IMPACT OF CLIMATE ON MORPHOLOGICAL CHARACTERISTICS, YIELD AND MOLECULAR PROFILING OF AONLA VARIETIES

Thesis

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By

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

Master of Science (Horticulture)

Fruit Science

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CERTIFICATE-1

This is to certify that the thesis entitled "**Impact of climate on morphological characteristics, yield and molecular profiling of Aonla varieties**" submitted in partial fulfillment of the requirements for the degree of **Master of Science (Horticulture)** with major in **Fruit Science** of the **College of Horticulture & Forestry** Post Graduate Studies, **Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya** is a record of *bonafide* research carried out by **Mr. Satyam Maurya, Id. No. H-14732/23,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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

Kumarganj, Ayodhya
Date: 26/08/2025

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Abbreviation	**Standard Form**
DNTP	Dinitro Triphosphate
Bp	Base Pairs
Kbp	Kilo Base Pairs
Ng	Nanogram
µg	Microgram
mg	Milligram
kg	Kilogram
ml	Microlitre
Pmol	Pico Mole
µm	Micro Mole
mM	Milli Molar
M	Molar
N	Normality
W/V	Weight/Volume
V/V	Volume/Volume
mm	Millimeter
cm	Centimeter
gm	Gram
CTAB	Cetyl Trimethyl Ammonium Bromide
EDTA	Ethylene Diamine Tetra Acetic Acid
$NaCl_2$	Sodium Chloride
$CaCl_2$	Calcium Chloride
PVP	Polyvinyl Pyrrolidone
TE	Tris EDTA Buffer
TAE	Tris Acetate EDTA Buffer
TBE	Tris-Boric Acid EDTA Buffer
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
EtBr	Ethidium Bromide
PCR	Polymerase Chain Reaction
O.D	Optical Density
LN2	Liquid Nitrogen
RAPD	Randomly Amplified Polymorphism
SCAR	Sequence Characterized Amplified Region Markers
SSR	Simple Sequence Repeat Markers
AFLP	Amplified Fragment Length Polymorphism
ISSR	Inter Simple Sequence Repeat Markers

Chapter-I

INTRODUCTION

Aonla (*Emblica officinalis* Gaertn.), Indian gooseberry or amla, is majorly play a vital role in Indian Ayurvedic and medicinal value and belongs to the Euphorbiaceae family. According to an old Indian legend, the "Amla″ tree was the first to grow on Earth. This tree, cultivated primarily for its sour fruits, has significant medicinal potential. It is also referred to by various local names, including amalika and amla (Sharma and Sumbali 2012). Aonla is an excellent source of ascorbic acid (300-900 mg/100 g), amino acids, and minerals along with phytochemicals such as polyphenols, tannins, emblicol, linoleic acid, corilagin, phyllemblin and rutin (Baliga and Dsouza, 2011).

The fruit of this tree is rich in various nutraceuticals, including calcium, vitamin C, lysine, minerals, methionine, nicotinic acid, phosphorus, riboflavin, and tryptophan. It holds significant importance in Ayurveda, an ancient Indian system of medicine, and is believed to possess immune-boosting properties against various diseases. Aonla is credited with medicinal value, such as antiscorbutic, diuretic, laxative, and antibiotic. The fruit also possesses pronounced expectorant, antiviral, cardiotonic, and hypoglycaemic activity (Mehta and Tomar 1979). The fruits are mainly used as raw material or for preparing different Ayurvedic and Unani medicines (Agarwal and Chopra 2004).

Aonla is one of the major fruit crops grown in arid and semi-arid regions (Jat *et al.,* 2013), Uttar Pradesh being a key area of cultivation, particularly in districts such as Ayodhya, Pratapgarh, Sultanpur, Azamgarh, Varanasi, Jaunpur, Rai Bareilly, Agra, and Bareilly. Indigenous to India, Aonla has extended its cultivation to various tropical and subtropical countries, encompassing Bangladesh, Malaysia, the Mascarene Islands, Myanmar, Pakistan, Sri Lanka, and Uzbekistan (Thilaga *et al*., 2013).

It is well-suited for growing in wastelands due to its drought resistance and its low susceptibility to pests and diseases. The crop spans over 105,000 hectares in India, with an

annual production of 1,276,000 metric tons (MoA & FW, 2022). Aonla is quite hardy, a prolific bearer, and highly remunerative even without much care under varied agro-climatic conditions. A large number of reports have appeared in the literature using RAPD patterns for differentiating varieties, species, etc. of crop plants.

Aonla tree is a medium-sized, highly branched tree, reaching heights of 10–20 meters. While typically evergreen in tropical regions, it acts as a deciduous tree, shedding its leaves completely. The tree produces new shoots in February or March before the leaves fall. The stem features smooth, exfoliating bark with a greenish-grey to brown hue. The Aonla tree exhibits a phyllanthoid branching pattern with two types of shoots, long (indeterminate) and short (determinate). The indeterminate shoots are longer, continue growing throughout the season, and do not bear flowers. In contrast, the determinate shoots emerge from the nodes of indeterminate shoots, with 3 to 5 appearing at each node in different cultivars. These determinate shoots bear small, closely arranged leaves, appearing similar to a pinnately compound leaf. The flowers are unisexual, with male flowers appearing first in clusters. The inflorescence is racemose, with male flowers positioned lower and female flowers at the upper nodes. The female flowers feature a hypogynous ovary, with 3–4 carpels and axile placentation. The fruit is nearly pedicellate, round, or oblate, with a slight indentation at the base. It is fleshy and smooth, usually with six lobes (Wali *et al*., 2015).

To overcome the limitations of morphological assessments, molecular characterization techniques have been increasingly adopted. These techniques evaluate the genetic makeup of plants using DNA-based markers, which are not influenced by environmental conditions. Among various types of molecular markers, Inter-Simple Sequence Repeat (ISSR) markers have gained popularity due to their simplicity, cost-effectiveness, and ability to detect high levels of polymorphism without prior genomic information.

ISSR markers amplify regions between microsatellites loci using a single primer composed of a repeat motif. This method produces a distinct fingerprint for each genotype, making it highly suitable for assessing genetic diversity, varietal identification, phylogenetic studies, and marker-assisted selection in plant breeding (Aryanegad *et al.,* 2013).

A comprehensive understanding of Aonla diversity requires the integration of both morphological and molecular tools. While morphological characterization helps in identifying traits of agronomic importance, molecular markers offer a reliable assessment of genetic variation at the DNA level. Together, these approaches provide a robust framework for the selection, improvement, and conservation of Aonla germplasm.

