MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF STRAWBERRY CULTIVARS UNDER SUBTROPICAL CONDITIONS OF JAMMU

by

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(J-21-MB-44)

Thesis submitted to
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MOLECULAR BIOLOGY AND BIOTECHNOLOGY

School of Biotechnology

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CERTIFICATE-I

This is to certify that the thesis entitled **"Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu"** submitted in partial fulfilment of the requirements for the degree of **Masters of Science in Agriculture (Molecular Biology and Biotechnology)** to the Faculty of Agriculture, 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. Isha Kotwal,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma. It is further certified that help and assistance received during the course of thesis investigation have been duly acknowledged.

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We, the member of the Advisory committee of Ms. **Isha Kotwal**, Registration No. **J-21-MB-44**, a candidate for the degree of **Masters of Science in Agriculture (Molecular Biology and Biotechnology)**, have gone through the manuscript of the thesis entitled **"Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu"** and recommend that it may be submitted by the student in partial fulfilment of the requirements for the degree.

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ABSTRACT

Title of the Thesis	:	**Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu**
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Major Subject	:	Molecular Biology and Biotechnology
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Abstract

A field trial was conducted in the experimental field of Advanced Centre for Horticulture Research, Udheywalla, Jammu in Randomised Block Design with three replications while the molecular research was carried out in the laboratory of School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, during the year 2022-23. A total of nineteen strawberry genotypes were evaluated. Among the cultivars, 'Chandler,' 'Winter Dawn,' 'Nabila,' and 'Selva' displayed the widest plant spread, most leaves per plant, and maximum leaf area. 'Chandler,' 'Nabila,' and 'Sweet Charlie' showed the highest number of runners. 'Fern' and 'Nabila' took the longest for runner formation. 'Chandler' had the longest flowering period. 'Cultivars 'Chandler,' 'Winter Dawn,' 'Sweet Charlie,' and 'Nabila' exhibited the highest fruit yield. 'Fairfox' had the most acidity. 'Chandler' had the maximum reducing and non-reducing sugars, along with the highest total sugar content. 'Selva' and 'Winter Dawn' had the highest TSS/acidity ratio. High heritability and high genetic advance as per cent of mean was observed in all the characters except for number of days to runners formation, flower size, number of days to flowering after planting.

ISSR markers used in the study generated a total of 78 amplicons out of which 73 were polymorphic, making up 93.58 percent of the total polymorphism.The Polymorphic information content value ranged from 0.20 to 0.43 with an average of 0.28.The Jaccard's similarity index was calculated based on UPGMA and dendrogram was made.The genotypes were grouped into two major Clusters. Cluster 1 included fifteen genotypes that was further divided into two sub clusters i.e.,1A and 1B .Sub cluster 1A had three genotypes and sub cluster 1B had twelve genotypes. Cluster 2 included four genotypes and it was further divided into two sub clusters i.e, sub cluster 2A and sub cluster 2B .Sub cluster 2A had three genotypes and sub cluster 2b had one genotype. The similarity coefficient of dendrogram made by ISSR primers range from 0.55 to 0.75 reflected the moderate intensity of variation present in the collected genotypes.

Keywords: Genotypes, heritability, genetic advance, polymorphism, polymorphic information content.

Vikas Tandon

Signature of Major Advisor

Isha Kotwal

Signature of Student

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LIST OF ABBREVIATIONS

Abbreviation		Terminology
%	:	Percentage
σ	:	Standard deviation
σ^2	:	Variance
µl	:	Micro litre
µM	:	Micro molar
^0C	:	Degree Celsius
bp	:	Base pair
cm	:	Centimetre
CTAB	:	Cetyl trimethyl ammonium bromide
CD	:	Critical difference
df	:	Degree of freedom
et al.	:	Et alia meaning ‘and others’
$MgCl_2$	:	Magnesium chloride
NaCl	:	Sodium chloride
NaOH	:	Sodium hydroxide
PCR	:	Polymerase chain reaction
RNase	:	Ribonuclease enzyme
rpm	:	Rotations per minute
ISSRs	:	Inter simple sequence repeats
TE	:	Tris EDTA

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CHAPTER-1

INTRODUCTION

The strawberry (*Fragaria x ananassa* Duch.) is a natural hybrid of two American species, *Fragaria chiloensis* and *Fragaria virginiana*. It is a widely grown hybrid species of the genus *Fragaria*, displaying an octaploid chromosomal makeup (2n=8x=56). Falling under the Rosaceae classification, this species holds significant commercial value as a consumable fresh fruit. The hybrid fruit is different from parents by their distinctive and desirable traits. It is well adopted to many different climates - moderate, Mediterranean, subtropical and even to high altitudes of tropical climate due to its genotypic diversity, highly heterozygous nature and broad range of environmental adaptations.

The fruit is grown mainly in Mahabaleshwar, Wai and Panchagani areas in India.The Panchgani-Mahabaleshwar belt contributes around 85% of the total production in the country. The rest comes from Himachal Pradesh and Jammu and Kashmir. In India it is generally cultivated in the hills. Its main center of cultivation are Nainital and Dehradun in Uttarakhand, Mahabaleshwar (Maharashtra), Kashmir Valley, Bangalore and Kalimpong (West Bengal). In recent years, strawberry is being cultivated successfully in plains of Maharashtra around Pune, Nashik and Sangali towns. Satara district famous for the country's 80% strawberry production. Currently, it is extensively cultivated in Samba, Kathua, Udhampur and Reasi, districts of Jammu and Kashmir. A Production of 20.35 lac MTs (2020-21) have been recorded in Jammu and Kashmir .The area under fruit cultivation is estimated 3.33 Lac Ha (2020-21) (Anonymous, 2021).

The strawberry, a perennial herbaceous plant, features an aggregate fruit structure where the fleshy part arises from the receptacle, not the ovaries. Each visible "seed," or achene, on the exterior represents a flower's ovary enclosing a seed. Typically holding about 200 seeds, strawberries mature to full crimson within 28–30 days post anthesis, boasting substantial size. Notably perishable and non-climacteric in nature (Coombey, 1976).

The captivating aroma of strawberries owes its allure to compounds like methyl hexanoate, ethyl heptanoate, ethyl hexanoate, ethyl butanoate, ethyl propionate, furanone, methyl butanoate, and linalool (Yamdagni and Sharma, 2000).

The vivid red hue finds its origin in anthocyanin, particularly pelargonidin 3-monoglucoside, accompanied by traces of cyanidin (Mitra, 1991). Research underscores strawberries role in averting cancer, cardiovascular ailments, and other health challenges (Capocasa *et al.*, 2008). The intrinsic plant phenol, ellagic acid, found in strawberries, exhibits potent anticancer properties. Consistent consumption also yields asthma control benefits (Mangal *et al.*, 1998). One can increase their dietary antioxidants by consuming strawberry (Cook & Samman, 1996). The abundant phytochemicals and their antioxidant potency, observed in various studies, offer safeguarding against chronic and degenerative diseases (Record *et al.*, 2001).

Earning a distinguished status on the global stage, strawberries reign as the foremost soft fruit, celebrated for their profound nutritional bounty (abundant in vitamins and minerals). Their allure resonates through captivating aroma, beguiling appearance, and invigorating flavor (Kher *et al.*, 2010). Remarkably, strawberry cultivation yields the swiftest economic rewards, making it a preferred choice, as its therapeutic attributes (anticancer, antidiabetic, and antioxidant) capture the attention of consumers across age groups (Asrey and Patel, 2003). The berries nutritional legacy is extraordinary, boasting a treasure trove of vitamins A, B, C, and niacin, alongside vital minerals such as phosphorus, potassium, calcium, and iron, thereby embodying a veritable powerhouse of nourishment (Karkara and Dwivedi, 2002).

This coveted fruit commands a significant presence, sought after both for its exquisite table appeal and its diverse applications in crafting jams, canning, ice cream, beverages, wines, and an array of premium creations. A treasure trove of pectin, primarily in the form of calcium pectate, makes it an exceptional ingredient for crafting delectable jellies of distinction (Mitra, 1991). A marvel of nature, strawberries boast an impressive 98% edible composition, embodying a fruit in its entirety. Within its succulent confines, strawberries reveal a composition of 87.8% water, 0.7% protein, 0.2% fat, and 0.3% iron,

encapsulating within 100g of fresh fruit a burst of vitality, yielding approximately 30 calories of invigorating energy.

Based on the morphological traits of leaves, blossoms, and fruits, strawberry cultivars have conventionally been distinguished (Dale 1996). However, effectiveness of this method is constrained, as morphological indicators can struggle to differentiate closely related cultivars. Environmental factors including production practices, can further complicate the manifestation of morphological traits. The consequences of inbreeding among strawberry cultivars manifest rapidly, inducing a decline in vigor, yield, and fruit dimensions (Morrow and Darrow, 1952; Spangelo, 1971).

In recent decades, the momentum of strawberry production and demand has surged, propelled by its health advantages and widespread integration into both the culinary and cosmetic sectors. In response to this escalating appetite, we are embracing genetic enhancement initiatives to match the growing demand for strawberries. The journey of refining the strawberry's genetic landscape commenced in the mid-18th century, and within the span of the last 50 years, dedicated breeding programs have orchestrated remarkable strides, propelling the genetic development of cultivated strawberries to unprecedented heights (Hancock *et al.*, 1996).

Due to the extensive array of cultivated strawberry cultivars, the need to promptly establish a dependable classification system and ascertain the genetic diversity within strawberry germplasm for precise breeding endeavors has become imperative. The distinctiveness among plants arises from the inherent fluctuations in their DNA, forming the essence of their genetic composition. Unveiling the potential within these genetic differences holds great promise for strawberry research and industry advancement, marking a pivotal avenue of exploration (Whitaker, 2011).

Molecular markers are one of the promising tools for detection and exploitation of DNA (deoxyribonucleic acid) polymorphism. One of the most important tools for strawberry research is the creation and application of DNA markers, and this topic has been covered by a number of authors, starting with Hokanson and Mass (2001). These markers wield their power in deciphering genetic diversity, pinpointing cultivars, unraveling

polymorphisms, and sculpting genetic maps.Therefore, molecular markers and associated technologies are being utilised to assess the genetic diversity of strawberry cultivars and to identify them (Chemda *et al.*, 2001).

Through the use of DNA-based markers and other molecular biology techniques, it is now possible to compare various genetic materials directly and without regard to outside factors (Weising *et al.*, 1995). The intricacies of genetic resemblance and relationships among the examined samples can be deciphered through a meticulous analysis of their banding patterns.

For genetic divergence investigations, a number of techniques that are mostly focused on DNA analysis are to be used. Genetic divergence research on strawberries use the markers ISSR and RAPD (Hussein *et al.,* 2008). The ISSR technique stands as a potentially invaluable instrument for identifying strawberry varieties. Its attributes of simplicity, affordability, rapidity, exceptional discriminatory capability, and steadfast reliability render it a compelling choice (Arnau *et al.*, 2003).

The present study entitled **"Morphological and Molecular characterization of strawberry cultivars under subtropical conditions of Jammu"** was undertaken with the following objectives:

1. To study the morphological and biochemical traits of different strawberry cultivars under subtropical conditions of Jammu.

2. Molecular characterization of the strawberry genotypes.

CHAPTER-2

REVIEW OF LITERATURE

The strawberry (Fragaria spp.) ranks among the most widely cultivated fruit crops worldwide, with production spanning nearly every continent and surpassing 4 million tonnes annually since 2007 (Wu *et al.*, 2012). Natural ploidy levels within strawberries encompass diploid, tetraploid, hexaploid, and octoploid forms, while some instances of rare decaploidy have also been documented (Hancock *et al.*, 1993; Di Meglio *et al.*, 2014). The base chromosome number of strawberries stands at seven, encapsulating a substantial genetic diversity spanning wild diploid to decaploid specimens (Stewart and Folta, 2010), fostering genotypic and phenotypic distinctions even within the same species. Because of its commercial value and popularity, the strawberry is a thoroughly documented fruit crop. Numerous researchers have meticulously studied diverse strawberry cultivars to unearth their distinctive attributes.

In this chapter, a comprehensive review delves into recent and relevant research pertinent to the ongoing project titled "Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu." The ensuing subheadings present a thorough review of the literature on morphological, biochemical, and molecular characterization.

2.1. Morphological characterization of cultivars

2.2. Biochemical characterization of cultivars

2.3. Molecular characterization of cultivars

2.1 Morphological characterization of cultivars

2.1.1 Plant growth characters

Different vegetative characteristics, such as runner production, number of runner, plant vigour, and plant spread, have been investigated by different scientists. Li *et al.*, (1993) found significant variations in plant heights between cultivars in the range of 25.5

cm and 28.5 cm, with the highest plant heights reported in ‘Festival,’ followed by ‘Camarosa’, and the lowest reported in’ Ofra’. Cultivars ‘Selva’ and ‘Ofra’ had substantially shorter plants than ‘Chandler’, and this variation was primarily due to the genetic makeup of the variety.

Ahad *et al.,* (2012) conducted a comparative study to identify the finest strawberry cultivars for boosting production of high-quality fruits. There were eight strawberry types (Chandler, Tioga, catskill, Confutura, Gorella, Pajaro, Selva, and ‘Fern) evaluated within polyhouse. ‘Chandler’ produced the most runners (8.54) and the plant spread (27.43 cm) that was the widest. And ‘Confutura’ (89.78 cm) produced maximum runner length. It was concluded that ‘Chandler’ and ‘Tioga’ performed well under polyhouse conditions for growth characters.

Sahu *et al.,* (2014) conducted a field trial of strawberry genotypes in the Himachal Pradesh region's mid-hill climate, and discovered that 'Festival' and 'Camarosa' stood out as the leading cultivars in relation to leaf area and plant height. 'Festival' and 'Camarosa' cultivars exhibited superior performance in terms of leaf area and plant height.

Das *et al.,* (2015) revealed that short-day strawberry cultivars were more pest-tolerant than day-neutral types, leading to higher yields during later fruit development stages. Optimal micro-climate conditions were crucial for superior plant growth, increased crop yield, and top-quality fruits. The 'Festival' variety stood out, excelling in fruit weight, yield, and marketable quality. Achieved remarkable growth metrics, including a maximum plant height, flowering duration, fruiting duration and a yield.

Thakur *et al.,* (2016) investigated nine strawberry varieties in the Kullu valley's mid-hills. 'Belrubi' displayed the tallest plant height (16.37 cm), 'Fern' achieved the widest plant spread (16.90 cm), 'Belrubi' exhibited the longest leaf length (16.90 cm), and 'Dana' showed the shortest (10.00 cm). ‘Chandler’ recorded the maximum leaf area, leaf blade length (6.27 cm), and leaf blade breadth (5.73 cm). 'Belrubi' and 'Chandler' were identified as the optimal cultivars for profitable cultivation in the mid-hill area of Kullu valley.

Kaur *et al.,* (2017) evaluated runners from five strawberry cultivars at 30x40 cm intervals on raised beds across three planting times: mid-October, end October, and mid-

November. The study's findings revealed that 'Chandler' excelled among the cultivars, producing the highest number of shoots, leaves, runners, flowers, and yield the most. Conversely, 'Selva' demonstrated the lowest performance across all the parameters. Mid-October emerged as the optimal planting time across all measured aspects.

Sonkar *et al.,* (2020) collected data for various strawberry varieties in the sub-tropical region of Western Malwa Plateau. In late October, runners from 12 varieties were planted. Results highlighted that 'Chandler' exhibited the highest plant height, longest leaf length (7.70 cm), and widest leaf, except for the number of runners per plant. 'Chandler' was identified as the optimal choice for the Western Malwa Plateau conditions in Madhya Pradesh.

Chawla *et al.,* (2020) Due to crucial photoperiod and temperature requirements, strawberry farming is determined by the suitability of cultivars in the growing zones. For yield-affecting factors like runner production, it is important to assess how well the various genotypes perform throughout vegetative growth. The cultivars 'Chandler', 'Camarosa', and 'Winter Dawn' were reported to have higher vegetative growth and lower mortality, whereas 'Sweet Charlie'and 'Hadar' were found to have poor adaptability and high mortality in the sub-mountainous region of Punjab.

Jan *et al.,* (2021) the growth characteristics of fifteen strawberry cultivars were examined. It was discovered that cultivar 'Catskill' had the highest maximum plant height (35.30 cm) and the most runners (8.82); 'Curaltar' had the highest average plant spread (34.16 cm); and cultivar 'Camarosa' had the largest maximum leaf area (42.86 cm^2). The number of leaves varied significantly among cultivars, ranging from 23.17 in 'Winter Dawn' to 53.87 in Everly, with 'Kimberly' and 'Catskill' coming in second and third, respectively, with 43.88 and 37.72 leaves. From 20.73 cm^2 in the cultivar 'Catskill' to 42.86 cm^2 in the cultivar 'Camarosa', the leaf area varied among varieties. The cultivar 'Catskill' generated the most runners (8.82), whereas cultivar 'Winter Dawn' yielded the fewest runners (3.96).

Kundu *et al.,* (2022) assessed eight strawberry cultivars (Barak, Crystal, Gilt, Hader, Sabrina, Sabrina 1, Sweet Charlie, and Winter Dawn) in a new alluvial zone.

Significant differences were observed in plant height, spread, runners, leaf area, and leaves per plant. 'Sweet Charlie' and 'Winter Dawn' were identified as superior performers in terms of growth.

Singh *et al.,* (2022) discovered that the effect of plant growth regulators and mulches on growth and yield of strawberry cultivar ‘Chandler’ is different for different vegetative characters. With seventeen treatments and three replications, the experiment was set up using a randomised block design. The outcomes demonstrated that different mulches and plant growth regulators have an impact on strawberry growth and output. An observation was made of the maximum plant height (24.4 cm), the number of leaves per plant (28.5), the length of the leaves (9.4 cm), and the petiole (12.4 cm).

2.1.2 Floral characters

In Dhaliwal and Singh's study (1983), comparisons of flowering times among strawberry varieties revealed significant variations. 'Red Coat' initiated flowering over the longest period (119 days), with a total flowering span of 66 days. 'Dilpasand' had the highest flower count per plant (45.94), followed by 'Pusa Early Dwarf' (37.50), 'Senga Sengana' (35.66), and 'Red Coat' had the lowest flower count per plant (12.55). Notably, 'Gorella' plants were reported to have 32 flowers each.

Kumar *et al.,* (2012) Field studies were performed to identify the best strawberry varieties for boosting the production of fruits of the highest caliber. There were not many strawberry varietals reviewed. After 97 days of planting, Tioga's first flower arose. The flowering plant with the most flowers per plant was ‘Chandler’ (27.23).

