	8th April, 2017	17th April, 2017
18.	SKJABh -18	5	19.8	9th April, 2017	15th April, 2017
19.	SKJABh -19	5	18.4	10th April, 2017	18th April, 2017
20.	SKJABh -20	5	10.7	10th April, 2017	15th April, 2017
21.	SKJAK -21	4	19.6	15th April, 2017	21st April, 2017
22.	SKJAK -22	4	18.8	16th April, 2017	22nd April, 2017
23.	SKJAK -23	4	11.9	17th April, 2017	23rd April, 2017
24.	SKJAK -24	5	16.9	16th April, 2017	23rd April, 2017
25.	SKJAK -25	5	18.1	15th April, 2017	22nd April, 2017
26.	SKJAK -26	5	22.4	17th April, 2017	23rd April, 2017
27.	SKJAK -27	6	25.0	15th April, 2017	21th April, 2017
28.	SKJAD -28	6	20.3	15th April, 2017	19th April, 2017

Cont…

29.	SKJAD -29	6	11.5	15th April, 2017	22nd April, 2017
30.	SKJAD -30	5	14.7	16th April, 2017	20th April, 2017
31.	SKJAD -31	5	13.4	15th April, 2017	21st April, 2017
32.	SKJAD -32	5	17.3	14th April, 2017	19th April, 2017
33.	SKJAD -33	4	22.1	15th April, 2017	20th April, 2017
34.	SKJAD -34	5	20.2	16th April, 2017	21st April, 2017
35.	SKJAD -35	6	22.1	15th April, 2017	20th April, 2017
36.	SKJAD -36	5	19.5	15th April, 2017	23rdApril, 2017
37.	SKJAN -37	4	21.8	13th April, 2017	19th April, 2017
38.	SKJAN -38	4	24.4	14th April, 2017	19th April, 2017
39.	SKJAN -39	5	18.5	15th April, 2017	20th April, 2017
40.	SKJAN -40	4	15.1	14th April, 2017	19th April, 2017
41.	SKJAN -41	4	21.1	14th April, 2017	20th April, 2017
42.	SKJAN -42	5	19.7	13th April, 2017	19th April, 2017
43.	SKJAM-43	5	22.8	12th April, 2017	18th April, 2017
44.	SKJAM-44	5	24.5	12th April, 2017	18th April, 2017
45.	SKAM-45	6	18.5	11th April, 2017	17th April, 2017
46.	SKJAM -46	6	19.3	12th April, 2017	19th April, 2017
47.	SKJAM -47	4	23.7	13th April, 2017	18th April, 2017
48.	SKJAC -48	4	25.1	15th April, 2017	21st April, 2017
49.	SKJAC -49	5	17.3	16th April, 2017	25th April, 2017
50.	SKJAC -50	5	17.7	17th April, 2017	27rd April, 2017
51.	**Mean**	4.86	18.86		
52.	**SD**	0.75	3.77		
53.	**CV**	15.43	19.98		

Table 8: Flower characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Regularity of flowering	Self Incompatibility	Bearing Habit	Age of first bearing (years)
1.	SKJAB -01	2	1	1	7
2.	SKJAB -02	2	1	1	6
3.	SKJAB -03	2	1	1	6
4.	SKJAB -04	2	1	1	7
5.	SKJAB -05	3	1	1	6
6.	SKJAT -06	3	1	4	7
7.	SKJAT -07	3	2	4	8
8.	SKJAT -08	3	2	1	8
9.	SKJAT -09	2	1	1	7
10.	SKJAG -10	2	1	4	6
11.	SKJAG -11	1	1	1	8
12.	SKJAG -12	1	2	4	7
13.	SKJAG -13	2	1	1	7
14.	SKJAG -14	2	1	1	7
15.	SKJAG -15	2	1	1	6
16.	SKJAG -16	3	1	4	7
17.	SKJAG -17	3	1	4	7
18.	SKJABh -18	3	1	4	8
19.	SKJABh -19	3	2	1	8
20.	SKJABh -20	3	2	1	7

Legend

Regularity of flowering	Note	Self Incompatibility	Note	Bearing Habit	Note
Regular	1	Incompatible	1	On spurs	1
Biennial	2	Partially compatible	2	On shoot tips	2
Irregular	3	Compatible	3	On old shoots	3
				Mixed	4

Cont…

Table 8: Flower characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Regularity of flowering	Self Incompatibility	Bearing Habit	Age of first bearing (years)
21	SKJAK -21	2	1	1	6
22	SKJAK -22	2	1	1	6
23	SKJAK -23	2	1	1	6
24	SKJAK -24	2	1	1	6
25	SKJAK -25	2	1	1	7
26	SKJAK -26	2	1	1	7
27	SKJAK -27	2	1	1	6
28	SKJAD -28	2	1	1	7
29	SKJAD -29	2	1	1	7
30	SKJAD -30	2	1	1	6
31	SKJAD -31	2	1	1	7
32	SKJAD -32	2	1	1	7
33	SKJAD -33	2	1	1	6
34	SKJAD -34	2	1	1	6
35	SKJAD -35	1	2	1	7
36	SKJAD -36	3	2	1	6
37	SKJAN -37	2	1	4	7
38	SKJAN -38	2	1	4	7
39	SKJAN -39	2	1	1	6
40	SKJAN -40	2	1	1	7

Legend

Regularity of flowering	Note	Self Incompatibility	Note	Bearing Habit	Note
Regular	1	Incompatible	1	On spurs	1
Biennial	2	Partially compatible	2	On shoot tips	2
Irregular	3	Compatible	3	On old shoots	3
				Mixed	4

Cont…

Table 8: Flower characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Regularity of flowering	Self Incompatibility	Bearing Habit	Age of first bearing (years)
41	SKJAN -41	2	1	1	6
42	SKJAN -42	2	1	1	6
43	SKJAM-43	2	1	1	6
44	SKJAM-44	3	2	1	7
45	SKJAM-45	3	2	1	6
46	SKJAM -46	2	1	4	8
47	SKJAM -47	2	1	1	7
48	SKJAC -48	2	1	1	7
49	SKJAC -49	2	1	4	8
50	SKJAC -50	2	1	4	7
	Mean				6.76
	SD				0.68
	CV				10.05

Legend

Regularity of flowering	Note	Self Incompatibility	Note	Bearing Habit	Note
Regular	1	Incompatible	1	On spurs	1
Biennial	2	Partially compatible	2	On shoot tips	2
Irregular	3	Compatible	3	On old shoots	3
				Mixed	4

Table 9: Summary of frequency of flower characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Trait	Category	No. of Genotypes	Percentage
Date of start of flowering	1st week of April	16	32
	2nd week of April	26	52
	3rd week of April	8	16
Date of end of flowering	3rd week of April	41	82
	4th week of April	9	18
Regularity of flowering	Regular	3	6
	Biennial	35	70
	Irregular	12	24
Self Incompatibility	Incompatible	50	100
	Partially compatible	-	-
	Compatible	-	-
Bearing Habit	On spurs	38	76
	On shoot tips	-	-
	On old shoots	-	-
	Mixed	12	24
Age of first bearing	At age 6 years	19	38
	At age 7 years	24	48
	At age 8 years	7	14

were categorized into two periods i.e., 3rd week of April and 4th week of April. A total of 41 genotypes (82.00 per cent) ended their flowering 3rd week of April, while 9 genotypes (18 per cent) ended their flowering in 4th week of April.

4.1.3.5 Regularity of flowering

Regularity of flowering were categorised as regular, biennial and irregular as presented in Table 8. The results showed that biennial flowering 35 genotypes (70 per cent) was observed in most of genotypes, except that 3 genotypes (6 per cent) were found regular in flowering and 12 genotypes (24 per cent) showed irregularity in flowering.

4.1.3.6 Self incompatibility

Self incompatibility was categorised as incompatible, partially compatible and compatible presented in Table 8. Out of fifty *Ambri* apple genotypes studied self incompatibility was observed in all 50 genotypes (100 per cent). No selected genotype showed partially compatibility and compatible self incompatibility.

4.1.3.7 Bearing habit

Inflorescence bearing habit were categorized as on spurs, on shoot tips, on old shoots and mixed among different *Ambri* apple genotypes (Table 8). Majority of genotypes 38 (76 per cent) showed bearing on spurs and 12 genotypes (24 per cent) exhibited mixed bearing habit. Whereas, no bearing on shoot tips and on old shoot tip was observed among fifty selected *Ambri* apple genotypes.

4.1.3.8 Age of first bearing (years)

The age of first bearing among fifty selected genotypes of *Ambri* apple varied from 6-8 years as presented in Table 8. The results showed that most of the genotypes 24 (48 per cent) started bearing at the age of seven years of planting and 19 genotypes (38 per cent) started bearing fruit at the age of six years of planting, while few selected genotypes 7 (14 per cent) were not precocious and started bearing fruit at the age of eight years of planting.

4.1.4 Fruit characters

Various fruit characteristics *viz.*, days to fruit harvest, fruit shape, fruit base, fruit base cavity depth, fruit apex, fruit length, fruit width, fruit weight, fruit ground colour,

fruit over colour: fruit skin lenticels, pulp texture, pulp taste, juiciness, productivity status and biotic stress susceptibility were recorded during two successive years 2016-2017 and 2017-2018. Characteristics of each selected genotypes studied are presented in Table 10, 11, Figure 3 and Plate 3(A), 3(B), 3(C) and 3(D).

4.1.4.1 Days to fruit harvest

A wide variation has been recorded in days to fruit harvest of fifty *Ambri* apple genotypes presented in Table 10. The data reveals that time taken to fruit harvest ranged from 145 to 158 days.

4.1.4.2 Fruit shape

Fruit shapes varied from globose, globose-conical, short-globose-conical, flat, flat-globose (oblate), conical, long-conical, intermediate-conical, ellipsoid, ellipsoid-conical (ovate), oblong, oblong-conical, oblong-waisted and other. The descriptive data presented in Table 11 showed that 19 genotypes (38 per cent) exhibited globose, 8 genotypes (16 per cent) showed globose conical, 10 genotypes (20 per cent) had conical and rest 13 genotypes (23 per cent) possessed long conical fruit shape.

4.1.4.2 Fruit base

Fruit base were categorized into narrow, intermediate and broad base. Out of fifty selected *Ambri* apple genotypes, 17 genotypes (34 per cent) had narrow base, 6 genotypes (12 per cent) had intermediate and rest 27 genotypes (54 per cent) had broad fruit base.

4.1.4.3 Fruit base cavity depth

Fruit base cavity depth varied from shallow to medium and deep. The descriptive data presented in Table 11 showed that 18 genotypes (36 per cent) exhibited shallow, 18 genotypes (36 per cent) showed medium and 14 genotypes (28 per cent) possessed deep fruit base cavity depth.

4.1.4.4 Fruit apex

Fruit apex varied from smooth, wrinkled, grooved and others. The data presented in Table 11 showed that 30 genotypes (60 per cent) possessed smooth apex and 20

Genotype 1

Genotype 2

Genotype 3

Genotype 4

Genotype 5

Genotype 6

Genotype 7

Genotype 8

Genotype 9

Genotype 10

Genotype 11

Genotype 12

Genotype 13

Genotype 14

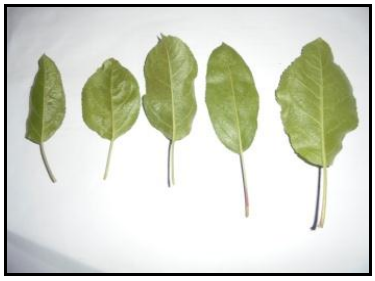
Genotype 15

Plate 2(A): Varaiation in leaf characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Genotype 16

Genotype 17

Genotype 18



Genotype 19

Genotype 20

Genotype 21

Genotype 22

Genotype 23

Genotype 24

Genotype 25

Genotype 26

Genotype 27

Genotype 28

Genotype 29

Genotype 30

Plate 2(B): Varaiability in leaf characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 31

Genotype 32

Genotype 33

Genotype 34

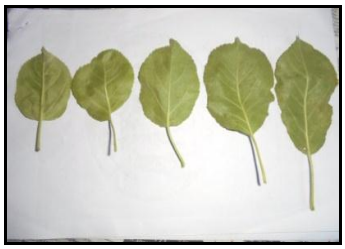
Genotype 35

Genotype 36

Genotype 37

Genotype 38

Genotype 39



Genotype 40

Genotype 41

Genotype 42

Genotype 43

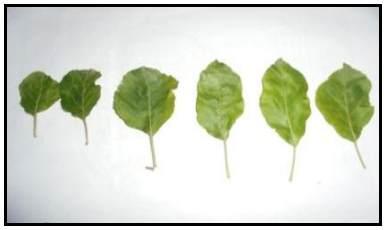
Genotype 44

Genotype 45

Plate 2(C): Varaiability in leaf characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

Genotype 46

Genotype 47



Genotype 48

Genotype 49

Genotype 50

Plate 2(D): Varaiability in leaf characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

genotypes (40 per cent) showed grooved fruit apex. Wrinkled and other category of fruit apex were not observed in selected genotypes of *Ambri* apple.

4.1.4.5 Fruit length (cm)

The fruit length of the selected genotypes studied varied from 4.35 cm to 6.37 cm. The descriptive data as presented in the Table 19 showed that among fifty genotypes maximum (6.37 cm) fruit length were observed in genotype SKJAD -29 and SKADM-45 which was at par with genotype SKJAD -30 (6.35 cm), while minimum fruit length (4.35 cm) was observed in SKJABh -18.

4.1.4.6 Fruit width (cm)

Mean fruit width ranged between 4.62-7.62 cm among studied *Ambri* apple genotypes. The minimum fruit width (4.62 cm) was recorded in SKJABh -18, whereas, maximum fruit width (7.62 cm) was recorded in genotype SKJAD -29.

4.1.4 .7 Fruit weight (g)

Perusal of results presented in the Table 19 reveal that mean fruit weight was in a ranged from 158.2g - 292.2 g among fifty selected *Ambri* apple genotypes. The minimum fruit weight (158.2 g) was observed in SKJABh -18 whereas maximum fruit weight (292.2 g) in SKJAD-29.

4.1.4.8 Fruit ground colour

Based on the ground colour of skin of fruits the genotypes were classified as cream white yellow, yellow and green yellow (Table 12). Among the fifty genotypes, 15 (30 per cent) had cream white yellow, 8 (16 per cent) had yellow and rest 27 (54 per cent) genotypes had green yellow fruit ground colour.

4.1.4.9 Fruit over colour

The fruit over colour was found to vary from yellow (golden), pink, green, orange, red, dark red, brown, purple, dark brown and other. The descriptive data presented in the Table 12 showed that 19 genotypes (38 per cent) showed yellow, 31 genotypes (62 per cent) had pink fruit over.

4.1.4.10 Fruit skin lenticels

Fruit skin lenticels were categorized into absent, low, medium and high (Table 12). Out of fifty *Ambri* apple genotypes 25 genotypes (50 per cent) lenticels were absent and 25 genotypes (50 per cent) had low fruit skin lenticels.

4.1.4.11 Pulp texture

Pulp texture was categorised into three categories i.e. soft, intermediate and firm. Among fifty *Ambri* apple genotypes 37 genotypes (74 per cent) had soft, 10 genotypes (20 per cent) showed intermediate and 3 genotypes (6 per cent) possessed firm pulp texture.

4.1.4.12 Pulp taste

The pulp taste ranged from acidic, sub acidic, medium sweet to sweet. Analysed data presented in Table 14 showed that 24 genotypes (48 per cent) had medium sweet and 26 genotypes (52 per cent) possessed sweet pulp taste.

4.1.4.13 Juiciness

The juice content in apple were categorised as less, medium and high (Table 14). Among fifty *Ambri* apple genotypes 43 genotypes(86 per cent) showed high juice content while 7 genotypes (14 per cent) showed medium juice content.

4.1.4.14 Productivity status

Productivity status recorded at the time of harvest was found to vary from low, medium to high. The descriptive data of productivity status per unit area reveals that among fifty genotypes, 47 genotypes (94 per cent) had medium, 3 genotypes (6 per cent) showed high productivity status and none were low in productivity.

4.1.4.15 Biotic stress susceptibility

The biotic stress susceptibility was categorised to very low or no visible sign of susceptibility, low, intermediate, high and very high. The data presented in Table 15 indicates that out of fifty selected genotypes, 39 genotypes (78 per cent) had very low or

Table 10: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Days to fruit harvest	Fruit shape	Fruit base	Fruit base cavity depth	Fruit apex
1.	SKJAB -01	150-153	1	7	3	1
2.	SKJAB -02	150-151	1	7	3	1
3.	SKJAB -03	150-152	1	7	3	1
4.	SKJAB -04	149-150	1	7	3	3
5.	SKJAB -05	145-148	7	7	5	1
6.	SKJAT -06	145-146	1	7	3	1
7.	SKJAT -07	145-147	7	7	5	1
8.	SKJAT -08	145-146	7	7	5	1
9.	SKJAT -09	145-148	7	7	5	1
10.	SKJAG -10	151-152	2	7	5	3
11.	SKJAG -11	151-153	2	7	5	3
12.	SKJAG -12	152-152	2	7	5	3
13.	SKJAG -13	151-153	2	3	7	3
14.	SKJAG -14	152-152	2	3	7	3
15.	SKJAG -15	151-153	2	3	7	3
16.	SKJAG -16	152-152	2	3	7	3
17.	SKJAG -17	151-153	2	3	7	3
18.	SKJABh -18	147-150	1	3	3	1
19.	SKJABh -19	146-150	6	7	7	3
20.	SKJABh -20	145-150	6	7	7	3

Legend

Fruit shape	Note	Fruit base	Note	Fruit base cavity depth	Note	Fruit apex	Note
Globose	1	Narrow	3	Shallow	3	Smooth	1
Globose conical	2	Intermediate	5	Medium	5	Wrinkled	2
Flat	4	Broad	7	Deep	7	Grooved	3
Conical	6					Others	99
Long conical	7						
Intermediate conical	8						

Cont…

Table 10: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Days to fruit harvest	Fruit shape	Fruit base	Fruit base cavity depth	Fruit apex
21.	SKJAK -21	155-158	7	7	5	1
22.	SKJAK -22	156-157	7	7	5	1
23.	SKJAK -23	154-156	7	7	5	1
24.	SKJAK -24	155-158	7	7	5	3
25.	SKJAK -25	156-157	7	7	5	1
26.	SKJAK -26	154-156	7	7	5	1
27.	SKJAK -27	155-158	1	7	5	1
28.	SKJAD -28	151-154	1	7	3	1
29.	SKJAD -29	152-153	7	7	5	3
30.	SKJAD -30	153-154	1	5	3	3
31.	SKJAD -31	151-152	1	7	3	3
32.	SKJAD -32	153-154	1	7	3	1
33.	SKJAD -33	151-152	1	3	3	3
34.	SKJAD -34	151-154	1	7	3	1
35.	SKJAD -35	152-153	1	7	3	3
36.	SKJAD -36	153-154	1	5	3	1
37.	SKJAN -37	145-147	6	3	7	1
38.	SKJAN -38	146-147	6	5	7	1
39.	SKJAN -39	145-148	6	5	7	1
40.	SKJAN -40	146-148	6	5	7	1

Legend

Fruit shape	Note	Fruit base	Note	Fruit base cavity depth	Note	Fruit apex	Note
Globose	1	Narrow	3	Shallow	3	Smooth	1
Globose conical	2	Intermediate	5	Medium	5	Wrinkled	2
Flat	4	Broad	7	Deep	7	Grooved	3
Conical	6					Others	99
Long conical	7						
Intermediate conical	8						

Cont…

Table 10: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Days to fruit harvest	Fruit shape	Fruit base	Fruit base cavity depth	Fruit apex
41.	SKJAN -41	145-147	6	7	3	3
42.	SKJAN -42	146-147	6	7	3	1
43.	SKJAM-43	144-145	1	7	7	1
44.	SKJAM-44	144-145	1	7	7	3
45.	SKJAM-45	144-145	6	7	3	1
46.	SKJAM -46	144-145	7	3	5	1
47.	SKJAM -47	144-145	1	5	7	3
48.	SKJAC -48	154-157	6	5	3	1
49.	SKJAC -49	153-154	1	5	7	1
50.	SKJAC -50	152-153	1	5	7	1

Legend

Fruit shape	Note	Fruit base	Note	Fruit base cavity depth	Note	Fruit apex	Note
Globose	1	Narrow	3	Shallow	3	Smooth	1
Globose conical	2	Intermediate	5	Medium	5	Wrinkled	2
Flat	4	Broad	7	Deep	7	Grooved	3
Conical	6					Others	99
Long conical	7						
Intermediate conical	8						

Table 11: Summary of frequency of fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Trait	Category	No. of Selections	Percentage
Fruit shape	Globose	19	38
	Globose conical	8	16
	Flat	-	-
	Conical	10	20
	Long conical	13	26
	Intermediate conical	-	-
Fruit base	Narrow	17	34
	Intermediate	6	12
	Broad	27	54
Fruit base cavity depth	Shallow	18	36
	Medium	18	36
	Deep	14	28
Fruit apex	Smooth	30	60
	Wrinkled	-	-
	Grooved	20	40

Table 12: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Fruit over colour	Fruit ground colour	Fruit skin lenticels
1.	SKJAB -01	2	3	0
2.	SKJAB -02	2	3	0
3.	SKJAB -03	1	3	0
4.	SKJAB -04	2	2	3
5.	SKJAB -05	2	3	3
6.	SKJAT -06	1	2	3
7.	SKJAT -07	1	2	3
8.	SKJAT -08	1	2	3
9.	SKJAT -09	1	2	0
10.	SKJAG -10	2	4	0
11.	SKJAG -11	2	4	0
12.	SKJAG -12	2	4	0
13.	SKJAG -13	2	4	0
14.	SKJAG -14	2	4	0
15.	SKJAG -15	2	4	0
16.	SKJAG -16	2	4	0
17.	SKJAG -17	2	4	0
18.	SKJABh -18	1	2	3
19.	SKJABh -19	2	4	3
20.	SKJABh -20	2	4	3

Legend

Fruit over colour	Note	Fruit ground colour	Note	Fruit skin lenticels	Note
Yellow	1	Cream white	1	Absent	0
Pink	2	Yellow	2	Low	3
Green	3	Green yellow	3	Medium	5
Orange	4	Green	4	High	7
Red	5	Orange	5		
Dark red	6	Red	6		
Brown	7	Others	99		
Purple	8				
Dark brown	9				
Others	99				

Cont..

Table 12: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Fruit over colour	Fruit ground colour	Fruit skin lenticels
21.	SKJAK -21	2	2	3
22.	SKJAK -22	1	4	3
23.	SKJAK -23	1	2	3
24.	SKJAK -24	2	4	0
25.	SKJAK -25	1	4	3
26.	SKJAK -26	2	4	0
27.	SKJAK -27	2	2	0
28.	SKJAD -28	1	4	3
29.	SKJAD -29	1	3	3
30.	SKJAD -30	2	2	3
31.	SKJAD -31	2	4	3
32.	SKJAD -32	1	4	3
33.	SKJAD -33	2	2	3
34.	SKJAD -34	1	3	3
35.	SKJAD -35	1	3	3
36.	SKJAD -36	1	4	3
37.	SKJAN -37	2	2	0
38.	SKJAN -38	2	2	0
39.	SKJAN -39	2	4	0
40.	SKJAN -40	2	4	3

Legend

Fruit over colour	Note	Fruit ground colour	Note	Fruit skin lenticels	Note
Yellow	1	Cream white	1	Absent	0
Pink	2	Yellow	2	Low	3
Green	3	Green yellow	3	Medium	5
Orange	4	Green	4	High	7
Red	5	Orange	5		
Dark red	6	Red	6		
Brown	7	Others	99		
Purple	8				
Dark brown	9				
Others	99				

Cont…

Table 12: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Fruit over colour	Fruit ground colour	Fruit skin lenticels
41.	SKJAN -41	2	4	0
42.	SKJAN -42	2	4	0
43.	SKJAM-43	1	4	0
44.	SKJAM-44	1	4	0
45.	SKAM-45	2	2	3
46.	SKJAM -46	2	4	0
47.	SKJAM -47	1	4	3
48.	SKJAC -48	2	3	0
49.	SKJAC -49	1	4	3
50.	SKJAC -50	2	2	0

Legend

Fruit over colour	Note	Fruit ground colour	Note	Fruit skin lenticels	Note
Yellow	1	Cream white	1	Absent	0
Pink	2	Yellow	2	Low	3
Green	3	Green yellow	3	Medium	5
Orange	4	Green	4	High	7
Red	5	Orange	5		
Dark red	6	Red	6		
Brown	7	Others	99		
Purple	8				
Dark brown	9				
Others	99				

Table 13: Summary of frequency of fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Trait	Category	No. of Selections	Percentage
Fruit over colour	Yellow	19	38
	Pink	31	62
	Green	-	-
	Orange	-	-
Fruit ground colour	Yellow	15	30
	Green yellow	8	16
	Green	27	54
Fruit skin lenticels	Absent	25	50
	Low	25	50

Table 14: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Pulp texture	Pulp taste	Juiciness	Productivity status	Biotic stress susceptibility
1.	SKJAB -01	3	3	5	5	1
2.	SKJAB -02	3	4	5	5	1
3.	SKJAB -03	3	4	5	5	1
4.	SKJAB -04	3	4	5	5	1
5.	SKJAB -05	3	3	5	5	3
6.	SKJAT -06	3	3	5	5	3
7.	SKJAT -07	3	3	5	5	1
8.	SKJAT -08	3	3	5	5	1
9.	SKJAT -09	3	3	5	5	1
10.	SKJAG -10	3	4	5	5	1
11.	SKJAG -11	3	4	5	5	1
12.	SKJAG -12	3	3	5	5	1
13.	SKJAG -13	3	4	5	5	1
14.	SKJAG -14	5	3	5	5	1
15.	SKJAG -15	5	3	5	5	5
16.	SKJAG -16	3	4	5	5	1
17.	SKJAG -17	3	4	7	5	1
18.	SKJABh -18	3	3	7	5	1
19.	SKJABh -19	3	3	5	5	1
20.	SKJABh -20	3	3	5	5	1

Legend

Pulp texture	Note	Pulp taste	Note	Juiciness	Note	Productivity status	Note	Biotic stress susceptibility	Note
Soft	3	Acidic	1	Less	3	Low	3	Very low or no visible sign of susceptibility	1
Intermediate	5	Sub acidic	2	Medium	5	Medium	5	Low	3
Firm	7	Medium sweet	3	High	7	High	7	Intermediate	5
		Sweet	4					High	7
								Very high	9

Cont…

Table 14: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Pulp texture	Pulp taste	Juiciness	Productivity status	Biotic stress susceptibility
21.	SKJAK -21	5	4	5	5	1
22.	SKJAK -22	5	4	5	5	1
23.	SKJAK -23	7	4	5	5	1
24.	SKJAK -24	7	3	5	5	3
25.	SKJAK -25	7	4	5	5	3
26.	SKJAK -26	5	3	5	5	3
27.	SKJAK -27	3	4	5	5	5
28.	SKJAD -28	3	3	7	5	1
29.	SKJAD -29	3	4	7	7	1
30.	SKJAD -30	3	4	7	5	1
31.	SKJAD -31	3	4	5	7	1
32.	SKJAD -32	3	4	5	5	1
33.	SKJAD -33	3	3	5	5	3
34.	SKJAD -34	5	3	5	5	3
35.	SKJAD -35	5	3	5	5	1
36.	SKJAD -36	3	4	5	5	1
37.	SKJAN -37	3	4	5	5	1
38.	SKJAN -38	3	3	5	5	1
39.	SKJAN -39	5	4	7	5	1
40.	SKJAN -40	5	4	5	5	1

Legend

Pulp texture	Note	Pulp taste	Note	Juiciness	Note	Productivity status	Note	Biotic stress susceptibility	Note
Soft	3	Acidic	1	Less	3	Low	3	Very low or no visible sign of susceptibility	1
Intermediate	5	Sub acidic	2	Medium	5	Medium	5	Low	3
Firm	7	Medium sweet	3	High	7	High	7	Intermediate	5
		Sweet	4					High	7
								Very high	9

Cont...