The combination of morphological traits and ISSR-based molecular profiling enables researchers to establish relationships among varieties, detect duplicates, and identify unique genotypes with potential for commercial cultivation or use in breeding programs. (Sinha *et al.,* 2020). This research evaluates the yield and quality of various aonla varieties under semi-arid conditions, focusing on cultivars like BSR-1, Krishna (NA-5), Kanchan (NA-4), NA-7, and Chakaiya. The study highlights differences in fruit characteristics and growth habits, aiding in the selection of appropriate cultivars for specific environmental conditions (Vijayalatha *et al.,* 2024)**.**

Morphological characterization refers to the evaluation of visible traits in plants, such as leaf shape and surface, flower morphology, fruit size, tree habit, and bark texture. These traits have traditionally been used to classify, describe, and distinguish plant varieties. In *Aonla,* morphological parameters are crucial in identifying genotypes with superior agronomic traits such as higher fruit yield, better quality, pest and disease resistance, and tolerance to abiotic stresses. However, morphological traits can be influenced by environmental factors, which may result in phenotypic plasticity. Therefore, although morphological evaluation is valuable for preliminary characterization and field-level selection, it may not always accurately reflect genetic differences (Godwin *et al.,* 1997).

In recent decades, plant molecular biology techniques such as RAPD, ISSR, SSR, CAPS, AFLP, and others have been extensively applied for molecular mapping, identifying genotypes of interest, and studying genetic diversity. Improved CTAB DNA extraction methods are particularly effective for PCR analysis, as they are simple, rapid, and efficient for handling large numbers of samples. Molecular Characterization: The analysis of genetic diversity and relationships within and between species, populations, and individuals is a key area of research in biological sciences. Over the last few decades, molecular techniques have supplemented

classical approaches, such as morphological and cytological studies, for plant classification and genetic evaluation. DNA-based molecular markers have revolutionized research in various fields, including taxonomy, ecology, genetics, and plant breeding. DNA fingerprinting, introduced by Alec Jeffrey's in 1985, allows for the simultaneous detection of multiple highly variable DNA loci. This technique has become invaluable for genotype identification, population genetics, and marker-assisted selection.

2.1 Objectives of this studies are:-

1. To assess Aonla varieties for morphological characteristics, ICAR-IIHR and ANDUAT, Ayodhya.

2. To generate DNA profiles of Aonla varieties using polymorphic ISSR/SRAP markers

3. To assess genetic relationships of varieties using morphological and molecular markers

Review of Literature

2.1 Tree Characters

Singh *et al.* (2006) conducted at the Experimental Farm of the Central Horticultural Experiment Station (CIAH) in Vejalpur, Panchmahals, Gujarat, during 2004 and 2006.the highest growth in terms of plant height and stem diameter, while NB-7 had the largest plant spread. Significant differences were observed among the genotypes for most growth traits.

Kumar *et al.* (2012) experiment was conducted at KVK, Panchmahal, Vejalpur (Godhra), Gujarat, to assess the vegetative and fruit traits of Aonla (*Emblica officinalis* Garten) varieties under rainfed hot semi-arid conditions. Varieties exhibited significant diversity in growth habit (tall drooping to upright spreading), plant height (3.64–5.69 m), trunk colour (grey to whitish green), and leaf characteristics (shape, apex, and size). Leaf dimensions ranged from 1.28 × 0.24 cm to 4.80 × 0.33 cm. Marked variation was observed in fruit setting and maturity times. NA-7 showed the highest fruit setting (51.95%) and retention (26.40%), while Francis had the lowest (36.56% and 11.43%). Fruit size ranged from small to large; NA-7 had the highest fruit weight, while Anand-2 recorded the lowest.

Singh *et al.,* (2016) evaluated eight *Aonla* cultivars were studied for various morphological traits, including fruit and tree characteristics, leaf shape, fruitlet shape, and flower count. The dendrogram analysis revealed that Francis was the most distinct cultivar. Chakaiya and NA-6 shared a similarity coefficient of 59, indicating some similarity. Banarasi and NA-10 were grouped together, showing similar morphological characteristics.

Kumar *et al.* (2021) studied the variation in growth, physicochemical characteristics, and yield among different Aonla cultivars. They observed plant shapes such as spreading (CHES 1, Chakaiya, Krishna, NA 6, NA 7, NA 10, BSR 1), drooping (NA 20), and upright (G1). Significant variability was noted in parameters like plant height (4.90-6.70 m), stem girth (46.45-95.37 cm), fruit weight (5.89-55.43 g), yield (36-102

kg/plant), and ascorbic acid (323-567 mg/100 g pulp). Fruit shapes varied from flattened round to triangular and oval, with differences in cavity and apex shape.

Akhtar *et al.* (2024) studied focusing on Assam lemon, a unique Citrus cultivar valued for its aroma and flavor, was studied for morphological, seeding pattern, and biochemical diversity across 132 populations from 22 districts of Assam. UPGMA clustering revealed two major groups, with Upper Assam populations closely related to the control. Tinsukia and Dhemaji showed strong similarity to the original stock. Bisexual and unisexual flowers were found, with unisexual having 40 anthers and bisexual 36. Seedlessness was noted in Tinsukia, Dhemaji, Lakhimpur, and nearby areas, while Golaghat served as a transition zone. Biochemical and soil nutrient variations were significant, supporting potential for breeding seedless, high-quality fruits.

Saroj & Kumar. (2024) The institute has developed several varieties of fruits and vegetables for commercial cultivation, promoted fruit-based cropping systems to reduce risks, and established techniques for vegetative propagation, canopy management, and integrated nutrient, water, and pest management. These efforts have improved productivity and sustainability in arid horticulture, with promising prospects amid climate change and growing demand for organic produce.

2.2 Leaf Characters

Chopra *et al.* (2010) studied the foliage of *Emblica officinalis* is dense, providing essential photosynthetic activity, which is vital for the plant's overall growth. The leaves are arranged oppositely, simple, and elliptical, typically measuring 3-8 cm in length. Foliage plays a key role in the plant's health and growth rate. Dense leaves are advantageous in hot climates as they help protect the plant from extreme conditions. The nature of the foliage is genetically controlled, and variations in leaf size and structure are observed among different cultivars. These traits can be targeted for selection in breeding programs.

Kumar *et al.* (2012) Evaluated leaf characters among the evaluated Aonla (*Emblica officinalis* Garten) varieties under rainfed hot semi-arid conditions at KVK, Panchmahal, and Gujarat. The leaf shape varied from oval oblong, oblong to elliptical,

while the apex ranged from obtuse to acute. Leaf size also exhibited wide diversity, with length and width ranging between 1.28 cm × 0.24 cm and 4.8 cm × 0.33 cm, respectively. Such morphological variability in leaf traits may serve as useful descriptors for varietal identification and selection in Aonla breeding programs.