Yadav *et al.,* (2014) the flowering characteristics of fifteen strawberry cultivars were examined. The cultivars ‘Pajaro’, ‘Howard’, ‘Catskill’, ‘Tioga’, ‘Dana’, and ‘Chandler’ were the first to flower. The fact that different strawberry cultivars have widely varying requirements for chilling and that plants of these cultivars were able to grow and produce early flowers without a prolonged chilling period may be the cause of the variation in the timing of flowering among strawberry cultivars (Craig and Brown, 1977; Nicoll and Galletta, 1987). In contrast to late blooming cultivars, which had a shorter flowering period, early blooming cultivars had a longer flowering season.

Chandel *et al.,* (2014) found that 'Sweet Charlie' and 'Ofra' were the earliest bloomers among thirteen genotypes, while 'Camarosa' and 'Sweet Charlie' had the longest flowering durations (106.5 days each). Investigating the flowering process, Jami *et al.,* (2015) evaluated cultivars including 'Fairfox', 'Ofra', 'Elista', 'Douglas', 'Blackmore', 'Shasta', 'Shimla Delicious', 'Sweet Charlie', 'Belrubi', and 'Chandler'. 'Sweet Charlie' had the highest flower count (35.07), followed by 'Chandler' (34.81) and 'Fairfox' (32.93), with the most berry set per plant (33.73). 'Ofra' exhibited the slowest flowering (132 days), while 'Sweet Charlie' showed the quickest (107.67 days). According to reports, 'Sweet Charlie' outpaced 'Fairfox' by 9.17 flowers per plant, with 35.07 to 25.90 respectively.

To investigate the variation in vegetative and floral characteristics, Kumar *et al.,* (2020) assessed strawberry varieties. Eleven genotypes were included in the experiment, which had a random block design layout. The findings indicated that the flowering traits varied noticeably between strawberry varieties. While the cultivars ‘Chandler’ produced the most flowers per runner and had the longest flowering period (36 days), cultivars ‘Gorella’, ‘Belrubi’, and ‘Brighton’ cultivars date of initiation of first flowering was recorded.

Sharma *et al.,* (2021) investigated on few strawberry cultivars and revealed that Everly's blossoming lasted for the longest amount of time (85.30 days), while Winter Dawn's flowering lasted the smallest amount of time (55.38 days). There were noticeable variations in the number of flowers per plant across the several strawberry varieties, ranging from 10.17 ‘Missionary’ to 26.55 ‘Camarosa’. ‘Chandler’ required the fewest number of days from flowering to harvest (33.20), but ‘Brighton’ required a longer period of time (53.90).

2.1.3 Fruit physical characters

Morgan (2006) found that 'Missionary' strawberries yielded the heaviest fruits (26.02), on par with 'Ofra' (25.61), 'Camarosa' (22.43), and 'Fairfox' (22.25). 'Senga Sengana' produced the lightest fruits (14.85). Pollination and fertilization during blooming influenced berry size and shape via achene count.

Rao *et al.,* (2010) the performance of 17 strawberry genotypes was evaluated under Garhwal agro-climatic conditions. The results revealed that the genotypes ‘Chandler’ (190.70 g) and ‘Senga Sengana’ (165.80 g) for yield per plant, berry length and berry width ‘Belrubi’ (16.80) and ‘Gorella’ (15.10) for number of fruit per plant were found superior as compared to other genotypes.

Chhetri *et al.,* (2016) evaluated strawberry genotypes to study the variability in fruit yield .The results showed that different strawberry cultivars exhibited marked variation in the fruiting characteristics. 'Dana' exhibited the greatest fruit breadth, number of fruits per plant, and yield per plant among all genotypes. On the other hand, 'Shimla Delicious' had the highest fruit length.

Gaikwad *et al.,* (2018) evaluated 'Camarosa', 'Winter Dawn', 'Nabila', and 'Seascape' cultivars using a randomized block design with four treatments and five replications. Parameters included plant height, spread, fruit characteristics (number, weight, and yield), TSS, and harvesting duration. 'Seascape' had the least height (22.9 cm), fruit weight (24.40 g), yield per plant (839 g), and yield per hectare (33.55 MT), comparable to 'Winter Dawn' and 'Nabila'. 'Nabila' displayed the tallest height (30.80 cm), while 'Winter Dawn' had the widest East-West (35.92 cm) and North-South (33.92 cm) spread, akin to 'Camarosa' (32.92 and 31.28 respectively) and 'Nabila' (32.00 cm North-South spread).

Hansra *et al.,* (2019) four strawberry cultivars-‘Sweet Charlie’, ‘Winter Dawn’, ‘Camarosa’, and ‘Festival’ were examined in a study on terrace gardening in subtropical climates. Important fruit quality indicators were chosen. In ‘Winter Dawn’ (16.5), the most fruits were collected per plant. However, ‘Sweet Charlie’ has the largest fruit size. ‘Winter Dawn’ had the maximum fruit yield per plant (211.2 g), ‘Sweet Charlie’ had the greater fruit weight (13.2 g).

Wani *et al.,* (2021) evaluated fruit length and diameter among all the strawberry cultivars it was highest in ‘Curaltar’ (4.28 cm) and ‘Camarosa’ (3.39 cm) .The cultivar ‘Chandler’ reported a length of 2.62 cm and a diameter of 2.65 cm, whereas cultivar ‘Brighton’ had a minimum berry length of 2.55 cm. The outcomes are consistent with

Sharma and Thakur's (2008) observations in ‘Chandler’, where they noted longer and wider berries. From 4.94 g (Catskill) to 16.30 g (Kimberly), the average berry weight was the highest, followed by 'Honeoye'. The cultivar ‘Camarosa’ produced the most fruit, measuring 13.90 cm^3, while cultivar 'Catskill' produced the least, and measuring 6.17 cm^3.

2.2 Biochemical characterization of cultivars

Chaturvedi *et al.,* (2011) found that 'Seascape' had the highest TSS (0.53 percent) and acidity (12.67 °B and 0.77 percent), while 'Addie' had the lowest TSS (9.73 °B). Among fresh fruits, 'Fern' had the highest ascorbic acid concentration whereas 'Seascape' had the lowest (62 mg/100 g).

Sharma *et al.,* (2014) evaluated fifteen strawberry cultivars for quality attributes. The results revealed that cultivar ‘Chandler’ exhibited maximum total sugar content (6.81%). 'Pajaro' exhibited higher TSS (12.17°B) and T.S.S/ acid ratio (14.02°B). Acidity was found highest (1.14%) in 'Catskill', while sugar/acid ratio was found highest (11.04) in 'Selva'. With regard to quality characters, cultivar ‘Chandler’ stand promising in mid hills of Himachal Pradesh.

Belakud *et al.,* (2015) evaluated 15 genotypes and discovered that 'Dana' had the highest juice content (89.98%). 'Belrubi' had the most total sugar (7.23%), while 'Phenomenal' and 'Selva' exhibited the highest acidity (0.19%). Among them, 'Sweet Charlie' had the lowest acidity (0.09%), yet it displayed the highest ascorbic acid levels (50.00%) and juice pH (5.92).

Mishra *et al.,* (2015) reported 'Sweet Charlie' and 'IC 318916' had high TSS (9.53 °B) and acidity (0.82%). 'Addie' showed reduced sugars (3.63%), while 'Swiss' had the maximum total sugar content (4.55%). 'IC 318916' exhibited the highest ascorbic acid (73.60 mg/100 g).

Asadpoor *et al.,* (2015) assessed six strawberry cultivars ('Kordestan', 'Parose', 'Marak', 'Queen', 'Selva', and 'Camarosa') in a tropical area in Iran. Biochemical characters, were determined i.e total soluble solid (TSS), juice acidity (TA), the sugar-acid ratio (TSS/TA). The results of variance analysis showed that the effects of cultivars on

biochemical parameters were significant. The highest total soluble solid (TSS), sugar-acid ratio (TSS/TA) were in Queen. Total acidity (TA), were significantly high in 'Camarosa' cultivar compared with the other cultivars.

Tohidloo *et al.,* (2018) evaluated a nutrient solution experiment for biochemical characteristics of strawberry genotypes under different potassium levels of nutrient solution. Potassium concentrations including 235 (control), 350, 450 and 600 mg L^{-1} applied to three strawberry genotypes of 'Camarosa', 'Selva' and 'Parus' under hydroponic culture. Fruit total soluble solids (TSS) in 'Camarosa' and 'Selva' were maximum in 350 and 450 mg L^{-1}.

Karuna *et al.,* (2020) carried out an experiment to demonstrate the biochemical characteristics of strawberry cv. 'Camarosa' by the use of chitosan, calcium chloride, and low temperature storage (2 °C). After each harvest, strawberries were treated with chitosan and calcium chloride to increase their biochemical qualities (TSS, Titrable acidity, anthocyanins, antioxidants, and ascorbic acid), which were then measured along with their organoleptic score at storage temperature (2 °C). Chitosan 6 g/L + 1.00% $CaCl_2$ application resulted in the greatest TSS (11.10 Brix) and total antioxidant capacity (21.13 mol TE g^{-1} FW). With the addition of chitosan 5 g/L + 1.50% $CaCl_2$, the pulp's maximum anthocyanin concentration (39.91 mg/100gm pulp) was found. Though treatment T11 was also associated with the lowest titrable acidity value (0.64%) and lowest PLW (6.20%) rates. In conclusion, strawberries kept at 2 °C for a longer period of time, roughly 13 days, maintained a usable quality.

Ahsan *et al.,* (2022) carried out an experiment in controlled conditions,employed a randomized complete block design with 20 treatment combinations and three replications. All soilless substrates significantly increased TSS, total sugars, reducing sugars, non-reducing sugars, and ascorbic acid content, except for Sand (M1). The substrate M2 (coco-peat) yielded the highest TSS (8.77 °Brix), total sugars (6.60%), reducing sugars (4.43%), non-reducing sugars (2.16%), ascorbic acid (25.27 mg/100 g), and organoleptic rating (4.02). The lowest values were observed in Sand (M1). Among the varieties, Kimberly displayed the highest TSS (8.16 °B), total sugars (5.68%), reducing sugars (4.05%), and ascorbic acid (38.90 mg/100 g). The combination of coco-peat substrate and Kimberly

variety demonstrated the best performance for soilless strawberry production in passively ventilated greenhouses.

2.3 Molecular characterization of cultivars

2.3.1 DNA isolation

DNA extraction is a technique for isolating DNA from a sample using physical or chemical means to purify the DNA. For the first instance, DNA isolation was done by Friedrich Miescher in 1869. The employment of a DNA isolation process should result in an effective extraction of DNA that is clean, abundant, and free of impurities like RNA and proteins. Problems might arise during various manipulations and applications, like as amplification, restriction digestion, and hybridization, due to variable DNA quality.

During the isolation and purification of high-quality DNA in plants, the most significant challenges are the existence of unwanted compounds such as polyphenols and polysaccharides (Nunes *et al.,* 2011). It is challenging to isolate genomic DNA from plants that have high levels of polysaccharides, phenolics, terpenoids, tannins, and metabolites because these substances bind to DNA and co-extract it (Michiels *et al.* 2003; Puchooa 2004). According to the procedure described by Doyle and Doyle (1990), leaf samples were used to isolate plant DNA (CTAB method).

2.3.2 Modified CTAB extension method

John (1992) proposed an effective DNA purification strategy to prevent degradation and eliminate contaminants, ensuring reproducibility and reliability in enzymatic reactions. Tissue lyophilization before purification mitigates degradation and extends storage, aided by the antioxidant poly-vinyl pyrrolidone (PVP) to prevent polyphenol adherence. Regardless of the technique used, genomic DNA purification should offer sufficient pure DNA, minimizing PCR inhibition. Incorporating lyophilization and PVP in maceration can enhance efficiency, reliability, and DNA yield, reducing challenges with unwanted components. This study aimed to assess a modified CTAB technique for genomic DNA extraction from strawberry leaves.

Allen *et al.*, (2006) outlined a modification to the DNA extraction technique where nucleic acids were extracted from plant tissues using cetyltrimethylammonium bromide (CTAB). The simplified CTAB approach has several advantages over the original CTAB method, including speed, omission of the selective precipitation and CsCl gradient phases, use of less expensive and hazardous reagents, requirement of just low-cost laboratory equipment, and ease of adaptation to high-throughput DNA extraction. Depending on the plant species and tissue supply, this technique produces between 5 and 30 lg of total DNA from 200 mg of fresh tissue. It can be finished in just 5 to 6 hours.

Ferreira *et al.*, (2011) observed the presence of undesirable compounds such as polyphenols and polysaccharides is one of the biggest problems faced during the isolation and purification of high quality DNA in plants. Therefore, achievement of fast and accurate methods for DNA extraction is crucial in order to produce pure samples. Leaves of strawberry genotypes (Fragaria ananassa) have high contents of polysaccharides and polyphenols which increase the sample viscosity and decrease the DNA quality, interfering with the PCR performance.

2.3.3 PCR amplifications conditions

Kary Mullis created this ground-breaking technique in the 1980s. The basis of PCR is the DNA polymerase's capacity to create new DNA strands that are complementary to the provided template strand. DNA polymerase requires a primer on which it may add the first nucleotide since it can only add a nucleotide onto an already existing 3'-OH group. This pre requisite allows us to identify a particular area of the template sequence that we want to amplify. The specified sequence will accumulate in billions of copies (amplicons) at the conclusion of the PCR procedure.

A significant development in the field of DNA fingerprinting was the introduction of PCR-based techniques. It was the isolation and purification of Taq polymerase, a thermostable DNA polymerase that led to the discovery. As only one enzyme addition was necessary, this made it possible to automate the cycling process. DNA fragments undergo enzymatic amplification between sets of DNA primers that hybridize to the sample DNA. The PCR product's characteristics and outcome are influenced by various factors, including

the primer DNA's quantity and quality, DNA polymerase quantity and stability, magnesium ion concentration, and temperature profile.

Arnau *et al.,* (2003) evaluated ISSR amplification for strawberry variety identification. 18 primers were tested, selected five informative ones, and used polyacrylamide gel electrophoresis to analyze 30 varieties, revealing 30% polymorphism from 390 bands. Genetic similarity was determined by Jaccard's coefficient and UPGMA analysis, aligning with pedigree data. ISSR effectively differentiated varieties, even related ones. Reproducible patterns were seen in different tissues and plants of the same variety. ISSR's simplicity, speed, cost-effectiveness, discrimination, and reliability highlighted its value for strawberry varietal identification.

Gupta *et al.,* (2012) conducted ISSR analysis using 25 µl PCR reactions with 75 ng template DNA, 1x PCR buffer (100 mM Tris, 500 mM KCl, 1% Triton X-100 with 15 mM MgCl2), 0.2 mM dNTPs mix, 2.5 mM MgCl2, 2U Taq DNA polymerase, and 0.5 µM primer. PCR involved initial denaturation at 94°C for 4 minutes, 35 cycles of denaturation (94°C, 45 seconds), annealing (Ta°C, 1 minute), and a final extension (72°C, 8 minutes). Amplicons were analyzed on 1.4% agarose and their size compared to a 100 bp DNA ladder.

Hussein *et al.,* (2014) conducted ISSR amplification in a 25 µl volume: 0.75 µl MgCl2 (50 mM), 0.5 µl dNTP (10 mM), 2.5 µl PCR buffer (10x), 0.2 µl Taq DNA polymerase (1 unit), 0.5 µl primer (100 mM), 1 µl template DNA (10 ng/µl), and 19.5 µl ddH2O. Amplified products were separated on 2% agarose gels in 0.5x TBE buffer, visualized with ethidium bromide (1.0 g/ml), and captured using a Gel documentation system with a UV transilluminator.

2.3.4 ISSR marker

Zurawicz *et al.,* (2004) identified the genetic connection of twenty strawberry varieties in Poland using RAPD and ISSR, using two PCR-based methods. Ninety tests with 18 ISSR primers unveiled polymorphism. ISSR data's higher accuracy (UPGMA percent technique) was confirmed through dendrogram analysis of polymorphic products.

Simultaneously using both data sets improved cultivar relationship estimates and reduced methodological errors.

Hussein *et al.,* (2008) Molecular characterisation and fingerprinting were performed on six strawberry varieties. To ascertain the genetic connections between the six strawberry varieties, the ISSRs method with nine primers was employed. Primers generated 102 amplified fragments in total, of which 86 (84.3%) were polymorphic fragments. According to a dendrogram-tree created during the experiment, six strawberry varieties were split into two groups. ‘Capitola’ was the only variety in the first cluster, while the second cluster included ‘Tamar’, ‘Chandler’, ‘Sweet Charlie’, ‘Rosa’, and ‘Diamond’. ‘Diamond’ and ‘Chandler’ have a genetic similarity value of 83% with the ‘Sweet Charlie’ variety. But ‘Tamar’ and ‘Capitola’, with a similarity rating of 45%, were the varieties with the highest genetic distance. Approach were reliable methods for classifying the six varieties of strawberries. The six strawberry kinds were successfully identified using the ISSR technique and the nine primers employed in this study.

Khanizadeh *et al.,* (2008) evaluated genetic diversity and relatedness of sixteen strawberry cultivars and eleven breeding lines in Canada with ISSR primers. Seventeen primers produced 225 polymorphic ISSR PCR bands.

Resende *et al.,* (2011) utilized ISSR markers to assess genetic similarity among eleven strawberry cultivars, aiming to identify promising hybrids. Strawberry cultivar DNA was extracted and amplified using ISSR primers via PCR, with DNA fragments separated on agarose gel for ISSR markers.

Generoso *et al.,* (2013) investigated genetic variability in strawberries using inter-simple sequence repeat (ISSR) markers. They analyzed 84 hybrids resulting from crosses between different progenitors ('Toyonoka' x 'Sweet Charlie', 'Camino Real' x 'Sweet Charlie', etc.). Each hybrid's genetic profile was diverse, attributed to the progenitors' high genome heterozygosity. Fourteen genotypes from each hybrid combination were evaluated. It highlighted the significant genetic diversity among strawberry hybrids due to the heterozygous nature of the progenitors' genomes.

Kumari *et al.,* (2019) evaluated genetic similarity among fifteen strawberry cultivars using RAPD and ISSR molecular markers. DNA from the cultivars was extracted and PCR-amplified with these markers. RAPD fragments were separated in agarose gel, and ISSR fragments in polyacrylamide gel. Jaccard coefficient estimated genetic similarity. Cultivars grouped into two clusters, with RAPD showing coherence with origin and genealogy. ISSR markers suggested similarity between 'Chandler' and 'Shasta', while RAPD showed 'Chandler' and 'Sweet Charlie' as closely related. Elyana stood out in molecular assessment, being distinct and suitable for local breeding due to its long flowering duration, high yield, and large fruits.