Table 14: Fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S.No.	Genotype number	Pulp texture	Pulp taste	Juiciness	Productivity status	Biotic stress susceptibility
41.	SKJAN -41	3	4	7	5	1
42.	SKJAN -42	5	3	5	5	1
43.	SKJAM-43	3	4	5	5	3
44.	SKJAM-44	3	3	5	5	1
45.	SKAM-45	3	3	5	5	1
46.	SKJAM -46	3	4	5	5	1
47.	SKJAM -47	3	3	5	7	5
48.	SKJAC -48	3	4	5	5	1
49.	SKJAC -49	3	3	5	5	1
50.	SKJAC -50	3	4	5	5	1

Legend

Pulp texture	Note	Pulp taste	Note	Juiciness	Note	Productivity status	Note	Biotic stress susceptibility	Note
Soft	3	Acidic	1	Less	3	Low	3	Very low or no visible sign of susceptibility	1
Intermediate	5	Sub acidic	2	Medium	5	Medium	5	Low	3
Firm	7	Medium sweet	3	High	7	High	7	Intermediate	5
		Sweet	4					High	7
								Very high	9

Table 15: Summary of frequency of fruit characters of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Trait	Category	No. of Selections	Percentage
Pulp texture	Soft	37	74
	Intermediate	10	20
	Firm	3	6
Pulp taste	Acidic	-	-
	Sub acidic	-	-
	Medium sweet	24	48
	Sweet	26	52
Juiciness	Less	-	-
	Medium	43	86
	High	7	14
Productivity status	Low		
	Medium	47	94
	High	3	6
Biotic stress susceptibility	Very low or no visible sign of susceptibility	39	78
	Low	8	16
	Intermediate	3	6
	High	-	-
	Very high	-	-

SKJAB -1

SKJAB-2

SKJAB-3

SKJAB -4

SKJAT-5

SKJT-6

SKJAT -7

SKJAT-8

SKJAT-9

SKJAT -10

SKJAT-11

SKJAT-12

Plate 3 (A): Varaiability in fruit characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

SKJAT -13

SKJAT-14

SKJAT-15

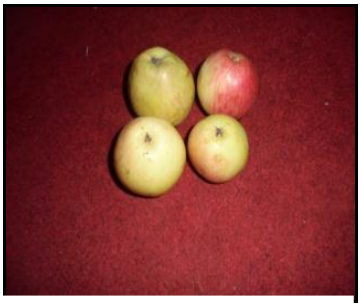
SKJAG-16

SKJAG-17

SKJABH-18

SKJABH -19

SKJABH-20



SKJAK-21

SKJABH -22

SKJAK-23

SKJAK-24

Plate 3 (B): Varaiability in fruit characters among different Ambri apple (*Malus × domestica* Borkh.) genotypes

SKJAK -25

SKJAK-26

SKJAK-27

SKJAD -28

SKJAD-29

SKJAD-30

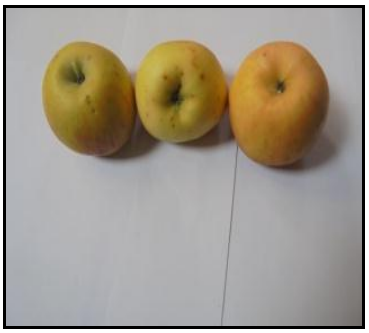
SKJAD -31

SKJAD-32

SKJAD-33

SKJAD -34

SKJAD-35



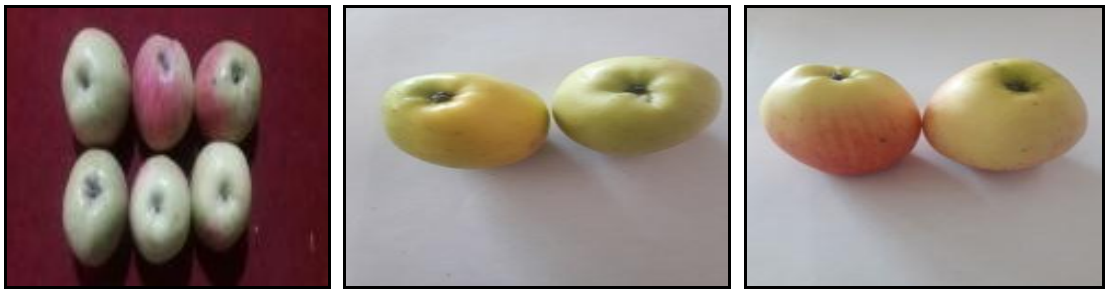
SKJAD-36

Plate 3 (C): Varaiability in fruit characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

SKJAN -37 **SKJAN-38** **SKJAN39**

SKJAN -40 **SKJAN-41** **SKJAN-42**

SKJAM -43 **SKJAM-44** **SKJAM-45**



SKJAM -46 **SKJAM-47** **SKJAC-48**

SKJAC -49 **SKJAC-50**

Plate 3 (D): Varaiability in fruit characters among different Ambri apple (*Malus* × *domestica* Borkh.) genotypes

no visible sign of susceptibility, 8 genotypes (16 per cent) had low sign of susceptibility and 3 genotypes (6 per cent) had intermediate sign of susceptibility to biotic stress.

4.1.5 Cluster analysis

The cluster of genetic divergence of morphological characters divided *Ambri* apple genotypes into 3 main clusters (Fig 5). The formation of large number of clusters with variable number of entries in each cluster is indicative of diversity with the help of dendrogram as showed in Fig. 5. The clustering of fifty *Ambri* apple genotypes are as presented in Table 16 revealed that the genotypes from different eco-geographical areas was apparently random. Cluster II contained the maximum (25) number of *Ambri* apple genotypes namely SKJAB-03, SKJAB-04, SKJAT -08, SKJAG -13, SKJAG -15, SKJAG -16 , SKJAG -17, SKJABh -18, SKJABh -20, SKJAK -22, SKJAK -23, SKJAK -24, SKJAK -25, SKJAK -26, SKJAD -28, SKJAD -31, SKJAD -33, SKJAD -35, SKJAN -38, SKJAN -39, SKJAN -40, SKJAM-43, SKJAM-44, SKJAC -48, SKJAC-49, whereas the cluster I contained lowest (11) number of genotypes namely SKJAB-01 SKJAB-02, SKJAG-11, SKJAK-21, SKJAK-27, SKJAD-34, SKJAD-36, SKJAN-37, SKJAM-46, SKJAM-47, SKJAC-50 fell in cluster I. Furthermore, cluster III contained (14) genotypes namely SKJAB -05, SKJAT -06, SKJAT -07, SKJAT -09, SKJAG -10, SKJAG -12, SKJAG -14, SKJABh -19 , SKJAD -29, SKJAD -30, SKJAD -32, SKJAN -41, SKJAN -42, SKJAM-45. Clusters with variable number of entries in each cluster indicating the presence of genetic diversity. In present investigation twenty six morphological traits of *Ambri* apple were studied. The intra cluster distances ranged from 27.96 (cluster III) to 45.34 (cluster II) as presented in Table 17. The maximum intra-cluster distance was observed in cluster II (45.34) followed by cluster I (33.75). The minimum intra cluster distance was observed in cluster III (27.96) which contain fourteen genotypes. The narrow range intra cluster distance depicted the presence of narrow range of diversity within a cluster. The inter cluster distance ranged from 167.33 to 124.39. The maximum inter cluster distance was observed between cluster II and cluster III indicated that genotypes falling in these clusters can be used in hybridization programme to get greater variability in the segregating generations. These genotypes can also be utilized for transfer of beneficial traits in the commercial *Ambri* apple cultivar.

Table 16: Clustering pattern of fifty Ambri apple (*Malus* × *domestica* Borkh.) genotypes for twenty six characters based on morphological parameters.

Cluster	Number of genotypes	Genotypes
I	11	SKJAB-01 SKJAB-02, SKJAG-11, SKJAK-21, SKJAK-27, SKJAD-34, SKJAD-36, SKJAN-37, SKJAM-46, SKJAM-47, SKJAC-50
II	25	SKJAB-03, SKJAB-04, SKJAT -08 , SKJAG -13, SKJAG -15, SKJAG -16 , SKJAG -17, SKJABh -18, SKJABh -20, SKJAK -22, SKJAK -23, SKJAK -24, SKJAK -25, SKJAK -26, SKJAD -28, SKJAD -31, SKJAD -33, SKJAD -35, SKJAN -38, SKJAN -39, SKJAN -40, SKJAM-43, SKJAM-44, SKJAC -48, SKJAC-49
III	14	SKJAB -05, SKJAT -06, SKJAT -07, SKJAT -09, SKJAG -10, SKJAG -12, SKJAG -14, SKJABh -19 , SKJAD -29, SKJAD -30, SKJAD -32, SKJAN -41, SKJAN -42, SKJAM-45

Table 17: Average intra and inter-cluster distances for three clusters in Ambri apple (*Malus* × *domestica* Borkh.) genotypes based on morphological parameters

Cluster number	I	II	III
I	33.7555	167.3337	51.0073
II		45.3432	124.3980
III			27.9654

Note: On diagonal value intra cluster distance and above diagonal inter cluster distance

Table 18: Principle component analysis for contribution of different morphological parameters towards variability

S. No.	Parameters	Principle Component								
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	Eigen value	3.8592	3.0996	2.7495	2.3148	1.6606	1.5742	1.2992	1.0741	1.0621
	Variance (%)	14.8432	11.9215	10.5751	8.9032	6.3868	6.0545	4.9970	4.1310	4.0851
	Cumulative variance (%)	14.8432	26.7647	37.3397	46.2430	52.6298	58.6842	63.6812	67.8123	71.8974
1.	Tree habit	-0.0795	0.2369	-0.4830	-0.0179	0.0163	0.3109	0.2259	-0.1043	0.3655
2.	Tree vigour	0.3858	0.2199	-0.3347	0.1294	-0.3082	0.0746	0.4784	0.0794	0.2519
3.	Tree height	-0.1455	0.6490	0.5880	0.1733	-0.0509	0.0111	-0.0507	0.0376	0.0088
4.	Trunk girth	-0.0564	0.2878	0.3520	0.4705	-0.1448	-0.1143	-0.2915	0.1311	0.5505
5.	Tree spread	-0.2685	0.2415	0.5758	0.4819	-0.1913	-0.0850	0.2203	-0.0389	0.0630
6.	Leaf size	0.1797	0.5865	0.0973	-0.2941	-0.0525	0.1539	0.1297	0.2388	-0.3579
7.	Leaf shape	0.0492	-0.5512	-0.2400	0.0499	-0.3607	0.2792	-0.0289	0.1334	-0.0662
8.	No of flower buds	0.2371	-0.2371	0.3019	0.3527	0.4986	-0.2459	-0.1282	0.0484	-0.1452
9.	Flower stalk length	-0.4136	0.2049	-0.0415	0.2221	0.4144	0.0717	0.2646	-0.1747	-0.0258
10.	Days to start of flowering	-0.4049	-0.6387	-0.1165	-0.1463	-0.3315	-0.1960	-0.0196	0.0259	-0.0074
11.	Days to end of flowering	-0.3907	-0.4893	0.2212	-0.1579	-0.4707	-0.2333	-0.0425	0.0463	0.2132
12.	Regularity of flowering	-0.1053	-0.5270	0.1378	0.4360	0.2327	0.1947	-0.0001	0.0553	0.0831
13.	Self Incompatibility	0.1686	-0.4491	0.2796	0.4554	0.0037	-0.0210	0.2705	-0.0845	-0.1907
14.	Bearing Habit	-0.4497	-0.2420	-0.4135	0.2766	0.2297	-0.0163	-0.2557	0.2374	-0.0291
15.	Age of first bearing	0.3275	0.0622	-0.3154	0.3897	0.0462	-0.1307	0.2385	0.5385	-0.0822
16.	Days to fruit harvest	0.2411	0.3311	0.0000	-0.4265	0.0567	-0.3894	-0.3760	0.3139	0.1269
17.	Fruit shape	0.0734	-0.0455	0.3378	0.0827	-0.0852	0.6928	-0.3231	-0.0121	0.1444
18.	Fruit base	0.4638	-0.2593	0.4918	-0.3669	0.0401	0.1142	0.0091	-0.3186	-0.0690
19.	Fruit base cavity depth	-0.0878	0.1581	-0.2932	0.5072	-0.1933	0.2838	-0.4450	0.0507	-0.3275
20.	Fruit apex	0.3144	0.0406	-0.2617	0.3344	-0.3042	-0.4959	-0.0316	-0.2118	-0.1021
21.	Fruit weight	0.7917	-0.0602	-0.0299	0.2973	-0.0155	-0.0704	0.0714	-0.0593	0.1370
22.	Fruit length	0.9012	-0.2593	-0.0462	0.0397	0.1016	0.0733	-0.0744	-0.0559	0.1089
23.	Fruit width	0.8309	-0.1593	-0.0568	-0.1050	0.1602	0.0163	-0.2002	0.0412	0.1418
24.	Fruit over colour	0.3431	-0.0122	0.3814	-0.0123	-0.4389	0.2699	0.0935	0.3079	-0.2210
25.	Fruit ground colour	0.2180	0.3289	-0.1166	0.2670	-0.3707	-0.2054	-0.2208	-0.3910	-0.2178
26.	Fruit skin lenticels	-0.0155	-0.2955	0.5807	-0.1203	0.0571	-0.1973	0.1684	0.2571	-0.0315

4.1.6 Principle Component Analysis

The principle component analysis is a technique that reduces the dimensionality of data set and revealed the predominant variables. In the present investigation the PCA for morphological traits revealed that the 1st nine principal components (PC's) possessed Eigen value > 1.0 (Table 18 and Fig.6). The nine components with Eigen values are able to explain the more than 71.00 per cent of total variation. PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8, PC9 accounted for 14.8 per cent, 11.92 per cent, 10.57 per cent, 8.90 per cent, 6.38 per cent, 6.05 per cent, 4.99 per cent, 4.13 per cent and 4.08 per cent. In particular the first component is positively and strongly associated with tree vigour but negatively associated tree habit, tree height, trunk girth and tree spread. Second component is positively and strongly associated with tree habit, with tree vigour tree height, trunk girth and tree spread, but negatively associated with leaf shape. Third component explains the positive and strong association with tree height, trunk girth and tree spread, leaf shape and negatively associated leaf size. Fourth component is positively and strongly associated with tree vigour, tree height, trunk girth, tree spread, leaf shape, but negatively associated with tree habit, leaf size. Fifth component is positively and strongly associated with tree habit but negatively associated with vigour, tree height, trunk girth, tree spread, leaf size and leaf shape. Sixth component is positively and strongly associated with tree habit, tree vigour, tree height, leaf size and leaf shape but negatively associated with trunk girth and tree spread. Seventh component is positively and strongly associated with tree habit, tree vigour, tree spread, leaf size but negatively associated with tree height, trunk girth and leaf shape. Eighth component is positively and strongly associated with tree vigour, tree height, trunk girth, leaf size and leaf shape but negatively associated with tree habit and tree spread. Ninth component is positively and strongly associated with tree habit, tree vigour, tree height, trunk girth, tree spread but negatively associated with leaf size and leaf shape. The PC analysis provided a simplified classification of the apple cultivars for collecting and breeding. Loading plots of PC clearly explains the variability towards the Ambri apple. The individual contribution towards variability of Ambri apple through morphological and genotypes (as a parameter) i.e.,14.84 per cent and 11.92 per cent respectively. Cumulative variability

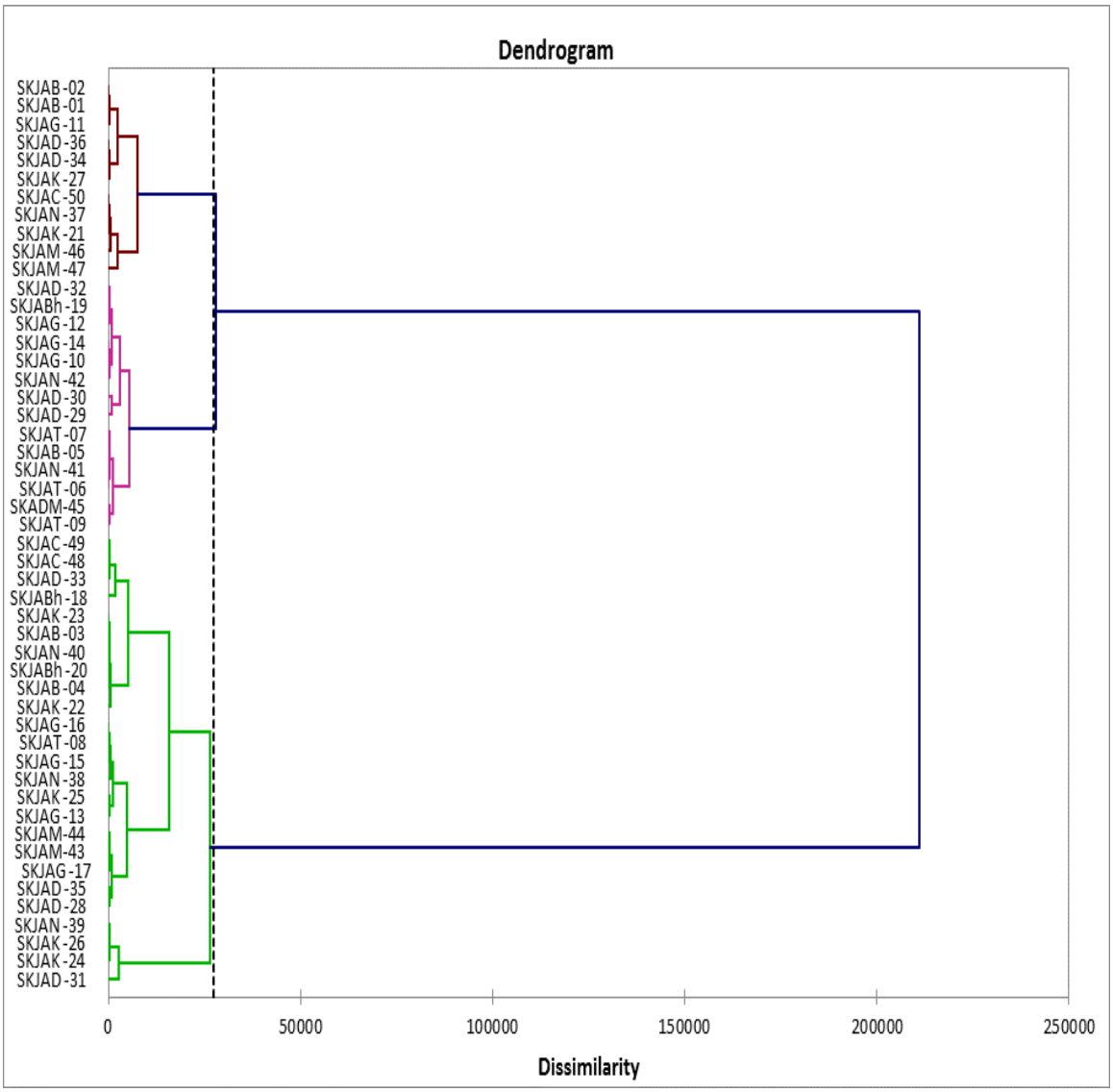


Fig. 5: Dendrogram showing clustering pattern of fifty Ambri apple (*Malus* × *domestics* Borkh.) genotypes based on morphological parameters.

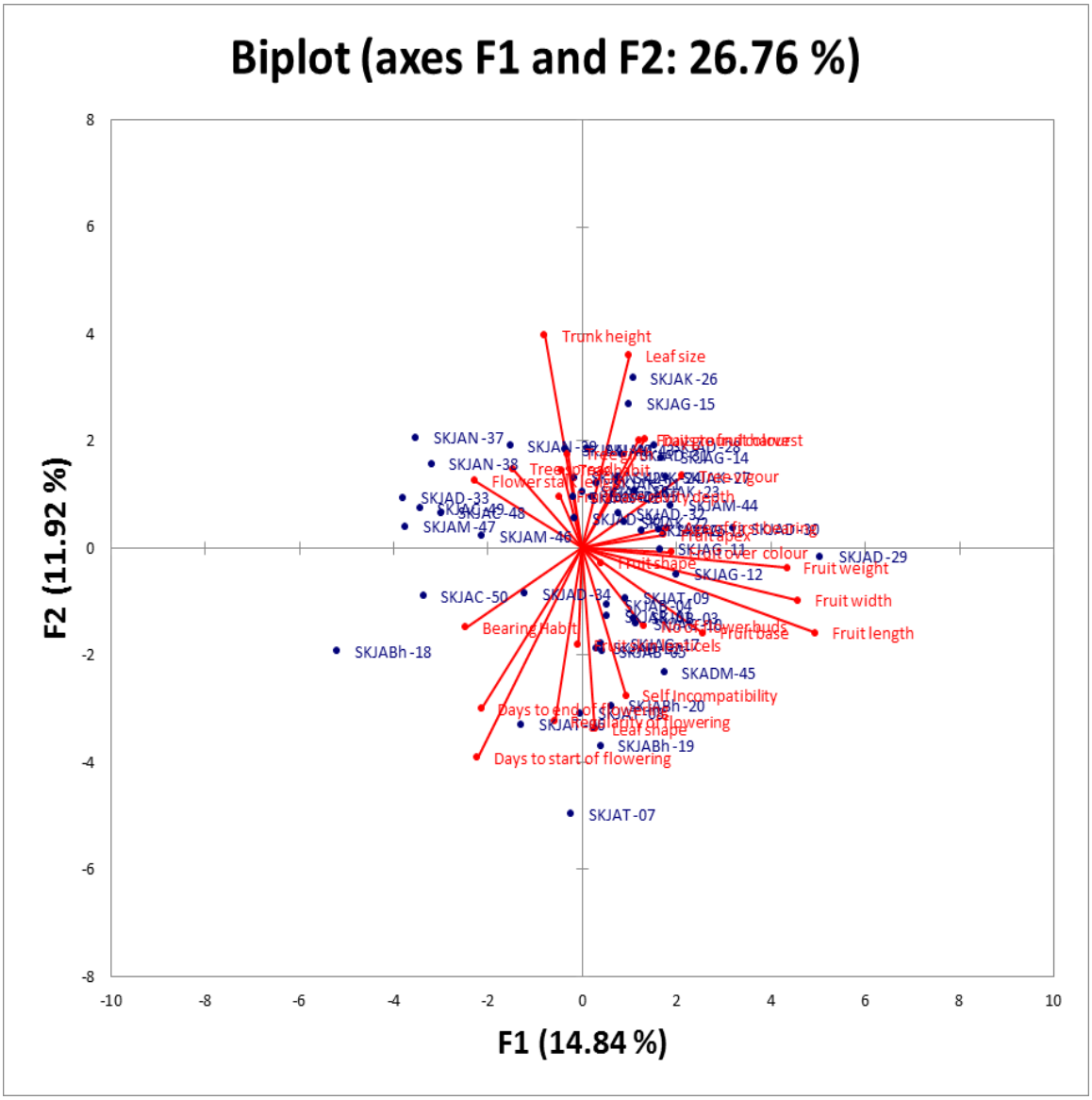


Fig. 6: Loading plot for morphological parameters and different genotypes of Ambri apple (*Malus* × *domestics* Borkh.) .

both morphological and genotypes towards the Ambri apple is 26.76 per cent respectively.