. Kumar et al. (2012) conducted an experiment at KVK, Panchmahal, Vejalpur (Godhra), Gujarat, to evaluate vegetative and fruit traits of *Emblica officinalis* varieties under rainfed hot semi-arid conditions. Significant variability was reported in leaf characteristics such as shape, apex, and size among the varieties. The leaf dimensions ranged from 1.28 × 0.24 cm to 4.80 × 0.33 cm, reflecting genotypic differences

Singh *et al.* (2015) studied the bael (*Aegle marmelos* Correa) Bael shows considerable phenotypic variation due to cross-pollination and different agroclimatic conditions across the country. However, farmers face challenges in identifying different cultivars and are often unfamiliar with their distinct characteristics. This highlights the need for morphological characterization, excluding fruit traits, to distinguish between bael varieties. Leaf morphology has been extensively used for identifying plant varieties, and this study aims to explore the diversity of bael varieties in rainfed, semi-arid regions of western India. This research will help in the effective use and improvement of bael genetic resources.

Pandey *et al.* (2020) evaluated nine cultivars of bael (*Aegle marmalos* Correa) were evaluated under subtropical conditions at the Experimental Research Farm of ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow, in a Randomized Block Design over two consecutive years (2016 and 2017). The maximum terminal leaf length (13.50 cm) and breadth (8.40 cm) were observed in cultivar CISH-B-2, with lateral leaf length (10.38 cm) also recorded in CISH-B-2, and lateral leaf breadth (6.54 cm) in NB-5.

Z and Jiang L *et al.* (2020) studied the shape and structure of a leaf's apex help in quickly draining rainwater, protecting the leaf from potential rain damage. As the apex changes from rounded to pointed and the leaf surface curves, water drains faster with less retention. In tropical plants like *Alocasia macrorrhiza*, the unique curvature at the tip turns capillary forces into drivers of water flow, improving drainage. This mechanism differs

from traditional water control methods and offers a precise way to manage liquid movement using specific shape parameters.

Kumar and Tripathi. (2024) experiment was conducted in the eastern region of Uttar Pradesh harbors rich but underutilized biodiversity in *Ber* (*Ziziphus mauritiana* Lamk.), with significant potential for commercial cultivation due to favorable agroclimatic conditions. The forty genotypes were collected and evaluated for morphological and quantitative traits, revealing wide phenotypic variation. Notable differences were observed in leaf shape (cordate, oval, obovate, and elliptic) and stone shape (obtuse, acute, oblong, round). Quantitative traits like stone weight (0.56–1.58 g), stem girth (25.90–62.63 cm), and tree height (3.43–5.50 m) also varied significantly. These variations present valuable targets for breeding programs aimed at developing improved *Ber* cultivars.

2.3 Flower Characters

Singh *et al.* (2006). Conducted an experiment on Experimental Farm of the Central Horticultural Experiment Station (CIAH) in Vejalpur, Panchmahals, Gujarat, during 2004 and 2006. They evaluated the growth and flowering behavior of bael genotypes, including CISH Bael-1, CISH Bael-2, NB-5, NB-7, NB-9, Pant Aparna, Pant Sujata, Pant Urvashi, Pant Shivani, Dhara Road, and PB-1 under rainfed conditions.The results showed that genotype CISH Bael-1 had Flower bud emergence started on April 30 and continued until June 23, with flowering beginning on May 15 and completing by June 24. Pollen dehiscence occurred just before the flowers opened, with anthers exposed on the interior petals. Pollen viability was above 95% in nearly all genotypes. Bud length varied from 10.00 to 43.00 mm, while flower length ranged from 12.00 to 19.00 mm, and flower width ranged from 25.00 to 35.00 mm across the evaluated genotypes.

Aulakh *et al.* (2013) evaluated six aonla cultivars (Chakaiya, Kanchan, Krishna, Amrit, Neelam, and Balwant) for varietal suitability. Chakaiya and Krishna flowered early, while Kanchan and Neelam were late bloomers. Neelam, Amrit, and Balwant had the highest number of flowers. Neelam recorded the largest pollen size and good germination, while Amrit had the highest pollen viability. Kanchan and Amrit matured early while Chakaiya matured late. Amrit and Krishna had heavier, larger fruits with higher yields.

Singh *et al.* (2015) evaluated bael (*Aegle marmelos* Correa) rich in riboflavin and has potential as an export commodity because all parts of the tree root, bark, leaf, flower, and fruit are used in traditional formulations. Bael shows considerable phenotypic variation due to cross-pollination and different agroclimatic conditions across the country. However, farmers face challenges in identifying different cultivars and are often unfamiliar with their distinct characteristics.

Pandey *et al.* (2020) evaluated nine cultivars of bael (*Aegle marmalos* Correa) were evaluated under subtropical conditions at the Experimental Research Farm of ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow,The flowering period began on May 15 and continued until June 29, with peak flowering occurring in the second and third weeks of June.

Meena *et al.* (2011) evaluated total of 24 diverse pomegranate genotypes, including both indigenous and exotic types, were evaluated for fruit yield and related traits under Delhi conditions during the ambe bahar season over two consecutive years. Significant variability was observed among genotypes in terms of fruit yield. 'Kandhari' recorded the highest fruit yield (11.60 kg/plant), followed by other promising genotypes, while 'Speen Danedar' showed the lowest yield (4.75 kg/plant). The genotypes showed considerable differences in plant habit and vigor, which indirectly influenced yield potential. The variation in fruit yield among genotypes reflects underlying genetic diversity and adaptation potential, offering scope for selection and improvement in breeding programs targeting high-yielding cultivars suitable for north Indian agroclimatic conditions.

2.4 Fruit Characters

Goyal *et al.* (2007) studied the physical and mechanical properties of three Aonla cultivars-Krishna, NA-7, and Chakaiya-were studied. Traits like size, sphericity, surface area, mass, and true density were measured, along with the effect of boiling on surface hardness, pulp firmness, and toughness. NA-7 fruits had lower size and surface area but higher mass than Krishna and Chakaiya. Chakaiya showed the highest rolling resistance vertically, while all cultivars had rolling resistance between 12°–28° radially. NA-7 and Chakaiya had larger seed size and surface area, but Krishna had higher seed sphericity.

Boiling reduced the firmness and toughness of all cultivars, indicating structural differences among them.