Correa *et al.,* (2020) employed Inter-simple sequence repeat (ISSR) markers to characterize 40 three-way hybrids developed by USBP. They evaluated the hybrids against commercial and heirloom cultivars, as well as single hybrids. Nine ISSR primers were used to genotype different genotypes. High polymorphism (100%), Nei genetic diversity (h = 0.34), and Shannon index (I = 0.51) indicated significant variability. Commercial cultivars had the highest diversity, followed by photoperiod-insensitive hybrids (PIH). Genotypes formed distinct clusters in the dendrogram, supported by further analyses. The USBP-developed three-way hybrids displayed unique genetic traits compared to existing cultivars, suggesting their potential as new strawberry varieties.

CHAPTER-3

MATERIALS AND METHODS

The current study, titled **"Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu".** The field trial was carried out at the Advanced Centre for Horticulture Research, Udheywalla and the molecular studies in the laboratory of School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, during 2022-23. This chapter contains relevant information pertaining research methodologies used for conducting the present study.

3.1 Experimental material

The vigorous, healthy, free from diseases, insect-pest and well rooted cultivars of all 19 varieties of strawberry were selected.

3.2 Collection of strawberry samples

Healthy strawberry cultivars procured from IARI Regional station Shimla, and recognized fruit nurseries of Himachal Pradesh.

Table 3.1: List of strawberry varieties used in the study

S.No.	Varieties	S.No.	Varieties	S.No.	Varieties
1.	Addie	8.	Douglas	15.	Selva
2.	Belrubi	9.	Etna	16.	Sea Scape
3.	Brighton	10.	Fairfox	17.	Shimla Delicious
4.	Camarosa	11.	Fern	18.	Sweet Charlie
5.	Capri	12.	Jutogh Special	19.	Winter Dawn
6.	Chandler	13.	Katrain Sweet		
7.	Dana	14.	Nabila		

Table 3.2: List of strawberry varieties, release, pedigree and developer used in the study.

S.No.	Varieties	Release	Pedigree	Developer
1.	Addie	1982	Senga x Pantagruella MDU3816	Italy
2.	Belrubi	-	Pocahontas x Red cote	Imported in India
3.	Brighton	-	Unknown	-
4.	Camarosa	1994	Douglas x Cal 85.218-605	University of California
5.	Capri	2005	CIVRI-30 x R6R1-26	S. Giuseppe di Comacchio Ferrara, Italy
6.	Chandler	1983	Douglas x Cal 72.361-105	University of California
7.	Dana	1982	Unknown	Europe and North America
8.	Douglas	1979	(Tioga x Sequoia) x Tufts	USA
9.	Etna	1986	Unknown	Europe and North America
10.	Fairfox	1925	Royal Sovereign ×Howard 17	Maryland
11.	Fern	1973	Tufts × Cal 69.63-103	University of California
12.	Jutogh Special	-	-	-
13.	Katrain Sweet	-	Unknown	Imported variety in Inda from America
14.	Nabila	2004	VENTANA× Q6Q8-26	S. Giuseppe di Comacchio Ferrara, Italy
15.	Selva	1983	Cal 70.3-117× Cal 71.98-605	Belgium
16.	Sea Scape	1992	Selva× Douglas	University of California
17.	Shimla Delicious	-	-	-
18.	Sweet Charlie	-	FL 80-456× Pajaro	University of Florida’s Gulf Coast Research and Education Center
19.	Winter Dawn	-	FL 93-103 ×FL 95-316	-

3.3 Experimental site

This study was conducted at Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, at the latitude of 32° 21' 41.0519"N and 74° 51' 41.5932"E longitude during 2022-23. This region falls in sub-tropical zone. The collected strawberry varieties were brought to Advanced Centre for Horticulture Research, Udheywalla-Jammu, where they were transplanted. A total of 19 strawberry varieties were transplanted in the last week of September 2022.

3.4 Experimental design and layout

The experiment was laid out in a randomized block design (RBD) design with three replications. Healthy strawberry cultivars were planted in field at 30cm x 30cm spacing and bed size 1.5m*1.5m with fertigation of recommended dose of fertilizers and maintained uniform cultural practices.

3.5 Observations recorded

All the experimental plants were uniformly maintained. Four plants per plot were randomly selected and tagged for observing the data. The morphological parameters (vegetative and floral characters), fruit yield and yield component parameters, biochemical analysis and molecular characterization of strawberry cultivars were recorded.

3.5.1 Morphological parameters

3.5.1.1 Plant growth characters

1. **Plant height (cm):** Plant height was measured in centimeters (cm) from the base of the stem (at the soil surface) to the apex of primary leaves using a measuring scale.

2. **Plant spread (cm):** This is the width of a mature specimen. In order to identify the precise plant spread, it was evaluated in both East-West (E-W) and north-south (N-S) directions.

3. **Number of leaves per plant:** The average no of leaves per plant was counted after dormancy.

4. **Leaf area per plot (cm^2):** On the graph sheet, measurements were taken using indirect method of leaf area estimation (Pandey *et al.*, 2011). Leaf area was measured using ten samples from each replication.

5. **Number of days to runners formation after planting:** The days to runner formation were counted from the planting day till the first runner was produced.

6. **Number of runners formation:** The quantity of runners produced by each cultivar was counted before the plant was taken out to make way for a new planting. We counted the number of runners that each plant produced.

3.5.1.2 Floral characters

1. **Flower size (cm):** It was measured using a vernier calliper crosswise the ends of any two opposing petals.

2. **Number of days to flowering after planting (days):** For the purpose of calculating the days to blooming, the interval between seeding and the appearance of the first flower for each cultivar was noted.

3. **Opening of first flower:** On this day, the first plant blossom began to open.

4. **Opening of last flower:** The day that a plant's last flower blossomed.

5. **Duration of flowering (days):** For each cultivars, the dates of the first and last flower openings were noted in order to calculate the length of the flowering period.

6. **Flower type:** The hermaphrodite, pistillate, and staminate floral types of each cultivar were determined by close inspection.

3.5.2 Yield and yield component parameters

1. Fruit length (cm)
2. Fruit breadth (cm)
3. Average berry weight (g)
4. No. of fruits per plant
5. Fruit yield per plant (g)

Plate-3.1: Field trial of strawberry cultivars at experimental field, Udheywaala-Jammu

Plate-3.2: Flowering of strawberry cultivars

Plate-3.3: Fruiting of strawberry cultivars

1. **Fruit length (cm):** It was measured with a vernier calliper from top to bottom of fruit.

2. **Fruit breadth (cm):** It was measured by vernier calliper through the shoulder's broader side of the fruit.

3. **Average berry weight (g):** It was determined by weighing the berry on a digital scale.

4. **Number of fruits per plant:** When the fruit reached maturity, the number of fruits on each pedicel was counted, and the result was expressed as the total number of fruits produced by each plant.

5. **Fruit yield per plant:** Each cultivar's yield per plant was calculated based on the proportion of entire fruits harvested from each plant.

3.5.3 Biochemical analysis

1. Total soluble solids (˚Brix)
2. Acidity (%)
3. Reducing sugar
4. Non-reducing sugars (%)
5. Total sugars (%)
6. TSS/acid ratio

1. **Total soluble solids (°Brix):** A drop of juice was placed to the prism of a hand refractometer to measure TSS. The results were presented as °Brix (Ranganna, 1986).

2. **Acidity (%):** The titratable acidity (TA) was determined by juice titration with 0.1 N NaOH and expressed in percent of citric acid per 100 ml juice.

3. **Reducing sugar (%):** Lane and Eynon (1923) volumetric method as detailed by Ranganna (1986) was followed for determining reducing sugar. Measured quantity

of sample (20 g) was taken in 250 ml volumetric flask to which 100 ml distilled water was added and neutralised with 40 per cent sodium hydroxide solution using phenolphthalein as indicator and clarified with 2 ml of 45 per cent natural lead acetate for about 30 minutes. Excess of lead acetate was removed by adding 5 ml of 22 per cent potassium oxalate. The volume was made to 250 ml and filtered through Whatman filter paper No. 4. To determine the amount of reducing sugars, the filtrate was titrated against 10 ml of standard Fehling's solution using methylene blue as an indicator, resulting in a brick red precipitate.

$$\text{Reducing Sugar} = \frac{0.5 \times \text{Volume made up}}{\text{Titre value} \times \text{Weight of sample}} \times 100$$

4. **Non-reducing sugar (%):** The amount of non-reducing sugars was calculated using the difference between total soluble sugars and reducing sugars.

   Non-Reducing sugar (%) = Total soluble sugar (%) - Reducing sugar (%) × 0.96

5. **Total Sugar (%):** A measured aliquot (100 ml) of the above filtrate was taken in 250 ml volumetric flask and was hydrolysed by adding 10 ml of 50 per cent hydrochloric acid, kept overnight for 24 h at room temperature followed by neutralization with 40 per cent sodium hydroxide solution using phenolphthalein as indicator. The volume was made to 250 ml and titrated against Fehling's solution, as above, for total sugars and expressed as per cent total sugar.

6. **Total soluble solids/acid ratio:** It can be measured by dividing the TSS with the titratable acidity.

3.5.4 Data analysis of morphological, yield components and biochemical traits

3.5.4.1 Analysis of variance

The significant difference among observations was estimated by employing ANOVA for RBD.

Table 3.3: ANOVA for RBD

Source of variation	Degree of freedom	Mean sum of squares (MSS)	Expected value of MSS
Replication (r)	r-1	M_1	$\sigma^2_e + g\sigma^2_r$
Genotypes (t)	g-1	M_2	$\sigma^2_e + \sigma^2_g$
Error	(r-1)(g-1)	M_3	σ^2_e
Total	rg-1		

Where, r = number of replications

σ^2_e = error variance

σ^2_r = variance due to genotypes

g = number of genotypes

M_1 = mean sum of squares due to replications

M_2 = mean sum of squares due to genotypes

M_3 = mean sum of squares due to error

3.5.4.2 Estimation of components of variance

Lush (1940) suggested the estimation of genotypic, phenotypic and environmental variances.

- **Genotypic variance**

Calculation of genotypic variance was done by using the method suggested by Johnson *et al.*, (1955) as below:

$$\sigma^2_g = \frac{MSG\text{-}ME}{R}$$

Where,

σ^2_g = Estimate of genotypic variance among the genotypes

MSG = Mean sum of squares for the genotypes

ME = Error mean sum of square, and

R = Number of replications

- **Phenotypic variance**

Phenotypic variance is a combination of variance due to environment and genotype .It is calculated by the formula devised by Johnson *et al.*, (1955) as below:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e$$

Where,

σ^2_p = Estimate of phenotypic variance among the genotypes

σ^2_g = Estimate of genotypic variance among the genotypes

σ^2_e = Estimate of error variance

- **Environmental variance**

The non- heritable phenotypical variation among genotype due to environment is defined as environment variance. It is equal to error mean sum of squares.

$$\sigma^2_e = ME$$

Where,

σ^2_e = Environment variance

ME = Error mean sum of squares

3.5.4.3 Heritability

The index of transmission of characters from parents to their off springs (Falconer and Mackay, 1983). It helps in identification of noble genotypes from diverse population. The applied formula was suggested by Lush (19490 and Burton and Devane (1953).

Where,

h^2 = Heritability

σ^2_p = Phenotypic variance

σ^2_g = Genotypic variance

3.5.4.4 Genotypic and phenotypic coefficient of variation

Both genotypic and phenotypic coefficient of variability were computed as per the method suggested by Burton and DeVane, (1953).

Genotypic coefficient of variability (GCV)

$$GCV\ (\%) = [(\sigma^2_g)^{½} / Mean] \times 100$$

Phenotypic coefficient of variability (PCV)

$$PCV\ (\%) = [(\sigma^2_p)^{½} / Mean] \times 100$$

3.5.4.5 Genetic advance

It determines the genetic gain of a character under particular selection pressure. The genetic advance for the mentioned population was estimated using the formula given by Johnson *et al.,* (1955).

$$\text{Estimated genetic advance} = [\sigma^2_g / \sigma^2_p] \times k \times \sigma_p = h^2 \times k \times \sigma_p$$

Where,

h^2 = Heritability

k = Selection differential (K= 2.06 at 5% selection intensity)

σ_p = Phenotypic standard deviation

3.5.5 Molecular characterization of cultivars

ISSR (Inter-simple sequence repeat) markers are used for polymorphic survey of strawberry cultivars.

3.5.5.1 Reagents and solutions

1. **1M Tris Cl pH 8.0:** A total of 121.1g of Tris base was dissolved in 800ml of water. pH was raised to 8.0 by adding 42ml of concentrated HCL. The solution was

allowed to cool to room temperature before making the final adjustment of pH. The final volume was made up to 1L with water and sterilize in an autoclave.

2. **0.5M EDTA:** A total of 186.1g EDTA was dissolved to 800ml of double distilled water. Sodium hydroxide were used to bring the pH to 8.0 followed by autoclave.

3. **5M NaCl:** About 292.2g of NaCl was dissolved in accurately 880 ml ultrapure water. The volume was adjusted to 1L with ultrapure water again and sterilized by autoclaving.

4. **10X TE Buffer:** About 100ml of Tris-Cl around with pH 7.5 and 20 ml of 0.5M EDTA was mixed and final volume was adjusted to 1L with distilled water.

5. **Chloroform: Isoamyl alcohol (24:1):** Approximately 24 volumes of chloroform were added using a measuring cylinder, and the mixture was then transferred to a dark bottle. The appropriate volume of isoamyl alcohol was measured and added to the mixture.

6. **Phenol: Chloroform: Isoamyl alcohol (25:24:1):** A mixture was prepared by adding approximately 25 volumes of phenol, 24 volumes of chloroform, and 1 volume of isoamyl alcohol. This mixture was then transferred to a dark bottle, thoroughly mixed, and stored.

7. **DNA loading dye:** About 30% (v/v) Glycerol, 0.25% (w/w) Bromophenol blue and 0.25% (w/v) Xylene cyanol were stirred and filtered.

8. **DNA extraction buffer:** The DNA extraction buffer was prepared using 1M Tris –Cl (10ml), NaCl(28ml), 0.5M EDTA(4ml), CTAB powder(2g), double distilled water(to make up volume to 100ml).

9. **TBE Buffer (10X):** Following constituents were used for preparing TBE buffer

   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 upto 100 ml by distilled water with final concentration 10x.

10. **Ethidium Bromide:** Ethidium bromide (EtBr) dye is employed for visualizing genomic DNA on agarose gels.

3.5.5.2 Genomic DNA isolation and purification

1. Genomic DNA extraction

The genomic DNA isolation was carried out by Doyle and Doyle (1990) method. About 5g young leaves of 3-4 weeks old plants form each genotype were taken for genomic DNA extraction. Leaf samples were taken and grinded to fine powder in liquid nitrogen using pestle and mortar. The powdered material was then transferred to 2ml Eppendorf tubes and 800µl of pre-warmed extraction buffer was added to each tube. These tubes were then incubated at 65ºC in a water bath for one hour with occasional stirring .After incubation an equal volume of Chloroform:Isoamylalcohol (24:1) was added to each tube and slowly mixed by inverting the tubes for atleast 10 minutes. The samples were then centrifuged at 10,000 rpm at 20ºC for 10 minutes After centrifugation the supernatant was transferred to another fresh autoclaved effendorf tube. After this 700-800µl volume of chilled isopropanol was added to the supernatant in each tube and the contents were mixed by gently inverting the tubes several times and kept overnight in -20°C. Next day, the samples were centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was discarded and the DNA pellet was washed with 70% ethanol (200-300 µl) to remove contamination and then air dried. DNA pellets were then dissolved in 200-300 µl of TE buffer and kept at room temperature (37°C) for overnight and stored at 4°C.

2. DNA purification

RNase treatment was given to sample by adding 2µl of RNase (10g/ml) to the sample (1ml TE/DNA mixture) and incubated at 37°C for 1hour in water bath. An equal volume of Phenol :Chloroform : Isoamylalcohol (25:24:1) was added and gently mixed for 1 minute and centrifuged at 12,000 rpm for 10 min. The supernatant was collected in

another tube and again equal 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 pellet was washed with 70 percent ethanol, centrifuged at 7000 rpm for 5 min, air dried ,dissolved in 100 µl of 1xTE (Tris-Cl,EDTA) buffer and stored at 4°C for further use.

3.5.5.3 DNA quantification

Quality and quantity of genomic DNA was estimated by using agrose gel electrophoresis and spectrophotometer.

1. Agrose gel electrophorosis

DNA of all genotypes was quantified by loading 3 µl of DNA of each genotype mixed with 2 µl of loading dye (0.25% w/v bromophrnol blue, 50% glycerol in strile water) on 0.8% agrose gel.Agrose gel was prepared by melting 0.8g of agrose in 10ml of 1X TBE (Tris Borate EDTA) buffer in a conical flask in the microwave for 3 min. It was allowed to cool for few minutes and then stained with 6 µl ethidium bromide for visualization of DNA bands and stirred for some time .The gel was then poured into gel casting trya with combs in it; and allowed to solidify for 20-30 minutes at room temperature. The electrophoresis was carried out at 80V for 1 hour. It was then viewed under gel documentation system .The concentration of DNA was determined by comparing intensity of fluorescence of genomic DNA bands with that of known standards. The quality of DNA samples were judged based on whether DNA formed a single high molecular weight band(good quality) or a smear(degrade/poor quality).

3.5.5.4 ISSR amplification

Total of twenty-six ISSR primers were used against nineteen strawberry cultivars.

1. Dilutions of primer

Each primer was dissolved ddH_2O in (100 µM) and diluted further to the working concentration of 5µM (20 µl primer + 80 µl ddH_2O).

Plate-3.4: Genomic DNA extraction of strawberry leaves

Table 3.4: List of ISSR primers used in PCR study

Primer name	Sequence	Annealing Temperature (ºC)
ISSR-1	AGCGCTAGCACACACACACAC	56
ISSR-2	GCTAGTGCTCACACACACACA	54
ISSR-3	GAGAGAGAGAGAGAGATC	42
ISSR-4	GCAGCTCTCTCTCTCTCTC	55
ISSR-5	CCTAGTAGCAGAGAGAGAGAGAGA	50
ISSR-6	CACACACACACAGT	36
ISSR-7	CACACACACACAGC	36
ISSR-8	CACACACACACAGG	36
ISSR-10	CTCTCTCTCTCTCTCTTG	42
ISSR-11	CACACACACACAAG	37
ISSR-12	CTCTCTCTCTCTCTCTAC	40
ISSR-13	GAGAGAGAGAGAGG	35
ISSR-14	CACCACCACGC	38
ISSR-15	GAGGAGGAGGC	35
ISSR-16	AGAGAGAGAGAGAGAGT	42
ISSR-17	AGAGAGAGAGAGAGAGC	48
ISSR-18	AGAGAGAGAGAGAGAGG	48
ISSR-19	GAGAGAGAGAGAGAGAT	46
ISSR-20	GAGAGAGAGAGAGAGAC	48
ISSR-21	CACACACACACACACAA	42
ISSR-827	ACACACACACACACACG	47
ISSR-834	AGAGAGAGAGAGAGAGGC	45
ISSR-845	CTCTCTCTCTCTCTCTG	42
ISSR-848	CACACACACACACACAAG	48
ISSR-849	GTGTGTGTGTGTGTGTC	47
ISSR-860	TGTGTGTGTGTGTGTGGA	48

3.5.5.6 PCR amplification

1. Components used for PCR reaction

DNA was amplified in 0.2 ml PCR tubes with a 15 µl reaction mixture. Template DNA, buffer, $MgCl_2$, dNTPs, primers, ddH_2O and Taq polymerase are the components used in a PCR reaction. The quantity of these components is given in Table 3.5.