4.2 Biochemical characterization

The biochemical characters of fifty selected genotypes of Ambri apple are presented in Table 19 while the range, mean, standard deviation and coefficient of variation are showed in Table 20.

4.2.1 Total soluble solids (ºBrix)

The data presented in the Table 19 depicts that TSS content among different Ambri apple genotypes varied from 12.57 ^{O}B (SKABh-18) to 15.91 ^{o}B in SKJAD-30. The maximum TSS (15.91^{O}B) was observed in genotype SKJAD-30 followed by SKJABh -19 (15.79 ^{O}B) and SKJAK-25 (15.75 ^{O}B). The minimum TSS was recorded in genotype SKABh-18. (12.57 ^{O}B).The mean total soluble solids content among all the genotypes was 15.01^{O}B.

4.2.2 Titratable acidity (per cent)

Data shows variation in titratable acidity among fifty selected genotypes under study. The minimum titratable acidity (0.30 per cent) was observed in genotype SKJAD-30 followed by the genotypes SKJAK -22 (0.31 per cent), SKJAD -32 (0.32 per cent) and SKJAD -31 (0.33 per cent), whereas, maximum titratable acidity (1.72 per cent) was recorded in genotype SKJAG-17. The average titratable acidity recorded among selected genotypes was 0.88 per cent.

4.2.3 Ascorbic acid (mg/100g)

The data presented in Table 19 revealed that ascorbic acid content ranges from 2.10 mg/100g (SKJAG -17) to 4.81mg/100g (SKJAN -39). The maximum ascorbic acid content was observed in genotypes SKJAN -39 (4.81mg/100g) followed by SKJAN -40 (4.80 mg/100g), SKJAN -37 (4.29 mg/100g) and SKJAD -36 (4.27 mg/100g). The average ascorbic acid recorded among selected genotypes was 2.77 mg/100g.

4.2.4 Total sugars (per cent)

The data presented in Table 19 revealed that total sugar content ranged 9.89 per cent (SKAD-31) to 13.15 per cent (SKAD-30). The maximum sugar content was observed in genotypes SKAD-30 and SKJAC -50 (13.15 per cent) followed by SKJAG -15 (13.12 per cent) and SKJAC -49 (13 per cent). The minimum per cent of total sugars was recorded in the genotype SKAB-01 (9.00 per cent). The average sugar content observed among selected genotypes was 11.41 per cent.

4.2.4.1 Reducing Sugars (per cent)

The data presented in Table 19 revealed that reducing sugar content ranges 5.63 per cent (SKAB-01) to 8.22 per cent (SKAD-30). The maximum reducing sugar content was observed in genotypes SKAD-30 and SKJAC -50 (8.22 per cent) followed by SKJAG -15 (8.20 per cent) and SKJAC -49 (8.13 per cent).The minimum reducing sugars was recorded in the genotype SKAB-01 (5.63 per cent). The average reducing sugar content observed among selected genotypes was 7.13 per cent.

4.2.4.2 Non-Reducing sugars (per cent)

The data presented in Table 19 revealed that non -reducing sugar content ranges 3.20 per cent (SKAB-01) to 4.68 per cent (SKJAD-30). The maximum non -reducing sugar content was observed in genotypes SKJAD-30 and SKJAC -50 (4.68 per cent) followed by SKJAG -15 (4.66 per cent) and SKJAC -49 (4.62 per cent).The minimum reducing sugars was recorded in the genotype SKJAB-01 (3.20 per cent). The average reducing sugar content observed among selected genotypes was 4.05 per cent.

4.2.5 Pectin content (per cent)

The data presented in the Table 19 revealed that pectin content of *Ambri* apple genotypes ranged from 1.32 per cent (SKJAN -38) to 1.00 per cent (SKJAG -17). The maximum pectin content was observed in the genotype SKJAN-38 (1.32 per cent) followed by SKJAN -37 (1.31 per cent), SKJAN -39 (1.31 per cent) and SKJAN -42 (1.30 per cent). Minimum pectin content (1.00 per cent) was recorded in genotypes SKJAG-17

Table 19: Chemical characters of various fruit traits in Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

S. No.	Genotype number	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Total soluble solids (^{0}Brix)	Titrable acidity (%)	Ascorbic acid (mg/100g)	Sugars (%)	Reducing sugar(%)	Non reducing sugar(%)	Pectin (%)	TSS/Acid ratio	Phenolics (mg/100g)
1.	SKJAB - 01	225.15	5.87	6.46	15.31	1.17	2.67	9.00	5.63	3.37	1.08	13.08	50.38
2.	SKJAB - 02	217.34	5.90	7.16	15.20	1.14	2.62	9.92	6.20	3.72	1.05	13.33	49.47
3.	SKJAB - 03	222.73	5.94	6.69	15.31	1.1	2.61	9.94	6.22	3.72	1.05	13.91	48.34
4.	SKJAB - 04	231.18	5.80	7.31	15.60	0.41	2.3	9.99	6.25	3.74	1.18	38.04	62.89
5.	SKJAB - 05	218.11	5.90	6.55	13.00	0.85	2.56	10.11	6.32	3.79	1.08	15.29	50.18
6.	SKJAT - 06	229.43	5.88	6.41	13.17	0.88	2.62	10.19	6.37	3.82	1.05	14.96	51.42
7.	SKJAT - 07	233.14	5.92	7.10	14.10	0.89	2.63	10.38	6.49	3.89	1.05	15.84	54.06
8.	SKJAT - 08	242.27	5.88	6.70	14.17	0.90	2.64	11.1	6.94	4.16	1.04	15.74	55.27
9.	SKJAT - 09	247.45	5.89	6.66	14.37	0.92	2.59	11.22	7.02	4.20	1.10	15.61	60.87
10.	SKJAG - 10	239.21	5.90	6.81	15.33	0.86	2.62	11.53	7.21	4.32	1.06	17.82	62.27
11.	SKJAG - 11	228.48	5.93	6.69	15.3	0.85	2.62	11.81	7.38	4.42	1.06	18.00	54.06
12.	SKJAG - 12	255.21	5.88	6.53	15.35	0.87	2.59	12.23	7.65	4.58	1.07	17.64	54.17
13.	SKJAG - 13	259.32	5.86	7.42	15.55	0.88	2.57	12.73	7.96	4.77	1.06	17.44	53.25
14.	SKJAG - 14	237.45	5.84	7.12	15.30	0.86	2.53	12.97	8.11	4.86	1.05	17.79	52.34
15.	SKJAG - 15	227.37	5.85	6.44	15.27	0.81	2.59	13.12	8.20	4.91	1.06	18.85	51.42
16.	SKJAG - 16	243.11	5.90	6.02	15.30	0.84	2.17	9.91	6.20	3.71	1.10	18.21	50.18
17.	SKJAG - 17	273.32	5.88	6.23	15.75	1.72	2.1	9.96	6.23	3.73	1.00	9.15	53.54
18.	SKJABh - 18	158.21	4.35	4.62	12.57	1.67	2.15	10.12	6.33	3.79	1.08	7.52	47.24
19.	SKJABh - 19	255.58	5.94	6.22	15.79	1.64	2.11	10.22	6.39	3.83	1.00	9.45	43.59

20.	SKJABh - 20	227.45	5.84	6.13	15.72	1.70	2.13	10.41	6.50	3.90	1.15	9.24	62.14
21.	SKJAK - 21	212.11	5.86	5.98	15.33	1.80	2.38	11.30	7.07	4.23	1.14	8.51	60.21
22.	SKJAK - 22	200.11	5.82	7.28	15.31	0.31	2.33	11.39	7.12	4.27	1.16	49.38	57.35
23.	SKJAK - 23	218.21	5.81	7.03	15.55	0.37	2.3	11.64	7.28	4.36	1.17	42.02	59.31
24.	SKJAK - 24	213.45	5.81	7.03	15.45	0.36	2.31	11.83	7.40	4.43	1.17	42.91	45.48
25	SKJAK - 25	256	5.80	6.96	15.75	1.15	2.29	11.95	7.47	4.48	1.17	13.69	55.35
26	SKJAK - 26	214.79	5.80	7.13	15.55	0.37	2.3	12.15	7.60	4.55	1.18	42.02	61.35
27	SKJAK - 27	231.18	5.80	7.31	15.60	0.41	2.3	12.45	7.78	4.67	1.18	38.04	62.89
28	SKJAD - 28	248.42	5.74	7.00	15.61	1.15	2.37	12.73	7.96	4.77	1.19	13.57	64.05
29	SKJAD - 29	292.23	6.37	7.62	15.35	0.80	2.59	12.45	7.78	4.66	1.09	8.52	46.72
30	SKJAD - 30	284.17	6.35	7.05	15.91	0.30	2.36	13.15	8.22	4.93	1.19	19.18	60.25
31	SKJAD - 31	238.41	5.73	5.79	15.60	0.33	2.35	9.89	6.18	3.70	1.19	47.27	57.32
32	SKJAD - 32	245.25	5.73	6.42	15.60	0.32	2.35	9.93	6.21	3.72	1.19	48.75	54.34
33	SKJAD - 33	200.51	4.68	4.81	15.32	0.35	3.33	9.98	6.24	3.74	1.08	43.77	51.38
34	SKJAD - 34	220.34	5.09	5.19	15.55	0.37	3.17	10.14	6.34	3.80	1.21	42.20	53.75
35	SKJAD - 35	237.31	5.17	5.50	15.60	1.14	3.11	10.29	6.43	3.85	1.23	13.68	55.35
36	SKJAD - 36	229.32	5.37	5.51	15.56	0.39	4.27	10.47	6.55	3.92	1.24	39.89	52.19
37	SKJAN - 37	194.21	4.62	4.95	14.31	0.81	4.28	10.82	6.77	4.05	1.31	17.66	47.29
38	SKJAN - 38	218.32	4.90	4.98	15.02	0.87	4.24	11.4	7.13	4.27	1.32	17.26	45.37
39	SKJAN - 39	224.34	5.11	5.21	15.02	0.87	4.81	11.62	7.27	4.35	1.31	17.26	52.58
40	SKJAN - 40	229.32	5.11	5.37	15.07	0.88	4.8	11.87	7.42	4.45	1.19	17.12	57.35
41	SKJAN - 41	237.39	5.41	5.45	15.10	1.10	4.11	11.99	7.50	4.49	1.18	13.73	50.37

42	SKJAN - 42	234.15	5.42	5.46	15.07	0.99	2.42	12.11	7.57	4.54	1.3	15.22	55.45
43	SKJAM- 43	267.21	5.73	6.48	15.47	1.11	2.43	12.29	7.68	4.60	1.3	13.93	55.25
44	SKJAM- 44	254.39	5.99.	6.49	15.30	1.02	2.41	12.44	7.78	4.66	1.04	15	50.15
45	SKJAM- 45	234.41	6.37	6.56	15.10	1.10	2.41	12.65	7.91	4.73	1.03	13.72	51.44
46	SKJAM - 46	197.34	5.37	5.69	14.37	0.81	2.42	12.78	7.99	4.79	1.04	17.74	54.09
47	SKJAM - 47	161.11	4.78	4.94	13.17	0.74	3.91	12.89	8.06	4.83	1.04	17.79	59.43
48	SKJAC - 48	203.32	5.03	5.58	14.37	0.81	3.82	12.97	8.11	4.86	1.15	17.74	57.43
49	SKJAC - 49	190.11	4.90	5.00	14.16	0.77	2.63	13.00	8.13	4.87	1.1	18.38	48.87
50	SKJAC - 50	187.13	4.92	5.09	13.17	0.77	2.62	13.15	8.22	4.93	1.01	17.1	54.32
General mean		228.93	5.60	6.22	15.01	0.88	2.77	11.46	7.99	4.19	1.12	22.02	54.31
SD		26.78	0.46	0.81	0.86	0.39	0.71	01.15	0.09	0.04	0.08	10.52	05.19
CV		11.74	8.21	13.02	5.72	44.31	25.92	9.57	6.54	7.72	7.9	47.77	9.6

Table 20: Range, Mean, Standard Deviation and Coefficient of variation of Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

Character	Range	Mean	Standard deviation	Coefficient of variation (%)
Fruit weight (g)	158.21-292.23	228.93	26.78	11.74
Fruit length (cm)	4.35-6.37	5.60	0.46	8.21
Fruit width (cm)	4.62-7.62	6.22	0.81	13.02
Total Soluble Solids (^{0}Brix)	13-15.91	15.01	0.86	5.72
Titrable acidity (%)	0.30-1.7	0.88	0.39	44.31
Ascorbic acid (mg/100g))	2.10-4.80	2.77	0.71	25.92
Reducing sugar (%)	6.18-8.22	7.99	7.99	6.54
Non reducing sugar (%)	3.70-4.93	4.19	4.19	7.72
Sugars (%)	9.89-13.15	11.46	1.15831	9.57
Pectin (%)	1.04-1.19	1.12	0.08975	7.90
TSS/Acid ratio	8.83-41.93	22.02	10.52	47.77
Phenolics (mg/100g)	46.72-64.05	54.31	5.19	9.60

and SKJABh-19. The average pectin content observed among selected genotypes was 1.12 per cent.

4.2.6 Phenolics (mg/100g)

Data presented in the Table 19 revealed that phenolics content ranges from 43.59 mg/100g in genotype (SKJABh-19) - 64.05 mg/100g in genotype (SKJAD-28). The maximum phenolics content was observed in genotype SKJAD-28 (64.05 mg/100g), followed by SKJAB -04 (62. 89 per cent), SKJAK -27 (62. 89 per cent) and SKJAG -10 (62.27 per cent). While minimum phenolics (43.59 mg/100g) was observed in genotype SKJABh-19. The average phenolics content observed among selected genotypes was 54.31 per cent.

4.2.7 TSS/Acid ratio

Perusal of data in Table 19 revealed that maximum (49.38) TSS/Acid ratio was observed in genotype SKJAK- 22 while minimum TSS/Acid ratio (8.52) was observed in genotype SKJAD-29. The average TSS/Acid ratio content observed among selected genotypes was 22.02per cent.

4.3 Analysis of Variance:

Analysis of variance was done in physico-chemical fruit traits and it was observed that coefficient of variance varies from 11.74 per cent in fruit weight, 8.21 per cent in fruit length, 13.02 per cent fruit width and 5.72 per cent in TSS respectively as shown in Table 20.

4.4 Cluster Analysis:

Biochemical data was classified into 4 main clusters i.e., cluster I, cluster II, cluster III and cluster IV as depicted in Figure 7. Cluster I contains 34 genotypes namely (SKJAB -01, SKJAB -02, SKJAB -03, SKJAB -04, SKJAB -05, SKJAT -06, SKJAT -07, SKJAT -08, SKJAT -09, SKJAG -10, SKJAG -11, SKJAG -14, SKJAG -15, SKJAG -16, SKJABh -20, SKJAK -21, SKJAK -22, SKJAK -23, SKJAK -24, SKJAK -26, SKJAK -27, SKJAD -28, SKJAD -31, SKJAD -32, SKJAD -33, SKJAD -34, SKJAD -35, SKJAD

-36, SKJAN -38, SKJAN -39, SKJAN -40, SKJAN -41, SKJAN -42, SKJAN -45), cluster II contains 8 genotypes namely (SKJAG -12, SKJAG -13, SKJAG -17,SKJAK -25, SKJAD -29, SKJAD -30, SKJAM-43, SKJAM-44), cluster III contains 7 genotypes namely (SKJABh -18, SKJAN -37, SKJAM-46, SKJAM-47, SKJAC-48, SKJAC-49, SKJAC-50) and cluster IV contains 1 genotype (SKJABh -19) respectively. Clustering pattern of morphological data of fifty *Ambri* apple genotypes for twenty six characters is presented in Table 21. Based on the clustering pattern of biochemical parameters the intra cluster distances ranged from 0.00 (cluster IV) to 17.61 (cluster I) as presented in Table 22. The maximum intra cluster distance was exhibited by cluster I (17.61) followed by cluster II (12.77). The minimum intra cluster distance was observed in cluster IV (0) which contain only one genotype. The inter cluster distance ranged from 38.96 to 80.20. The maximum inter cluster distance was observed between cluster II and cluster III.

4.5 Principle Component Analysis:

The proportion of the total biochemical variation accounted for by each PC was calculated from the Eigen values (>1) (Table 23 and Fig. 8). The four components with Eigen values are able to explain the more than 76.00 per cent of total variation. PC1, PC2, PC3, and PC4 accounted for 32.61 per cent, 18.17 per cent, 13.97 per cent and 11.98 per cent. In particular the first component is positively and strongly associated with fruit weight, fruit length, fruit width, TSS, titratable acidity, sugars and phenolics but negatively associated with ascorbic acid, pectin and TSS/ACID ratio. Second component second is positively and strongly associated fruit weight, fruit length, fruit width, TSS, ascorbic acid, sugars, pectin, TSS/Acid ratio, Phenolics but negatively associated with fruit length and titratable acidity. Third component is positively and strongly associated with fruit width, sugars, TSS/Acid ratio, Phenolics but negatively associated with fruit weight, fruit length, TSS, titratable acidity, ascorbic acid and pectin. Fourth component is positively and strongly associated with TSS, titratable acidity, pectin, TSS/Acid ratio but negatively associated with fruit weight, fruit length, fruit width, ascorbic acid, sugars and Phenolics. Loading plots of PC as shown in Figure 8 clearly explains the variability towards the *Ambri* apple. The individual contribution towards variability of *Ambri* apple through biochemical and genotypes i.e., 32.62 per cent and 18.17 per cent respectively.

Table 21: Clustering pattern of fifty Ambri apple (*Malus* × *domestica* Borkh.) genotypes based on biochemical parameters.

Cluster	Number of genotypes	Genotypes
I	34	SKJAB -01, SKJAB -02, SKJAB -03, SKJAB-04, SKJAB -05, SKJAT -06, SKJAT -07, SKJAT -08, SKJAT -09, SKJAG -10, SKJAG -11, SKJAG -14, SKJAG -15, SKJAG -16, SKJABh -20, SKJAK -21, SKJAK -22, SKJAK -23, SKJAK -24, SKJAK -26, SKJAK -27, SKJAD -28, SKJAD -31, SKJAD -32, SKJAD -33, SKJAD -34, SKJAD -35, SKJAD -36, SKJAN -38, SKJAN -39, SKJAN -40, SKJAN -41, SKJAN -42, SKJAN -45
II	8	SKJAG-12, SKJAG-13, SKJAG-17, SKJAAK -25, SKJAAD -29, SKJAAD -30, SKJAAM-43, SKJAAM-44
III	7	SKJABh-18, SKJAN-37, SKJAM-46, SKJAM-47, SKJAC-48, SKJAC-49, SKJAC-50
IV	1	SKJABh -19

Table 22: Average intra and inter-cluster distances for three clusters in Ambri apple (*Malus* × *domestica* Borkh.) genotypes for biochemical parameters

Cluster number	I	II	III	IV
I	17.6145	38.9679	41.4804	41.6933
II		12.7726	80.2033	37.7634
III			16.6196	75.9228
IV				0

Note: On diagonal value intra cluster distance and above diagonal inter cluster distance

Table 23: Principle component analysis for contribution of different bio-chemical parameters towards variability

S.No.	Parameters	Principle component			
		1	**2**	**3**	**4**
	Eigenvalue	3.2619	1.8174	1.3980	1.1989
	Variability (%)	32.6195	18.1738	13.9799	11.9890
	Cumulative (%)	32.6195	50.7933	64.7732	76.7622
1.	Fruit weight (g)	0.7847	0.0585	-0.5102	-0.1054
2.	Fruit length (cm)	0.9465	-0.1035	-0.0195	-0.0295
3.	Fruit width (cm)	0.8945	0.0519	0.1636	-0.0405
4.	Total soluble solids (^{o}Brix)	0.5913	0.4807	-0.3497	0.3007
5.	Titrable acidity (%)	0.0164	-0.6234	-0.1284	0.0973
6.	Ascorbic acid (mg/100g)	-0.6654	0.2976	-0.4483	-0.2218
7.	Sugars (%)	0.0235	0.3870	0.4049	-0.6618
8.	Pectin (%)	-0.2628	0.7296	-0.4285	0.1246
9.	TSS/Acid ratio	-0.1238	0.3115	0.4752	0.7495
10.	Phenolics (mg/100g)	0.2696	0.5596	0.4445	-0.1446

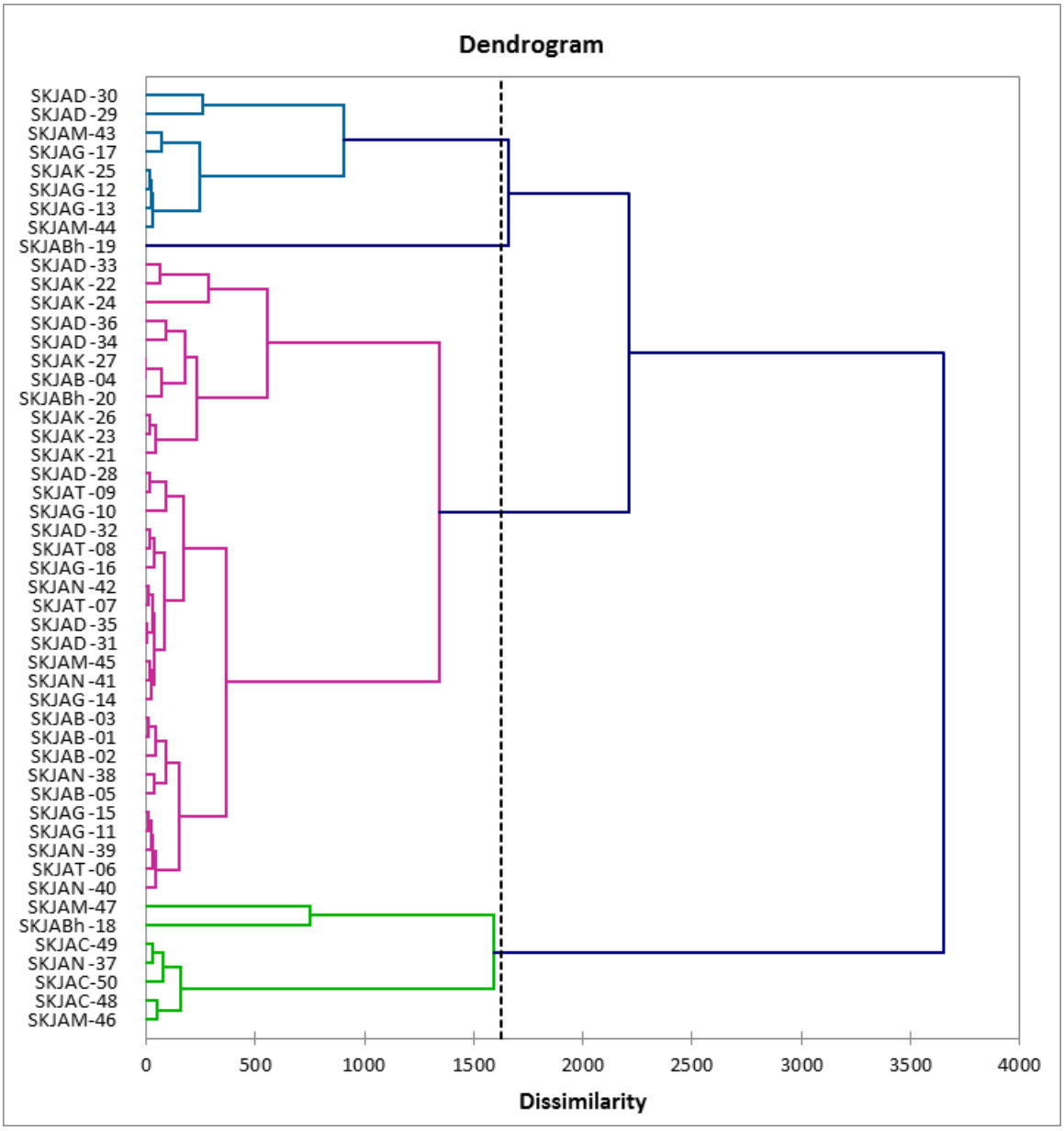
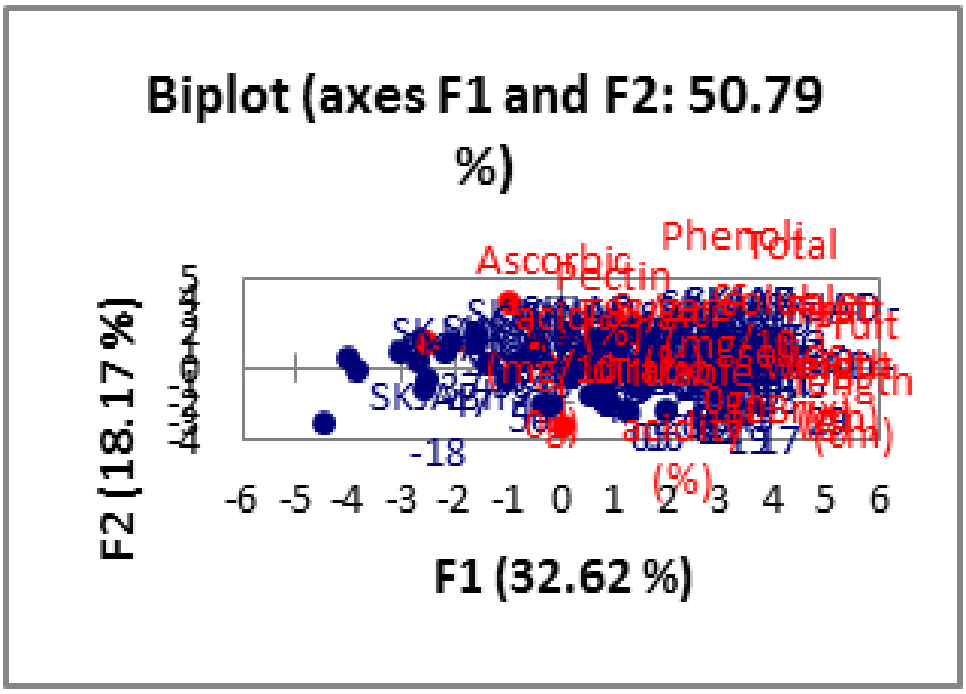


Fig. 7: Dendrogram showing clustering pattern of fifty Ambri apple (*Malus* × *domestics* Borkh.) genotypes based on biochemical parameters.