Meena *et al.* (2011) studied the taste of Aonla fruit is sour and slightly astringent due to its high vitamin C content. This taste profile makes it suitable for medicinal use and for processing into various products such as jams, juices, and candies. Highlighted that the fruit's high acidity contributes to its therapeutic properties, making it popular in traditional medicine. While the taste may not appeal to everyone in its raw form, its medicinal and culinary uses are highly valued. Breeding programs aim to select cultivars with less intense sourness to improve palatability for fresh consumption.

Singh *et al.* (2016) studied the variability in physical and biochemical traits among 39 Aonla genotypes from different regions of northeastern India (Manipur, Meghalaya, Assam, and Nagaland) during 2014-15. They observed wide variability in traits such as fruit weight (1.39-10.59 g), fruit length (1.26-2.53 cm), fruit girth (4.16-8.10 cm), and stone weight (0.28-1.50 g). The genotype T26 was found to be superior for fruit weight, girth, length, and breadth, while T12, T14, T4, and T36 showed superior qualitative traits.

Yadav *et al.,* (2017) evaluated surface of the Aonla fruit is smooth but with a slight roughness that can be felt when touched. Concluded that the texture aids in reducing water loss and preventing microbial invasion. The surface also features small lenticels, which are involved in gas exchange, contributing to the fruit's respiration. Noted that the smoothness of the fruit surface is one of the key characteristics for processing, as it impacts both the texture and shelf life of processed products. The surface texture can vary slightly between cultivars, with some having a more pronounced roughness.

Chiranjeevi *et al.* (2018) conducted an experiment in farm of ICAR-NBPGR, New Delhi, aimed to identify promising ber (*Ziziphus mauritiana*) genotypes with minimal stone percentage. Twelve genotypes were evaluated during 2017–18 and 2018–19 for key horticultural traits. Significant variability was observed in fruit maturity, shape, color, and stone size. 'Gola' was the earliest maturing genotype, while 'Umran' was the latest. 'Umran' also exhibited the highest fruit (35.40 g) and pulp weight (28.08 g), whereas 'Aliganj Selection' recorded the lowest fruit weight (17.4 g). The genotype IC-0625596 showed the least stone weight (1.70 g), the highest TSS (17° Brix), and the most desirable

pulp-to-stone ratio (21.15), making it a potential candidate for future breeding programs focused on improving fruit quality and reducing inedible stone content.

Fallik. and Ilic. (2018) studied on postharvest losses from national and international organizations highlight issues with the production systems of horticultural commodities. The lack of knowledge about maturity, proper harvesting, and postharvest management is a major factor contributing to these losses, especially in developing countries. Horticulturists are increasingly aware of the importance of preserving the postharvest quality of produce. Proper postharvest management begins from harvesting and continues until the product reaches the consumer. Judging the right maturity stage for harvesting is crucial and varies by crop. This process is irreversible and directly impacts both internal and external quality. Understanding harvesting methods, timing, and their effects on the commodity's physiological processes helps horticulturists select the best practices for harvesting and postharvest management. This chapter explores the importance of maturity assessment, postharvest treatment, and maintaining quality standards for the success of horticultural crops.

Singh *et al.* (2018) evaluated bael (*Aegle marmelos* Correa), bael trees have been observed to bear fruits on woody stems aged from one to eight years, confirming their cauliflorous and ramiflorous nature, especially common in varieties like Goma Yashi and NB-16. Vivipary, though rare in bael, leads to poor-tasting fruits with visible germinated seeds inside, often linked to high humidity and warm weather. Pollen grains can influence fruit traits such as size, shape, development speed, and ripening time, likely due to metaxenia. Additionally, variation is seen in the number of petals, sepals, leaflets, and thorns among genotypes.

Singh *et al.* (2020) conducted a study on six aonla cultivars-Krishna, Kanchan, Neelum, Chakaiya, Balwant, and desi-at different stages of fruit growth.Their study reported that fruit size, weight, and stone weight showed exponential growth up to harvest. Among the cultivars, Kanchan had the largest fruits, while desi had the smallest. Based on physico-chemical parameters, the varieties were categorized into early, mid, and late-season groups.

Pandey *et al.* (2020) evaluated nine cultivars of bael (*Aegle marmalos* Correa) Fruit characteristics varied widely, with fruit weight ranging from 0.746 kg to 1.80 kg, fruit

circumference from 37.5 to 50.01 cm, and fruit length from 9.10 to 17.20 cm.. Pulp percentage varied between 57.63% and 82.39%. They demonstrated significant variability among bael cultivars, which can be used for improving production and productivity.

Jahed & Hirst. (2023) studied fruit growth and development are controlled by various internal and external factors. These processes unfold in a sequence over the growing season. To fully understand fruit development, it's essential to consider earlier events like flowering, pollination, fertilization, and fruit set, as these processes are interlinked. Recent advances in high-throughput sequencing, combined with improved statistical methods, have allowed scientists to identify key molecular components involved in regulating fruit growth. These developments have linked genotypic differences with phenotypic traits. Various techniques, including transcriptomic analysis, QTL mapping, whole-genome approaches, and epigenetics, have been used to explore the genetic basis of these processes. This review provides an in-depth look at the molecular, genomic, and epigenetic factors involved in apple fruit growth and development, which ultimately determine the fruit size. It also offers a brief overview of the events leading up to fruit growth.

Pandey *et al.* (2024) explored the performance of *Emblica officinalis* Gaertn. (Aonla) genotypes under alkaline soil conditions to identify accessions suitable for climate-resilient cultivation. Among the 11 genotypes evaluated**,** CISH-A-33 and CISH-A-31 exhibited significantly higher fruit yield (53.99 ± 1.97 kg/tree and 44.17 ± 0.91 kg/tree, respectively), indicating better adaptability. Morphologically, fruit weight showed a strong positive correlation with fruit diameter and overall yield, suggesting that larger fruits contribute directly to productivity. Principal component analysis (PCA) revealed two major components explaining a co mbined variability of 95.56%, highlighting the diversity among genotypes in terms of fruit yield and size. These findings confirm the potential of CISH-A-33 and CISH-A-31 as promising genotypes for breeding programs aimed at enhancing fruit yield under alkaline and stress-prone soils.

Sachan and Kumar. (2025) studied the fruit colour of *Emblica officinalis* (amla) changes significantly during its growth and maturation, serving as an indicator of harvest maturity. In a study on the ‘Chakaiya’ variety, fruits harvested between 135 to 270 days after fruit set (DAFS) showed a progressive increase in yellowness index, indicating a shift

in fruit skin color with ripening. These changes correlated with enzymatic and chemical transformations, such as increased ascorbic acid and decreased phenolic content. The optimal fruit color and maturity for harvesting were recorded around 210 DAFS, aligning with peak nutritional and physical quality.