Table 3.5: List of the concentrations and quantities used in one PCR reaction

S.No.	Reagents	Working Concentration	Volume
1.	Buffer	5X	3µl
2.	$MgCl_2$	25mM	1.2µl
3.	DNTPs	10mM	0.35µl
4.	Primer	5uM	1.5µl
5.	Taq polymerase	1 unit	0.15µl
6.	Sterile water	----	6.8µl
7.	Genomic DNA	50ng	2.0ul
	Total		**15µl**

2. PCR amplication programme

The most common method of amplification for DNA sequences is Polymerase Chain Reaction (PCR), which is carried out in a thermal cycler. In PCR tubes, the master mix and DNA template were thoroughly mixed before being exposed to the thermal profile. In a thermal cycler, the amplication reaction was handled.

Table 3.6: Thermal profile used for DNA amplification

Steps	Cycles	Temperature	Duration
Initial Denaturation	1	94°C	04 min
Denaturation	40	94°C	30 sec
Annealing		34-56°C (vary for every primer used)	30 sec
Extension		72°C	90 sec
Final Extension	1	72°C	10 min
Hold		4°C	Until removed

Plate-3.5: PCR amplification and loading of amplified products on 2% agrose gel

3. PCR amplified product visualization

The amplified product was examined using agarose gel electrophoresis. To accomplish this, a 2% agarose gel will be created by combining agarose powder with a 1X TBE buffer.

4. ISSR-PCR banding profile

Using a horizontal agarose gel electrophoresis, the amplified products were separated electrophoretically. A 2% agarose gel was created in 1X TBE buffer, and ethidium bromide was used to stain it. Each PCR tube was placed into a different well after receiving 2µl of loading dye. Using a 100 bp DNA ladder as a molecular weight marker, the molecular weights of the ISSR-based PCR bands were calculated. Two hours of 120 volt electrophoresis were completed. A gel documentation system was used to record the gel's visual inspection under UV light. The banding patterns of amplified PCR products were scored after viewing. Additionally, a dendrogram is produced using UPGMA software.

5. Scoring of bands

The ISSR-PCR bands were examined under ultra violet transilluminator and photographed under gel documentation unit. The ISSR bands were counted and scored manually as 1 for their presence and 0 for their absence to generate the binary data for diversity analysis. The sizes of the bands were estimated by compared with using 100bp standard marker. The presence and absence of bands in all the genotypes for the primers were used to generate Bi-nominal data using excel sheet. Bands were marked as present only if the DNA amplification produced the fragment of a particular sequence and absent if the DNA amplification lacked the fragment. The banding patterns of all the genotypes against each primer were compared. Bands present in one genotype and absent in another genotype were regarded as variable and used to score for polymorphism. In order to check the informativeness and discriminatory power of ISSR primers utilized in this content and number of alleles were calculated.

a) Percentage polymorphism

It was calculated by dividing the polymorphic bands by the total number of scored bands:

$$\frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

b) Polymorphism information content (PIC) value

The markers with more alleles have larger polymorphism information content. Average PIC indicates the ability of utilized markers to differentiate the genotypes. It was calculated as proposed by Roldan –Ruiz *et al.*, (2000)

$$PIC = 2f\ (1\text{-}f)$$

Where, f = Frequency of bands present

1-f = Frequency of bands absent

CHAPTER-4

RESULTS

The present investigation entitled **"Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu"** was carried out at the Advanced Centre for Horticulture Research, Udheywalla and the biochemical and molecular studies were carried out in the laboratory of School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, during the year 2022-23. To enhance genetic resources and develop innovative cultivars, it is imperative to employ modern breeding techniques that incorporate desirable traits from both traditional varieties and their wild counterparts. Evaluating diversity through the use of morphological and molecular markers will eventually become indispensable for crop advancements. The extent of crop improvement hinges on the magnitude of genetic variation present within the accessible germplasm. The wider the range of genetic diversity within the germplasm, the greater the likelihood of identifying superior genotypes. By harnessing this extensive genetic variety, breeders can unlock a vast array of potential traits and characteristics that can contribute to the development of superior cultivars. Therefore, the integration of diverse germplasm and the utilization of advanced evaluation methods are pivotal in driving crop development and ensuring a sustainable and prosperous future in agriculture.

An array of markers, including morphological, biochemical, and molecular markers, has been devised to discern genetic diversity. However, biochemical and morphological markers exhibit inadequate discriminatory power when it comes to distinguishing closely related genotypes. Moreover, these markers are significantly influenced by environmental factors and developmental stages, impeding their efficacy. Morphological markers necessitate the maturity of plants, entailing time-consuming observations contingent upon specific environmental conditions. The paucity of biochemical markers further restricts investigations to a mere handful of proteins.

Consequently, the utilization of morphological and biochemical markers to scrutinize genetic diversity poses an arduous task. Thus, in the modern era, molecular markers reign supreme as the most precise means to detect DNA polymorphism. By targeting specific DNA sequences, molecular markers offer unrivaled accuracy, making them indispensable in contemporary genetic analysis. Their implementation enables the differentiation of closely related genotypes and empowers researchers to unravel intricate genetic variations. Ultimately, molecular markers have revolutionized the field, surpassing other markers in their ability to unravel the complexities of genetic diversity and serving as an invaluable tool in the study of DNA polymorphism.

The results obtained in the present investigations are presented under following headings:

4.1 Analysis of variance

4.2 Mean performance of different morphological characters of strawberry cultivars

4.3 Mean performance of yield and yield parameters of different strawberry cultivars

4.4 Mean performance of biochemical characters of different strawberry cultivars

4.5 Estimation of genetic parameters

4.6 Molecular characterization of different strawberry cultivars

4.1 Analysis of variance

Analysis of variance for different traits is presented in table 4.1 and 4.2. All the morphological characters were found significant at 1% except the flower size which was found significant at 5% significance level. While most of the yield and biochemical characters were found significant at 1% level (number of fruits per plant, average berry weight, fruit yield, Non-reducing sugars, total sugars, total soluble solids/acidity ratio) and some were found significant at 5% level (fruit length, fruit breadth, total soluble solids, acidity, reducing sugars) respectively.

Table 4.1: ANOVA for morphological characters of different strawberry cultivars

Source of variation	Mean sum of squares									
	Df	**PH**	**PS**	**NOLPP**	**LA**	**NORF**	**NODTRF**	**FS**	**NODTFAP**	**DOF**
Replications	2	1.06	1.49	0.97	0.73	0.16	2.63	0.02	0.30	17.78
Treatment	18	381.10**	459.47**	493.86**	21,515.97**	101.89**	3,734.21**	2.28*	2,417.88**	1,304.66**
Error	36	16.88	23.88	12.93	6.98	7.54	159.36	1.12	151.70	123.54

**, * represents sgnificance at 1 % and 5 % levels respectively

Df = Degree of freedom, PH = Plant height, PS = Plant spread, NOLPP = Number of leaves per plant, LA = Leaf area, NORF = Number of runners formation, NODTRF = Number of days to runners formation, FS = Flower size, NODTFAP = Number of days to flowering after planting, DOF = During of flowering

Table 4.2: ANOVA for yield and biochemical traits of different strawberry cultivars

Source of variation	Mean sum of squares											
	Df	**FL**	**FB**	**NOFPP**	**ABW**	**FY**	**TSS**	**A**	**RS**	**NRS**	**TS**	**TSS / AR**
Replications	2	0.14	0.96	0.31	1.06	4.05	0.34	0.10	0.20	0.06	0.12	0.35
Treatment	18	14.04*	6.01*	256.40**	91.12**	27,207.43**	64.24*	2.22*	21.16*	44.35**	95.53**	82.34**
Error	36	1.07	1.51	3.12	15.88	40.83	2.32	1.07	1.04	3.67	1.11	1.12

**, * represents significance at 1 % and 5 % levels respectively

Df = Degree of freedom, FL = Fruit length, FB = Fruit breadth, NOFPP = Number of fruit per plant, ABW = Average berry weight, FY = Fruit yield, TSS = Total soluble solids, A = Acidity, RS = Reducing sugars, NRS = Non-reducing sugars, TS = Total sugars, TSS / AR = Total soluble solids / Acidity ratio

4.2 Mean performance of different morphological characters of strawberry cultivars

The performance of different cultivars with respect to their plant growth parameters is discussed as follows:

4.2.1 Plant growth parameters

The data related to plant growth parameters are presented in Table 4.3 and Table 4.4.

4.2.1.1 Plant height

The varietal differentiations pertaining to the average plant height manifested considerable statistical significance across all cultivars, as eloquently depicted in the illustrious Table 4.3.The plant height varied between 17.20 cm to 25.20 cm among all the cultivars. The maximum plant height was observed in cultivar ‘Selva’ (25.20 cm), closely followed by ‘Chandler’ (25.00 cm) and ‘Winter Dawn’ (24.70 cm) which was statistically at par with the plant height recorded in ‘Etna’ (24.10 cm) and ‘Sweet Charlie (24.00 cm). Conversely, the cultivars ‘Jutogh Special’ and ‘Shimla Delicious’ show cased a contrasting scenario, with their plants reaching the lower echelons of the height spectrum. 'Jutogh Special' stooped to a diminutive 17.20 cm, while 'Shimla Delicious' fared slightly better, registering a modest 17.40 cm.

4.2.1.2 Plant spread

The analysis of plant spread among different strawberry cultivars, as presented in Table 4.3 also revealed significant variations. The maximum plant spread was found in cultivar ‘Chandler’ (29.70cm) which was statistically at par with cultivars ‘Winter Dawn’ (29.50cm) followed by ‘Nabila’ (28.91cm) and ‘Selva’ (28.89 cm) and the minimum plant spread was found in cultivars ‘Shimla Delicious’ (21.00cm), ‘Etna’ (21.50cm) followed by cultivar ‘Seascape’ (22.20cm).

4.2.1.3 Numbers of leaves per plant

The tabulated data presented in Table 4.3 reveals a substantial variation in the number of leaves per plant across the evaluated strawberry cultivars. Notably, this trait

exhibited a significant difference among the cultivars, ranging from a minimum of 21.08 leaves per plant to a maximum of 29.93 leaves per plant. The maximum number of leaves was observed in the cultivar ‘Chandler’ (29.93), and ‘Winter Dawn’ (29.60), followed by ‘Selva’ (29.00) and ‘Nabila’ (28.98) which were also statistically at par with each other while the minimum leaves per plant were observed in the cultivar ‘Jutogh Special’(21.08) followed by the cultivars ‘Katrain Sweet’ (21.16) and ‘Shimla Delicious’ (21.60).

Table 4.3: Plant height, plant spread, number of leaves per plant and leaf area per plant of different strawberry cultivars

Cultivars	Plant height (cm)	Plant spread (cm)	Number of leaves/plant	leaf area/plant (cm^2)
Addie	19.50	25.30	21.70	151.40
Belrubi	19.10	24.30	26.93	148.40
Brighton	20.50	26.90	24.80	153.70
Camarosa	22.30	26.40	23.60	131.70
Capri	18.60	24.80	22.40	146.30
Chandler	25.00	29.70	29.93	186.00
Dana	19.10	23.90	23.10	156.76
Douglas	22.30	25.30	24.36	145.70
Etna	24.10	21.50	21.40	170.00
Fairfox	18.60	22.50	24.14	159.21
Fern	21.56	22.83	23.30	138.30
Jutogh Special	17.20	20.60	21.08	127.10
Katrain Sweet	22.50	24.10	21.16	160.00
Nabila	22.59	28.91	28.98	184.95
Seascape	18.80	22.20	23.30	139.90
Selva	25.20	28.89	29.00	184.30
Shimla Delicious	17.40	21.00	21.60	130.40
Sweet Charlie	24.00	27.40	26.50	180.70
Winter Dawn	24.70	29.50	29.60	185.60
CD value (5%)	1.13	1.35	0.99	0.73

4.2.1.4 Leaf area per plant

The leaf area per plant, as presented in Table 4.3, exhibits significant variability among the different strawberry cultivars. Among the evaluated strawberry cultivars, 'Chandler' and 'Winter Dawn' exhibited the largest leaf areas, measuring 186 cm^2 and 185.60 cm^2 respectively. Following closely behind were 'Nabila' with a leaf area of 184.95 cm^2 and 'Selva' with 184.30 cm^2 which were all statistically at par with each other. In contrast, 'Jutogh Special' had the smallest leaf area recorded at 127.10 cm^2.

4.2.1.5 Number of days to runner formation after planting

Table 4.4 provides data on the number of days required for runner production among the various strawberry cultivars. Notably, the cultivars 'Fern' and 'Nabila' exhibited the most prolonged duration for runner formation after planting, taking an extensive 189 days. These figures were statistically similar to the durations observed in 'Douglas' (187 days) and 'Winter Dawn' (186 days). In stark contrast, the cultivar 'Selva' showcased the shortest time for runner formation after planting, requiring a mere 161 days. However cultivars 'Etna' (165 days), 'Fairfox' (167 days), and 'Belrubi' (169 days) are at par with each other.

4.2.1.6 Number of runner formation after planting

The number of runners formed per plant is expressed by the data in Table 4.4. The number of runners per plant varied from 5.60 to 10.10 runners in all the cultivars. The cultivar 'Chandler' (10.10) had the most runners formed, and it was statistically at par with the cultivars 'Nabila' (9.96) and 'Sweet Charlie' (9.94). The cultivars 'Shimla Delicious' (5.60) and 'Seascape' (5.80) exhibited the minimum runner production and were statistically at par with each other.

The data pertaining to days to runner formation after planting and number of runner formation after planting of different strawberry cultivars has been presented in table 4.4.

Table 4.4: Days to runner formation after planting and number of runner formation after planting of different strawberry cultivars

Cultivars	No. of days to runner formation after planting	No. of runner formation after planting
Addie	176.00	8.90
Belrubi	169.00	7.90
Brighton	173.00	7.60
Camarosa	182.00	8.50
Capri	184.00	6.60
Chandler	171.00	10.10
Dana	178.00	8.00
Douglas	187.00	8.70
Etna	165.00	7.60
Fairfox	167.00	8.11
Fern	189.00	8.00
Jutogh Special	179.00	7.00
Katrain Sweet	180.00	6.88
Nabila	189.00	9.96
Seascape	172.00	5.80
Selva	161.00	9.40
Shimla Delicious	170.00	5.60
Sweet Charlie	176.00	9.94
Winter Dawn	186.00	9.90
CD value (5%)	3.49	0.76

4.2.2 Floral character

The floral characters of Strawberry are presented in Table 4.5 and Table 4.6.

4.2.2.1 Flower size

The data on flower size of different cultivars is presented in Table 4.5. The flower size ranged between 2.15 cm to 2.83 cm among different strawberry cultivars. The maximum flower size was observed in cultivar ‘Chandler’ (2.83 cm) and ‘Sweet Charlie’(2.81cm) which was statistically at par with ‘Nabila’ (2.80 cm), while the minimum flower size was exhibited by cultivar ‘Belrubi’ (2.15 cm) which was at par with ‘Shimla Delicious’(2.19cm)

4.2.2.2 Opening of first flower

Table 4.5 shows the estimated first flowering dates for all plants, which range from 18 to 20 weeks. In the third week of January of 2023, the majority of the cultivars bloomed. The cultivar ‘Shimla Delicious’ bloomed first (19-01-2023), while ‘Chandler’ (5-02-2023) and ‘Katrain Sweet’ (01-02-2023) were the last to start flowering.

4.2.2.3 Opening of last flower

The information on the last flower's opening is shown in Table 4.5 which demonstrates that for the majority of cultivars, the last flower opened 4-5 weeks after the first flower did. The first cultivar to finish flowering among all the others was ‘Shimla Delicious’ (36 days), while cultivars like ‘Chandler’, ‘Sweet Charlie’, ‘Katrain Sweet’ and ‘Selva’ continued to bloom until the first week of March.

The data pertaining to flower size, opening of first and last flower of different strawberry cultivars has been presented in table 4.5.

Table 4.5: Flower size, opening of first and last flower of different strawberry cultivars

Cultivars	Flower size (cm)	Opening of first flower	Opening of last flower
Addie	2.72	24-01-23	05-03-23
Belrubi	2.15	24-01-23	05-03-23
Brighton	2.69	29-01-23	11-03-23
Camarosa	2.56	26-01-23	07-03-23
Capri	2.51	20-01-23	01-03-23
Chandler	2.83	05-02-23	30-03-23
Dana	2.57	28-01-23	10-03-23
Douglas	2.67	26-01-23	07-03-23
Etna	2.48	31-01-23	13-03-23
Fairfox	2.23	29-01-23	11-03-23
Fern	2.65	23-01-23	04-03-23
Jutogh Special	2.59	21-01-23	08-03-23
Katrain Sweet	2.63	01-02-23	14-03-23
Nabila	2.80	31-01-23	13-03-23
Seascape	2.63	30-01-23	12-03-23
Selva	2.76	31-01-23	13-03-23
Shimla Delicious	2.19	19-01-23	28-02-23
Sweet Charlie	2.81	02-02-23	15-03-23
Winter Dawn	2.78	27-01-23	08-03-23
CD value (5%)	0.29		

4.2.2.4 Number of days to flowering after planting

All cultivars exhibited significant variation in number of days to flowering after planting. Different cultivars took between 110-134 days after planting for flower initiation.

The data recorded on number of days to flowering after planting is depicted in Table 4.6. Cultivar 'Shimla Delicious' took maximum number of days to flowering (134) followed by 'Katrain Sweet' (129.36), and 'Jutogh Special' (129), while the minimum number of days taken to flowering was observed in cultivar 'Sea Scape' (110.73) followed by 'Nabila'(112).