(C)

Fig. 8: Loading plots for biochemical parameters and different genotypes of Ambri apple (*Malus* × *domestics* Borkh.)

Cumulative variability as shown in Figure 8 by both biochemical and genotypes (as a parameter) towards the *Ambri* apple is 32.62 per cent respectively. No transformations were used because the data was already following normal distribution. The statistical functions were performed using SPSS version 23.0 (IBM Corp 2015).

4.6 Molecular characterization

The present study on "Morphological, Biochemical and Molecular Characterization of *Ambri* apple in Doda and Kishtwar districts of J&K" was carried out to determine genetic diversity among seedling origin *Ambri* apple genotypes. A total of fifty *Ambri* apple genotypes were selected for present investigation. Genomic DNA isolation followed by quantification of all fifty genotypes of *Ambri* apple. The DNA samples were further subjected to PCR amplification using twenty nine SSR markers. The scored data based on polymorphism was subjected to cluster analysis. The quantification of DNA of fifty *Ambri* apple genotypes was done and presented in Table 24. The quantification of DNA was performed with the help of spectrophotometer and the quantity of DNA (ug/ml) was calculated. The quantity of DNA obtained through this process varied from 23.00 ug/ml in genotype SKJAB -05 and 989.7 ug/ml in genotype SKJAC -49 respectively.

4.6.1 SSR analysis

A total number of twenty nine SSR primer pairs were used to evaluate the genetic diversity of fifty *Ambri* apple genotypes Table 2. Out of twenty nine SSR markers, seventeen revealed clear and consistent amplification profile in the entire genotype set. A total of 137 alleles were amplified and the number of alleles ranged from 2-10 with an average of 4.72 alleles per locus.

4.6.2 Similarity coefficient

The fifty genotype presented multi way similarity coefficient values ranging from 0.14 to 0.74. The highest similarity coefficient value (0.74) was observed between genotype SKJAN-39 and SKJAN-40, while lowest coefficient value (0.14) was observed between genotype SKJAG-12 and SKJABh-20. Genotype SKJABh- 19 and SKJAM-45 also observed coefficient value (0.14) as presented in Appendix I.

4.6.3 Principle co-ordinate analysis

The fifty genotypes were subjected to principle co-ordinate analysis presented in Figure 10. The values of principle co-ordinate analysis ranging from -0.40 to 0.32 and -0.24 to 0.32in in co-ordinate 1 and co-ordinate 2 respectively. The highest dissimilarity coefficient value was -0.32 to 0.32 observed between genotypes SKJABh-18, SKJAM-45 and same -0.47 to 0.32 was observed between SKJAM-47, SKJAG-14 while the lowest dissimilarity value (-0.16 to -0.16) was observed in SKJAD-31, SKJAD-36, SKJAN-39 and SKJAN-37 as shown in Figure .

4.6.4 Cluster analysis

The allelic diversity data was used to produce a dendrogram by using a distance matrix by UPGMA method that revealed the genetic relationship among all selected *Ambri* apple genotypes. (Figure 9). The UPGMA dendrogram classified all fifty apple genotypes into 2 major clusters i.e., cluster I and cluster II with further sub clusters. The cluster I contained of 27 genotypes namely (SKJAB-01, SKJAB-02, SKJAB-05, SKJAT-06, SKJAG-11, SKJAG-10, SKJAT-09, SKJAT-08, SKJAT-07, SKJAB-04, SKJAB-03, SKJABh-19, SKJABh-18, SKJAG-17, SKJAAG-15, SKJAG-16, SKJAG-14, SKJAG-13, SKJAK-26, SKJAK-27, SKJAK-25, SKJAK-24, SKJAK-23, SKJAK-22, SKJAK-21, SKJABh-20 and SKJAG-12) and cluster II contained 23 genotypes namely (SKJAC-50, SKJAC-49, SKJAM-48, SKJAM-47, SKJAM-46, SKJAM-45, SKJAM-44, SKJAM-43, SKJAN-42, SKJAN-41, SKJAN-40, SKJAN-39, SKJAN-38, SKJAN-37, SKJAD-35, SKJAD-34, SKJAD-33, SKJAD-36, SKJAD-32, SKJAD-31, SKJAD-30, SKJAD-29 and SKJAD-28). Cluster I was further divided into two sub clusters contained (8) and (19) genotypes respectively. While as cluster II is also divided into two sub cluster contained (9) and (14) genotypes respectively.

4.6.5 Polymorphism of SSR primers

A total of 137 alleles were detected across the fifty genotypes through the use of these twenty nine SSR markers Table 25 each of loci differed significantly in their ability to determine variability among the genotypes.

Table 24: Quantification of DNA of Ambri apple (*Malus × domestica Borkh*.) genotypes.

Sample No.	Quantity of DNA (ug/ml)
1.	366.5
2.	437.0
3.	279.0
4.	254.2
5.	23.7
6.	49.2
7.	105.5
8.	37.0
9.	147.5
10.	243.7
11.	131.1
12.	274.1
13.	552.2
14.	927.9
15.	744.6
16.	695.3
17.	681.5
18.	199.3
19.	176.6
20.	224.2
21.	138.1
22.	302.5
23.	117.6
24.	74.8
25.	271.7
26.	255.0
27.	191.4
28.	866.8
29.	110.5
30.	139.0
31.	223.5
32.	230.2
33.	775.6
34.	774.1
35.	729.1
36.	850.9
37.	605.5
38.	680.3
39.	653.3

40.	212.3
41.	127.9
42.	305.5
43.	840.0
44.	307.6
45.	91.5
46.	663.0
47.	213.2
48.	365.9
49.	989.7
50.	693.5

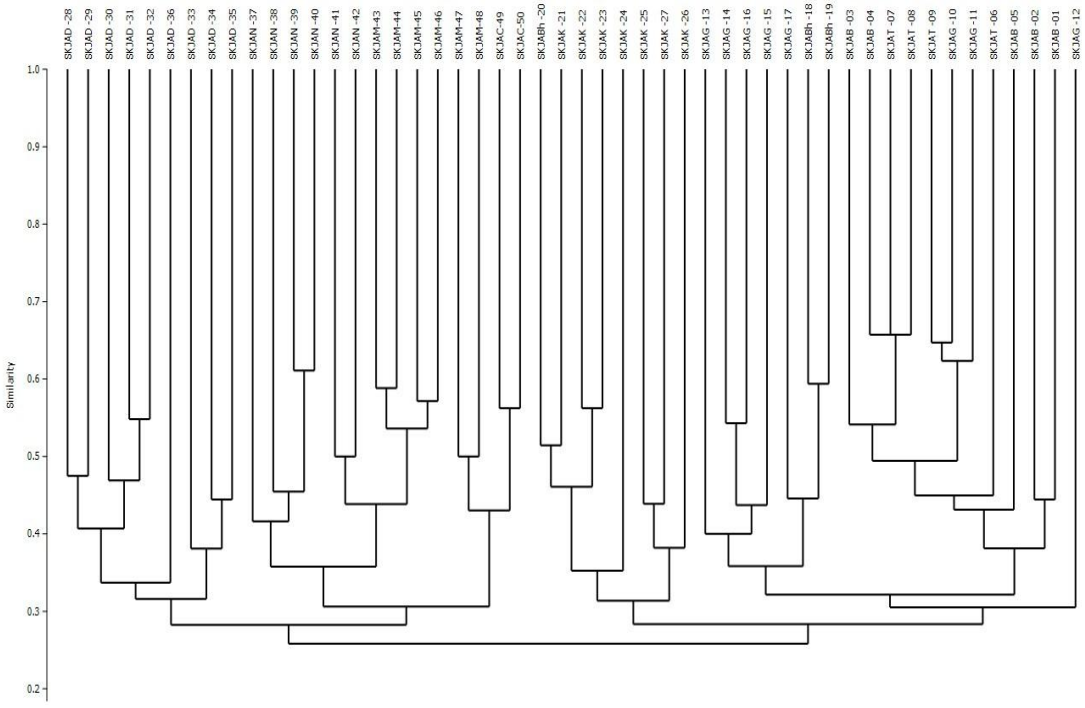


Fig. 9: Dendrogram showing clustering pattern of fifty Ambri apple (*Malus* × *domestics* Borkh.) genotypes using UPGMA analysis based on SSR genotyping.

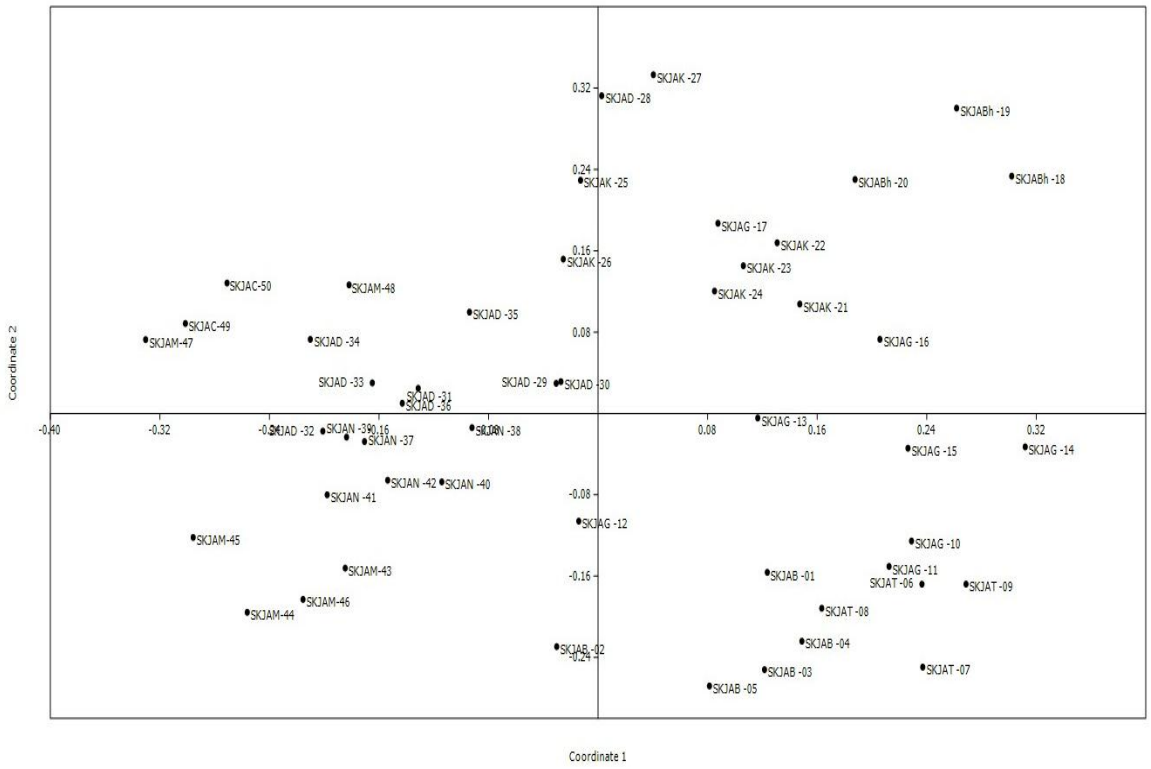


Fig. 10: Principle Co-ordinate Analysis showing fifty Ambri Apple (*Malus* × *domestics* Borkh.) genotypes

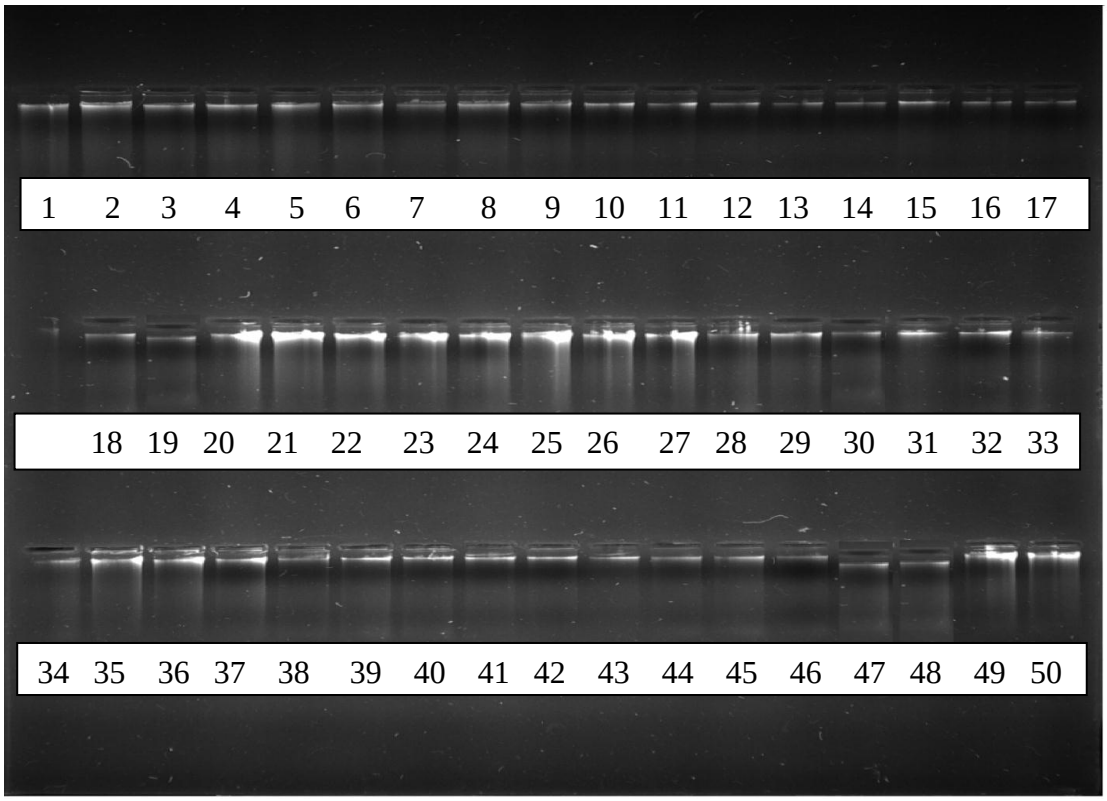


Plate 4: Agarose gel image of DNA of Ambri apple (*Malus* × *domestica* Borkh.) 1 to 50 genotypes.

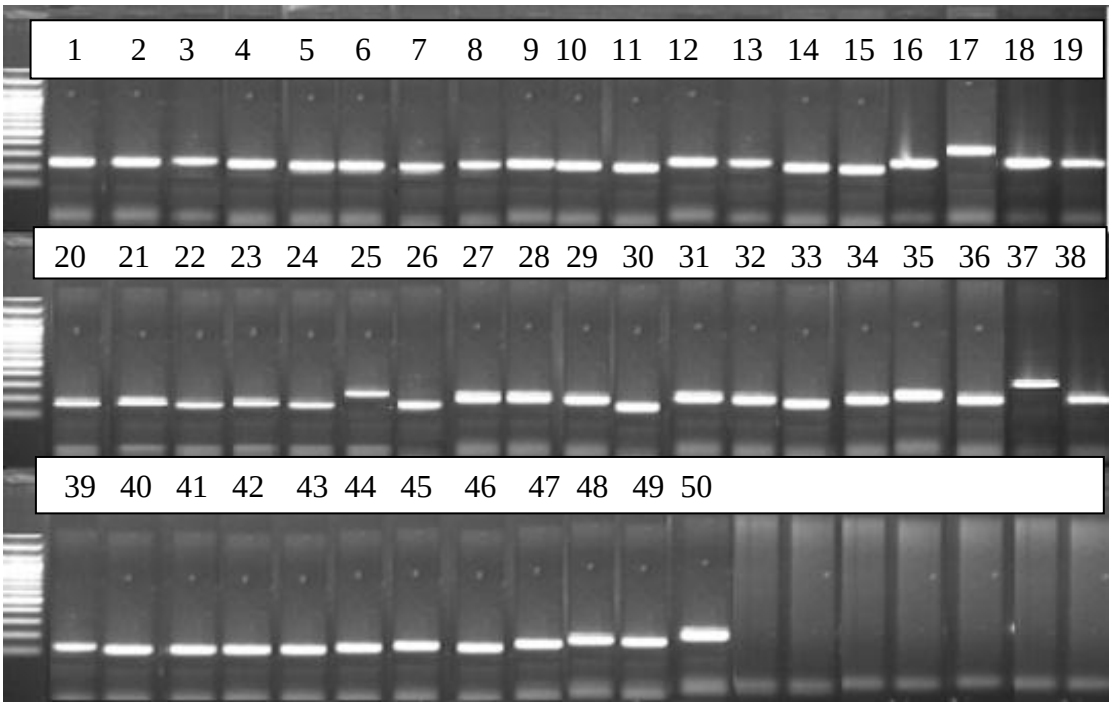


Plate 5: SSR based DNA amplification pattern of Ambri apple germplasm with primer CH05d11 and 100 bp DNA ladder, whereas 1 to 50 represent 50 Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

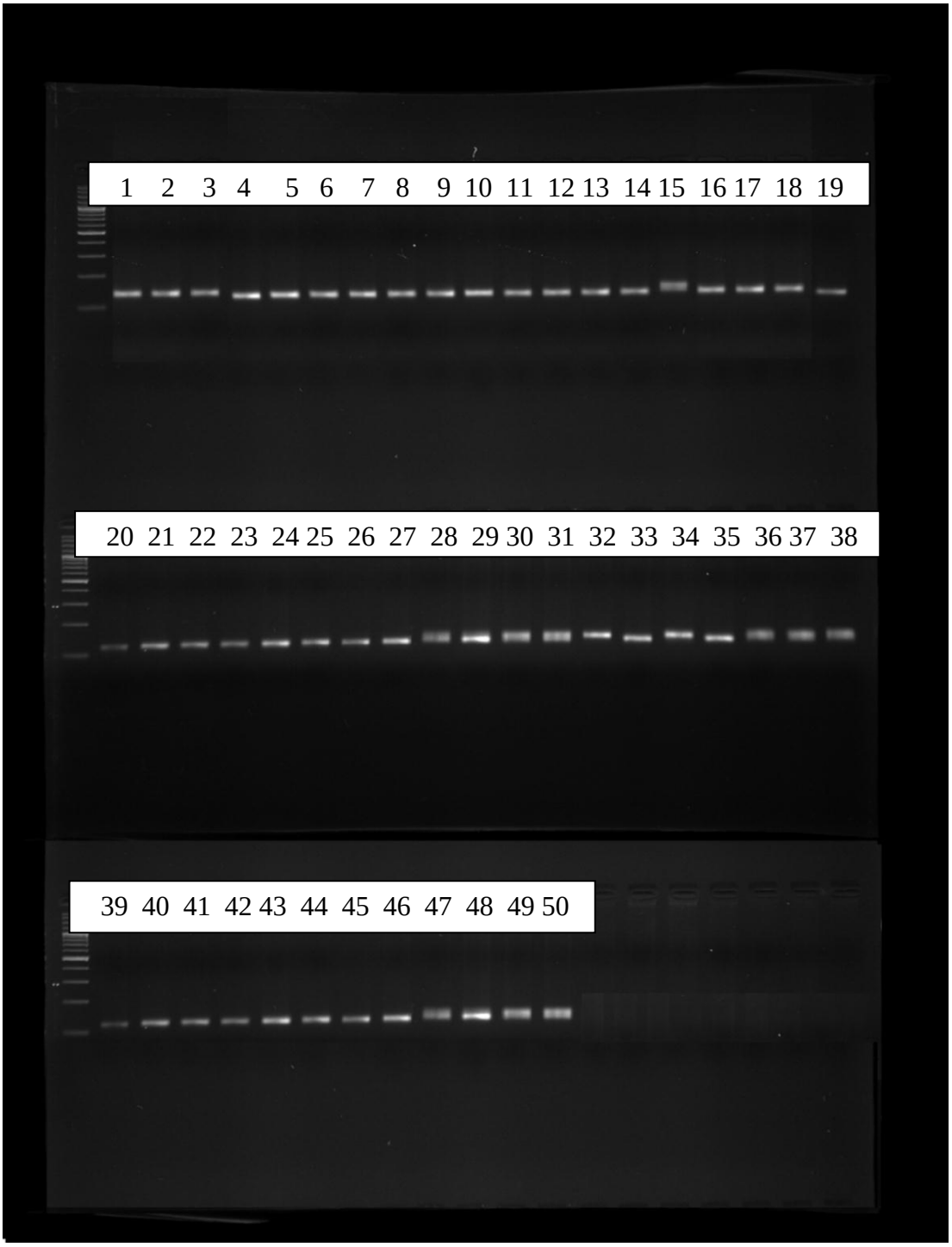


Plate 6: SSR based DNA amplification pattern of Ambri apple germplasm with primer Hi02d04 and 100 bp DNA ladder, whereas 1 to 50 represent 50 Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

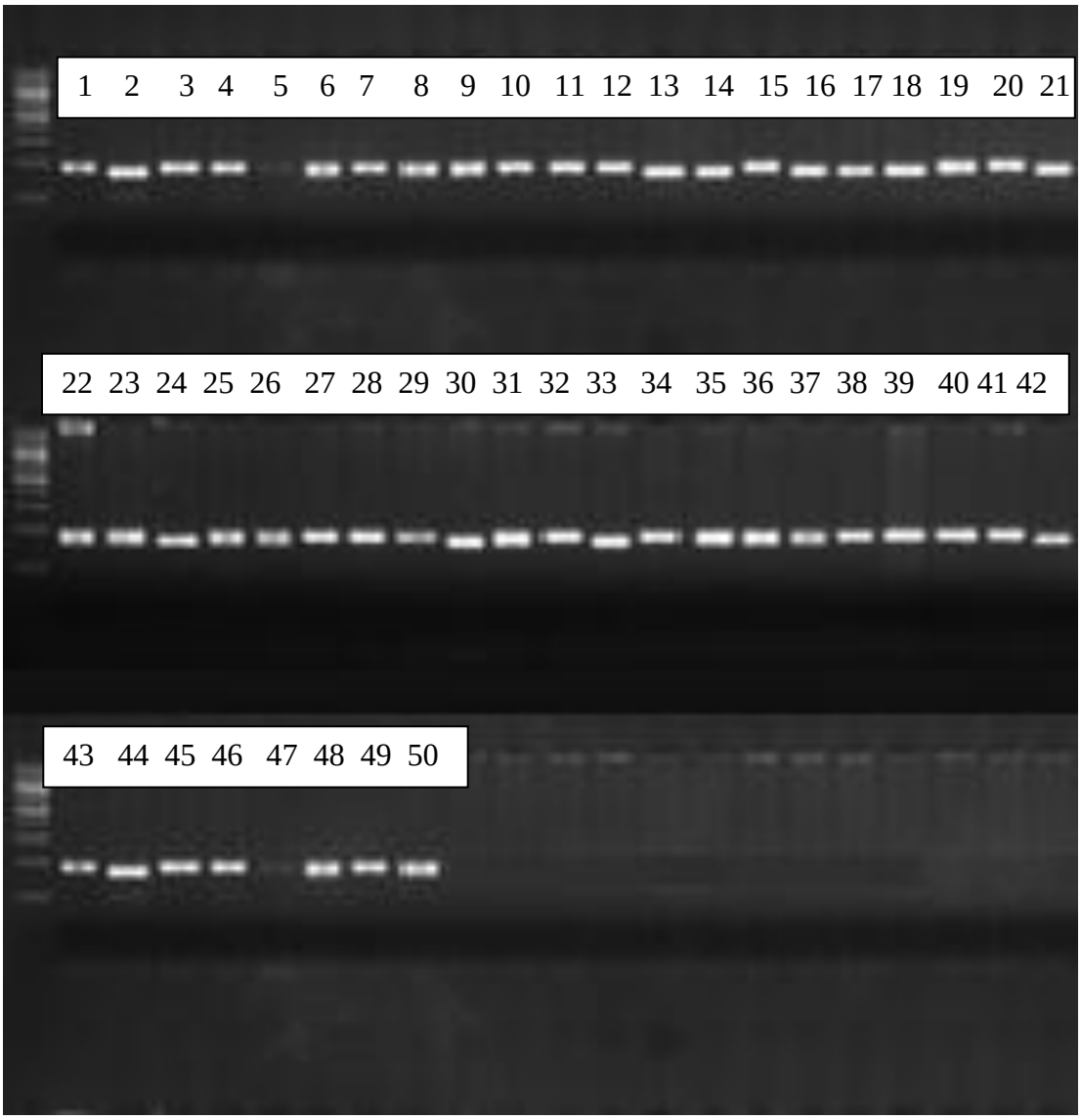


Plate 7: SSR based DNA amplification pattern of Ambri apple germplasm with primer CH03e03 and 100 bp DNA ladder, whereas 1 to 50 represent 50 Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