Singh *et al.* (2025) assessed the genetic diversity of 80 wild bael genotypes and two commercial cultivars (NB-5 and NB-9) using 16 pomological traits. Due to rising temperatures, bael is promising for rainfed areas because of its drought tolerance. Among the wild types, JMU-Bael (Sel-27) showed superior fruit traits like highest length, width, weight, and pulp content. Strong correlations were found among traits, especially with fruit weight. PCA revealed high genetic variability, with two main components explaining 63.98% of variation. Cluster analysis grouped genotypes into two major clusters, highlighting their potential for future breeding and climate adaptability.

2.5 Seed Characters

Pandey *et al.* (2020) evaluated nine cultivars of bael (*Aegle marmalos* Correa) Seed percentage ranged from 0.36% to 1.08%, and shell weight varied from 0.150 g to 0.548 g. Shell percentage ranged from 14.46% to 21.54%, and shell thickness ranged from 1.6 mm to 3.4 mm.

Singh *et al.* (2015) experiments was conducted under rainfed hot semi-arid conditions of western India evaluated *Emblica officinalis* (Aonla) varieties for vegetative and fruit traits. Notable variation was found in fruit stones (seeds), which differed in shape (triangular, oval-round) and size (large, medium, small) among cultivars. Stone weight ranged from 1.97 to 2.08 g, indicating significant genetic variability in seed traits under the given agro-climatic conditions.

Singh *et al.* (2016) studied the variability in physical and biochemical traits among 39 Aonla genotypes from different regions of northeastern India (Manipur, Meghalaya, Assam, and Nagaland) during 2014-15. They observed wide variability in traits stone weight (0.28-1.50 g, while T12, T14, T4, and T36 showed superior qualitative traits.

2.6 Yields Characters

Kumar *et al.* (2015) studied the comparative performance of Aonla (*Emblica officinalis)* cultivars under Parbhani conditions. They found that Kanchan produced the highest yield, with 3459 fruits per tree and 99.79 kg of fruit per tree. NA-7 had the largest fruits, while Krishna produced smaller fruits. The heaviest fruit was recorded in NA-7 (42.44 g). Based on their findings, Kanchan was considered the superior variety for the Parbhani agro-climatic conditions due to its high yield and good-quality fruit.

Prasanna *et al.* (2023) significant variation was observed for fruit, yield, and biochemical traits. The highest fruit diameter (54.87 mm) and length (54.95 mm) were recorded in TAL/94 the maximum number of segments per fruit (12.33) was noted in BKS-4, while TAL/94-14. The highest fruit yield per tree (232.72 kg) was observed in Petlur Pulusu nimma. TAL/94-14 also had the highest rind thickness (1.66 mm).

Panday *et al.* (2025) examined the potential of *Emblica officinalis* (aonla) cultivation in various soil types, focusing on alkaline soils, to improve biodiversity, sustainable agriculture, and climate resilience. Among 11 Aonla genotypes tested, CISH-A-33 and CISH-A-31 showed the highest fruit yields (53.99 ± 1.97 kg/tree and 44.17 ± 0.91 kg/tree, respectively), indicating better adaptability to alkaline conditions. CISH-A-33 also exhibited higher levels of beneficial biochemical traits such as total soluble solids (9.70 ± 0.10°B), The promising genotypes CISH-A-33 and CISH-A-31 are suitable for enhancing aonla cultivation in alkaline soils, offering potential for breeding resilient varieties with higher productivity and fruit quality.

2.7 Molecular Work

Andersen and Fairbanks. (1990) examined the use of molecular markers like RFLP and RAPD for plant genetic resource characterization, especially in developing core collections and monitoring genetic changes during conservation. RAPD markers, which involve the polymerase chain reaction amplification of random DNA segments, map to the same locations as RFLPs. Unlike RFLPs, RAPDs are species-independent, offering a versatile tool for genetic analysis across different plant species.

Badenes and Parfitt. (1995) used chloroplast DNA (cpDNA) restriction-site mutations to study the phylogenetic relationships among seven cultivated Prunus species. The analysis revealed polymorphisms in amplified regions of cpDNA, which were analyzed using parsimony and cluster methods. The species pairs P. persica-P. dulcis, P. domestica-P. Salicina, and P. cerasus-P. fruticosa formed monophyletic groups, with the subgenus Cerasus being the most diverged. There suggested a higher mutation rate in Cerasus chloroplast genomes compared to other subgenera.

Ben-Meir. (1996) assessed the use of DNA fingerprinting with mini- and microsatellite sequences for identifying genotypes and determining genetic distances in carnation and rose. Their study reported very low probabilities (1.8x10^-6 for carnation and 2x10^-8 for rose) of offspring from similar genotypes sharing identical DNA fingerprints. DFPs provided a perfect match with genetic relationships based on known histories. The study also identified a RAPD marker linked to the "non-bullheaded" flower trait in carnation, offering future applications for breeding and trait selection.

Ballard *et al.* (1996) identified DNA markers for rose cultivar identification using RFLP and RAPD patterns. Their results demonstrated the effectiveness of both methods for cultivar identification and patent protection. Additionally, a genetic mapping program was initiated to find molecular markers linked to blackspot resistance. Preliminary RAPD analysis showed significant genetic polymorphism between resistant amphidiploids and susceptible tetraploid rose cultivars, supporting the creation of genetic maps for resistance traits.

Ament *et al.* (2000) used DNA amplification fingerprinting (DAF) to identify the pollen donor of open-pollinated seedlings from a *Cornus florida* 'Cherokee Chief' tree. The analysis included potential pollen donors such as 'Cherokee Brave', 'Cherokee Daybreak', and others. Thirteen out of 15 seedlings (87%) were confirmed to be progeny of 'Cherokee Brave' and 'Cherokee Chief'. The study demonstrated the effectiveness of DAF in verifying parentage in open-pollinated *C. florida* cultivars.

Anand *et al.* (2000) discussed the significance of cultivar identification and relatedness for horticultural breeders. Accurate characterization is crucial for understanding genetic diversity, phylogenetic relationships, and ensuring plant variety

protection. A variety of methods, including cytology, morphology, physiology, phenolic compounds, and DNA markers, are used for plant characterization, each with its strengths and limitations. The study emphasizes the need to tailor characterization programs based on specific requirements and highlights the application of these techniques in ornamental crops.