Table 4.6: Number of days to flowering after planting, duration of flowering and flower type of different strawberry cultivars

Cultivars	No. of days to flowering after planting (days)	Duration of flowering (days)	Flower type
Addie	125.00	41.00	Hermaphrodite
Belrubi	117.00	41.00	Hermaphrodite
Brighton	122.00	47.00	Hermaphrodite
Camarosa	124.00	43.00	Hermaphrodite
Capri	128.00	37.00	Hermaphrodite
Chandler	115.00	53.00	Hermaphrodite
Dana	119.00	46.00	Hermaphrodite
Douglas	119.00	43.00	Hermaphrodite
Etna	128.00	49.00	Hermaphrodite
Fairfox	113.00	47.00	Hermaphrodite
Fern	122.00	42.00	Hermaphrodite
Jutogh Special	129.00	38.33	Hermaphrodite
Katrain Sweet	129.36	50.00	Hermaphrodite
Nabila	112.00	49.00	Hermaphrodite
Seascape	110.73	48.66	Hermaphrodite
Selva	127.00	49.00	Hermaphrodite
Shimla Delicious	134.00	36.00	Hermaphrodite
Sweet Charlie	120.00	51.00	Hermaphrodite
Winter Dawn	115.00	44.00	Hermaphrodite
CD value (5%)	3.41	3.08	

4.2.2.5 Duration of flowering

Duration of flowering also exhibited considerable variation among different strawberry cultivars. The duration of flowering varied between 36-53 days in different strawberry cultivars and the data is presented in Table 4.6. Maximum flowering duration was observed in cultivar ‘Chandler’ (53 days). ‘Sweet Charlie’ (51 days) and ‘Katrain Sweet’ (50 days) was at par with each other. Whereas, Cultivar ‘Shimla Delicious’ (36 days) recorded minimum flowering duration which was at par with ‘Capri’(37 days),and ‘Jutogh Special’(38.33 days).

4.2.2.6 Flower type

The information in Table 4.6 demonstrated that all cultivars had hermaphrodite flowers. This meant that the flowers of every cultivar had both male and female parts.

4.3 Mean performance of yield and yield parameters of different strawberry cultivars

The yield and yield component parameters data for strawberries are shown in Tables 4.7 and 4.8. The parameters evaluated were fruit length (cm), fruit breadth (cm), average berry weight (g), number of fruits per plant, and fruit yield per plant (g).

4.3.1 Fruit length

The information pertaining to fruit length is shown in Table 4.7. Regarding fruit length, there was a sizable variation among all the cultivars.The maximum fruit length was recorded in cultivar ‘Sweet Charlie’ (3.72 cm). Whereas ‘Chandler’ (3.70 cm), ‘Selva’ (3.69 cm), ‘Winter Dawn’ (3.68cm) were statistically at par with each other. The minimum fruit length was recorded in cultivar ‘Shimla Delicious’ (2.31cm) which was at par with ‘Jutogh Special’ (2.35cm).

4.3.2 Fruit breadth

The fruit breadth of various strawberry cultivars varied, and data relating to fruit breadth are shown in Table 4.7.The maximum fruit breadth was recorded in the fruit of cultivar ‘Chandler’ (3.13 cm), ‘Sweet Charlie’ (3.12 cm), ‘ Nabila’(3.11cm) which were statistically at par with each other .The minimum fruit breadth was observed in the

cultivar‘Shimla Delicious’(2.21cm), ‘Katrain sweet’ (2.25 cm) followed by ‘Douglas’ (2.29 cm) .

4.3.3 Average berry weight

Fruit yield is directly related to average berry weight and number of fruits per plant. The information on average berry weight is shown in Table 4.7, which shows that it varied between 6.98 g and 9.50 g among various cultivars. Cultivar ‘Winter Dawn’ recorded the highest berry weight (9.50 g) which was at par with berry weight recorded in ‘Selva’ (9.49g) and ‘Sweet Charlie’ (9.46 g). The minimum berry weight was measured for the cultivar ‘Shimla Delicious’ at 6.98 g, followed by ‘Jutogh Special’ (7.56 g), ‘Dana’ (7.73 g), and ‘Capri’ (7.77 g).

Table 4.7: Fruit length and fruit breadth of different strawberry cultivars

Cultivars	Fruit length (cm)	Fruit breadth (cm)	Average berry weight (g)
Addie	3.57	3.04	8.27
Belrubi	3.65	2.55	7.88
Brighton	3.57	2.52	8.31
Camarosa	3.40	3.01	7.90
Capri	3.33	2.85	7.77
Chandler	3.70	3.13	9.28
Dana	2.39	2.67	7.73
Douglas	3.60	2.39	8.07
Etna	3.62	2.96	7.80
Fairfox	2.63	2.39	8.01
Fern	3.07	2.86	8.30
Jutogh Special	2.35	2.31	7.56
Katrain Sweet	3.54	2.25	8.98
Nabila	3.68	3.11	9.42
Seascape	2.90	3.05	8.64
Selva	3.69	3.03	9.49
Shimla Delicious	2.31	2.21	6.98
Sweet Charlie	3.72	3.12	9.46
Winter Dawn	3.68	3.09	9.50
CD value (5%)	0.27	0.34	1.10

4.3.4 Number of fruits per plant

The number of strawberries per plant varied slightly between strawberry cultivars. The number of fruits per plant varied between (15.50 and 23) among various cultivars. The data presented in Table 4.8 exhibits that the maximum number of fruits per plant was produced by the cultivar ‘Chandler’ (20.80) which was statistically at par with ‘Winter Dawn’ (20.14), followed by ‘Nabila’(20.10) and 'Sweet Charlie' (20.10). The minimum number of fruits per plant was recorded in the cultivar ‘Jutogh Special’ (15.50) followed by ‘Capri’ (15.90) and ‘Fern’ (16.00).

Table 4.8: Number of fruits per plant and fruit yield per plant of different strawberry cultivars

Cultivars	No. of fruits per plant	Fruit yield per plant (g)
Addie	20.09	166.14
Belrubi	17.28	136.16
Brighton	20.00	166.20
Camarosa	17.30	136.67
Capri	15.90	123.54
Chandler	20.80	193.02
Dana	17.10	132.18
Douglas	19.00	153.33
Etna	18.10	133.40
Fairfox	17.18	141.18
Fern	16.00	132.80
Jutogh Special	15.50	117.18
Katrain Sweet	17.40	156.25
Nabila	20.10	189.34
Seascape	17.00	146.88
Selva	19.80	187.80
Shimla Delicious	16.76	116.98
Sweet Charlie	20.10	190.14
Winter Dawn	20.14	191.33
CD value (5%)	0.49	2.77

4.3.5 Fruit yield per plant

The data on yield per plant is shown in Table 4.8 showed that the maximum fruit yield was observed in the cultivar ‘Chandler’ (193.02 g) which was at par with ‘Winter Dawn’ (191.33g), ‘Sweet Charlie’ (190.14 g) and ‘Nabila’ (189.34g). However, the minimum fruit yield was observed in the cultivar ‘Shimla Delicious’ (116.98 g) followed by ‘Jutogh Special’ (117.18g).

4.4 Mean performance of biochemical character of different strawberry cultivars

The data pertaining to biochemical characteristics i.e., Total soluble solids (TSS), acidity, reducing sugars, non-reducing sugars, total sugars and TSS/acidity ratio, of strawberry cultivars are presented in Table 4.9 , Table 4.10 and Table 4.11.

4.4.1 Total Soluble Solids (TSS)

The information in Table 4.9 shows that the Total Soluble Solids ranged from 8.05 to 11.60°B. The fruits of the cultivars ‘Selva’ (11.60°B) and ‘Chandler’ (11.49°B) had the highest TSS, followed by ‘Winter Dawn’ (11.32°B) and ‘Sweet Charlie’ (11.30°B). The fruits of the cultivar ‘Shimla Delicious’ had the lowest TSS (8.05°B), but ‘Camarosa’ (8.63°B) and ‘Capri’ (8.64°B) are on par with one another.

4.4.2 Acidity

The data on fruit acidity is presented in Table 4.9. The acidity ranged between (0.64 % - 0.88 %) all the cultivars exhibited little variation in terms of fruit acidity. The highest acidity was recorded in the fruits of cultivar ‘Fairfox’ (0.88 %) which was statistically at par with ‘Nabila’ (0.87 %) and ‘Brighton’ (0.87 %). The minimum acidity was recorded in the fruit of cultivar ‘Shimla Delicious’ (0.64%).However ‘Capri’ (0.70 %),‘Seascape’ (0.71 %)and ‘Camarosa’ (0.73 %)are at par with each other.

Table 4.9: Total soluble solids and acidity of different strawberry cultivars

Cultivars	Total Soluble Solids (°Brix)	Acidity (%)
Addie	10.30	0.84
Belrubi	9.10	0.77
Brighton	10.11	0.86
Camarosa	8.63	0.73
Capri	8.64	0.70
Chandler	11.49	0.83
Dana	9.43	0.76
Douglas	9.60	0.81
Etna	10.02	0.84
Fairfox	8.81	0.88
Fern	9.00	0.76
Jutogh Special	9.18	0.85
Katrain Sweet	9.90	0.77
Nabila	11.17	0.87
Seascape	9.54	0.71
Selva	11.60	0.75
Shimla Delicious	8.05	0.64
Sweet Charlie	11.30	0.82
Winter Dawn	11.32	0.80
CD value (5%)	0.42	0.07

4.4.3 Reducing sugars

The information on reducing sugars is shown in Table 4.10, which shows variation between cultivars. The maximum reducing sugar was recorded in the fruit of cultivar ‘Chandler’ (4.97 %), ‘Sweet Charlie’ (4.91 %) and ‘Winter Dawn’ (4.87 %). The minimum reducing sugar was recorded in the fruit of cultivar ‘Shimla Delicious’ (3.10 %), ‘Seascape’ (3.17 %) and ‘Etna’ (3.20 %).

4.4.4 Non-reducing sugar

The information in Table 4.10 illustrates the non-reducing sugars that can be found in the fruits of various strawberry cultivars. The fruits of the cultivars ‘Chandler’ (3.75%) and ‘Winter Dawn’ (3.72%) had the highest non-reducing sugar levels, followed by ‘Sweet Charlie’ (3.70%). The fruits of cultivar 'Seascape' (1.02%) and 'Shimla Delicious' (1.31%) had the lowest non-reducing sugar levels.

Table 4.10: Reducing sugars and non-reducing sugars of different strawberry cultivars

Cultivars	Reducing sugars (%)	Non-reducing sugars (%)
Addie	4.50	3.68
Belrubi	3.80	2.97
Brighton	3.85	3.34
Camarosa	3.76	3.21
Capri	3.73	3.20
Chandler	4.97	3.75
Dana	3.77	2.12
Douglas	3.30	1.98
Etna	3.20	2.00
Fairfox	3.71	2.10
Fern	4.20	3.16
Jutogh Special	3.29	1.41
Katrain Sweet	3.33	2.50
Nabila	4.71	3.55
Seascape	3.17	1.02
Selva	4.00	3.50
Shimla Delicious	3.10	1.31
Sweet Charlie	4.91	3.70
Winter Dawn	4.87	3.72
CD value (5%)	0.28	0.53

4.4.5 Total sugars

The information in Table 4.11 shows the total sugar content in fruits of strawberry cultivars. The data showed that the fruit from the cultivar ‘Chandler’ had the highest total sugar content (8.17%), followed by ‘Sweet Charlie’ (8.10%), while the fruits from the cultivar ‘Shimla Delicious’ had the lowest total sugar content (4.37%), and followed by ‘Seascape’ (4.46%).

Table 4.11: Total sugars and TSS/acidity ratio of different strawberry cultivars

Cultivars	Total sugars (%)	TSS/Acidity ratio
Addie	8.00	12.26
Belrubi	7.64	11.81
Brighton	7.33	12.18
Camarosa	6.03	11.82
Capri	6.02	12.34
Chandler	8.17	13.84
Dana	5.79	12.40
Douglas	6.50	11.85
Etna	5.40	11.92
Fairfox	5.62	10.01
Fern	7.20	11.84
Jutogh Special	4.55	10.76
Katrain Sweet	5.83	12.96
Nabila	8.05	12.83
Seascape	4.46	13.43
Selva	7.60	15.45
Shimla Delicious	4.37	12.57
Sweet Charlie	8.10	13.78
Winter Dawn	8.03	14.11
CD value (5%)	0.29	0.29

4.4.6 Total soluble solids/acidity ratio

The data presented in Table 4.11 showed the Total soluble solids /acid ratio present in the fruit. The highest TSS/acidity ratio was observed as (15.45%) in 'Selva' followed by 'Winter Dawn' (14.11%) and 'Sweet Charlie' (13.78%) while the lowest ratio was observed in 'Fairfox' (10.01%).However 'Jutogh Special' (10.76 %) and 'Brighton' (11.75 %) was at par with each other.

4.5 Estimation of genetic parameters

The genetic parameters *viz.*, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability, genetic advance as percent of mean were estimated for all the traits and presented in Table no. 4.12 and 4.13.

Table 4.12: Estimates of genetic parameters for different morphological traits

Parameters	Genotypic coefficient of variance	Phenotypic coefficient of variance	Heritability (%)	Genetic advance as (%) of mean
Plant height (cm)	12.38	12.52	97.80	25.22
Plant spread (cm)	11.49	11.64	97.40	23.36
No. of leaves per plant	12.22	12.30	98.70	25.01
Leaf area (cm^2)	12.72	12.72	99.90	26.20
No. of days to runners formation	4.66	4.71	97.90	9.49
No. of runners formation	16.57	16.89	96.30	33.51
Flower size (cm)	7.39	7.93	86.80	14.19
No. of days to flowering after planting	5.41	5.50	96.90	10.98
Duration of flowering	10.66	10.92	95.30	21.43

The data presented in Table 4.12 reveals phenotypic coefficient of variation to be higher than the genotypic coefficient of variation for all the traits under study indicating considerable impact of environment on the expression of traits. The value of phenotypic coefficient of variation and genotypic coefficient of variation was found highest for number

of runners formation followed by leaf area, plant height and number of leaves per plant.

High heritability was observed for leaf area (99.90) followed by number of leaves per plant (98.70), number of days to runners formation (97.90), and plant height (97.80).

High genetic advance as percent of mean was recorded for number of runner formation (33.51) followed by leaf area (26.20), plant height (25.22) and number of leaves per plant (25.01).

High heritability and high genetic advance as per cent of mean was observed in all the characters except for number of days to runners formation, flower size, number of days to flowering after planting .Suggests that the trait having high heritability and high genetic advance as per cent of mean are governed by additive gene effect and selection would be effective.

Table 4.13: Estimates of genetic parameters for yield and yield parameters

Parameters	Genotypic coefficient of variance	Phenotypic coefficient of variance	Heritability (%)	Genetic advance as (%) of mean
Fruit length (cm)	15.39	15.52	98.30	31.43
Fruit breadth (cm)	12.24	12.33	98.50	25.02
Average berry weight (g)	14.17	14.84	91.30	27.90
No. of fruits per plant	11.73	11.77	99.40	24.10
Fruit yield	14.93	14.94	99.90	30.77

The data presented in Table 4.13 reveals phenotypic coefficient of variation to be comparatively higher than the genotypic coefficient of variation for all the traits under study indicating considerable impact of environment on the expression of traits. The value of phenotypic coefficient of variation and genotypic coefficient of variation was found highest for fruit length followed by fruit yield and average berry weight.

High heritability was observed for fruit yield (99.90) followed by number of fruits per plant (99.40), and fruit breadth (98.50).

High genetic advance as per cent of mean was recorded for fruit length (31.43) followed by fruit yield (30.77) and average berry weight (27.90).

High heritability and high genetic advance as per cent of mean was observed in all the characters suggesting that the traits are governed by additive gene effect and selection would be effective.

4.6 Molecular characterization of cultivars

In the present study, evaluation of genetic diversity of nineteen strawberry genotypes at molecular level was done using PCR based ISSR markers. For the molecular characterization of nineteen strawberry genotypes, extraction of genomic DNA was done by using agrose gel electrophoresis. After the DNA extraction of all the nineteen strawberry genotypes, PCR amplification was carried out by using ISSR markers. A total of 26 ISSR markers were used. Out of twenty-six markers 15 were employed in the current study.