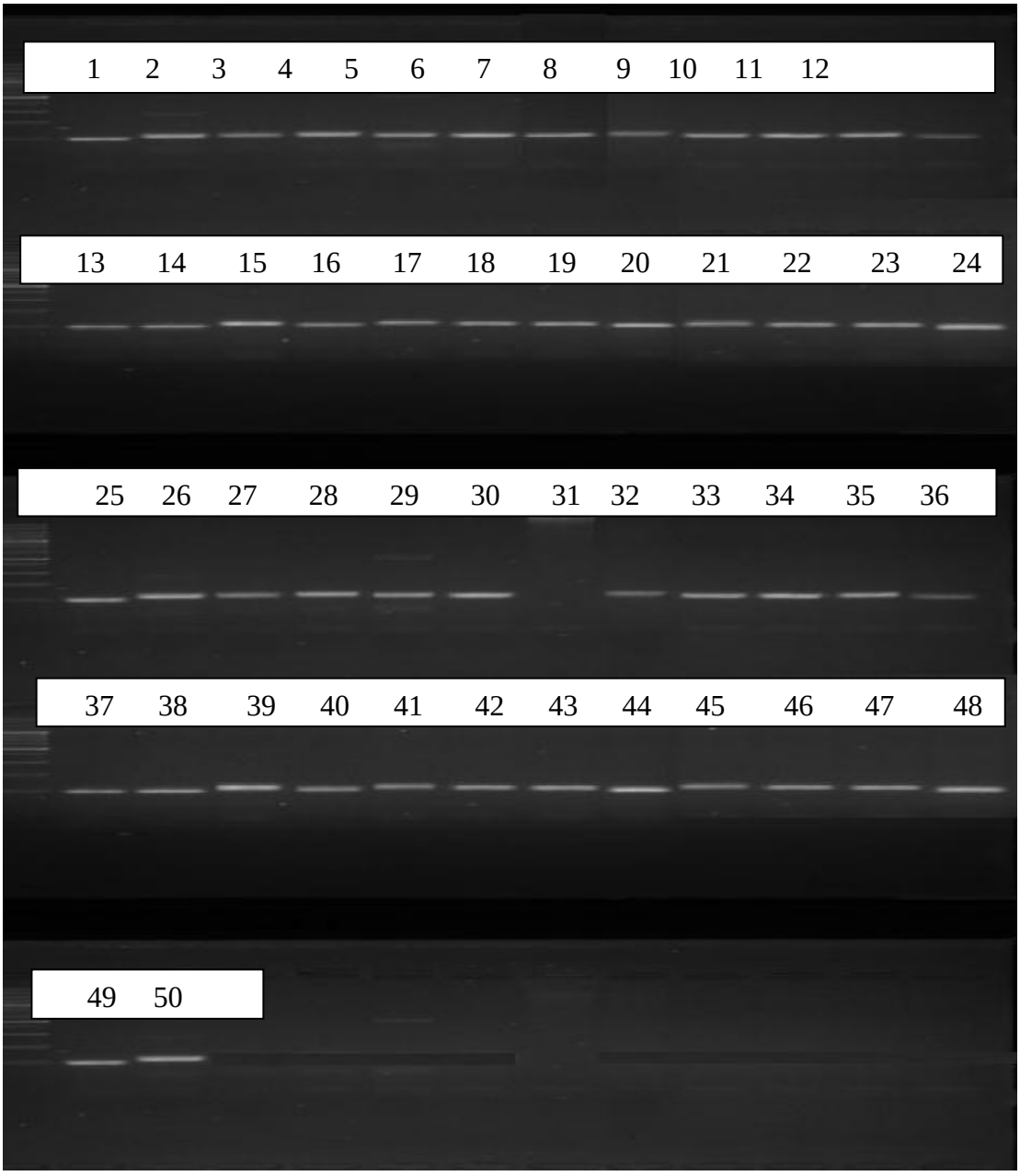


Plate 8: SSR based DNA amplification pattern of Ambri apple germplasm with primer CH02c02 and 100 bp DNA ladder, whereas 1 to 50 represent 50 Ambri apple (*Malus* × *domestica* Borkh.) genotypes.

4.6.6 PIC (Polymorphism Information Content)

Polymorphism information content (PIC) value is reflection of allelic diversity and frequency among the genotypes, any value exceeding 0.5 reflects polymorphism. The PIC values provide an estimate of the discriminating power of a marker by taking into account not only the no of alleles at a locus but also relative frequencies of those alleles in the genotypes. The PIC value varied from 0.02 (CH03c02) to 0.8 (CH05d11) presented in Table 25. Maximum PIC value (0.8) was observed in primer CH05d11and Hi03a03 while minimum PIC value (0.02) was observed in primer (CH03c02).

4.6.7 PP (Polymorphism percentage)

Polymorphism percentage among fifty *Ambri* apple genotypes are presented in Table 25 revealed that the highest polymorphic percentage (62.50) was observed in primer CH04g10 while lowest polymorphic percentage (14.20) was observed in primer CH03d12.

4.6.8 No. of alleles per locus

A total of 137 alleles were amplified by 29 polymorphic SSR loci and the number of allels ranged from 2-10 with an average of 4.72 alleles per locus presented in Table 25.

4.7 Comparison of promising seedling genotypes of *Ambri* apple with commercial cultivar

4.7.1 Morphological characters

The tree and leaf characteristics *viz.*, type of plating material, tree height, trunk girth, tree spread, tree habit, tree vigour were recorded on visual observations and are given in Table 26.

4.5.1.1 Tree height

The tree height of genotype SKJAD-29 and SKJAD-30 was observed 7.74 m and 7.62 m and height of the Red Delicious was observed 6.00 m respectively presented in Table 26.

4.5.1.2 Trunk girth

Trunk girth was observed 191.29 cm and 160.45 cm in genotype SKJAD-29 and SKJAAD-30. While it was observed 195.45 cm in Red Delicious showed in Table 26. .

4.5.1.3 Tree spread

Tree spread was observed 5.46 m in genotype (SKJAD-29) and 5.60 m in genotype (SKJAD-30). Red Delicious showed tree spread of (5.27 m) showed in Table 26.

4.5.1.4 Tree habit

Tree habit was observed upright in genotypes SKJAD-29 and spreading SKJAAD-30 whereas, it was upright in Red Delicious.

4.5.1.5 Tree vigour

Tree vigour was observed intermediate in genotype SKJAD-29 whereas, it was vigorous in genotype SKJAD-30. Red Delicious had intermediate tree vigour.

4.5.2 Leaf characters

The observations regarding various leaf characteristics *viz.*, leaf size and leaf shapes are presented in Table 26.

4.5.2.1 Leaf size

Leaf size was large in genotypesSKJAD-29 and SKJAD-30 while it was observed medium in Red Delicious.

4.5.2.2 Leaf shape

Leaf shape was observed oval in genotypes SKJAD-29 and broad elliptical in genotype SKJAD-30, while it was observed oval in Red Delicious.

4.5.3 Flower characters

The observations regarding flower characteristics are presented in Table 27.

Table 25: PIC value, polymorphic percentage and alleles per locus of SSR primers.

S.No.	Primer	PIC (Polymorphism Information Content) value	PP (Polymorphic Percentage)	Allele per locus
1.	CH03g07	0.49	28.57	7
2.	CH04e03	0.50	50.00	6
3.	CH04g10	0.40	62.50	8
4.	CH05c02	0.50	20.30	5
5.	CH05d11	0.80	40.00	10
6.	CH05e03	0.70	20.00	5
7.	CH02h11a	0.60	33.33	3
8.	CH03d12	0.70	14.20	7
9.	CH03e03	0.70	33.31	9
10.	CH03g12z	0.04	50.00	2
11.	CH04a12	0.11	33.60	3
12.	CH05d04	0.03	50.50	2
13.	CH05e03	0.07	50.50	2
14.	Hi01d06y	0.18	50.50	2
15.	Hi02d04	0.76	33.33	3
16.	Hi03a03	0.21	25.00	4
17.	Hi03e03	0.71	33.30	3
18.	CH03c02	0.02	25.01	4
19.	Hi02b10	0.60	40.00	5
20.	Hi02d04	0.70	25.00	5
21.	CH05c07	0.62	33.38	3
22.	CH03a02	0.52	33.35	3
23.	CH02b12	0.33	60.00	5
24.	Hi03e03	0.23	33.60	3
25.	CN444636	0.20	25.00	4
26.	CH05g07	0.50	40.00	5
27.	CH02c02	0.61	42.85	7
28.	Hi03a03	0.80	25.00	8
29.	CN444542	0.61	25.00	4
	Range	0.02-0.8 (Av 0.45)	14.20-62.50 (Av 35.76)	2-10 (Av 4.72)

Table 26: Comparison of tree and leaf characters of promising genotypes of Ambri apple with Red Delicious.

S.No.	Genotype number	Tree habit	Tree vigour	Tree height (m)	Trunk girth (cm)	Tree spread (m)	Leaf size	Leaf shape
1	SKJAD -29	3	7	7.74 m	191.29	5.46	7	3
2	SKJAD -30	5	7	7.62 m	160.45	5.60	7	3
3	Red Delicious	3	5	6.00 m	195.45	5.27	5	3

Legend

Type of planting material	Note	Tree habit	Note	Tree vigour	Note	Leaf size	Note	Leaf shape	Note
Seedling	1	Upright	3	Extremely weak	1	Small	3	Oval	1
		Spreading	5	Weak	3	Medium	5	Broad elliptic	3
		Dropping	7	Intermediate	5	Large	7	Others	99
				Vigrous	7				
				Very vigorous	9				

4.5.3.1 Number of flower bud per inflorescence

Number of flower bud per inflorescence was observed 6 in genotype (SKJAD-29) and 5 in genotype (SKJAD-30). It was observed 6 in Red Delicious apple showed in Table 27.

4.5.3.2 Flower stalk (pedicel) length

Flower stalk length was observed (11.5 mm) in genotype SKJAD-29 and (14.7 mm) in genotype SKJAD-30, while it was observed (14.2 mm) in Red Delicious apple.

4.5.3.3 Date of start of flowering

Date of start of flowering in genotype SKJAAD-29 and SKJAAD-30 were observed 16th of April while it was 9th of April in Red Delicious apple showed in Table 27.

4.5.3.4 Date of end of flowering

Date of end of flowering in genotypes SKJAD-29 and SKJAD-30 were observed 20th of April while it was observed 15th of April in Red Delicious apple showed in Table 27.

4.5.3.5 Regularity of flowering

Genotypes SKJAD-29 and SKJAD-30 were observed biennial flowering while same pattern i.e. biennial flowering was observed in Red Delicious apple showed in Table 28.

4.5.3.6 Self incompatibility

Self incompatibility was observed in genotype SKJAD-29 and SKJAD-30 and same was observed in Red Delicious apple showed in Table 28.

4.5.3.7 Bearing habit

Bearing habit of genotype SKJAD-29 and SKJAD-30 were observed on spurs while Red delicious was observed the same pattern of bearing habit i.e. spurs showed in Table 28.

4.5.3.8 Age of first bearing

Age of first bearing of genotype SKJAD-29 and SKJAD-30 were observed five years and six years while it was observed five years in Red Delicious apple showed in Table 28.

4.5.4 Fruit characters

The various fruit characteristics *viz.*, days to fruit harvest fruit shape, fruit base, fruit base cavity depth, fruit apex, fruit length, fruit width, fruit weight, fruit ground colour, fruit over colour, fruit skin lenticels, pulp texture, pulp taste, juiciness, productivity status, biotic stress susceptibility, days to fruit harvest, fruit shape, fruit base, fruit base cavity depth, fruit apex, fruit length, fruit width, fruit weight, fruit ground colour, fruit over colour, fruit skin lenticels, pulp texture, pulp taste, juiciness, productivity status, biotic stress susceptibility were recorded and presented in Table 29 and Table 30.

4.5.4.1 Days to fruit harvest

Days to fruit harvest of genotype SKJAD-29 and SKJAD-30 were observed 152-153 days, and 153-154 days. While it was observed 131-140 days in Red Delicious showed in Table 28.

4.5.4.2 Fruit shape

Globose fruit shape was observed in genotype SKJAD-29 and SKJAD-30 it was globose conical in Red Delicious apple showed in Table 29.

4.5.4.3 Fruit base

Genotypes SKJAD-29 and SKJAD-30 were observed broad base while Red Delicious apple was observed intermediate fruit base.

4.5.4.4 Fruit base cavity depth

Fruit base cavity depth of genotype SKJAD-29 and SKJAD-30 were observed shallow while Red Delicious was also observed shallow fruit base cavity depth showed in Table 29.

Table 27: Comparison of flower characters of promising genotypes of Ambri apple with Red Delicious

S.No.	Genotype number	No of flower buds per inflorescence	Flower stalk (pedicel) length (mm)	Date of start of flowering (dd/mm/yy)	Date of end of flowering (dd/mm/yy)
1	SKJAD -29	6	11.5	15th April, 2017	22nd April, 2017
2	SKJAD -30	5	14.7	16th April, 2017	20th April, 2017
3	Red Delicious	5	12.5	10th April, 2017	15th April, 2017

Table 28: Comparison of flower characters of promising genotypes Ambri apple with Red Delicious

S.No.	Genotype number	Regularity of flowering	Self Incompatibility	Bearing Habit	Age of first bearing (years)	Days to fruit harvest
1.	SKJAD -29	2	1	1	7	152-153
2.	SKJAD -30	2	1	1	6	153-154
3.	Red Delicious	1	1	**1**	5	131-140

Regularity of flowering	Note	Self incompatibility	Note	Bearing Habit	Note
Regular	1	Incompatible	1	On spurs	1
Biennial	2	Partially compatible	2	On shoot tips	2
Irregular	3	Compatible	3	On old shoots	3
				Mixed	4

4.5.4.5 Fruit apex

Grooved fruit apex was observed in genotypes SKJAD-29 and SKJAD-30 were observed grooved and same fruit apex was observed in Red Delicious.

4.5.4.6 Fruit length

Genotype SKJAD-29 observed fruit length (6.37 cm) and genotype SKJAD-30 were observed (6.35 cm) fruit length and while Red Delicious apple was observed (5.80 cm) fruit length showed in Table 29.

4.5.4.7 Fruit width

Fruit width of genotypes SKJAD-29 and SKJAD-30 were observed (7.62 cm) and (7.05 cm) respectively, while fruit width of Red Delicious was observed (6.31 cm).

4.5.4.8 Fruit weight

Fruit weight of genotypes SKJAD-29 and SKJAD-30 were observed (292.23 g) and (284.17 g) respectively, while fruit weight of Red Delicious was observed 231.18 g.

4.5.4.9 Fruit ground colour

Fruit ground colour was observed yellow in genotypes SKJAD-29 and SKJAD-30 while, fruit ground colour was observed greenish in Red Delicious.

4.5.4.10 Fruit over colour

Fruit over colour of genotypes SKJAD-29 and SKJAD-30 were observed pink in colour while fruit over colour in Red Delicious was observed red in colour.

4.5.4.11 Fruit skin lenticels

Low fruit skin lenticels were observed in genotypes SKJAD-29 and SKJAD-30. In Red Delicious also low fruit skin lenticels were observed.

4.5.4.12 Pulp texture

Genotypes SKJAD-29 and SKJAD-30 were observed soft pulp texture while same i.e. soft pulp texture was observed in Red Delicious presented in Table 30.

4.5.4.13 Pulp taste

Pulp taste of genotypes SKJAD-29 and SKJAD-30 were observed sweet. Red Delicious also showed sweet pulp taste presented in Table 30.

4.5.4.14 Juiciness

Juiciness of genotypes SKJAD-29 and SKJAD-30 were observed high. While medium juiciness was observed in Red Delicious showed in Table 30.

4.5.4.15 Productivity status

Productivity status of genotype SKJAD-29 was observed intermediate and in genotype SKJAD-30 was observed high while it was observed intermediate in Red Delicious showed in Table 30.

4.5.4.16 Biotic stress susceptibility

Biotic stress susceptibility of genotypes SKJAD-29 and SKJAD-30 were observed very low or no visible signs of susceptibility and same was observed in Red Delicious showed in Table 30.

4.5.5 Biochemical characters

Apart from morphological characters, the biochemical characters particularly TSS, acidity, ascorbic acid plays an important role in deciding the market value. Maximum TSS (15.91 ^{o}B) was observed in genotype SKJAD-30 which was followed by (15.35 ^{o}B) in genotype SKJAD-29. TSS was observed (15.11 ^{o}B) in Red Delicious apple (Table 31). Titratable acidity was observed (0.3 per cent) in genotype SKJAD-30 followed by (0.5 per cent) in Red delicious apple. Ascorbic acid was observed (2.59 per cent) in SKJAD -29 followed by (2.41 per cent) in Red Delicious apple. Sugars was recorded as (12.45 per cent), (13.15 per cent), (13.00 per cent) in SKJAD-29, SKJAD-30 and Red Delicious respectively. Reducing sugars (7.78 per cent), (8.22 per cent), (8.11 per cent) in SKJAD-29, SKJAD-30 and Red Delicious respectively. Non-reducing sugars was observed as (4.42 per cent) in SKJAD-29, (4.68 per cent) in SKJAD-30 and (4.64 per cent) in Red Delicious. Pectin was found (1.09 per cent), (1.19 per cent), (1.13 per cent)

Table 29: Comparison of fruit characters of promising genotypes Ambri apple with Red Delicious

S.No.	Genotype number	Fruit shape	Fruit base	Fruit base cavity depth	Fruit apex	Fruit ground colour	Fruit over colour	Fruit skin lenticels
1.	SKJAD -29	7	7	5	3	4	3	3
2.	SKJAD -30	1	5	3	3	2	2	3
3.	Red Delicious	1	5	3	3	2	3	5

Legend

Fruit shape	Note	Fruit base	Note	Fruit base cavity depth	Note	Fruit apex	Note	Fruit ground colour	Note	Fruit skin lenticels	Note
Globose	1	Narrow	3	Shallow	3	Smooth	1	Cream white	1	Absent	0
Globose conical	2	Intermediate	5	Medium	5	Wrinkled	2	Yellow	2	Low	3
Flat	4	Broad	7	Deep	7	Grooved	3	Green yellow	3	Medium	5
Conical	6					Others	99	Green	4	High	7
Long conical	7							Orange	5		
Intermediate conical	8							Red	6		

Table 30: Comparison of fruit characters of promising genotypes Ambri apple with Red Delicious

S.No.	Genotype number	Pulp texture	Pulp taste	Juiciness	Productivity status	Biotic stress susceptibility
1.	SKJAD -29	3	4	7	7	1
2.	SKJAD -30	3	4	7	5	1
3.	Red Delicious	3	3	5	5	3

Legend

Pulp texture	Note	Pulp taste	Note	Juiciness	Note	Productivity status	Note	Biotic stress susceptibility	Note
Soft	3	Acidic 1		Less	3	Less	3	Very low or no visible signs of susceptibility	1
Intermediate	5	Sub acidic	2	Medium	5	Medium	5	Low	3
Firm	7	Medium sweet	3	High	7	High	7	Intermediate	5
		Sweet	4					High	7
								Very high	9
								Very low or no visible signs of susceptibility	1

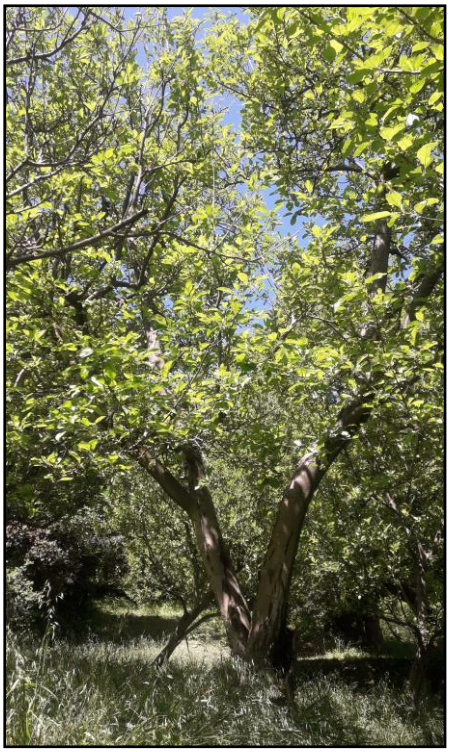
in genotype SKJAD-29, SKJAD- 30 and Red Delicious respectively. TSS/acid ratio was observed as (19.18), (53.03) and (32.32) in SKJAD-30, SKJAD-29 and Red Delicious respectively. Phenolics was observed as (46.72 mg/100mg), (60.25 mg/100g) and (67.84 mg/100g) in genotype SKJAD-29, SKJAD- 30 and Red Delicious respectively.

Table 31: Comparison of fruit and biochemical characters of promising genotypes of Ambri apple with Red Delicious

S. No.	Selection No.	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Total soluble solids (°Brix)	Titrable acidity (%)	Ascorbic acid (mg/100g)	Sugars (%)	Reducing sugar(%)	Non reducing sugar (%)	Pectin (%)	TSS/ Acid ratio	Phenolics (mg/100g)
1	SKJAD -29	292.23	6.37	7.62	15.35	0.80	2.59	12.45	7.78	4.66	1.09	19.18	46.72
2	SKJAD -30	284.17	6.35	7.05	15.91	0.30	2.36	13.15	8.22	4.93	1.19	53.03	60.25
3	Red Delicious	200.00	5.74	6.35	12.93	0.40	4.54	13.00	8.11	4.89	1.13	32.32	67.84
General mean		258.8	6.15	7.00	14.73	0.53	3.16	12.86	8.03	4.82	1.13	34.84	58.27
SD		51.08	0.35	0.63	1.58	0.21	1.19	0.36	0.18	0.11	0.05	17.06	10.69
CV		19.73	5.82	9.08	10.75	40.02	37.90	2.86	2.24	2.28	4.45	48.98	18.35

PROMISING AMBRI APPLE GENOTYPE SKJAD-29

Name of the Farmer: Zubair Ahmed **Latitude: 28^0 08.751**
Village: Lonepora, Dool (Kishtwar) **Longitude: 075^0 46.580**



A

B

C

Plate 9 (A): A: Mature tree B: Leaf C: Fruit

PROMISING AMBRI APPLE GENOTYPE SKJAD-30

Name of the Farmer: Zubair Ahmed **Latitude: 28^0 08.751**
Village: Lonepora, Dool (Kishtwar) **Longitude: 075^0 46.580**

A

C

D

Plate 9 (B): A: Mature tree B: Leaf C: Fruit

DISCUSSION

CHAPTER-5

DISCUSSION

The apple is an important fruit tree in temperate regions of the world and is grown for its commercial value. In addition Jammu and Kashmir has genetically abundant areas in apple germplasm. Historically most of the apple trees in these areas were propagated by seeds and thus apple population in these areas exhibit a wide range of morphological and genetical variation. The present investigation was conducted in one of the important apple growing regions of Jammu. Despite the low access in the studied areas, the results of our investigation indicate that valuable *Ambri* apple genotypes, especially desirable fruit characteristics, exist in these areas. Being perennial, cross pollinated and growing in wide range of different soil and climatic conditions, *Ambri* apple reveals excessive variability in both pomological and phenological traits. A broad variation is exhibited by *Ambri* apple with respect of growth habit, leaf characters, flower characters and fruit characters. For successful planning and execution of crop improvement programme in *Ambri* apple basic information on vegetative, floral and fruit character is mandatory tool. This knowledge is helpful in identification of best combiners for future breeding programmes. Observations of phenotypes has been classical approach to study variation. The explanation of the plant morphology is an invaluable source of information of genetic diversity nevertheless; morphological traits are influenced by environmental conditions. The subsequent development of biochemical markers represented a significant improvement since they offer great variability. DNA markers have authorize characterization of genotypes independent of the influences of environmental growth conditions, physiological age of plant and type of tissue being examined. In the present investigation, fifty *Ambri* apple genotypes were characterized systematically by means of NBPGR descriptor and analyzed for polymorphic markers based on SSR analysis.

5.1 Morphological characterization

5.1.1 Tree characters

The morphological traits provide basic information for breeding programmes, management of genetic resources, protection of cultivars and selection of genotypes to diversify local production. Most of the *Ambri* apple trees selected for the present study were naturally growing which were never pruned and trained. Fully bearing mature trees were selected from different locations of the study area, with different topographical features. Variation within and among selected traits in different environmental conditions of *Ambri* apple genotypes growing sites has been reported. The morphological parameters *viz.,* tree height, trunk girth, leaf shape and leaf size etc. are helpful in the identification of the genotypes. The bearing capacity of a plant is very much influenced by the variation in these attributes. Significant variability was observed in different genotypes with respect to tree characteristics.