Kuma *et al.* (2001) this case study involved the identification of spurious chilli seeds marketed under an elite variety's brand name, referred to a court in India for investigation. To differentiate the four disputed samples, highly reproducible molecular marker assays, ISSR-PCR and FISSR-PCR, were used. A total of 17 ISSR anchored primers, including nine di-nucleotide and eight tri-nucleotide primers, were employed, yielding 212 bands with di-nucleotides and 288 bands with tri-nucleotides. Five di-nucleotide and four tri-nucleotide primers successfully distinguished all four disputed samples. The sensitivity of the assay was enhanced by FISSR-PCR, which produced 566 bands using a subset of primers, reliably differentiating the samples. They highlights the importance of modern DNA technologies in protecting Plant Breeder's Rights and preventing seed fraud in developing countries like India.

Bakker *et al.* (2001) investigated the genetic variation in autochthonous populations of *Quercus robur* (pedunculate oak) and *Q. petraea* (sessile oak) in ancient, coppiced, and grazed woodlands. Using microsatellite and AFLP™ analysis, 14 unique genotypes were identified from 80 trees. Clonal structures were observed in both species, with the largest clone diameters (up to 5.8 m) found in *Q. robur*. The clone sizes may reflect the age of the trees, providing insights into the genetic dynamics of these populations.

Abdel-Sattar *et al.* (2024) *conducted a study on Indian jujube (Ziziphus mauritiana) cultivars, aiming* to evaluate both morphometric characteristics and genetic diversity using molecular markers such as matK barcoding and ISSR. The research found considerable variation in the fruit’s morphometric traits, including geometric diameter, surface area, sphericity, shape index, fruit length, fruit diameter, fruit weight, and seed weight, across the eleven cultivars. Additionally, color values also varied among cultivars, highlighting the morphological differences. Molecular analysis using matK barcoding and ISSR

markers revealed discrepancies in genetic diversity among the cultivars, with distinct clusters identified. A hierarchical clustering heatmap indicated that cultivars like ‘Zytoni’ and ‘Um-Sulaem’, characterized by their spiny fruits, were grouped as monoclades, separate from other cultivars. These differences were linked to gene expression variations. The findings suggest that both morphometric and molecular analyses, particularly matK barcoding and ISSR markers, should be used together for accurate cultivar identification. This approach would enhance the effectiveness of local germplasm conservation and help in the better exploitation of Indian jujube cultivars.

Pharmawati *et al.* (2005*) evaluated genetic variation and relationships among 30 Leucadendron cultivars using ISSR (Inter-Simple Sequence Repeat) markers in this study*. A total of 64 ISSR primers were screened, and 25 were selected based on their ability to produce clear and reproducible banding patterns. This resulted in the amplification of 584 bands, ranging from 305 to 2400 bp, with 97% polymorphism. The Unweighted Pair Group Method with Arithmetic Average (UPGMA) dendrogram revealed that the cultivars clustered into two main groups. Twenty-four out of the 30 cultivars were easily differentiated, while three pairs-Katie's Blush' and 'Silvan Red', 'Highlights' and 'Maui Sunset', and Yellow Crest and Yellow Devil-had identical profiles. The study highlighted that ISSR profiling is an effective method for identifying and classifying *Leucadendron* cultivars, and a fingerprinting key was created using two ISSR primers (UBC856 and UBC857). Additionally, cultivar-specific ISSR bands were identified for 17 of the 30 cultivars. This work provides a foundation for better understanding the genetic relationships and diversity within *Leucadendron* cultivars.

Sangeeta *et al.* (2005) the genetic diversity of ten commercially significant Indian papaya (*Carica papaya*) cultivars was assessed using three Single-Primer Amplification Reaction (SPAR) techniques: RAPD, ISSR, and DAMD. A total of 134, 74, and 21 DNA fragments were generated by 18 RAPD, 7 ISSR, and 2 DAMD primers, respectively, with polymorphism levels of approximately 51%, 61%, and 57%. Each method produced unique clustering patterns in Neighbor Joining (NJ) dendrograms in papaya. But the combined analysis across all methods aligned cultivar grouping with traits like fruit pulp color and plant height. Among the three methods, ISSR-PCR proved most effective, exhibiting the highest polymorphism (61%) and thus offering superior resolution for genetic diversity analysis

Wang *et al.* (2009) investigated the genetic diversity and phylogenetic relationships among 31 *Dendrobium* species from the Yunnan region of China using ISSR (Inter-Simple Sequence Repeat) markers. A total of 2368 bands were amplified by 17 ISSR primers, producing 278 loci with 100% polymorphism at the genus level. The species were distinctly identified using ISSR fingerprinting, with species-specific markers found in nine of the 31 species. The Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) analysis grouped the species into six clusters, indicating a polyphyletic nature of the genus with several well-supported lineages. The high polymorphism and consistent amplification across species underscore the effectiveness of ISSR markers for species diagnosis and studying the genetic diversity of *Dendrobium*.

Wang *et al.* (2009) studied genetic diversity in *Cymbidium goeringii* cultivars was evaluated using the Inter-Simple Sequence Repeat (ISSR) technique. A total of 25 ISSR primers were selected, resulting in 224 ISSR loci. The analysis revealed significant genetic variation across 50 tested cultivars, with a Nei's gene diversity of 0.2241 and 93.75% polymorphic loci. ISSR fingerprinting was able to unequivocally distinguish all cultivars, and cultivar-specific markers were identified in seven of the 50 cultivars. The Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) and Principal Coordinates Analysis (PCA) grouped the cultivars into two main clusters: one composed of cultivars from Japan, and the other containing three subclusters of cultivars mainly from China. Two of the Chinese subclusters were consistent with horticultural classification, while the third included cultivars from various groups. The findings suggest that ISSR markers are an effective tool for cultivar identification and understanding the genetic relationships within *C. goeringii* cultivars.

Chaurasiya *et al.* (2009) used RAPD markers to assess genetic variability among commercially cultivated Aonla (Indian Gooseberry) varieties. Four decamer primers successfully distinguished these varieties, which was difficult using morphological markers. Cluster analysis identified three distinct groups based on their region of origin. RAPD markers also differentiated varieties of the same origin or from the same parents, providing valuable information for variety identification and crop improvement programs.