Table 4.14: List of 15 ISSR markers used in present study

S.No.	Primer name	Primer sequence	Annealing temperature (ºC)
1.	ISSR-1	AGCGCTAGCACACACACACACAC	56
2.	ISSR-2	GCTAGTGCTCACACACACACACA	54
3.	ISSR-3	GAGAGAGAGAGAGAGATC	50
4.	ISSR-4	GCAGCTCTCTCTCTCTCTC	55
5.	ISSR-7	CACACACACACAGC	36
6.	ISSR- 8	CACACACACACAGG	36
7.	ISSR- 17	AGAGAGAGAGAGAGAGC	48
8.	ISSR-19	GAGAGAGAGAGAGAGAT	46
9.	ISSR- 20	GAGAGAGAGAGAGAGAC	48
10.	ISSR- 21	CACACACACACACACAA	42
11.	ISSR- 827	ACACACACACACACACG	47
12.	ISSR- 834	AGAGAGAGAGAGAGAGGC	45
13.	ISSR- 845	CTCTCTCTCTCTCTCTG	42
14.	ISSR-849	GTGTGTGTGTGTGTGTC	47
15.	ISSR-860	TGTGTGTGTGTGTGTGGA	48

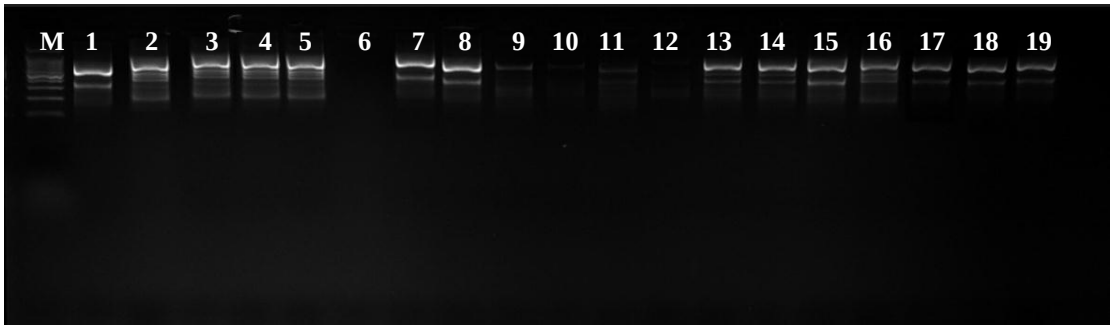


Plate-4.1: Banding pattern of ISSR- 1 primer

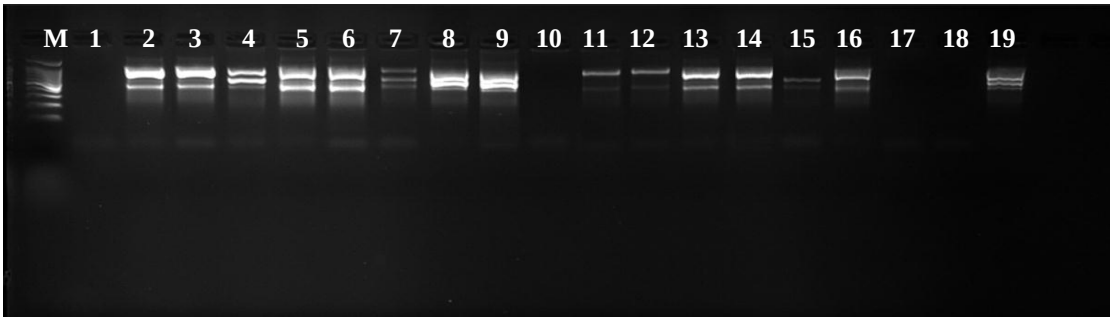


Plate-4.2: Banding pattern of ISSR- 2 primer

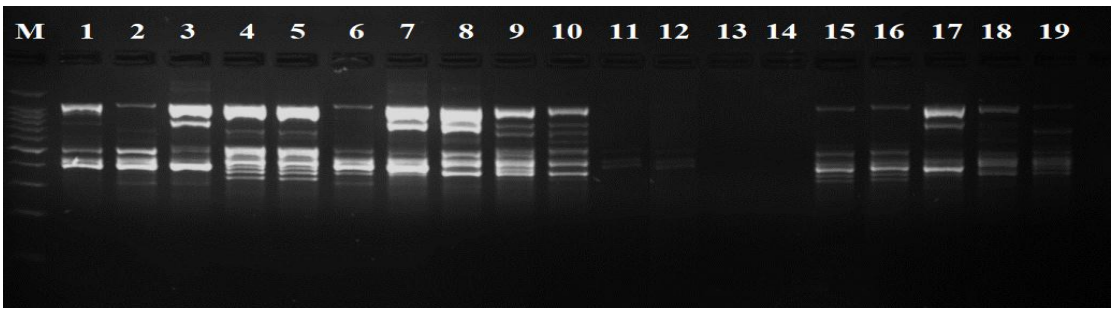


Plate-4.3: Banding pattern of ISSR- 3 primer

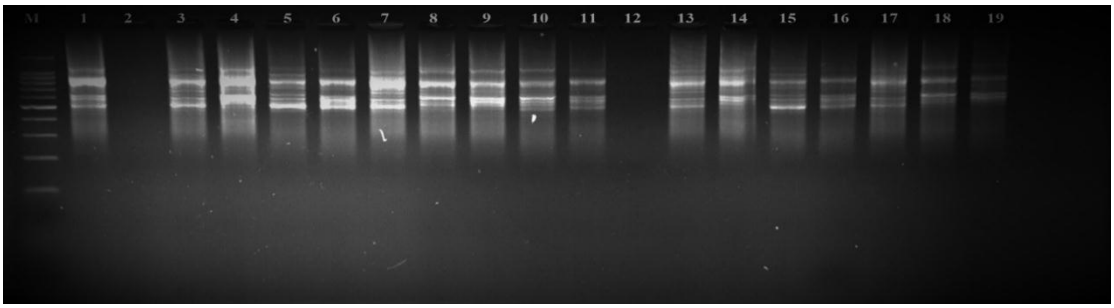


Plate-4.4: Banding pattern of ISSR- 4 primer

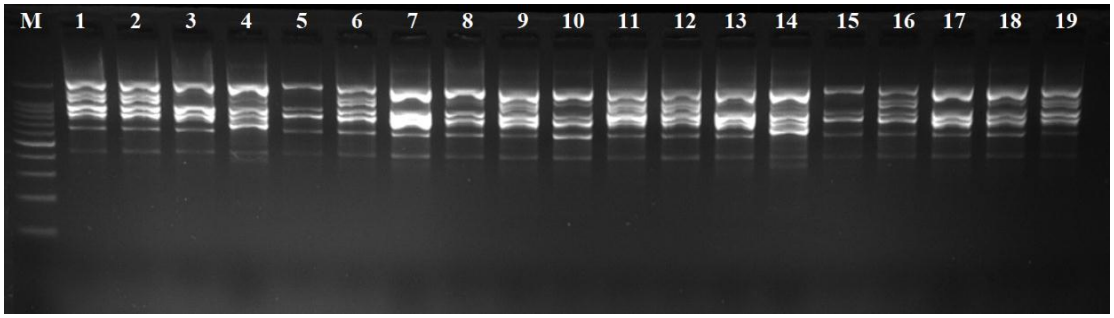


Plate-4.5: Banding pattern of ISSR- 7 primer

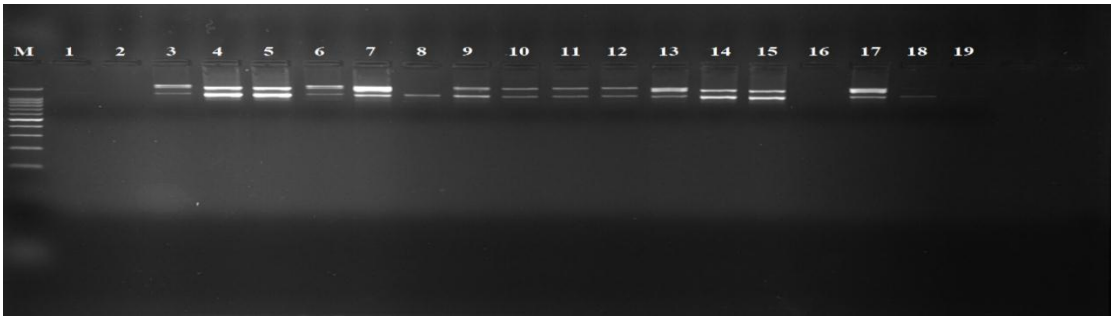


Plate-4.6: Banding pattern of ISSR- 8 primer

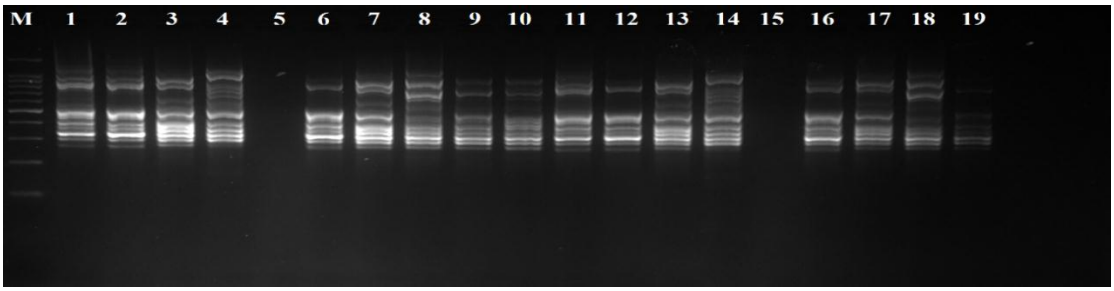


Plate-4.7: Banding pattern of ISSR- 17 primer

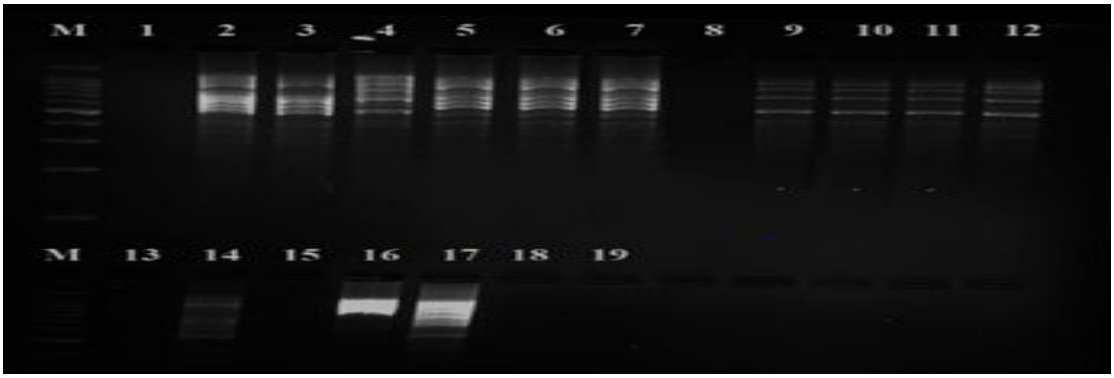
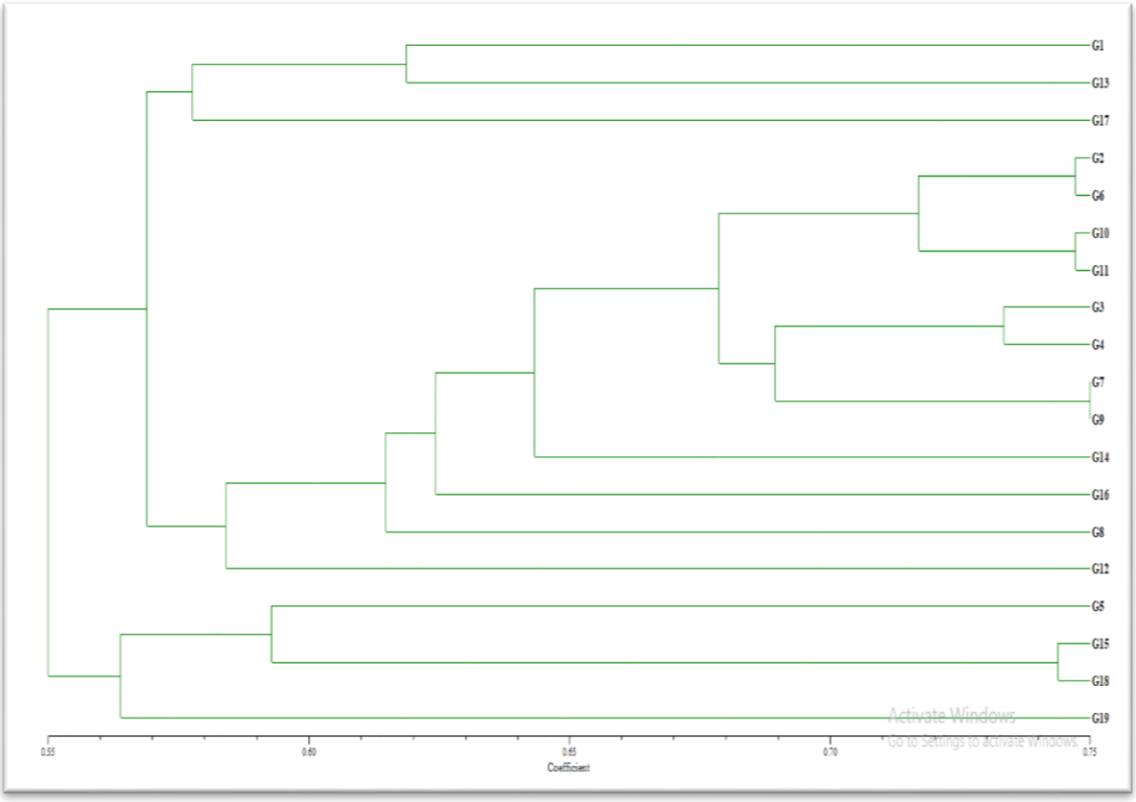


Plate-4.8: Banding pattern of ISSR- 19 primer

4.6.1 Genetic relationship among strawberry genotypes

The Jaccard's similarity index was calculated using NTSYS-pc version v2.10e software based on UPGMA (Unweighted pair group method with arithematic mean) and dendrogram was constructed. The dendrogram showcased distinct clusters corresponding to different genotypes, which have been organized based on their genetic similarity. Figure 4.1 visually presented the outcome of the clustering analysis. Notably, the dendrogram unveils two prominent clusters: Cluster 1 and Cluster 2. Cluster 1 composed of fifteen genotypes, further stratified into two sub clusters, namely sub cluster 1A and sub cluster 1B. Sub cluster 1A had three genotypes and sub cluster 1B had twelve genotypes. Cluster 2 included four genotypes and it was further divided into two sub clusters i,e sub cluster 2A and sub cluster 2B. Sub cluster 2A had three genotypes and sub cluster 2B had one genotype.



G1;Addie , G2; Belrubi, G3; Brighton, G4; Camarosa, G5; Capri, G6; Chandler, G7; Dana, G8; Douglas, G9; Etna, G10; Fairfox , G11; Fern, G12; Jutogh Special, G13; Katrain Sweet, G14; Nabila, G15; Selva, G16; Sea Scape, G17; Shimla Delicious, G18; Sweet Charlie, G19; Winter Dawn.

Fig. 4.1: Dendrogram based on ISSRs banding pattern showing the relationship among 19 strawberry cultivars

The two main clusters formed at a 55% similarity level, and subsequent cluster divisions that resulted in sub clusters formed a 0.58 similarity coefficient. The similarity coefficient of dendrogram made by ISSR primer range from 0.55 to 0.75 reflected the moderate intensity of variation present in the collected genotypes.

4.6.2 Polymorphism survey

The gel was evaluated depending on whether bands were missing or present. The bands were given scores and then matched against each other to evaluate the genetic diversity among the various genotypes of strawberry based on the polymorphism. The details of banding pattern observed in fifteen primers have been given in plates 4.1 to 4.15 with highest polymorphism being depicted in plate - 4.14.Genetic diversity and relatedness among all genotypes were evaluated using the Polymorphic Information Content (PIC) value and Percentage polymorphism.

4.6.2.1 Percentage polymorphism

Table 4.15: Summary of amplified products produced in 19 strawberry genotypes using 15 ISSR primers

Total number of primers taken into account	26
Number of informative primers used	15
Total number of amplified bands	78
Number of polymorphic bands	73
Number of monomorphic bands	5
Polymorphic percentage	93.58

Fifteen of the 26 primers were successful in causing amplification during PCR analysis, making them useful. Five bands were found to be monomorphic, and the remaining 73 of the 78 bands generated were polymorphic, making up 93.58 percent of the total polymorphism.