Out of fifty genotypes studied almost 4 per cent genotypes had very vigorous, 38 per cent had vigorous, 50 per cent possessed intermediate and rest 8 per cent genotypes exhibited weak tree vigour. These findings are in agreement with the findings of Hassan *et al.* (2017) who reported that tree vigour of apple varies from weak in 27.27 per cent accessions, medium in 27.27 per cent accessions, and strong in 45.45 per cent accessions. The wide variation in tree characteristics viz., tree vigour can be attributed to the difference in the age of trees, different growing habitats and environmental and edaphic factors. It was observed that those trees which were exposed to better sunlight had vigorous tree growth as compared to dense canopies.

Tree habit i.e., upright, spreading and drooping also varied among the genotypes under study. Out of fifty selected genotypes, the upright 22 (44 per cent) and spreading 23 (46 per cent) type growth habit remained predominant among studied genotypes and rest were drooping 5 (10 per cent). The results are in accordance with the line of Hassan *et al.* (2017), where they found upright tree habit (36.36 per cent) and spreading tree habit (63.63 per cent) among selected accessions. Similar variations in tree characters were also observed by Ahmed (2008) who reported that pear genotypes showed variability in

their tree behaviour and tree size. He further reported that most of genotypes were pyramidal in shape with medium to large tree size, some were larger in size with broadly spreading branches while, limited number of genotypes were found in upright in growth with small tree size. Shyamali (2006) also reported huge variation in tree characters of different pear genotypes and these variations might be due to attributed genetic makeup of the pear cultivar/rootstock. Variation in tree habits among Asian pear genotypes was observed as spreading and upright (Bhat 2012; Griggs and Iwakiri 1977).

Among the selected fifty *Ambri* apple genotypes, the tree height ranged from 5.23 m to 9.51 m, trunk girth ranged from 105. 28 cm to 277.78 cm and tree spread ranged from 2.10 m to 6.80 m. Similar observations has been reported by Kumar *et al.* (2018) in apple cultivars. They reported that *cv.* Lal *Ambri* exhibited maximum plant height (480 cm), annual extension growth (60 cm), plant spread (N-S= 122.33 cm and E-W= 115.00 cm) and plant volume (23.70M3) during investigations. Verma *et al.* (2014) also studied genetic variability in pear *cv.* Kashmiri Nakh and reported that the height ranged from 3.15 to 4.15 m whereas, tree spread ranged from 2.45 m to 15.56 m. Variation in morphological attributes within and among cultivars may be attributed to the fact that the morphological traits are greatly influenced by environmental conditions such as temperature, rainfall and solar radiation, etc. (Rotondi *et al.*, 2003). Therefore, study of the morphological traits can be useful for diversity analysis and establishing phylogenetic relationship within and between the cultivars (Cantini *et al.*, 1999; Rana *et al.*, 2015).

In the present study, bearing habit of *Ambri* apple genotypes showed wide variation among the selected genotypes. Out of fifty selected genotypes 38 genotypes (76 per cent) had on spur bearing habit and rest 12 genotypes (24 per cent) had mixed bearing habit. Similar results has been reported by Hassan *et al.* (2017) according to them apple bearing habit was recorded as 75.75 per cent on spurs and long shoots, 18.18 per cent on spurs and 6.06 per cent on long shoots among the selected genotypes.

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. Variation in foliage characters such leaf size and leaf shape

is considered to be important for the identification and characterization of any fruit crop germplasm. Leaf size was large (48 per cent) in majority of genotypes, medium (44 per cent) and small (8 per cent) leaf size in rest of genotypes. Our results are supported by the results of Hassan *et al.* (2017), they showed a significant range in leaf size of an apple i.e., leaf blade length (6.26-12.06 cm) and leaf blade width (2.73-7.23 cm). Similar results were observed by Bist *et al.* (2003) they reported that Gola cultivar of pear had higher leaf length as compared to Patharnakh cultivar. They also reported that the cultivar Tumariya showed higher leaf breath as compared to Gola and Patharnakh cultivars of pear. Parallel results were observed by the Reim *et al.* (2012) they reported no pubescence on leaf surface (43 per cent) or only few hairs (37 per cent) in *Malus sylvestris*.

The leaf of *Ambri* apple was broad elliptic (60 per cent) in majority genotypes and rest genotypes (40 per cent) exhibited oval leaf shape. Our results are close conformity with the results of Kumar *et al.* (2018) who studied morphological and biochemical diversity among the *Malus* species including indigenous Himalayan wild apples and reported that the frequency of ovate leaf shape was observed maximum in selected apple genotypes. Similar results were observed by Elshihy *et al.* (2004) they reported that leaves were serrated and the leaf index (leaf width/leaf length) ranged from 0.35 to 0.78 in Syrian pear genotypes. Variation in leaf shape and size were also observed by Raina *et al.* (2011). Rana *et al.* (2015) also observed wide variability among the genotypes on the basis of leaf characteristics in pome fruit trees. Variability with respect to leaf characters might be due to their genetic makeup and interaction with the environmental conditions. *Ambri* apple genotypes exhibited quantitative diversity in foliar dimensions.

5.1.3 Flower characters

The knowledge of flowering period is important in the execution of successful characterization and also for successful hybridization programme. Genotypes vary in their flowering depending upon the prevailing climatic conditions. From the investigation, duration of flowering ranged from 19-21 days and majority of genotypes started flowering from 2nd week of April and end of flowering was recorded in 4th week of April. Similar results were also reported by Mratinic and Aksic (2011) they reported

that the earliest initial bloom was recorded in some apple cultivars on 22nd April and lasted till 6th May and also reported an approximate 16 day of difference in full bloom between the earliest and latest cultivars. Kumar *et al.* (1997) evaluated six apple cultivars and found that flowering time varied from the last week of March to the first week of April. They also revealed that percentage anthesis, dehiscence increased from 8.00 to 12.00 hrs, then it decreased slightly until 14.00 hrs and then declined rapidly. Bhat (2012) also observed that variability in flowering time among the pear genotypes and it may be attributed to the difference in chilling hours required for breaking flower bud dormancy. Elshihy *et al.* (2004) also reported perfect flowers bloom on two years or older spurs between April and May in the south of Syria. Kumar *et al*. (1997) reported that flowering time in apple started from last week of March to first week of April. However, most of the accessions in present study were mid bloomers but late bloomers should be favoured because of its possibility to avoid freezing injury.

On the basis of regularity of flowering genotypes were categorized as regular, biennial and irregular. Among the fifty selected genotypes 35 genotypes (70 per cent) were biennial, 12 genotypes (24 per cent) were irregular and rest 3 genotypes (6 per cent) were regular in flowering pattern. Fioravanco and Czermainsk (2018) studied biennial bearing in apple cultivars and reported Gala cultivars showed BBI (Biennial Bearing Indices) ranging from 0.28 to 0.35 and the Fuji from 0.26 to 0.38. Milatoviæ and Duroviæ (2012) also obtained indices of 0.17 and 0.26 for the cultivars Royal Gala and Gala Must of 0.49 and 0.55 for the Fuji Naga-fu 6 and Fuji Naga-fu 2. Similarly, Crassweller *et al*. (2005) estimated indices of 0.57 and 0.59 for Gala Supreme and Fuji. Flower and fruit thinning is considered a fundamental management practice to reduce the intensity of production alternation in apple trees (Bukovac *et al*., 2006), especially when carried out in the year of high production (Tromp, 2000). This is very important for assuring regular production every year and good sized fruits, and for avoiding unbalanced relationship between production and vegetative growth of the plant.

Variability in flowering may probably be due to topography, elevation and mean temperature of the area where the genotypes were growing. Flowering in temperate deciduous fruits are linked with the flowering behaviour, previous crop load, flower bud

formation and prevailing climatic conditions (Kappel and Neilsen, 1994; Kumar *et al.*, 2004). Flower bud formation is a intricate phenomenon. Flowering takes place by the transformation of vegetative apex into a reproductive structure under the control of endogenous and exogenous elements. When the apex of the vegetative bud receives a signal for differentiating as a flower bud, a sequence of events takes place. The mitotic activity increases in the central meristem of apex changing its histological structure. Pome fruits crops start their flower bud development during the previous season and flowers in next spring. In apple initiation generally starts with the ceasation of shoot growth. Large number of factors that influence the proportion of buds giving rise to flowers. Warm temperature advances the flower bud differentiation in apple. Thus understanding the processes of flower induction and flower bud development are important for a lot of horticultural activities particularly in fruit cultivation.

Considerable amount of variability was observed in self incompatibility, number of flower bud per inflorescence and flower stalk (pedicel) length. Self compatibility was observed in all selected *Ambri* apple genotypes. Flower bud per inflorescence was observed six in (11 genotypes) followed by five in (21 genotypes) and four in (18 genotypes). Flower stalk length showed wide variation (10.7 mm -25.15 mm) in the selected genotypes SKJAC -48 and SKJABh -20 respectively. Hegedus (2006) studied self incompatibility in apple and reported that apple showed ribonuclease mediated type of self incompatibility. Broothaerts and Nerum (2003) studied self-incompatibility in apple genotypes and reported the *S*-genotypes of 130 North-American, European and Asian apple cultivars are incompatible groups. Variation in length and width of pedicel has also been reported by various workers in pear (Shyamali 2006).

Inflorescence bearing habit were categorized as on spurs, on shoot tips, on old shoots and mixed among different *Ambri* apple genotypes. Majority of genotypes 38 (76 per cent) showed bearing on spurs and 12 genotypes (24 per cent) exhibited mixed bearing habit. Whereas on shoot tips and on old shoot tip bearing habit were not observed among fifty selected *Ambri* apple genotypes. These results are in agreement with the results of Hassan *et al.* (2017) who found that the bearing habit of apple was 75.75 per cent on spurs and long shoots**,** 18.18 per cent was observed on spurs only and 6.06 per

cent was observed on long shoots in genotypes. In case of number of flower buds per inflorescence and flower stalk (pedicel) length a wide range of variability was observed and the findings are in conformity with the results obtained by Rana *et al.*(2015) who studied genetic diversity of pear genotypes in Himalayan region based n morphological and molecular markers and recorded wide variation in traits of pear. Variability in length and thickness of pedicel has also been observed by various other workers in pear (Shyamali, 2006; Bhat 2012; Jan *et al.,* 2016).

5.1.4 Fruit characters

Variability in fruit attributes such fruit size, fruit shape, fruit weight and fruit width is considered to be important for identification and characterization of any fruit crop germplasm. Days to fruit harvest in the majority of selected genotypes was observed above 150 days. Genotype SKJAN-27 showed maximum days to fruit harvest i.e. 155-158 days. These observations are similar to the find findings of Tripathi *et al.* (1984) they observed 124-129 days from full bloom to maturity in Red Delicious apple cultivar for determining its maturity. The fruit length of the selected genotypes ranged from 4.35 cm to 6.37 cm and fruit width ranged from 4.62 cm to 7.62 cm and fruit weight ranged from 158.21 g to 292.23 g. These results are in agreement with the results of Sharma *et al.* (2016) they found wide range of diversity in *Ambri* apple in respect of fruit weight (86.90-334.97 g), fruit length (59.13-84.00 mm), fruit width (68.21-94.31 mm). The data clearly showed wide variability with respect to weight. 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). Fruit size is an important parameter for selection of superior genotypes through breeding programmes (Westwood and Blaney, 1963). The mechanism of fruit development is influenced by environmental and genetic factors (Harada *et al.,* 2005; Mandic *et al.,* 2011).Variation in fruit size might be under control of genetic factors involving their phylogenic behaviours. The mechanisms of fruit development are influenced by cultural and genetic factors (Cowan *et al.,* 2001 and Harada *et al.,* 2005). Fruit size is also an important marketing parameter, determining economical value in horticultural crops especially for apples and pears

(Gillaspy *et al.,* 1993). The fruit growth is characterized by an initial period of rapid cell division followed by long period of cell expansion. Bigger the size of fruits might be the inherent ability of a genotype to utilize the available resources efficiently to achieve a certain fruit size. (Stanley *et al.,* 2000).

In the present study, the selected genotypes showed wide variation in fruit shapes of *Ambri* apple. Among the selected fifty *Ambri* apple genotypes none of the genotypes had flat fruit shape and intermediate conical fruit shape, while 19 genotypes (38 per cent) exhibited globose fruit shape, 8 genotypes (16 per cent) had globose conical fruit shape, 10 genotypes (20 per cent) had conical fruit shape and rest 13 genotypes (26 per cent) had long conical fruit shape. Fruit base were categorized into narrow, intermediate and broad base. Out of fifty selected *Ambri* apple genotypes, 17 genotypes (34 per cent) had narrow base, 6 genotypes (12 per cent) had intermediate and rest 27 genotypes (54 per cent) had broad fruit base. Fruit base cavity depth varied from shallow to medium and deep. Out of fifty *Ambri* apple genotypes 18 genotypes (36 per cent) exhibited shallow, 18 genotypes (36 per cent) showed medium and 14 genotypes (28 per cent) possessed deep fruit base cavity depth. These findings are in agreement with the findings of Hofer *et al*. (2012) in apple. They found that majority of apple accessions possess flat globose (65 per cent), flat globose in (14 per cent) and globose in (11 per cent) accessions.

Fruit skin lenticels of *Ambri* apple genotypes were studied and it was observed that 50 genotypes (50 per cent) had no fruit skin lenticels and rest 50 genotypes (50 per cent) had low in fruit skin lenticels. Our results are in close conformity with the Hassan *et al.* (2017) they reported few numbers of lenticels (42.42 per cent), medium numbers of lenticels (30.30 per cent) and many number of lenticels (27.27 per cent) in selected accessions of apple under study.

In the present study fruit over colour was found yellow in 15 genotypes (30 per cent), green yellow in 8 genotypes (16 per cent) and green in 27 genotypes (54 per cent). Fruit ground colour was observed as yellow in 15 genotypes (30 per cent), green yellow in 8 genotypes (16 per cent) and green in 27 genotypes (54 per cent). Similar results were reported by Mratinic and Aksic (2012) they reported in apple that background colour varies from cream white (38.89 per cent), over yellow (16.67 per cent) to green yellow

(44.44 per cent), while over colour ranged from red (50.0 per cent) to dark red or purple (5.56 per cent) in some *Malus* species in South Serbia. 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 and responsible for fruit colour (Marini *et al.*, 1991). When the apple fruit gains optimum size, ground colour of skin changes from green to green yellow or yellow on the tree then it is ready for harvesting. Beginning of fruit ripening was evaluated on the basis of fruit size, change in colour and overall visual observations.

Pulp texture, pulp taste and juiciness also showed wide variation among the selected genotypes. Out of fifty *Ambri* apple genotypes, 37 genotypes (74 per cent) had soft pulp texture, 26 genotypes (52 per cent) had sweet pulp taste and 43 genotypes (86 per cent) had medium juice content. These findings are also in agreement with those of Verma *et al.* (2006) they reported that all European type of pears exhibited soft pulp texture and low grittiness. Out of them EC552668 and EC 027809 had russet free yellow skin color with pink tinge and possessed high juice content. Similar results were found by Dzhangaliev *et al*. (2003), they reported that the fruits of both *Maluskirghisorum* and *Malus sieversii* are diverse in flavour. The pulp taste of both species were recorded as sour-sweet to acid and sweet to bitter in flavour. The present findings of the study are also in line with the earlier workers (Vaysse *et al.*, 2005; and Chen *et al.*, 2007).

Productivity among the selected genotypes were estimated and out of fifty selected genotypes 47 genotypes (94 per cent) had medium productivity status and 3 genotypes (6 per cent) had high productivity status. None of the selected genotypes showed low productivity status. Parallel study was carried out by Reighard *et al.* (2008) they reported that cumulative yield efficiency (kg/cm^2) was significantly different among pear cultivars with Atago. They further reported that cultivar Atago was more yield efficient than Hosui. Variability in productivity may be attributed to several factors like number of blossoms, final fruit set, fruit number, fruit size and tree size. Productivity is reduced by the number of reject fruit and one criterion for rejection is blemishes with low

or zero tolerance e.g. russeting, superficial insect pest and disease damage, leaf rub, bird damage, extensive (>1cm2) pest or disease damage (O.E.C.D., 1983). Tree yield is a function of the number of both fruit on the tree and their weight. The number of fruit depends on the flower density (number of flower clusters per trunk cross-sectional area (TCA), the tree size and percentage of set (Lombard *et al.*, 1988). When fruit set is too large for the tree size, it becomes necessary to thin to ensure that the remaining fruit reach commercial size. Percentage of the fruit set decreases as the flower density increases, but their relationship is heavily influenced by climate (Dennis, 1986).

In present investigation biotic stress susceptibility was categorised into five heads i.e., very low or no visible sign of susceptibility, low, intermediate, high and very high. Among the fifty selected genotypes, majority of genotypes had no signs of susceptibility. These results are in accordance with the findings of Janik and Moore (1975) and Bell *et al.* (2004), where they that reported considerable differences exist in gene frequencies for disease resistance in various wild species of fruit trees. Ahmed (2008) studied that among pear accessions RTII (Glass), RT12 (Bagugosha), RT13 (Frashishi), Bg19 (Kotharmul), BG24 (Bagugosha), BG25 (Frashishi), were highly affected by the diseases (fire blight and pear scab).

5.2 Biochemical characterization

Quality is a human construct comprising many properties including sensory properties, nutritive values, chemical constituents, functional properties and defects (Abbott, 1999). The fruit quality is determined by internal composition such as contents of sugars, acids and other characteristics like texture, firmness and flavour change in taste, firmness. Appearance of fruits can be consequences of changes in sugar contents, sugar acid ratio, organic acids etc.

Chemical aspects of fruits such as total soluble solid, acidity and sugars provide important information to the consumers in terms of recognizing a more nutritious fruit (Drogoudi *et al.,* 2008). Data procured from study on the parameter showed a great variability on TSS, reducing sugars, non reducing sugar, total sugar, acidity, ascorbic acid, pectin and phenolics. The maximum TSS (15.91 ^{o}B) was recorded in genotype

SKJAD-30 whereas, minimum TSS (12.57 ^{o}B) was recorded in genotype SKJABh-18. Similar observations were recorded by Sharma *et al.* (2016) they revealed that among the thirty four collected variants of *Ambri* apple, a wide range of TSS (10.90 to 20.00 ^{o}Brix) was observed. Bostan (2009) reported that SS (Soluble Solids) values from 10.50 to 15.00 per cent in apple. Mratinic (2012) also reported in apple that soluble solid content (SS) varied from 12.55 to19.24 per cent and total solids (TS) were between 8.65 and 12.18 per cent. TSS is influenced by environmental factors such as temperature, light (duration and intensity), rainfall/supply of water and locations (Ahmed, 2008).

Titratable acidity among the different *Ambri* apple genotypes showed wide variability. Genotype SKJAG-17 exhibited maximum titratable acidity (1.72 per cent) while minimum titratable acidity (0.30 per cent) was observed in genotype SKJAD-29 followed by the genotypes SKJAK -22 (0.31 per cent), SKJAD -32 (0.32 per cent) and SKJAD -31 (0.33 per cent). These results are also in consonance with the findings of earlier study conducted by Sharma *et al.* (2016) on *Ambri* apple and observed that titratable acidity varies from 0.31 to1.78 per cent among the thirty four collected variants of *Ambri* apple. Mratinic (2012) also reported that titratable acidity (TA) content was observed between 0.10 and 0.82 per cent in apple. Hassan *et al.* (2017) they studied morphological characterization of apple accessions observed that acidity varied from 0.06 to 0.79 per cent in Kashmir region.

Ascorbic acid content ranges from 2.10 mg/100g (SKJAG -17) to 4.81mg/100g (SKJAN-39). Similar results were obtained by Ahmed *et al*. (2011) who found significant differences among the genotypes regarding Vitamin C. They further reported ascorbic acid content ranged from 1.46 to 5.94 mg/100 ml of juice, depicting diversity in pear genotypes. Vitamin C content in fruits can be regulated by various factors such as genotypic variation, pre-harvest climatic factors, maturity and harvesting methods. Alteration in day temperature at different localities might be responsible for variability for vitamin C contents. Fruits of high altitude with low temperatures showed better in terms of ascorbic acid than low lying and warm localities.

An insight of data revealed that maximum TSS/Acid ratio (49.38) was observed in genotype SKJAK- 22 while minimum TSS/Acid ratio (8.52) was observed in genotype

SKJAD-29. These results are close conformity with the results of Misger *et al.* (2014) they reported that Coscia cultivar of pear exhibited maximum TSS/acid ratio (48.80) whereas, minimum TSS/acid ratio (28.260 was found in Red Anjou.

The data obtained in the present study revealed a high variability among the genotype regarding reducing (8.22 per cent), non reducing (4.93 per cent) and total sugar (13.15 per cent) was in genotype SKJAD-30whereas, minimum reducing (5.63 per cent) , non reducing (3.37 per cent) and total sugar was recorded in genotype SKJAB-01 (9.89 per cent). Similar results were obtained by Ahmed *et al.* (2011) they found significant differences among the genotypes regarding total sugars which ranged from 4.77 to 12.34 per cent. Such variability might be due to variability in rainfall or maturity level at the time of fruit harvesting. Hudina and Stampar (2005) also reported that excessive water supply reduces the sugar content in pears fruits and vice versa. Sugar is an important component of fruits which correlates with sweetness and is basic ingredient of fruit quality (aroma, texture and flavour).

Pectin content of *Ambri* apple genotypes varied from 1.00 per cent in genotype (SKJAG -17) to 1.32 per cent in genotype (SKJAN -38). Gautam *et al*. (1986) also observed calcium pectate level in nine cultivars of apple and observed calcium pectate level ranged from 0.63 to 1.15 per cent. Kawabata and Sawayama (1974) also reported pectin content ranged from 0.32 to 0.72 per cent in seven cultivars of apple. Ross *et al.* (1985) also observed a range of pectin in cultivars of apple varied 0.39 to 0.49 per cent. Virk and Sogi (2004) also observed d 1.21 per cent pectin in apple peel.

Phenolics content ranged from 43.59 mg/100g in genotype (SKJABh-19) to 64.05 mg/100g in genotype (SKJAD-28). Our results are in close conformity with the results of Duric *et al.* (2015) they reported maximum total extractable phenolic content in the cv. Poljakinja (17.70 mg/GE/g) while the minimum amount of phenols was observed in cv. Miljevicka (30.70 mg/GE/g).

5.3 Coefficient of variation

The knowledge of coefficient of variation is necessary for the improvement of a crop which helps to measure the extent the variability in the characters among genotypes.

Wide the variability, huge are the chances of selecting desired genotypes (Vavilov, 1951). Most of the economic traits, which are of interest to plant breeders are quantitative in nature and mostly influenced by environment. Among the fruit chemical parameters coefficient of variability was highest for TSS/Acid ratio (47.77 per cent) followed by titratable acidity (44.31 per cent) and ascorbic acid (25.92 per cent) the coefficient of variation was lowest (5.72 per cent) for TSS content, followed by (9.6 per cent) for phenolic content. The coefficient of variation is estimated to assess the variability. The These results are concurrent with the results Hassan *et al.* (2017) they reported the coefficient of variation in apple among the different parameters viz., TSS (7.34 per cent), acidity (5.29 per cent), fruit length (17.23 per cent), fruit diameter (16.04 per cent) and fruit weight (3.66 per cent). Similar results were observed by Mir (2015) in wild apple. For diversity studies coefficient of variation was also studied by Baisati (2012) in apple. The higher the coefficient of variation greater would be the variability. In other words, when the coefficient of variation is high the sample is more variable and when it is low the sample is less variable. Analysis of variance for the various traits under study and the data revealed significant differences at genotypic level among genotypes which indicates that the material selected was genetically diverse for all the traits. The magnitude of variability present in various quantitative traits under study revealed existence of wide range of diversity for all the traits. Knowledge of extend of genetic variation in fruit morphology, quality and maturity helps in subsequent identification of adapted superior genotypes as potential donors for yield and quality improvement.

5.4 Cluster analysis

In present study cluster analysis for twenty six morphological traits of *Ambri* apple are grouped into main two clusters with variable number of entries in each cluster indicating the presence of genetic diversity. The intra cluster distances ranged from 27.96 (cluster III) to 45.34 (cluster II). The maximum intra-cluster distance was observed in cluster II (45.34) followed by cluster I (33.75). The minimum intra cluster distance was observed in cluster III (27.96) which contain fourteen genotypes. The inter cluster distance ranged from 167.33 to 124.39. The maximum inter cluster distance was observed between cluster II and cluster III indicated that genotypes falling in these

clusters can be used in hybridization programme to get greater variability in the segregating generations. These genotypes can also utilized for transfer of beneficial traits in the commercial *Ambri* apple cultivar.

Based on the clustering pattern of biochemical parameters the intra cluster distances ranged from 0.00 (cluster IV) to 17.61 (cluster I). The maximum intra cluster distance was exhibited by cluster I (17.61) followed by cluster II (12.77). The minimum intra cluster distance was observed in cluster IV (0) which contain only one genotype. The inter cluster distance ranged from 38.96 to 80.20. The maximum inter cluster distance was observed between cluster II and cluster III. These results are in conformity with the Sharma *et al*. (2015) who studies assessment in variation in twenty three pear genotypes on the basis of total variability were grouped into 3 main clusters. Genotypes belonging to clusters separated by genetic distance may be used in hybridization programme to obtain a wide spectrum of diversity among the segregates. Genotypically distant parents are able to exert high heterosis (Farhad *et al.,* 2010). Ahmad (1999) observed that clustering pattern reflected considerable influence on genetic diversity. The grouping pattern of genotypes based on morphological attributes confirmed maximum divergent clusters were expected to manifest maximum heterosis. The inter cluster distances were larger than the intra cluster distances indicating a wider genetic diversity between genotypes of clusters with respect to the trait considered. Maximum inter cluster distance indicate that genotypes falling in these clusters had wide diversity and can be used for hybridization programme to get better recombinants in the segregating generations. Low levels of intra cluster distances were the indicative of narrow genetic variation within the cluster. Genotypes of the same cluster would not yield desirable recombination.