Nagarajan *et al.* (2011) studied modified Cetyltrimethylammonium bromide (CTAB) protocol for DNA isolation was developed from leaf tissues of Phyllanthus emblica for obtaining high-quality genomic DNA. Fresh leaves of three different maturity stages were analyzed for yield and quality of DNA. Acidity was determined in three different maturity stages of leaves, viz. tender, intermediate, and mature, and their influence on DNA quality was determined. A drastic reduction of pH was the primary cause of poor quality of DNA. However, high-quality DNA isolation was achieved by stabilizing the pH with the addition of NaOH during different stages of the DNA isolation process. The present protocol yields high-quality intact DNA for genetic fingerprinting as well as for amplification of chloroplast genes for molecular analysis

Elmeer, K. and Almalki. (2011) The study explored the genetic diversity within and between populations of *Prosopis cineraria* and *Prosopis juliflora* collected from various locations in Qatar using ISSR and RAPD markers. A total of 109 bands were generated using 29 ISSR primers, and 19 bands from 7 RAPD primers, showing more than 99% polymorphism across all genotypes. ISSR markers were more effective in distinguishing between the two species, with 21 specific bands. In contrast, only three RAPD bands differentiated the species. The dendrograms generated from the genetic similarity analysis grouped the individuals into two highly related clusters, indicating clear genetic variation between the species. This study highlights the effectiveness of ISSR and RAPD markers in identifying genetic diversity among *Prosopis* accessions.

Bhat *et al.* (2010) studied the markers have been essential tools in plant classification, helping to easily and reliably identify traits of an organism. These traits, which can be traced through a mapping population, are linked to economically significant characteristics under polygenic control. Markers are classified into morphological markers (naked eye polymorphisms) and molecular markers. Morphological markers are visible traits such as plant height, disease resistance, or the shape and color of flowers, fruits, or seeds. Molecular markers, on the other hand, are based on biochemical constituents and are less influenced by environmental factors. In fruit crops, the limitations of morphological markers include long generation times and the large size of the plants, making molecular markers a more suitable option. Molecular markers are widely used in fruit crop improvement, aiding in genetic diversity studies, varietal identification, gene tagging,

disease diagnostics, pedigree analysis, hybrid detection, sex differentiation, and marker-assisted selection.

Kebour *et al.* (2012) assessed the genetic relationships among 10 *Pistacia vera* varieties from Algerian semi-arid regions were assessed using six ISSR primers. Good amplification was achieved with primers based on guanine-adenine (GA), cytosine-adenine (CA), and GAA repeats. However, primers based on cytosine-tyrosine (CT) and CAA repeats produced fewer significant bands and were excluded from the final analysis. A total of 111 bands were amplified, of which 60 (54.04%) were polymorphic, with an average of seven bands per primer. The number of amplified fragments ranged from five to ten, and the polymorphic fragments varied from four to seven. Genetic similarity ranged from 0.84 to 1, and the UPGMA dendrogram classified the genotypes into two main clusters. The study found low genetic diversity among the tested cultivars and confirmed that ISSR-PCR is a rapid, reliable method for large-scale DNA fingerprinting. This is the first application of the ISSR technique in characterizing Algerian pistachio cultivars of Syrian origin.

Singh *et al.* (2014) studied the genetic relationships among popular Aonla cultivars developed by N.D.U.A. & T., Faizabad, and those existing in the region using RAPD markers. DNA was extracted from young leaves of ten cultivars, and six RAPD primers (OPA-7, OPA-8, OPZ-4, OPZ-14, OPY-1, OPY-2) were used for analysis. OPZ-14 provided the most distinct banding pattern for cv. Banarasi. The dendrogram revealed four major groups, with sub-groups IA (NA-04 and NA-10) and IB (cv. Francis), IIA (NA-5, NA-6, NA-9), IIB (NA-7 and Chakaiya), and III & IV representing Banarasi and Anand, respectively.

Singh *et al.* (2014) studied eight varieties of *P. emblica* (Anand-2, Banarasi, Chakaiya, Francis, Krishna, Kanchan, NA7, and NA10). Morphological and physiological characteristics were insufficient to differentiate the varieties. Ten RAPD primers revealed an average polymorphism of 56.18% and 83.1% PIC, demonstrating their potential as powerful markers for genetic diversity. They highlighted the utility of the ITS rDNA region as a reliable phylogenetic marker and DNA barcode for the genetic cataloging and improvement of *P. emblica*.

Singh *et al.* (2015) studied the Indian gooseberry, *Phyllanthus emblica* is an important minor fruit crop having commercial significance. Due to great tolerance to salinity and sodicity it is well adapted to arid conditions of Rajasthan. Genetic diversity studies of eight varieties, Anand-2, Banarasi, Chakaiya, Francis, Krishna, Kanchan, NA7, and NA10, were carried out. The morpho-physiological characterization proved insufficient to distinguish the studied varieties of *P. emblica*. Ten RAPD primers exhibited an average of 56.18 % polymorphism in banding pattern and 83.1 % PIC. They can be used as powerful markers to reveal genetic diversity in *P. emblica.* The present study validates the utility of the ITS rDNA region being third third-generation molecular marker as a reliable indicator of phylogenetic interrelationships, especially for ITS regions as DNA barcode at higher levels can serve as an additional approach for identification and genetic cataloguing of *P. emblica* germplasm for crop improvement.

Ravishankar *et al.,* (2017) in this study, diversity was analyzed using ISSR markers in 18 AAB genomic group banana and plantain cultivars, along with two AA types and two BB wild accessions for comparison. The results revealed that AAB cultivars formed a distinct group. Dendrogram analysis showed that the subgroups 'Plantain,' 'Silk,' and 'Mysore' were positioned between the AA and BB types. Interestingly, the ten cultivars from the 'Pome' subgroup were placed in a separate cluster, indicating their uniqueness. This study successfully identified the subgroups within the AAB group and clarified their genetic relationships, with cultivars such as Rasthali and Nendran being distinctly separated. The Pome subgroup cultivars appeared to be grouped based on their geographical origin.

Pathirana *et al.* (2018) experiment was conducted for DNA extraction in tropical tree species rich in phenolic compounds is often hindered by low yield and the presence of PCR inhibitors. To overcome this, a study was conducted using three fruit crops-Bael (*Aegle marmelos*)**,** Pomegranate (*Punica granatum***)**, and Mango (*Mangifera indica*)-representing varied secondary metabolic profiles. A modified CTAB method and two commercial kits (Promega Wizard® and QIAGEN DNeasy®) were compared for DNA quality and quantity. The modified CTAB method consistently yielded superior DNA. Furthermore**,** addition of spermidine (0.8 μM) in PCR reactions significantly improved amplification using rbcL**,** SSR, and ISSR primers. This protocol provides a cost-effective and reliable method for molecular work in phenol-rich tropical plants.

Maeda *et al.* (2018) experiment conducted to develop a reliable method for quantitatively analyzing persimmon (*Diospyros kaki*) fruit shapes and to understand their development patterns across cultivars. The analysis was conducted using 153 cultivars and two wild *Diospyros* relatives, evaluating two-dimensional pictures of fruit sections with elliptical Fourier descriptors (EFDs) and the SHAPE program. Principal component analysis (PCA) of the EFDs identified two main components of shape variability: the length-to-diameter ratio and the shape of the fruit apex. Seasonal analysis of the first principal component highlighted the coordinated development of fruit shapes in the early stages. Additionally, correlations between the shapes of persimmon fruit, seeds, and leaves were found. The study concludes that combining EFDs and PCA provides a robust method for analyzing persimmon organ shapes, offering insights into the quantitative relationships among these traits.