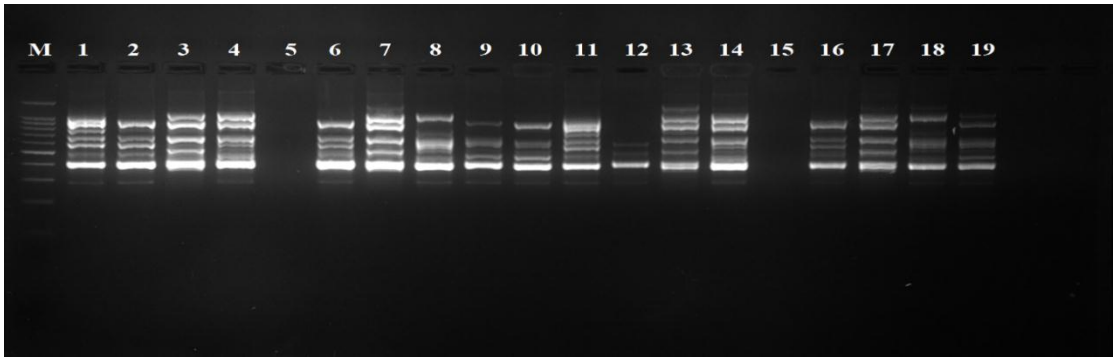


Plate-4.9: Banding pattern of ISSR- 20 primer

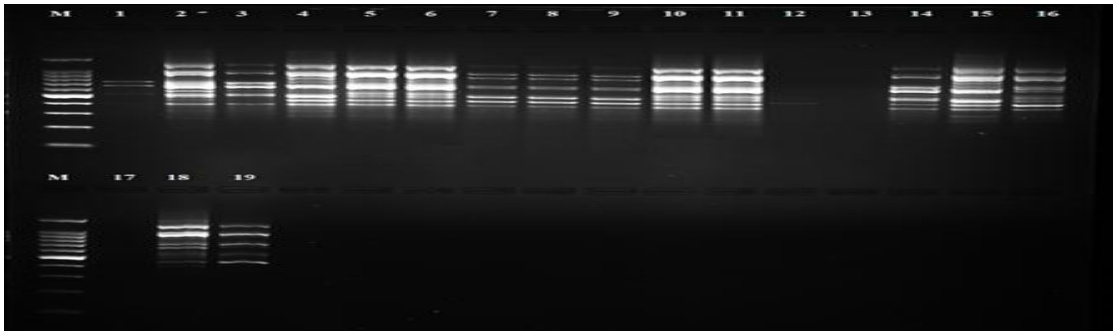


Plate-4.10: Banding pattern of ISSR- 21 primer

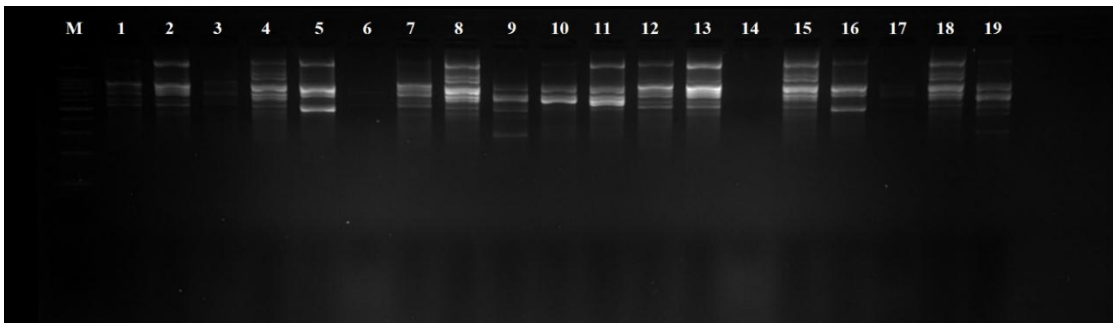


Plate-4.11: Banding pattern of ISSR-827 primer

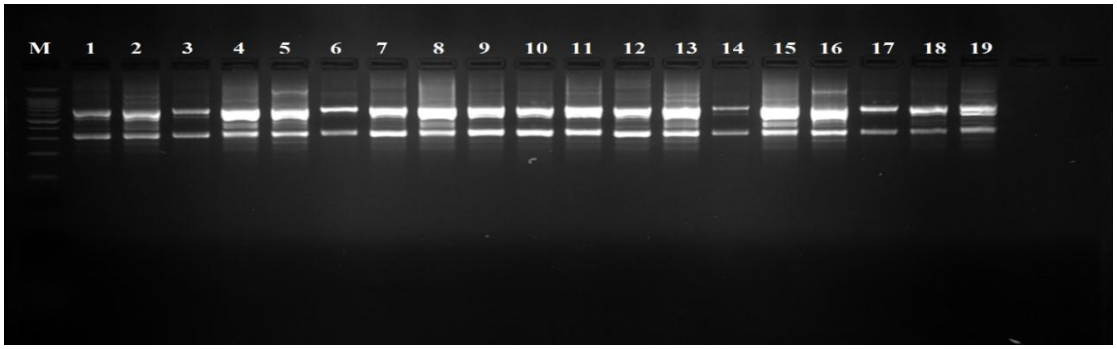


Plate-4.12: Banding pattern of ISSR-834 primer

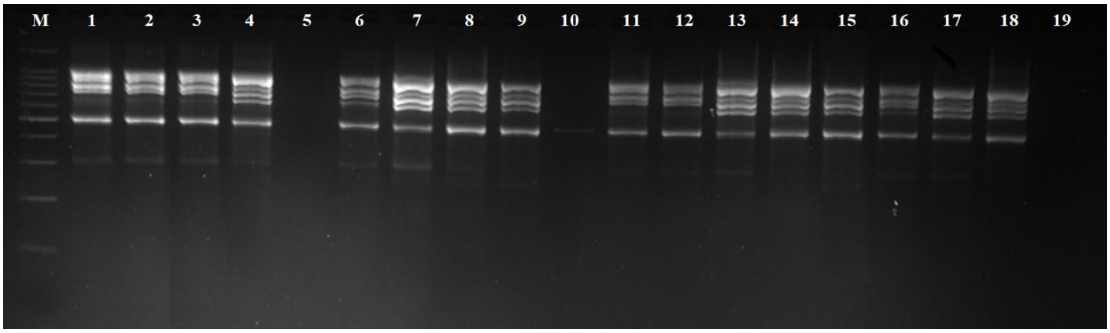


Plate-4.13: Banding pattern of ISSR- 845 primer

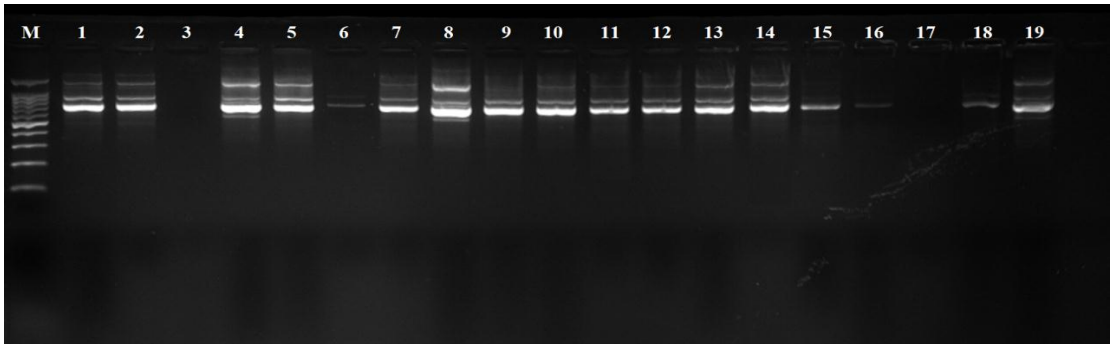


Plate-4.14: Banding pattern of ISSR-849 primer

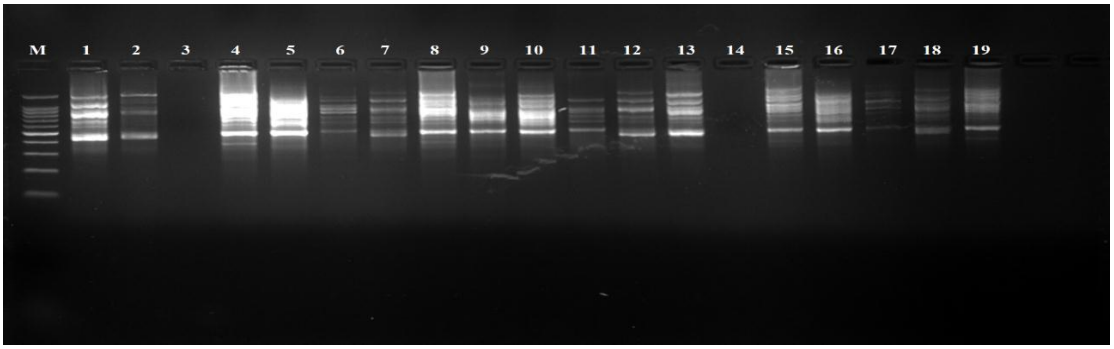


Plate-4.15: Banding pattern of ISSR-860 primer

4.6.2.2 Estimate of polymorphism information content and number of alleles.

Polymorphic information content (PIC) measures the ability of a marker to detect polymorphism among the genotypes.

Table 4.16: PIC value of 15 ISSR markers used in study

S.No.	Primer name	Primer sequence	PIC value	No. of Alleles
1.	ISSR-1	AGCGCTAGCACACACACACACAC	0.32	2
2.	ISSR-2	GCTAGTGCTCACACACACACACA	0.35	2
3.	ISSR-3	GAGAGAGAGAGAGAGATC	0.30	7
4.	ISSR-4	GCAGCTCTCTCTCTCTCTC	0.26	4
5.	ISSR-7	CACACACACACAGC	0.20	6
6.	ISSR- 8	CACACACACACAGG	0.32	2
7.	ISSR- 17	AGAGAGAGAGAGAGAGC	0.27	8
8.	ISSR-19	GAGAGAGAGAGAGAGAT	0.34	4
9.	ISSR- 20	GAGAGAGAGAGAGAGAC	0.40	8
10.	ISSR- 21	CACACACACACACACAA	0.31	7
11.	ISSR- 827	ACACACACACACACACG	0.28	3
12.	ISSR- 834	AGAGAGAGAGAGAGAGGC	0.28	3
13.	ISSR- 845	CTCTCTCTCTCTCTCTG	0.28	3
14.	ISSR-849	GTGTGTGTGTGTGTGTC	0.43	3
15.	ISSR-860	TGTGTGTGTGTGTGTGGA	0.26	4
	Average		0.28	4.4

For different ISSR marker analysis, amplified fragments of different sizes were considered as different alleles. The DNA bands that were amplified by a given primer were scored as present (1) or absent (0) for all the genotypes under study. In order to determine the utility of these markers polymorphic information content (PIC) values of individual primers were calculated. Among the fifteen primers employed, maximum Polymorphic information content value was observed in ISSR 849 (0.43) while minimum Polymorphic information content value was observed in ISSR 7 primer (0.20). A total of 66 amplicons were found with an average of 4.4 alleles per genotype.

CHAPTER-5

DISCUSSION

Strawberry is an important fruit crop in the sub - tropical and temperate regions of the world. It is herbaceous perennial fruit crop having short vegetative life cycle. The subtropical and intermediate zone of Jammu region is favourable for the production of quality strawberry fruits. It is the most significant soft fruit in the globe due to its great nutritional value (rich source of vitamins and minerals), alluring aroma, attractive appearance, and refreshing flavour (Kher *et al.,* 2010).

Genetic improvement of cultivated strawberry began in the mid-18th century and breeding programmes have produced tremendous advancements in the genetic development of the cultivated strawberry over the past 50 years (Hancock *et al.,* 1996).

Molecular markers and associated technologies are being utilised to assess the genetic diversity of strawberry cultivars (Chemda *et al.,* 2001). The use of DNA-based markers and other molecular biology techniques compare various genetic materials directly without regard to outside factors (Weising *et al.,* 1995).

For genetic divergence investigations, a number of techniques are used that mostly focus on DNA analysis. A variety of DNA-based approaches are available. Inter Simple Sequence Repeats (ISSR) markers are effective for genetic divergence investigations in strawberries.

5.1 Morphological characters of different strawberry cultivars

The present study was carried out for the evaluation of nineteen strawberry cultivars with respect to their morphological, yield, biochemical and molecular traits of plants.

5.1.1 Plant growth characters

5.1.1.1 Plant height, plant spread, number of leaves per plant, leaf area

As illustrated in Table 4.3, the maximum plant height was observed in cultivar ‘Selva’ (25.20 cm), ‘Chandler’ (25.00 cm), ‘Winter Dawn’ (24.70 cm) which was

statistically at par with the plant height recorded in ‘Nabila’ (24.10 cm). The maximum plant spread was found in cultivar ‘Chandler’ (29.70cm) which was statistically at par with cultivars ‘Winter Dawn’ (29.50cm) followed by ‘Nabila’ (28.91cm). These findings align harmoniously with previous research by Gupta (1998), Sharma and Sharma (2002), Singh *et al.,* (2008), Dolgun (2006), Ram and Yadav (2006), Rao and Lal (2010), Garg (2013), and Sharma *et al.,* (2014), all of whom concluded that 'Chandler' outperformed other cultivars in terms of plant height and spread.

Plant height and spread are genetic features that can also be modified by the existing agronomic and climatic conditions, which explains the observed variations in the current study and variations observed by earlier workers. The observed variation could also be related to the varied cultivars used in the study. Variation in vegetative traits among cultivars can be attributed to genetic characteristics of individual cultivars.

As shown in Table 4.3, the maximum number of leaves were observed in ‘Chandler’ (29.93), followed by cultivar ‘Winter Dawn’ (29.60), and ‘Selva’ (29.00). This variation in leaves number per plant could be genetic variation in the germplasms, cultivation site, cultural practices and climatic conditions (Li *et al.,* 1993). These findings echo the discoveries made by Singh *et al.,* (2008) and Garg (2013), who reported a leaf count of 33.30 in 'Chandler', while differing from Asrey and Singh (2004), who recorded a lower leaf count in 'Chandler'.

The maximum leaf area was also observed in ‘Chandler’ (186 cm^2) which was at par with cultivar ‘Winter Dawn’ (185.60 cm^2). The variability in leaf area can be attributed to how these genotypes respond to light, photoperiod, temperature, soil nutrition, and the allocation of free metabolites to above-ground plant parts. Further, the altitude of experiment sites also affected the leaf areas per plant and fruit yield (Crespo *et al.,* 2010). Sharma and Sharma (2002), Garg (2013), and Sharma *et al.,* (2014) consistently highlighted 'Chandler's' superiority in leaf area, Sahu and Chandel (2014) presented contrasting results, favoring 'Festival' and 'Camarosa' as the best performers.

5.1.1.2 Number of days to runner formation and number of runner formation after planting

As depicted in Table 4.4, a significant spectrum of runner output is unveiled, for number of runners per plant and days to runner formation after. This intriguing range could be attributed to the distinctive genetic makeup of the cultivars under study or their adaptive responses to varying climatic conditions, either independently or in unison. Higher or lower number of runners might be due to the differences in the prevailing agro climatic conditions, inherent potential of varieties for runner production and appropriate cultural practices adopted for strawberry culture.

The maximum runners were formed in the cultivar ‘Chandler’ followed by the cultivar ‘Nabila’ and 'Sweet Charlie’. These findings align with the research conducted by Das *et al.,* (2007) and Garg (2013).This variation in number of runners per plant may be due to potential of cultivars to produce runners.

5.1.2 Floral characteristics

5.1.2.1 Flower size, opening of first flower and last flower

As delineated in Table 4.5, the maximum flower size was observed in cultivar ‘Chandler’ (2.83 cm) and ‘Sweet Charlie’ (2.81cm) which was statistically at par with ‘Nabila’ (2.80 cm) .All cultivars took a time interval of between 18-20 weeks for the first flowering and flowering lasted between 4-5 weeks after first flowering to the opening of last flower in all the cultivars under study. Similar values for the trait for different cultivars were registered by Garg (2013) who found maximum flower size in ‘Selva’. This particular trait has strong genetic base and is less prone to the changing agro-climatic conditions. Flowering was early, according to Gunduz and Ozdemis (2008), which is identical to the results of the study under discussion. The observations by Sharma *et al.,* (2002), marking the bloom initiation for all cultivars in the early days of January's third week, mirror the current study, reinforcing a timeless synchrony.

5.1.2.2 Number of days to flowering after planting and duration of flowering, flower type

As outlined in Table 4.6, Number of days to flowering was estimated in between 110-134 days after planting and duration of flowering was estimated between 36-53 days

in all the strawberry cultivars .Strawberry cultivars exhibited significant differences in flowering behaviour, which could be attributable to the temperatures prevailing during the growing period (Joolka and Badiyala, 1983). Flowering behaviour is influenced by factors such as the current climate, planting period, cultivar juvenility, cultural practises, and inherent cultivar vigour, which all influence flowering behaviour individually and together. The flower type was observed as hermaphrodite in all the cultivars under study shown in Table 4.6. These findings blend harmoniously with the conclusions drawn by Gupta (1998) and Garg (2013). Additionally, a resonance of these observations can be found in the work of Hofer *et al.,* (2012), who likewise encountered a consistent flower type across a diverse array of cultivars.

5.2 Yield and yield parameter

5.2.1 Fruit length, fruit breadth and average berry weight

As presented in Table 4.7, maximum fruit length was observed in the strawberry cultivar ‘Sweet Charlie’ (3.72 cm), followed by ‘Chandler’ (3.70 cm). Increase in fruit length may be due to environmental factors such as increase in day length and sunlight which enhance the cell expansion and development of the fruit. Initiation of secondary fruits reduces the fruit length among different strawberry cultivars. The significant variation observed among these cultivars may be a result of genotypic differences, as observed by Dhaliwal and Singh (1983). Similar findings were reported by Kumar and Ahad (2012) in their study of strawberry yield characteristics in South Kashmir, where the cultivar 'Tioga' exhibited the maximum berry length (5.10 cm). Likewise, Negi and Upadhyay (2016) observed the highest fruit length (45.38 mm) in the cultivar 'Camarosa'. The observations unveiled in the present study seamlessly align with those of Singh *et al.* (2016), who elucidated significant size disparities among various cultivars, with Chandler reigning supreme in terms of fruit length.

The largest fruit breadth was observed in the strawberry fruits of 'Chandler' (3.13 cm), 'Sweet Charlie' (3.12 cm), and 'Nabila' (3.11 cm), which were statistically comparable. The increase in fruit breadth across different strawberry cultivars is influenced by their genetic makeup and adaptability to the prevailing climatic conditions (Singh *et al.,* 2007). The agro-climatic conditions directly impact fruit development and size variation among

different cultivars. Variation in fruit diameter was reported by Flanagan *et al.,* (2020) in Costal Virginia and found that cultivar Florida Radiance have maximum fruit diameter (33.6mm) in Chesapeake, cultivar Albion (38.3mm)in Virginia Beach and cultivar Benicia (39.2mm) in Westmoreland country. The current findings are closely matched with the work of Lies *et al.,* (2012) .Similar results are obtained by Singh *et al.,* (2016) who observed that cultivar Camarosa (3.34 cm) and cultivar Chandler (3.26 cm) have maximum fruit diameter among different strawberry cultivars under Punjab conditions.

Fruit size stands as a distinctive trait to each cultivar, delineating unique differentiations among them. Notably, Galletta *et al.,* (1981) have unveiled that even a mere 10°C reduction in soil temperature can yield a substantial augmentation in fruit size, spanning an increase of 0.9 to 1.6 grams per berry in day-neutral varieties. A multitude of factors influence fruit size, including plant vigor, competition among growing fruits, the number of flowers per inflorescence, the quantity and size of developed achenes, prevailing climatic conditions, and the overall plant and nutrient status (Moore *et al.,* 1970 and Janick and Eggert, 1968).

Notably, 'Winter Dawn' emerged as the cultivar with the highest berry weight, recorded at 9.50g, which closely mirrored the berry weight of 'Selva' (9.49 g) and 'Sweet Charlie' (9.46 g). The variation in fruit weight among strawberry cultivars in relation to achene number may be attributed to the distinct activity of achenes in producing growth-promoting hormones and the varying sensitivity of receptacle tissues, as suggested by Moore *et al.,* (1970). These findings resonate with the work of Masny and Zurawicz (2009), who reported an average berry weight ranging from 7.56 to 12.24 g.

5.2.2 Number of fruits per plant and fruit yield

As depicted in Table 4.8, the maximum number of fruits per plant was produced by the cultivar ‘Chandler’(20.80) which was statistically at par with ‘Winter Dawn’ (20.14), followed by ‘Nabila’(20.10)and Sweet Charlie (20.10). The substantial response of strawberry cultivars concerning the total number of fruits per plant illuminates the profound influence of their genetic makeup. These results strikingly align with the conclusions drawn by Luitel *et al.,* (2012) in tomato cultivars. The highest fruit yield per

plant was estimated in the cultivar ‘Chandler’ (193.02 g) which was at par with ‘Winter Dawn’ (191.33g), ‘Sweet Charlie’ (190.14 g) and Nabila (189.34g). Yield per plant is intricately influenced by various environmental factors, including temperature, photoperiod, and light intensities. According to Rao and Lal (2010) and Sharma and Thakur (2008), the cultivars 'Chandler' and 'Selva' exhibit the most promising yields in Himachal Pradesh. The current findings differ from those of Kumar *et al.,* (2011) and Dolgun (2006), who found that ‘Chandler'’ had both highest yield and yield per plot. The characteristics of the cultivar, the management techniques employed, the location of the cultivation, and their individual or combined impact on cultivar yield are all potential causes of the observed variation.

5.3 Biochemical characteristics of fruits

5.3.1 Total soluble solids (TSS) and acidity

The data presented in Table 4.9, pertaining to the total soluble solids among different strawberry cultivars, reveals significant variation among all the cultivars. Maximum TSS was recorded in cultivar 'Selva' (11.60°B) and 'Chandler' (11.49°B) had the highest TSS, followed by 'Winter Dawn' (11.32°B) and 'Sweet Charlie' (11.30°B). Total soluble solids (TSS) levels exhibited a range spanning from 6.70°B to 11.39°B in the cultivars examined by Sharma and Chauhan (1986) and Dhaliwal and Singh (1983), mirroring the consistent trends observed in the present study. TSS content fluctuation was also higher in 'Selva,' according to Garg (2013). According to Shaw (1990), environmental factors during growth and development had a greater influence on the Total Soluble Solids content than genetic inheritance. Asrey and Singh (2004), Nagre *et al.,* (2005) and Sharma and Thakur (2008), in their different work evaluated cultivar 'Chandler' recorded higher TSS.