5.5 Principle Component Analysis

Principal component analysis (PCA) model was performed to provide an easy visualization of the complete data set in a reduced dimension plot. Principal component analysis (PCA) has been used previously to evaluate germplasm of apple (Currie *et al.,* 2000). Principle component analysis (PCA) was performed to achieve parsimony and reduce the dimensionality of original multivariate component data. PCA, one of the

multivariate statistical procedures, has been used to study correlations among fruit traits and to establish genetic relationships among cultivars within sets of apple cultivars. Associations between traits emphasized by this method may correspond to genetic linkage between loci controlling traits or a pleiotropic effect (Oraguzie *et al.,* 2001).

In the present investigation the PCA for morphological traits revealed that the 1st nine principal components (PC's) possessed Eigen value > 1.0. The nine components with Eigen values are able to explain the more than 71.00 per cent of total variation. PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8, PC9 accounted for 14.8 per cent, 11.92 per cent, 10.57 per cent, 8.90 per cent, 6.38 per cent, 6.05 per cent, 4.99 per cent, 4.13 per cent and 4.08 per cent. The individual contribution towards variability of *Ambri* apple through loading plot of morphological and genotypes (as a parameter) was exhibited 14.84 per cent and 11.92 per cent with cumulative variability of 26.76 per cent respectively (Fig. 6). Similar results were reported by Ganopoulos *et al.* (2018) they found that the distribution of cultivars upon PC1 and PC2 is correlated to phenotypic variation between the tested cultivars, showing the magnitude of their dispersion along both axes. Seven significant components were obtained and which showed 85.56 per cent of total variation. Hussain *et al.* (2016) also reported the PCA in walnut and observed that the first four PC's revealed maximum variation in walnut genotypes and PC1 and PC2 contributed total variance of 41.65 per cent and 23. 42 per cent respectively.

The proportion of the total biochemical variation accounted for by each PC was calculated from the Eigen values (>1). The four components with Eigen values are able to explain the more than 76.00 per cent of total variation. PC1, PC2, PC3, and PC4 accounted for 32.61 per cent, 18.17 per cent, 13.97 per cent and 11.98 per cent. The individual contribution towards variability of *Ambri* apple through loading plot of biochemical (32.62 per cent) and genotypes (18.17 per cent) with cumulative variability of 50.79 per cent (Fig. 6). Similar results were obtained by Verma *et al.* (2014) who reported that first PCA accounts 33.58 per cent of total variation and second PCA expressed 23.76 per cent of total variation in pear. PCs accounted for the greatest amount of variation for each trait and each cultivar. No transformations were used because the data was already following normal distribution. s

5.6 Molecular characterization:

The genetic improvement of the crops is limited due to narrow genetic base of germplasm. Germplasm placed in gene banks can assist in the expansion of higher yielding varieties but for using such genetic resources the comprehensive knowledge and understanding of genetic diversity of cultivated as well as wild genotypes is a pre requisite. The genetic variability in different genotypes can be obsessed by several means out of which molecular markers hold promising place. Molecular markers are of various types among them SSRs are considered to be markers of choice because of several advantages like ample polymorphism, co-dominance, locus specificity, multi-allelic, reproducibility. SSRs are short tendem repeats of DNA sequences that are distributed throughout the genome of an organism. Keeping in view the numerous advantages of SSRs we carried out a study with one of the objective "Morphological, Biochemical and Molecular characterization of *Ambri* apple in Doda and Kishtwar districts of J&K." to analyze polymorphism among *Ambri* apple genotypes of Jammu province at molecular level. The present study could pave the way for detailed research to understand all the aspects of this genetic divergence. Many researchers all over the world have successfully used SSR technique for determination of genetic variation that PCR based assays can be used to analyze the variation among different cultivar. SSR markers are mainly suitable for breeding programs where large number of genotypes have to be analyzed. SSR markers provide genuine and reproducible molecular data. SSR technique has been successfully applied in various taxonomic and genetic diversity studies. Due to abundance and coverage of SSR markers of whole genome, reliable estimates of relationship among cultivars can be obtained.

In this study an effort was made to assess genetic diversity among fifty seedling origin *Ambri* apple genotypes using a total of 29 SSR primers. The DNA was extracted from fresh leaf material of all genotypes and quantified. Significantly, high concentration of DNA was obtained by CTAB method. The amplified products were resolved on gel and photographed. The polymorphic bands were scored based on alleles. Genetic variation was determined by constructing a dendrogram using UPGMA.

5.6.1 SSR analysis

Variation in DNA sequences between individuals in a primer binding site results in formation of bands at relatively different positions. However, SSR like assays can search large genomic portions due to their abundant distribution in the genome and thereby presenting a more precise picture of genetic variation within the crop plants. The number of markers used, their abundance in the genome and the degree of precision with which the results are analysed determine the accuracy to distinguish the genotypes. SSR have been used (Topcu *et al*., 2016 and Wang *et al*; 2008). The results obtained in the present study are discussed below:

5.6.2 Polymorphism

Polymorphism among individual genotype is observed due to variation in DNA sequences present in their chromosomes. Higher polymorphic bands of primers indicate their efficiency to study genetic diversity and discrimination of genotypes (Pradhan *et al*., 2004). The selected primers varied greatly in their ability to resolve variability among the genotypes. Primers with higher polymorphic bands were more efficient to discriminate the genotypes.

Parameters like PIC (polymorphic information content) allele per locus were also estimated to distinguish levels of polymorphism among all genotypes. The PIC value in the present study ranged from 0.031 to 0.801 with an average of 0.45. Maximum (0.801) PIC value was observed in primer CH05d11 which showed this primer have power for analyzing the genetic variability in *Ambri* apple genotypes. In similar study conducted Galli*et al*. (2005) also screened the 66 commercial apple cultivar with six SSR (Simple Sequence Repeat) markers for molecular identification. 55 polymorphic alleles were detected at the 6 SSR loci (average 9.2 alleles per locus) and the polymorphism information content (PIC) averaged 0.72. Farrokhi *et al.* (2011) also reported polymorphism information content (PIC) was varied from 0.18 to 0.76. The mean PIC value for all loci was 0.49. Bhat *et al.* (2013) reported that PIC value ranged from 0 to 0.84 with an average of 0.53 across 11 pear genotypes. Markers with high PIC values such as CH03c02, CH03g12z, CH05d04, Hi01d06y and Hi02d04 could be effectively

used in genetic diversity studies of apple. Dequigiovani *et al.* (2012) observed that PIC values ranged from 0.48 (KA14) and 0.87 (CH01H01). The PIC values provide an estimate of the discriminating power of a marker by taking into account not only the alleles at a locus but also relative frequencies of those alleles in the genotypes. PIC is reflection of allelic diversity and frequency among the genotypes, any value exceeding 0.5 reflects polymorphism.

5.6.3 Number of alleles per locus

The number of alleles per locus generated by these SSR markers varied from 2(CH03g12z, CH05d04, CH05e03, Hi01d06y), 3(CH02h11a, CH04a12, Hi02d04, Hi03e03, CH05c07, CH03a02, Hi03e03), 4(Hi03a03, CH03c02, CN444636, CN444542), 5(CH05e03, Hi02b10, Hi02d04, CH02b12), 7(CH03g07, CH02c02, CH03d12), 8(CH04g10, Hi03a03), 10(CH05d11). Primers showing more alleles per locus *i.e.*, CH04g10, Hi03a03 and CH05d11 showed that these primers are more efficient in studying diversity at particular locus. Similar results were obtained by Galli *et al.* (2005) in commercial apple cultivars, they reported that 55 polymorphic alleles were detected at the 6 SSR loci (average 9.2 alleles per locus) and the polymorphism information content (PIC) averaged 0.72.

5.6.4 Polymorphism percentage

Polymorphism percentage among fifty *Ambri* apple genotypes revealed the highest polymorphic percentage (62.50) in primer CH04g10 while lowest polymorphic percentage (14.20) was observed in primer CH03d12. Similar results were obtained by Cokran*et al*. (2019) in apple where thirty RAPD primers amplified 207 bands, of which 91 were polymorphic (40.1 per cent), 10 SSR primers produced 33 bands and 26 of them were polymorphic (78.78 per cent) and 5 AFLP combinations amplified 183 bands of which 88 were polymorphic (48.08 per cent).

5.6.5 Cluster analysis

Genetic diversity is known by observing polymorphism among the genotypes revealed by SSR profiles. More the polymorphic bands among genotypes more will be

expected diversity. Dendrograms are efficient means of summarizing microsatellite data can disclose relationships including identical genotypes. This dendrogram made it visible to visualize the diversity among the genotypes. It was observed that SSR markers were able to differentiate all the genotypes effectively. The UPGMA dendrogram exhibited that all fifty *Ambri* apple genotypes were grouped into two main cluster. Genotypes collected from different parts of Jammu province showed diverse clustering, which may be due to cross pollinated nature of *Ambri* apple.

5.6.6 Similarity matrix

A similarity matrix using SSR data revealed that similarity coefficients ranged from 0.14 to 0.74. Our results are in close conformity with the results of Farrokhi *et al.* (2011) they reported Jaccard's similarity coefficient 0.19 to 0.74 among apple cultivars and landraces which indicated a broad genetic base. Similar results were reported by Grauke *et al*. (2003) they observed highest dissimilarity (0.87) among pear nut genotypes between Brooks Hirschi while lowest 0.035 was observed between *Carya myristiciformis* (MYR) and Western cultivars. It could be concluded that *Ambri* apple germplasm used for present study have wide genetic base owing to cross pollination nature of *Ambri* apple. SSR markers proved to be very informative in the evaluation of genetic variation. The analysis with SSR markers discovered wide variation within seedling *Ambri* apple genotypes. The present findings further strengthened previous reports that SSR can be used more efficiently to evaluate genetic differences among genotypes and are in agreement with the results of Shah *et al.* (2016) in walnut in which the studied genotypes were discriminated according to the gene pool level by UPGMA based cluster analysis. Moreover, recent studies revealed the potential of SSRs in providing intra-specific diversity within genus.

5.6.7 Principle co-ordinate component Analysis

The fifty genotypes were subjected to principle co-ordinate analysis and genotypes were distributed at an average distance of 0.2 along the X-axsis and 0.18 along the Y-axsis. Distance between the genotypes is inversely proportional to similarity of characters among the genotypes. More the difference between the genotypes less they are belonging

to each other and vice versa. It was clearly observed that the genotypes SKJAK-7, SKJABh-19, SKJABh -18, SKJAM-45 are distantly arranged with the genotypes SKJAB-05, SKJAB-03, SKJAT-07 and SKJAB-04 along the Y-axsis. Similar pattern is shown between SKJAM-45 and SKJAG-14, SKJAM-44 and SKJAG-15 along the X- axsis. The genotypes SKJAT-06 and SKJAT-09 have less distance between them this shows the similarity among the given genotypes. We observe the same pattern between SKJAN-30 and SKJAN-37, SKJAN-42 and SKJAN-40. Shaili *et al*. (2016) studied molecular variance and principal coordinate analysis in guava and reported that analysis of molecular variance showed variation at three levels, among individual total variance is 59 per cent, among population is 1 per cent and within individual its percentage vary upto 40 per cent. Prinicipal co-ordinate analysis does not show any significant differences among the individual population, which varies only 1 per cent.

5.7 Comparison of promising seedling genotypes of *Ambri* apple with commercial cultivar

In order to designate any seedling genotype as superior, it is essential to compare its performance and characteristics with already available local variety. Characters of two best seedling genotypes i.e., SKJAD-29 and SKAD-30 were compared characters of Red Delicious apple (Plate 9A and 9B).

5.7.1. Morphological characters

5.7.1.2 Tree characters

Tree vigour was observed vigorous in both genotypes i.e., SKJAD-29 and SKJAD- 30, while it was observed intermediate in Red delicious. Tree habit of selected genotypes SKJAD-29 and SKJAD-30 was recorded as upright and spreading while, in Red Delicious apple it was upright growth habit. These findings are in agreement with the findings of Hassan *et al.* (2017) they reported tree vigour of apple varies from weak (27.27 per cent) accessions, medium in (27.27 per cent) accessions, and strong in (45.45 per cent) accessions.

5.7.1.3 Leaf characters

Leaf characteristics *viz.*, leaf size and leaf shape varied as large in the selected genotypes of SKJAD-29 and SKJAD-30 while, leaf size was observed medium in Red Delicious apple. Leaf shape varied as broad elliptic in genotypes SKJAD-29, SKJAD-30 and in Red Delicious apple. Our results are supported by the results of Hassan *et al.* (2017), they showed a significant range in leaf size of an apple i.e., leaf blade length (6.26-12.06 cm) and leaf blade width (2.73-7.23 cm). Similar results were reported by Kumar *et al.* (2018) who studied morphological and biochemical diversity among the *Malus* species including indigenous Himalayan wild apples and reported that the frequency of ovate leaf shape was observed maximum in selected apple genotypes.

5.7.1.4 Floral characters

Based on flowering characters number of flower bud per inflorescence was observed six and five in genotypes SKJAD-29 and SKJAD-30 while it was observed six in Red Delicious apple. Flower stalk (pedicel) length of genotypes SKJAD-29 and SKJAD-30, was observed as 11.5 mm and 14.7 mm while it was observed 14.2 mm in Red Delicious apple. Date of start of flowering in genotypes SKJAD-29 and SKJAD-30 were observed on 16th of April while it was 9th of April in Red Delicious apple. Date of end of flowering in genotypes of SKJAD-29 and SKJAD-30 were observed 20th of April while it was observed 15th of April in Red Delicious apple. Biennial flowering was recorded in genotype SKJAD-29 and genotype SKJAD-30 regular flowering pattern was observed in Red Delicious apple. Self incompatibility was observed both the genotypes of SKJAD-29 and SKJAD-30 and same was recorded in Red Delicious apple. Bearing habit of selected genotypes of SKJAD-29 and SKJAD-30 were observed on spurs while Red Delicious was also observed the same pattern. Age of first bearing of selected genotypes of SKJAD-29 and SKJAD-30 were observed five years and six years while it was observed five years in Red Delicious apple.

5.7.1.5 Fruit characters

Days to fruit harvest of selected genotypes of SKJAD-29 and SKJAD-30 were observed 152-153 days, and 153-154 days. While it was observed 131-140 days in Red

Delicious apple. Fruit shape of genotypes SKJAD-29 and SKJAD-30 were observed globose while it was globose conical in Red Delicious apple. Fruit base ofgenotypesSKJAD-29 and SKJAD-30 were observed broad base while Red Delicious apple observed intermediate fruit base. Fruit base cavity depth of genotypesSKJAD-29 and SKJAD-30 were observed shallow while Red Delicious apple was observed also shallow fruit base cavity depth. Fruit apex ofgenotypesSKJAD-29 and SKJAD-30 were observed grooved and same was observed in Red Delicious apple. Fruit length of genotypesSKJAD-29 and SKJAD-30 were observed 6.37 cm and 6.35 cm length and while Red Delicious apple was observed 5.80 cm fruit length. Fruit width of genotypeSKJAD-29 and SKJAD-30 were observed 7.62 cm and 7.05 cm while it was observed 6.31 cm in Red Delicious apple. Fruit weight ofgenotypesSKJAD-29 and SKJAD-30 were observed fruit weight of 292.23 g and 284.17 g while Red Delicious apple was observed 231.18 g. Fruit ground colour of genotypesSKJAD-29 and SKJAD-30 were observed yellow in colour while it was greenish in Red Delicious apple. Fruit over colour of genotypesSKJAD-29 and SKJAD-30 were observed pink in colour while it was observed red in Red Delicious apple. Fruit skin lenticels of genotypes SKJAD-29 and SKJAD-30 were observed low while medium was observed in Red Delicious apple. Pulp texture of genotypesSKJAD-29 and SKJAD-30 were observed soft pulp texture while same i.e., soft pulp texture was observed in Red Delicious apple. Pulp taste of genotypesSKJAD-29 and SKJAD-30 were observed sweet. While sweet was also observed in Red Delicious apple. Juiciness of genotypes SKJAD-29 and SKJAD-30 were observed high. While medium juiciness was observed in Red Delicious apple. Productivity status of genotypesSKJAD-29 and SKJAD-30 were observed intermediate and high while it was observed intermediate in Red Delicious apple. Biotic stress susceptibility of genotypes SKJAD-29 and SKJAD-30 were observed very low or no visible signs of susceptibility and same was observed in Red Delicious apple. Hassan *et al.* (2017) also showed similar variation in fruit length (1.14-5.21cm), fruit diameter (1.17-6.42 cm), length of fruit stalk (0.60-5.20 cm), fruit weight (1.06-81.34 g), number of seeds (2.12-10.00) per fruit. Sharma *et al.*(2016) also recorded similar findings on thirty four collected variants of *Ambri* apple, and reported wide range of diversity in

respect of fruit weight (86.90-334.97 g), fruit length (59.13-84.00 mm) and fruit width (68.21-94.31 mm).

5.7.2 Biochemical characterization

Apart from morphological characters, the biochemical characters particularly TSS, acidity, ascorbic acid plays an important role in deciding the market value. Maximum TSS (15.91 ^{o}Brix) was observed in genotype SKJAD-30 which was followed by (15.35^{o}B) in genotype SKJAD-29. TSS was observed (15.11^{o}Brix) in Red Delicious apple. Titratable acidity was observed (0.3 per cent) in genotype SKJAD-30 followed by (0.5 per cent) in Red Delicious apple. Ascorbic acid was observed (2.59 per cent) in SKJAD -29 followed by (2.41 per cent) in Red Delicious apple. Sugars was recorded as (12.45 per cent), (13.15 per cent), (13.00 per cent) in SKJAD-29, SKJAD-30 and Red Delicious respectively. Reducing sugars (7.78 per cent), (8.22 per cent), (8.11 per cent) in SKJAD-29, SKJAD-30 and Red Delicious respectively. Non-reducing sugars was observed as (4.42 per cent) in SKJAD-29, (4.68 per cent) in SKJAD-30 and (4.64 per cent) in Red Delicious. Pectin was found (1.09 per cent), (1.19 per cent), (1.13 per cent) in genotype SKJAD-29, SKJAD- 30 and Red Delicious respectively. TSS/acid ratio was observed as (19.18), (53.03) and (32.32) in SKJAD-29, SKJAD-30 and Red Delicious respectively. Phenolics was observed as (46.72 mg/100mg), (60.25 mg/100g) and (67.84 mg/100g) in genotype SKJAD-29, SKJAD- 30 and Red Delicious respectively. Similar results were observed by Hemmaty *et al.* (2006) who reported Red Delicious apples had lower total soluble solids and total soluble solids/titratable acids ratio (14.70 per cent and 98.2 per cent, respectively) than Golden Delicious apples (15.08 per cent and 86.5). Our results are also in close conformity with the results of Ashraf *et al.* (2018) who reported the TSS (14.77 ^{o}B), acidity (0.23 per cent), TSS/acid ratio (63.04) in Red Delicious. They further reported total sugar (10.80 per cent), reducing sugars (8.38 per cent) and non reducing sugar (2.42 per cent) in Red Delicious apple. Hassan *et al.* (2017) also observed variation in TSS from (6.70-16.30 ^{o}Brix) and acidity varied from (0.06-0.79 per cent) in apple. Sharma *et al.* (2016) also showed variation in TSS (10.90-20.00 ^{o}Brix) and titratable acidity (0.31to1.78 per cent) in apple accessions.

SUMMARY AND CONCLUSIONS

CHAPTER-6

SUMMARY AND CONCLUSION

The present investigation entitled "Morphological, Biochemical and Molecular characterization of *Ambri* apple in Doda and Kishtwar districts of J&K" was carried out to access the magnitude of genetic variability and characterize fifty *Ambri* apple genotypes with regard to morphological, biochemical and molecular diversity. To exploit the available genetic variation, characterization and evaluation of genetic potential and variability is must. For incremental improvement of *Ambri* apple, a diverse gene pool is essential. Characterization of genotype at genetic at genetic level supplemented with morphological characters could be first step towards efficient conservation, maintenance and utilization of existing genetic diversity. Assessment of diversity has traditionally been achieved through morphological characters, chemical composition and cytological characters. Usually, the easiest assessment of genetic variation is through systematic morphological or phenotypic measures. NBPGR descriptors are an important means for the standardization of apple characterization worldwide and provide an easy and rapid way to discriminate between apple genotypes.

Morphological characters are often limited in number and may not be reliable to discriminate between closely related apple genotypes. Simple sequence repeat (SSR) markers have been proven to be a very powerful tool to analyze genetic variability and relatedness at different hierarchical levels. As co-dominant and locus specific markers, SSR have been widely used as tool in genotype identification and population genetic powerful tool used for studying genetic variability, pedigree analysis, linkage maping, cultivar identification and for other purposes. Therefore, the present study was undertaken with the objectives to characterize *Ambri* apple genotypes on the basis of morphological, biochemical and molecular markers. An effort was made to investigate the feasibility of using SSR markers for studying the genetic variation and to estimate genetic relationships among different *Ambri* apple genotypes of Jammu with respect to different morphological diversity analysis and molecular characterization using SSR

technique. To achieve the objectives, the observations were recorded on morphological features of NBPGR guidelines (NBPGR, 2002). From these fifty genotypes were selected on the basis of fruit weight, fruit length, fruit width and on cluster analysis. The fifty genotypes were analyzed for biochemical and molecular characterization. For molecular characterization 29 simple sequence repeat (SSR) loci were used. The results obtained are briefly summarised and presented as under:

- Among the tree characters studied majority of genotypes had intermediate 25 (50 per cent), followed by vigorous 19(38 per cent), very vigorous in 2 (4 per cent) and rest weak 4(8 per cent) tree vigour. Growth habit of majority of trees were observed spreading 23 (46 per cent), rest had upright in 22 (44 per cent), and few had dropping 5(10 per cent) growth habit. Tree height ranged from 5.23 m (SKJAT -08) to 9.51 m (SKJAM-43), trunk girth from 105.28 cm (SKJAT -08) to 287.09 cm (SKJAK -24) and tree spread 3.20 m (SKJAT -08) to 6.80 m (SKJAD -28).

- Perusal of data related to leaf characters revealed that leaf size varied from small 4 (8 per cent), medium 22 (44 per cent) and large in 24 (48 per cent). Leaf shape in majority of genotypes 30 (60 per cent) had broad elliptic followed by oval in 20 (40 per cent) genotypes.

- Variation observed among flower characters of *Ambri* apple genotypes revealed that number of flower buds per inflorescence 6 number of flower buds per inflorescence was recorded in genotypes SKJAB -04, SKJAT -07, SKJAT -08 SKJAG -16 SKJAG -17, SKJAK-27, SKJAK -28 SKJAK -29, SKJAD-35 SKJAM-45, SKJAM-46 followed by 5 number of flower buds per inflorescence in genotype SKJAB -05, SKJAT-09 SKJAG-10 SKJAG-11 SKJABh -18 SKJABh -19 SKJABh -20, SKJAK -24, SKJAK -25, SKJAK -26, SKJAD -30, SKJAD -31, SKJAD -32, SKJAD -34, SKJAD -36, SKJAN -39, SKJAN -42, SKJAM -43,SKJAM -44, SKJAC -49 and SKJAC -50 and followed by 4 number of flower buds per inflorescence SKJAB -01, SKJAB -02, SKJAB -03 , SKJAT-06, SKJAG -12, SKJAG -13, SKJAG -14, SKJAG -15 , SKJAK -21, SKJAK -22, SKJAK -

23, SKJAD -33, SKJAN-37, SKJAN -38, SKJAN -40, SKJAN -41, SKJAM -47 and SKJAC -48.

- Flower stalk (pedicel) length showed wide variation (25.15 mm) in genotype SKJAC -48 followed by (25.0 mm) in genotype SKJAK -27, (23.1 mm) in genotype SKJAG -14, (22.0 mm) in genotype SKJAT-07. While the minimum flower stalk (pedicel) length (10.7 mm) was observed in genotype SKJABh -20 followed by (10.9 mm) in genotype SKJAB -03, (11.5 mm) in genotype SKJAD -29 and (11.9 mm) in genotype SKJAK -23.

- Date of start of floweringwas observed during blooming period and it is evident from the table 6 that the flowers opened during first to third week of April. Time of flower bud brust among the genotypes ranged from 21 to 25 days. A total of 16 (32.00 per cent) genotypes were early bloomers and flowering started 7th April to 10th April, while 26 (52.00 per cent) genotypes from total of fifty genotypes were mid bloomers and beginning of flowering varied from11th to 15th April and rest 8 (16.00 per cent) genotypes were late bloomers and flowering stared from 16th to 17th April. The date of beginning of flowering was early in SKJAG-15 and late in SKJAC-50.

- The trees were observed during complete blooming period. It was evident from Table 7 that the end of flowering (85-90 per cent of flowering) was during third to fourth week of April. Out of fifty *Ambri* apple genotypes the end of flowering were categorized into two periods i.e., 3rd week of April and 4th week of April. A total of 41 genotypes (82.00 per cent) ended their flowering 3rd week of April, while 9 genotypes (18 per cent) ended their flowering in 4th week of April.

- Regularity of flowering were categorized as regular, biennial and irregular. The results showed that biennial flowering 35 genotypes (70 per cent) was observed in most of genotypes, except that 3 genotypes (6 per cent) were found regular in flowering and 12 genotypes (24 per cent) showed irregularity in flowering.

- Self incompatibility was categorized as incompatible, partially compatible and compatible presented in Table 9. Out of fifty *Ambri* apple genotypes studied self

incompatibility was observed in all 50 genotypes (100 per cent). No selected genotype showed partially compatibility and compatible self incompatibility.

- Bearing habit of *Ambri* apple had three categories *viz.*, on spurs, on shoot tips, on old shoots and mixed. Majority of genotypes 38 (76 per cent) showed bearing on spurs and 12 genotypes (24 per cent) exhibited mixed bearing habit. Whereas on shoot tips and on old shoot tip bearing habit were not observed among fifty selected *Ambri* apple genotypes.

- Age of first bearing was recorded as number of years to attain first fruiting. The results showed that most of the genotypes 24 (48 per cent) started bearing at the age of seven years of planting and 19 genotypes (38 per cent) started bearing fruit at the age of six years of planting, while few selected genotypes 7 (14 per cent) were not precocious and started bearing fruit at the age of eight years of planting. Majority of *Ambri* apple genotypes attained age of their first bearing at the age of seven years followed by six and some had five years.

- Perusal of data related to fruit characters revealed that a wide variation has been recorded in days to fruit harvest of fifty *Ambri* apple genotypes, ranging from 145 to 158 days were recorded as a days to fruit harvest. Fruit shape of majority of *Ambri* apple genotypes had 19 genotypes (38 per cent) globose, followed by 8 genotypes (16 per cent) had globose conical and 10 genotypes (20 per cent) had conical and rest 13 genotypes (23 per cent) possessed long conical fruit shape. Majority of *Ambri* apple genotypes had broad base 27 (54 per cent) followed by narrow base 17 (34 per cent) and rest had intermediate 6 (12 per cent) base. Medium fruit base cavity depth was observed in 18 (36 per cent) genotypes followed by deep fruit base cavity depth in 14 (28 per cent) genotypes**.** Majority of *Ambri* apple genotypes had smooth fruit apex 30 (60 per cent) and rest genotypes had grooved fruit apex 20 (40 per cent).

- A wide range of variation was observed among different genotypes of *Ambr*i apple for various biochemical attributes. The dendrogram of the fifty *Ambri* apple genotypes was constructed based on morphological data in order to examine the

variability in *Ambri* apple. Main 3 clusters were obtained viz., Cluster I Cluster II and Cluster III. Cluster I contained maximum number of genotypes (11) comprising of genotype SKJAB -01 SKJAB -02 SKJAG -11 SKJAK -21 SKJAK -27 SKJAD -34 SKJAD -36 SKJAN -37 SKJAM -46 SKJAM -47 SKJAC -50, cluster II consisted of 25 genotypes SKJAB -03 SKJAB -04 SKJAT -08 SKJAAG -13 SKJAG -15 SKJAG -16 SKJAG -17 SKJABh -18 SKJABh -20 SKJAAK -22 SKJAK -23 SKJAK -24 SKJAK -25 SKJAK -26 SKJAD -28 SKJAD -31 SKJAD -33 SKJAD -35 SKJAN -38 SKJAN -39 SKJAN -40 SKJAM-43 SKJAM-44 SKJAC -48 SKJAC -49, while cluster III consisted of genotypes 14 SKJAB -05, SKJAT -06, SKJAT -07, SKJAT -09, SKJAG -10, SKJAG -12, SKJAG -14, SKJABh -19, SKJAD -29, SKJAD -30, SKJAD -32, SKJAN -41, SKJAN -42 and SKADM-45 respectively. The inter cluster distances were larger than the intra cluster distances indicating wider genetic diversity between cluster with respect to traits considered. Therefore the genotypes falling in these clusters can be used for hybridization programme to get better recombinants in the segregating generations.

- Principal component analysis (PCA) was performed to achieve parsimony and reduce the dimensionality of original multivariate component data. The contribution of different morphological parameters towards variability is (71.8974 per cent) and the contribution of different biochemical parameters towards variability is (76.7622 per cent) through principle component analysis.

- For molecular characterization twenty nine SSR markers revealed clear and consistent amplification profile. Out of 29 SSR markers used, 5 SSR markers namely CH04e03, CH05d11, Hi02610, CH05q07 and CH0c02 were more efficient based on their PIC (Polymorphic Information Content), PP (Percent Polymorphism) and alleles per locus values. The PIC value ranged from 0.02 to 0.8 with an average of 0.458 across fifty *Ambri* apple genotypes. Seventeen out of twenty nine SSR markers revealed PIC value of more than 0.5 and in remaining two markers it was less than 0.5. Marker CH05d11 had a highest PIC value of 0.801 while marker CH05d04, CH05e03, CH03g12z and CH03c02 had the lowest

PIC value of 0.031, 0.078, 0.041 and 0.021 and 0.48 respectively. UPGMA based dendrogram was constructed based on SSR analysis which grouped fifty selections into 2 major clusters.

- Based characterization two best seedling *Ambri* apple genotypes (SKJAK-29 and SKJAK-30) were compared with Red delicious. Above all, it is fruit characters that decide the superiority of any genotype, so morphological and bio-chemical studies revealed that two best seedling genotypes performed better than already commercialized varieties of *Ambri* apple. Molecular study revealed they are genetically diverse.

- Based on the findings of the present investigations, it can be concluded the estimates of coefficient of variation indicate that there is appreciable diversity among the selected seedlings of *Ambri* apple genotypes. Clusters obtained from the morphological characterization were not correlated with the clusters obtained from molecular characterization. Based on biochemical analysis two genotypes namely SKJAK-29 and SKJAA-30 were regarded as best. Out of twenty nine simple sequence repeats (SSR) markers used, seventeen were found polymorphic and cluster analysis grouped the studied seedling *Ambri* apple genotypes into 2 clusters. Morphological and biochemical investigation had already established that two seedling *Ambri* apple genotypes SKJAK-29 and SKJAK-30 from Dool Kishtwar were best for various tree, leaf, flower and fruit characters. On the basis of comparison studies between seedling *Ambri* apple genotypes SKJAK-29, SKJAK-30 and Red Delicious in which morphological and biochemical studies revealed that seedling *Ambri* apple genotypes were better than Red delicious. However, morphological and biochemical traits alone still cannot be regarded as critical indicator to know diversity among seedling *Ambri* apple. Therefore the use of morphological descriptors and biochemical analysis was backed with DNA markers for efficient and reliable genetic diversity studies and germplasm management to present the best picture of diversity among seedling *Ambri* apple.

- Based on similarity coefficient genotypes the highest similarity value (0.74) was recorded in genotype SKJAT-40 and genotype SKJAN-39and the lowest (0.14)

between genotype SKJAM-45, SKJABh-19 and between SKJABh-20, SKJAG-12 showing highest dissimilarity coefficient value between them suggested that a rich genetic variation exists between them and they can be used prospective parents in further breeding programme to get segregates.

- Molecular studies established that these SKJAM-45, SKJABh-19 SKJABh-20 and SKJAG-12 *Ambri* apple seedling genotypes are genetically diverse and do not belong to any of the commercial varieties. So, these SKAM-45, SKJABh-19 SKJABh-20 and SKJAG-12 *Ambri* apple genotypes with excellent traits of interest and high genetic dissimilarity can be used directly as cultivar after completing all codal formalities for variety release or can be further used in breeding programmes to get segregates.

Based on the findings of the present investigation, the following conclusion it can be concluded that genotypes SKJAD-29 and SKAD-30 are diverse at morphological and biochemical level and have good fruit attribute like fruit weight, fruit length, fruit width TSS, and acidity content. Both the genotypes found to be more diverse than Red Delicious with respect to different fruit characters. It is further recommended that these promising seedling strains are having the potential for quality *Ambri* apple production and may be used for further multiplication and breeding programmes in order to enhance the production and quality of *Ambri* apple in province of J&K.

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APPENDIX

Jaccards similarity coefficient

	SKJAB -01	SKJAB -02	SKJAB -03	SKJAB -04	SKJAB -05	SKJAT -06	SKJAT -07	SKJAT -08	SKJAT -09	SKJAG -10	SKJAG -11	SKJAG -12	SKJAG -13	SKJAG -14	SKJAG -15	SKJAG -16	SKJAG -17	SKJABh -18	SKJABh -19	SKJABh -20	SKJAK -21	SKJAK -22	SKJAK -23	SKJAK -24	SKJAK -25	SKJAK -26	SKJAK -27	SKJAD -28	SKJAD -29	SKJAD -30	SKJAD -31	SKJAD -32	SKJAD -33	SKJAD -34	SKJAD -35	SKJAD -36	SKJAN -37	SKJAN -38	SKJAN -39	SKJAN -40	SKJAN -41	SKJAN -42	SKJAM-43	SKJAM-44	SKJAM-45	SKJAM-46	SKJAM-47	SKJAM-48	SKJAC-49
SKJAB -01																																																
SKJAB -02	0.45																																															
SKJAB -03	0.348837	0.512195																																														
SKJAB -04	0.380952	0.409091	0.512195																																												
SKJAB -05	0.333333	0.454545	0.488372	0.560976																																											
SKJAT -06	0.4	0.304348	0.333333	0.428571	0.47619																																											
SKJAT -07	0.425	0.418605	0.487805	0.605263	0.5	0.512821																																										
SKJAT -08	0.363636	0.454545	0.52381	0.6	0.5	0.47619	0.702703																																									
SKJAT -09	0.435897	0.333333	0.463415	0.538462	0.47619	0.380952	0.594595	0.55																																								
SKJAG -10	0.311111	0.431818	0.4	0.465116	0.444444	0.418605	0.47619	0.547619	0.648649																																							
SKJAG -11	0.333333	0.363636	0.428571	0.463415	0.44186	0.414634	0.512821	0.512195	0.611111	0.605263																																							
SKJAG -12	0.285714	0.318182	0.288889	0.318182	0.395349	0.4	0.357143	0.395349	0.272727	0.404762	0.365854																																						
SKJAG -13	0.288889	0.319149	0.265306	0.347826	0.488372	0.395349	0.355556	0.391304	0.333333	0.431818	0.333333	0.348837																																					
SKJAG -14	0.447368	0.340909	0.282609	0.340909	0.355556	0.357143	0.45	0.418605	0.5	0.395349	0.425	0.309524	0.404762																																				
SKJAG -15	0.368421	0.333333	0.365854	0.333333	0.380952	0.384615	0.527778	0.45	0.459459	0.425	0.421053	0.3	0.365854	0.606061																																				
SKJAG -16	0.244444	0.395349	0.333333	0.395349	0.409091	0.348837	0.475	0.44186	0.414634	0.564103	0.348837	0.435897	0.395349	0.461538	0.542857																																				
SKJAG -17	0.222222	0.372093	0.404762	0.372093	0.386364	0.295455	0.414634	0.452381	0.357143	0.428571	0.425	0.375	0.340909	0.333333	0.394737	0.628571																																				
SKJABh -18	0.302326	0.363636	0.363636	0.304348	0.347826	0.318182	0.404762	0.409091	0.414634	0.452381	0.414634	0.217391	0.363636	0.5	0.588235	0.567568	0.628571																																				
SKJABh -19	0.27907	0.311111	0.372093	0.340909	0.297872	0.390244	0.414634	0.386364	0.357143	0.395349	0.357143	0.222222	0.340909	0.435897	0.514286	0.540541	0.513514	0.676471																																				
SKJABh -20	0.333333	0.304348	0.276596	0.276596	0.265306	0.26087	0.404762	0.291667	0.348837	0.326087	0.318182	0.142857	0.25	0.425	0.421053	0.414634	0.461538	0.567568	0.5																																				
SKJAK -21	0.35	0.45	0.348837	0.288889	0.333333	0.272727	0.357143	0.363636	0.333333	0.404762	0.302326	0.285714	0.348837	0.375	0.368421	0.473684	0.410256	0.473684	0.410256	0.555556																																				
SKJAK -22	0.384615	0.45	0.380952	0.288889	0.363636	0.365854	0.357143	0.304348	0.272727	0.340909	0.272727	0.285714	0.318182	0.341463	0.444444	0.435897	0.341463	0.513514	0.447368	0.4	0.542857																																				
SKJAK -23	0.435897	0.395349	0.304348	0.395349	0.377778	0.45	0.404762	0.347826	0.348837	0.355556	0.380952	0.302326	0.363636	0.357143	0.459459	0.414634	0.325581	0.414634	0.461538	0.380952	0.473684	0.6																																				
SKJAK -24	0.363636	0.391304	0.422222	0.306122	0.346939	0.347826	0.4	0.346939	0.291667	0.326531	0.291667	0.25	0.306122	0.418605	0.380952	0.409091	0.418605	0.512195	0.487805	0.44186	0.463415	0.463415	0.377778																																				
SKJAK -25	0.386364	0.382979	0.3	0.354167	0.367347	0.4	0.391304	0.367347	0.3125	0.375	0.369565	0.355556	0.354167	0.347826	0.372093	0.431818	0.377778	0.4	0.44186	0.369565	0.452381	0.452381	0.615385	0.425532																																				
SKJAK -26	0.292683	0.425	0.295455	0.325581	0.404762	0.341463	0.365854	0.311111	0.27907	0.348837	0.375	0.325	0.325581	0.285714	0.342105	0.375	0.35	0.375	0.35	0.309524	0.472222	0.432432	0.527778	0.404762	0.538462																																				
SKJAK -27	0.297872	0.354167	0.333333	0.391304	0.346939	0.347826	0.369565	0.404255	0.347826	0.413043	0.377778	0.276596	0.326531	0.291667	0.282609	0.377778	0.355556	0.369565	0.44186	0.431818	0.525	0.355556	0.431818	0.367347	0.511111	0.5																																				
SKJAD -28	0.272727	0.276596	0.428571	0.363636	0.347826	0.380952	0.439024	0.377778	0.45	0.418605	0.414634	0.272727	0.304348	0.295455	0.384615	0.380952	0.5	0.526316	0.540541	0.414634	0.4	0.435897	0.487179	0.409091	0.369565	0.410256	0.47619																																				
SKJAD -29	0.318182	0.44186	0.44186	0.47619	0.454545	0.363636	0.564103	0.488372	0.463415	0.465116	0.463415	0.348837	0.319149	0.311111	0.4	0.463415	0.512821	0.395349	0.439024	0.363636	0.414634	0.380952	0.5	0.306122	0.413043	0.425	0.454545	0.621622																																				
SKJAD -30	0.292683	0.325581	0.390244	0.390244	0.439024	0.341463	0.435897	0.372093	0.527778	0.526316	0.447368	0.261905	0.325581	0.317073	0.416667	0.341463	0.35	0.375	0.35	0.341463	0.325	0.325	0.410256	0.255319	0.333333	0.268293	0.282609	0.527778	0.583333																																				
SKJAD -31	0.25	0.282609	0.282609	0.340909	0.355556	0.390244	0.380952	0.418605	0.461538	0.463415	0.425	0.309524	0.282609	0.272727	0.292683	0.295455	0.302326	0.295455	0.244444	0.212766	0.27907	0.222222	0.295455	0.173077	0.265306	0.285714	0.297872	0.357143	0.475	0.542857																																				
SKJAD -32	0.243902	0.341463	0.35	0.35	0.4	0.238095	0.358974	0.333333	0.444444	0.410256	0.405405	0.282051	0.309524	0.268293	0.324324	0.368421	0.416667	0.292683	0.238095	0.232558	0.243902	0.214286	0.261905	0.212766	0.288889	0.315789	0.26087	0.368421	0.459459	0.580645	0.5																																				
SKJAD -33	0.22449	0.28	0.306122	0.391304	0.404255	0.291667	0.369565	0.375	0.377778	0.354167	0.347826	0.333333	0.333333	0.244898	0.26087	0.347826	0.355556	0.265306	0.22	0.215686	0.2	0.22449	0.347826	0.178571	0.34	0.229167	0.245283	0.347826	0.444444	0.475	0.452381	0.513514																																				
SKJAD -34	0.227273	0.288889	0.26087	0.318182	0.333333	0.272727	0.23913	0.276596	0.333333	0.404762	0.4	0.421053	0.234043	0.222222	0.181818	0.333333	0.341463	0.244444	0.170213	0.166667	0.255814	0.227273	0.217391	0.176471	0.297872	0.292683	0.333333	0.302326	0.318182	0.261905	0.410256	0.428571	0.395349																																				
SKJAD -35	0.27907	0.282609	0.340909	0.311111	0.270833	0.357143	0.348837	0.355556	0.425	0.463415	0.390244	0.25	0.204082	0.272727	0.292683	0.325581	0.333333	0.325581	0.333333	0.325581	0.27907	0.222222	0.295455	0.244898	0.347826	0.285714	0.418605	0.425	0.404762	0.384615	0.513514	0.378378	0.386364	0.486486																																				
SKJAD -36	0.342105	0.27907	0.309524	0.375	0.390244	0.358974	0.421053	0.357143	0.394737	0.435897	0.472222	0.342105	0.309524	0.333333	0.4	0.325	0.3	0.292683	0.268293	0.261905	0.243902	0.243902	0.358974	0.266667	0.348837	0.282051	0.234043	0.325	0.375	0.515152	0.444444	0.5	0.390244	0.342105	0.444444																																				
SKJAN -37	0.333333	0.333333	0.333333	0.302326	0.348837	0.317073	0.341463	0.348837	0.317073	0.390244	0.421053	0.333333	0.302326	0.232558	0.315789	0.285714	0.358974	0.255814	0.204545	0.285714	0.333333	0.268293	0.317073	0.26087	0.340909	0.342105	0.282609	0.317073	0.4	0.378378	0.394737	0.484848	0.380952	0.485714	0.432432	0.53125																																				
SKJAN -38	0.380952	0.409091	0.409091	0.377778	0.333333	0.333333	0.355556	0.361702	0.363636	0.431818	0.363636	0.26087	0.291667	0.255319	0.302326	0.363636	0.372093	0.333333	0.311111	0.333333	0.380952	0.414634	0.363636	0.306122	0.27451	0.23913	0.27451	0.363636	0.409091	0.390244	0.349909	0.341463	0.306122	0.288889	0.311111	0.447368	0.435897																																				
SKJAN -39	0.363636	0.422222	0.422222	0.391304	0.375	0.347826	0.369565	0.404255	0.319149	0.413043	0.347826	0.304348	0.361702	0.22	0.288889	0.291667	0.386364	0.347826	0.326087	0.319149	0.395349	0.363636	0.377778	0.32	0.313725	0.311111	0.34	0.44186	0.454545	0.404762	0.452381	0.325581	0.375	0.363636	0.418605	0.5	0.526316	0.615385																																				
SKJAN -40	0.365854	0.395349	0.428571	0.395349	0.347826	0.348837	0.372093	0.377778	0.318182	0.418605	0.348837	0.272727	0.333333	0.212766	0.285714	0.318182	0.357143	0.318182	0.325581	0.288889	0.365854	0.365854	0.348837	0.319149	0.340426	0.25	0.340426	0.348837	0.395349	0.341463	0.357143	0.261905	0.319149	0.333333	0.390244	0.432432	0.459459	0.666667	0.742857																																				
SKJAN -41	0.380952	0.512195	0.377778	0.291667	0.361702	0.304348	0.386364	0.333333	0.25	0.340426	0.276596	0.318182	0.409091	0.340909	0.333333	0.363636	0.404762	0.333333	0.311111	0.304348	0.414634	0.414634	0.363636	0.422222	0.354167	0.390244	0.3	0.363636	0.44186	0.325581	0.282609	0.309524	0.254902	0.208333	0.204082	0.27907	0.333333	0.512195	0.5	0.5																																				
SKJAN -42	0.348837	0.44186	0.44186	0.319149	0.391304	0.428571	0.452381	0.361702	0.304348	0.369565	0.304348	0.348837	0.319149	0.255319	0.333333	0.304348	0.372093	0.304348	0.340909	0.304348	0.318182	0.414634	0.333333	0.333333	0.326531	0.295455	0.333333	0.461538	0.512195	0.461538	0.340909	0.317073	0.306122	0.254043	0.311111	0.309524	0.272727	0.44186	0.5	0.5	0.631579																																				
SKJAM-43	0.473684	0.395349	0.363636	0.333333	0.44186	0.380952	0.404762	0.347826	0.380952	0.355556	0.288889	0.244444	0.311111	0.295455	0.317073	0.318182	0.266667	0.288889	0.266667	0.288889	0.333333	0.365854	0.414634	0.347826	0.369565	0.309524	0.291667	0.348837	0.395349	0.410256	0.325581	0.368421	0.265306	0.191489	0.295455	0.394737	0.317073	0.463415	0.409091	0.348837	0.428571	0.463415																																				
SKJAM-44	0.318182	0.409091	0.347826	0.377778	0.422222	0.276596	0.326087	0.361702	0.363636	0.431818	0.333333	0.348837	0.409091	0.282609	0.272727	0.363636	0.404762	0.276596	0.229167	0.22449	0.26087	0.26087	0.276596	0.254902	0.3	0.23913	0.254902	0.304348	0.409091	0.390244	0.349909	0.459459	0.391304	0.288889	0.255319	0.341463	0.4	0.409091	0.422222	0.333333	0.47619	0.409091	0.538462																																				
SKJAM-45	0.333333	0.333333	0.304348	0.304348	0.319149	0.234043	0.311111	0.319149	0.318182	0.326087	0.348837	0.244444	0.304348	0.23913	0.227273	0.26087	0.295455	0.208333	0.14	0.26087	0.272727	0.217391	0.288889	0.24	0.369565	0.195652	0.291667	0.234043	0.355556	0.309524	0.325581	0.368421	0.377778	0.302326	0.325581	0.394737	0.35	0.333333	0.409091	0.348837	0.333333	0.363636	0.487179	0.538462																																				
SKJAM-46	0.365854	0.395349	0.333333	0.333333	0.377778	0.288889	0.372093	0.409091	0.380952	0.418605	0.348837	0.244444	0.363636	0.325581	0.285714	0.318182	0.325581	0.26087	0.212766	0.234043	0.272727	0.272727	0.288889	0.291667	0.26	0.195652	0.24	0.234043	0.333333	0.341463	0.390244	0.368421	0.347826	0.272727	0.295455	0.358974	0.35	0.395349	0.409091	0.348837	0.463415	0.395349	0.526316	0.621622	0.567568																																				
SKJAM-47	0.162791	0.285714	0.255814	0.2	0.272727	0.268293	0.232558	0.217391	0.209302	0.27907	0.209302	0.25	0.2	0.186047	0.170732	0.3	0.307692	0.238095	0.214286	0.209302	0.25	0.282051	0.238095	0.244444	0.295455	0.25641	0.244444	0.405405	0.317073	0.4	0.342105	0.4375	0.333333	0.351351	0.342105	0.342857	0.263158	0.285714	0.365854	0.268293	0.35	0.459459	0.405405	0.35	0.333333	0.333333																																				
SKJAM-48	0.266667	0.326087	0.297872	0.270833	0.3125	0.255319	0.363636	0.340426	0.311111	0.377778	0.340909	0.325581	0.326087	0.26087	0.341463	0.372093	0.45	0.372093	0.318182	0.282609	0.295455	0.357143	0.340909	0.285714	0.361702	0.333333	0.3125	0.475	0.452381	0.435897	0.380952	0.394737	0.369565	0.295455	0.348837	0.35	0.27907	0.297872	0.431818	0.311111	0.418605	0.525	0.404762	0.418605	0.372093	0.439024	0.558824																																				
SKJAC-49	0.268293	0.244444	0.217391	0.272727	0.208333	0.227273	0.27907	0.288889	0.255814	0.266667	0.227273	0.238095	0.191489	0.232558	0.219512	0.285714	0.261905	0.255814	0.204545	0.2	0.238095	0.238095	0.35	0.208333	0.372093	0.275	0.26087	0.35	0.333333	0.342105	0.358974	0.371429	0.348837	0.268293	0.325	0.324324	0.25	0.333333	0.325581	0.285714	0.272727	0.302326	0.459459	0.365854	0.421053	0.35	0.411765	0.410256																																				
SKJAC-50	0.27907	0.282609	0.255319	0.255319	0.244898	0.23913	0.26087	0.297872	0.295455	0.333333	0.23913	0.25	0.229167	0.244444	0.232558	0.325581	0.333333	0.295455	0.191489	0.266667	0.25	0.25	0.295455	0.270833	0.319149	0.227273	0.297872	0.390244	0.340909	0.35	0.333333	0.416667	0.386364	0.341463	0.333333	0.3	0.292683	0.340909	0.355556	0.295455	0.340909	0.372093	0.461538	0.475	0.5	0.461538	0.545455	0.487179	0.65625

VITA

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CERTIFICATE-IV

Certified that all necessary corrections as suggested by the external examiner and advisory committee have been duly incorporated in the thesis entitled **"Morphological, Biochemical and Molecular Characterization of Ambri apple in Doda and Kishtwar Districts of J&K"** submitted by **Ms. Koushalya Devi**, Registration No. **J-15-D-248-A.**

16/8/21

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Place: Jammu

Date: 23-09-2021

23/09/2021

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