Despite the significant hereditary influence on the trait, it is crucial to recognize that the growing conditions, whether hot or cold, exert a considerable impact on the Total Soluble Solids (TSS) content of strawberries. Specifically, temperatures exceeding 25°C have been associated with adverse effects on fruit set, leading to a decline in fruit soluble solids content and hastened fruit development (Abdelrahman in 1984; Hellman and Travis, 1988). Conversely, cooler environments promote the accumulation of higher TSS content

compared to warmer conditions (Darrow, 1966; Dana, 1980). Variations in cultivar composition and culture conditions, according to Voca *et al.,* (2008), caused changes in TSS. Environmental factors during the growth and development phase had a greater influence on the Total Soluble Solids content than genetic inheritance.

The highest acidity was recorded in the fruits of cultivar ‘Fairfox’ (0.88 %) which was statistically at par with ‘Nabila’ (0.87 %) and ‘Brighton’ (0.87 %). In contrast to the current study, the research conducted by Hassan *et al.,* (2001) revealed that 'Chandler' exhibited the highest acid concentration, while the highest acid content was found in 'Fairfox.' This observed variation in acidity content among different cultivars may be attributed to the unique genetic makeup inherent to each cultivar, as elucidated by Dhaliwal and Singh (1983).

Titratable acidity undergoes modifications influenced by factors such as maturity period, harvesting practices, and prevailing climatic conditions, as underscored by Kidmose *et al.,* (1996). The observed decrease in acidity within strawberries could potentially be linked to variations in the cultivation environment, which encompasses daytime temperatures spanning from 28 to 32°C during the fruit's maturation phase.

Wysocki (2012) reported that acidity in strawberry fruits is not a fixed characteristic of the varieties; rather, it can vary depending on numerous environmental parameters encountered during cultivation. The acidity levels are significantly influenced by agricultural factors, implying that cultivation practices, growing conditions, and management strategies play a substantial role in shaping the acidity profiles of different strawberry varieties.

5.3.2 Reducing sugars and non-reducing sugars

As shown in Table 4.10, the maximum reducing sugar was recorded in the fruit of cultivar ‘Chandler’ (4.97 %) and ‘Sweet Charlie’ (4.91 %) and ‘Winter Dawn’ (4.87 %), while the fruits of the cultivars ‘Chandler’ (3.75%) and 'Winter Dawn' (3.72%) had the highest non-reducing sugar levels. Das *et al.,* (2007) and Kader (1991) in their separate work on evaluating strawberry cultivars recorded highest reducing sugar in cultivar ‘Chandler’.

The present findings are consistent with those of Singh *et al.* (2008) and Lal *et al.,* (2010), supporting the notion that 'Chandler' has relatively higher sugar content. The current findings differ from the works of Shukla *et al.,* (1980) and Dhaliwal and Singh (1983), who observed substantial variations in reducing sugars (ranging from 3.81 to 9.96 percent) and non-reducing sugars (ranging from 0.13 to 1.61 percent) among different cultivars. Sharma and Thakur (2008) identified a similar association, with 'Chandler' recording a relatively higher value.

Jami *et al.,* (2015) and Sharma *et al.,* (2008) reported higher sugar content in 'Ofra' and 'Chandler', respectively, aligning with the present investigation's findings for 'Chandler'. The sugar composition within strawberry cultivars is influenced by a multitude of cultivation conditions, encompassing factors such as irrigation (particularly crucial during flowering and fruit setting stages), fertilization (primarily NPK), and the overall health status of the plantation (Skupien 2003, Ohtsuka *et al.,* 2004, and Koszanski *et al.,* 2005).

5.3.3 Total sugars and TSS/acidity ratio

Among all the cultivars present in table 4.11, the cultivar ‘Chandler’ had the highest total sugar content (8.17%), followed by 'Sweet Charlie' (8.10%), while the highest TSS/acidity ratio was observed as (15.45%) in ‘Selva’ followed by ‘Winter Dawn’ (14.11%) and ‘Sweet Charlie’ (13.78%). The elevated TSS: acid ratio observed in this study could potentially be attributed to the higher TSS content detected in the 'Selva' cultivar in comparison to other cultivars. These results are in accordance with findings obtained by Saima *et al.,* (2014) and Neetu and Sharma (2018).

The observed variances in total sugar magnitude can be related to a variety of agro-climatic circumstances, cultivar genetic makeup, and plantation location, as sugar levels are often higher in cooler areas than in exposed ones. Such differences are frequently observed during evaluation with different cultivars and under different ecological situations, and research on such parameters needs to be carried out for longer periods of time and under similar conditions for better cultivar performance and interpretation of observed variation.

5.4 Variability analysis for morphological and yield and yield parameters

Genetic variability is the basis of all plant improvement programmes. The amount of genotypic and phenotypic variability that exists in a species is the utmost importance in breeding better varieties and in initiating a breeding program. The estimates of genetic variability parameters for all the traits were worked out. It was evident from the result that the phenotypic variance is greater than genotypic variance indicating the influence of environment. The value of phenotypic coefficient of variation and genotypic coefficient of variation was found highest for number of runners formation followed by leaf area, plant height and number of leaves per plant, fruit length, fruit yield and average berry weight.

The high values of PCV and GCV indicating that selection may be effective on these traits. The magnitude of genotypic and phenotypic coefficient of variability within a species holds immense importance in the development of superior varieties and the initiation of successful breeding programs, Ara *et al.,* (2009).

Kumar *et al.,* (2012) highlighted that phenotypic correlation variance was higher than genotypic correlation coefficient of variation for all the traits viz. plant height, plant spread, number of leaves per plant, number of flower per plant, number of fruit per plant, fruit length, fruit breadth, fruit yield per plant, average berry weight. Heritable variation is useful for permanent genetic improvement (Singh, 2000). Heritability serves as a reflection of the proportion of genetic variability that is transmitted from parent to offspring, contributing to the perpetuation of favorable traits.

High heritability was observed for leaf area (99.90), number of leaves per plant (98.70), number of days to runners formation (97.90), plant height (97.80), fruit yield (99.90), number of fruits per plant (99.40), and fruit breadth (98.50). The findings were almost supported by Kumar *et al.,* (2012) who observed high estimates of heritability for number of leaves per plant, number of flower per plant, number of fruit per plant, fruit breadth, fruit yield per plant, average berry weight. Ara *et al.,* (2009) also, observed high value of heritability with respect to trait like plant height, number of flower per plant and fruit yield per plant, similar results have also been reported by Karim, (2007). High value of heritability was observed in number of leaves per plant, number of flower per plant,

number of fruit per plant, fruit length, fruit breadth, fruit weight and fruit yield per plant reported by Mishra *et al.,* (2015) and similar result was found by Sah *et al.,* (2010).

Breeder should consider heritability estimates along with the genetic advance because heritability alone is not a good indicator of the usable genetic variability as reported by Masood, (1986). High genetic advance was recorded for number of runners formation (33.51), leaf area (26.20), plant height (25.22), number of leaves per plant (25.01), fruit length (31.43), fruit yield (30.77) and average berry weight (27.90).

When combined with heritability estimates, the GA estimate is more effective as a selection tool (Johnson *et al.,* 1955). High heritability and genetic advance might be attributed to additive gene action, and so selection would be successful for this trait.

5.5 Molecular characterization

Understanding the genetic diversity and the interconnectedness among different strawberry cultivars holds utmost significance to secure the enduring success of strawberry improvement initiatives. Populations characterized by rich genetic diversity serve as valuable resources for expanding the genetic foundation in breeding endeavors. Molecular markers have emerged as highly efficient and potent tools in this context, enabling the elucidation of genetic diversity, quantification of proportions, and unveiling the phylogenetic interrelationships among diverse strawberry cultivars. These tools play a pivotal role in advancing our comprehension of the intricate genetic landscape and fostering progress in strawberry breeding programs.

DNA extraction was performed utilizing the CTAB technique as outlined by Doyle and Doyle (1987). The extraction procedure involved the isolation of DNA from young and freshly harvested leaves of the strawberry plants. Due to the higher presence of secondary metabolites in strawberry leaves, the extracted DNA carried a substantial phenolic content. To mitigate this challenge, the DNA extraction buffer was supplemented with PVP (polyvinylpyrrolidone) and β-mercaptoethanol. This methodology was adopted considering analogous challenges faced by Majeed *et al.,*(2009), Thakur (2013) and Soni and Kaur (2014). For DNA purification, the extracted DNA was subjected to RNase treatment, followed by subsequent C:I and P:C:I procedures. Subsequently, the DNA's

purity was assessed by electrophoresis. The DNA samples were loaded onto a 0.8 percent agarose gel (stained with ethidium bromide) and electrophoresis was conducted at 100 V for one hour in I X TBE buffer and visualized using a UV-transilluminator. This process allowed for the evaluation of DNA purity and the detection of any relevant bands or patterns on the gel.

As depicted in Table 4.14 and Table 4.15, a comprehensive set of 15 ISSR markers was employed in this investigation. Scoring was executed based on the bands produced by the amplified PCR products, with a notation of present (1) or absent (0) assigned to each strawberry variety. This scoring methodology enabled the assessment of relationships among the examined varieties. To identify similarities among the cultivars, a dendrogram was constructed using the Un-weighted Pair-Group Method using Arithmetic Averages (UPGMA), a technique validated by Sokal and Michener (1985). The UPGMA clustering technique assumes uniform evolutionary rates among accessions or clones.

The current study's outcomes unveiled a substantial level of genetic relatedness among the strawberry cultivars and lines under scrutiny. The identification of diversity among different genotypes holds pivotal significance in crop enhancement endeavors. Employing PCR-based markers for molecular assortment analysis presents an economical and expedient avenue for characterizing relationships among diverse genotypes (Ramu *et al.*, 2013). This approach enhances our understanding of genetic variations and aids in refining crop improvement strategies.

Fifteen of the 26 primers were successful in causing amplification during PCR analysis, making them useful. Five bands were found to be monomorphic, and the remaining 73 of the 78 bands generated were polymorphic, making up 93.58 percent of the total polymorphism. These findings are in line with the work of Kumari et.,al (2019) who reported 85% of polymorphism in strawberry genotypes using ISSR markers.

The mean similarity assessed by ISSR markers for nineteen strawberry genotypes was 55%, which are in line with the work done by Morales et.al.,(2011). The highest PIC value (0.43) was obtained ISSR 849 followed by ISSR 20 (0.40) .The study reveals that

ISSR 849 were more powerful for diversity analysis as compared to those with lower PIC values. These findings align with the research conducted by Shahin *et al.,(*2018).

Despite the constrained genetic diversity within commercial strawberries, their extensive history of cultivation and domestication, as documented by Dale (1987) and Hancock *et al.,* (2002), underscores the importance of comprehensive genetic resource evaluation. Given the challenge of phenotypic differentiation among strawberry genotypes, the utilization of ISSR data emerges as particularly relevant. This data illuminates the distinctiveness of genotypes, thereby offering a robust tool for uncovering genetic relationships and informing cultivar enhancement initiatives.

The present findings of this study concluded that a significant genetic variation exists among selected ninteen genotypes of strawberry which can further be utilized in molecular breeding programmes.

CHAPTER-6

SUMMARY AND CONCLUSIONS

The present investigation entitled **"Morphological and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu"** was carried out on nineteen strawberry cultivars to ascertain the morphological traits and molecular characterization at the Advanced Centre for Horticulture Research in Udheywalla, the trial was set up using a randomized block design with three replications, and molecular research was conducted in the lab of the School of Biotechnology at SKUAST-Jammu. Diverse cultivars from various origins were grown together.

This study holds significance in leveraging the genetic differences within each strawberry type and the overall variety among these strawberries. This can aid in choosing and developing new and better strawberry varieties that will perform exceptionally well in the field. In simpler words, the study helps us find and create improved strawberries for better farming outcomes.

The present study was undertaken with the following objectives:

1. To study the morphological and biochemical traits of different strawberry cultivars under subtropical conditions of Jammu.
2. Molecular characterization of the strawberry genotypes.

The main conclusions drawn from the current surveys are condensed here under the following headings:

6.1 Evaluation of cultivars

6.2 Molecular characterization

6.1 Evaluation of cultivars

Cultivar 'Selva' (25.20 cm), 'Chandler' (25.00 cm), and 'Winter Dawn' (24.70 cm) displayed the highest plant heights, while 'Jutogh Special' and 'Shimla Delicious' exhibited the lowest plant height. The maximum plant spread, number of leaves per plant, leaf area, was found in cultivar 'Chandler' followed by the cultivars 'Winter Dawn', 'Nabila' and

‘Selva’. Runners formation were exhibited by cultivars ‘Chandler’ (10.10) and was statistically on par with 'Nabila' (9.96) and 'Sweet Charlie' (9.94). The cultivars ‘Fern’ and ‘Nabila’ exhibited the most prolonged duration for runner formation after planting. The minimum plant spread was found in cultivars ‘Shimla Delicious’, ‘Etna’ followed by cultivar ‘Seascape’, Conversely, the lowest leaf count per plant was recorded in the 'Katrain Sweet' cultivar. followed by the cultivars ‘Jutogh Special’ and ‘Shimla Delicious’. The cultivar 'Jutogh Special' had the smallest leaf area recorded, while the cultivar "Selva" showcased the shortest time for runner formation after planting. The cultivars 'Shimla Delicious' and 'Seascape' produced the smallest runner production. The maximum flower size was observed in cultivar ‘Chandler’ (2.83 cm) and ‘Sweet Charlie’(2.81cm) which was statistically at par with ‘Nabila’ (2.80 cm), while the minimum flower size was exhibited by cultivar ‘Belrubi’ (2.15 cm) which was at par with ‘Shimla Delicious’ (2.19cm).

All the cultivars exhibited a time span of 18 to 20 weeks before the onset of their first flowering.In the third week of January of 2023, the majority of the cultivars bloomed. The cultivar 'Shimla Delicious' bloomed first while ‘Chandler’ and ‘Katrain Sweet’ were the last to start flowering. The first cultivar to finish flowering among all the others was ‘Shimla Delicious’. Cultivar ‘Shimla Delicious’ took maximum number of days to flowering (134) while the minimum number of days taken to flowering was observed in cultivar ‘Seascape’ (110.73). Maximum flowering duration was observed in cultivar ‘Chandler’ whereas, Cultivar ‘Shimla Delicious’ recorded minimum flowering duration. 'Cultivar Sweet Charlie' achieved the highest fruit length (3.72 cm), while 'Cultivar Shimla Delicious' displayed the minimum. In terms of fruit breadth, 'Cultivar Chandler' recorded the maximum (3.13 cm), and 'Cultivar Shimla Delicious' exhibited the minimum (2.21 cm).

Cultivar ‘Winter Dawn’ recorded the highest berry weight (9.50 g) while the minimum berry weight was measured for the cultivar ‘Shimla Delicious’. The cultivar 'Chandler' yielded the highest number of fruits per plant (20.80), followed by 'Winter Dawn' (20.14) and 'Nabila' (20.10).The lowest number of fruits per plant was observed in the 'Jutogh Special' cultivar (15.50).The maximum fruit yield was observed in the cultivar ‘Chandler’ (193.02 g) followed by ‘Winter Dawn’ (191.33g), ‘Sweet Charlie’ (190.14 g)

and 'Nabila' (189.34g). However, the minimum fruit yield was observed in the cultivar ‘Shimla Delicious’ (116.98 g) followed by ‘Jutogh Special’ (117.18g).

The cultivar 'Selva' exhibited the highest TSS (11.60°B), while 'Shimla Delicious' recorded the lowest (8.05°B). The most elevated acidity was found in 'Fairfox' (0.88%), whereas the lowest was in 'Shimla Delicious' (0.64%). The cultivar 'Chandler' had the highest reducing sugar content, while 'Shimla Delicious' had the lowest.Cultivar 'Chandler' displayed the highest non-reducing sugar levels, whereas 'Seascape' cultivar had the lowest non-reducing sugar levels.

The fruit from the cultivar ‘Chandler’ had the highest total sugar content (8.17%), while the fruit from the cultivar 'Shimla Delicious' had the lowest total sugar content (4.37%). The highest TSS/acidity ratio was recorded in ‘Selva’(15.45%) , followed by ‘Winter Dawn’ (14.11%) while the lowest ratio was recorded in ‘Fairfox’ (10.01%).

The value of phenotypic coefficient of variation and genotypic coefficient of variation was found highest for number of runners formation followed by leaf area, plant height and number of leaves per plant. High heritability was observed for leaf area (99.90) followed by number of leaves per plant (98.70), number of days to runners formation (97.90), and plant height (97.80). High genetic advance as percent of mean was recorded for number of runner formation (33.51) followed by leaf area (26.20), plant height (25.22) and number of leaves per plant (25.01). High heritability and high genetic advance as per cent of mean was observed in all the characters except for number of days to runners formation, flower size, number of days to flowering after planting .The value of phenotypic coefficient of variation and genotypic coefficient of variation was found highest for fruit length followed by fruit yield and average berry weight. High heritability was observed for fruit yield (99.90) followed by number of fruits per plant (99.40), and fruit breadth (98.50).High genetic advance as per cent of mean was recorded for fruit length (31.43) followed by fruit yield (30.77) and average berry weight (27.90).High heritability and high genetic advance as per cent of mean was observed in all the characters suggesting that the traits are governed by additive gene effect and selection would be effective.

6.2 Molecular characterization

The DNA of cultivars was amplified using thirteen ISSRs primers in the present study. Scoring was conducted based on the presence (1) or absence (0) of the bands.On PCR analysis, five bands were found to be monomorphic, and the remaining 73 of the 78 bands generated were polymorphic, making up 93.58 percent of the total polymorphism.

Jaccard's similarity index was calculated using NTSYS-pc version v2.10e software with UPGMA, and a dendrogram was constructed. The dendrogram depicted distinct clusters representing different genotypes based on their genetic similarity. Figure 4.1 visually showcased the results, revealing two prominent clusters: Cluster 1 (divided into sub clusters 1A and 1B) and Cluster 2 (with sub clusters 2A and 2B). The main clusters formed at 55% similarity, and subsequent divisions resulted in sub clusters at a 0.58 similarity coefficient. The similarity coefficient ranged from 0.55 to 0.75. .

Among the fifteen primers employed, maximum Polymorphic information content value was observed in ISSR 849 (0.43) while minimum Polymorphic information content value was observed in ISSR 7 primer (0.20). A total of 66 amplicons were found with an average of 4.4 alleles per genotype.

When it comes to developing new cultivars, accurately assessing the available genetic resources is crucial. Distinguishing strawberry genotypes solely based on physical traits can be challenging. Nevertheless, the use of ISSR (Inter-Simple Sequence Repeat) data provides clear evidence that these genotypes are distinct from one another while also sharing some degree of relatedness. This highlights the complexity of genetic relationships within the strawberry varieties under study.

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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 and Molecular Characterization of Strawberry Cultivars under Subtropical Conditions of Jammu"**, submitted by **Ms. Isha Kotwal**, Registration No. **J-21-MB-44**.

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