Rathore *et al.* (2018) ISSRs have provided valuable insights into the molecular characteristics of *Emblica officinalis*, assisting in the identification of superior genotypes with enhanced traits. The use of these markers is beneficial for improving fruit quality, disease resistance, and overall yield, which are essential for successful Aonla cultivation (Patel et al., 2017). Molecular characterization through ISSRs enables better management and breeding of Aonla for increased productivity.

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Rathore *et al.* (2018) studied ISSRs have provided valuable insights into the molecular characteristics of *Emblica officinalis*, assisting in the identification of superior genotypes with enhanced traits. The use of these markers is beneficial for improving fruit quality, disease resistance, and overall yield, which are essential for successful Aonla cultivation (Patel et al., 2017). Molecular characterization through ISSRs enables better management and breeding of Aonla for increased productivity.

Sharifi *et al.* (2018) the date palm (*Phoenix dactylifera* L.) is a vital fruit crop in arid regions, particularly in the Middle East and North Africa. However, the genetic structure of date palm remains poorly understood, and knowledge of genetic variability is crucial for effective breeding. This study analyzed the genetic variability and population structure of 14 date palm cultivars collected from 28 locations in Iran. Additionally, publicly available chloroplast genome sequencing data for 47 cultivars were examined. The results indicated significant genetic variability among the cultivars. Interestingly, the genetic grouping of cultivars was not linked to their geographical distribution, and no correlation was found between genetic distance and geographical distance. The population structure analysis suggested that the low degree of shared alleles among cultivars might point to potential local adaptations influencing the genetic composition of date palms.

Amulya *et al.* (2022) a study on *Aegle marmelos* (bael), an underutilized fruit crop in India, to assess genetic diversity among 25 trees selected from a population of 356 based on distinct fruit morphological traits in Chikkamagalur, Karnataka. Genetic variation was evaluated using RAPD and ISSR molecular markers, both of which revealed considerable polymorphism. The Jaccard's similarity values ranged from 0.00 to 0.95 (RAPD) and 0.06 to 0.56 (ISSR), indicating a moderate level of genetic diversity. This findings demonstratede the effectiveness of RAPD and ISSR markers in identifying genetic relationships, which can aid in bael improvement and conservation programs.

Ali *et al.* (2023) focused on the ampelographic and genetic characterization of seven different grapevine cultivars: Red Globe, Autumn Royal, Crimson Seedless, Thompson, Perlette, King Ruby, and Sundar Khani (from Pakistan). Morphological characteristics such as berry shape, fruit skin color, flesh color, sweetness, compactness, and grape bunch weight were analyzed. The molecular diversity was assessed using Inter-Simple Sequence Repeat (ISSR) markers. Six primers were used, generating 84 bands ranging from 150 bp

to 1200 bp. The polymorphism information content (PIC) values ranged from 0.233 to 0.457, with polymorphism varying from 83% to 100%. The results demonstrated that ISSR markers are an effective tool for identifying and evaluating the genetic diversity among grape cultivars.

Abdel-Sattar *et al.* (2024) studied of Indian jujube cultivars aimed to assess their genetic diversity and relationships through both morphometric characteristics and molecular markers, such as mat K barcoding and ISSR markers. The analysis revealed notable variation in fruit characteristics, including geometric diameter, surface area, sphericity, shape index, fruit length, and seed weight across different cultivars. Additionally, significant differences were found in the color values of the fruits. Molecular marker analysis showed discrepancies in genetic diversity, and hierarchical clustering indicated that cultivars such as 'Zytoni' and 'Um-Sulaem', which have spines, form distinct mono-clades. This finding, related to gene expression variations, highlights the importance of using both morphometric and molecular approaches for accurate cultivar identification and maximization of local germplasm conservation and utilization.

Tran *et al.* (2025) explored the genetic diversity of 59 peach accessions from northern Vietnam using ISSR (Intersimple Sequence Repeat) markers. The results revealed significant polymorphism (92.5%) across six ISSR primers, allowing for the evaluation of genetic variation among the peach varieties. The accessions were grouped into two main clusters based on a genetic similarity coefficient threshold of 0.674, with no significant correlation between genetic and geographic distances. There study emphasizes the value of ISSR markers for understanding genetic relationships and conserving germplasm resources. It also highlights how genetic drift, influenced by the trade and exchange of varieties among farmers, may lead to the development of regionally named varieties. This research provides important insights for peach breeding and agricultural development in Vietnam.

Abhang *et al.* (2025) addressed the challenge of propagating true-to-type pomegranate plants by evaluating the genetic fidelity of micro propagated *Punica granatum* (Bhagwa cultivar). The micropropagation was carried out using nodal explants on MS medium with specific growth regulators for culture establishment and shoot multiplication. Rooting was achieved with 0.5 mg/L IBA and 0.5 mg/L NAA. The plantlets

underwent in vitro hardening and were transferred to greenhouse conditions. Genetic fidelity was assessed using molecular markers-RAPD, ISSR, and SSR-across a set of 48 SSR, 20 RAPD, and 12 ISSR primers. A subset of 14 SSR, 10 RAPD, and 9 ISSR primers generated distinct bands, confirming genetic uniformity with 100% homogeneity across all micropropagated plantlets. This study ensures the propagation of genetically identical pomegranate plants, providing quality assurance for Bhagwa pomegranate growers and the horticultural industry.

Chapter-III

MATERIALS AND METHODS

The present investigation, entitled **'Impact of Climate on Morphological Characteristics, Yield, and Molecular Profiling of Aonla Varieties,'** was conducted from 2024 to 2025 at the Indian Institute of Horticultural Research, Hessarghatta, Bengaluru, Karnataka, and Main Experiment Station (MES) Horticulture, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya (UP). This chapter presents the materials used and methods followed.

Indian gooseberry (*Emblica officinalis* Gaertn)

I.Subject

These test guidelines shall apply to all varieties and hybrids of Aonla *(Embilica officinalis Gaertn.).*

I.Planting material required

1.The Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA) shall decide on the quantity and quality of the planting material(s) required for testing the variety and when and where it is to be delivered for registration under the Protection of Plant Varieties and Farmers' Rights (PPV & FRA) Act, 2014. Applicants submitting such planting material(s) from a country other than India shall make sure that all customs and quarantine requirements stipulated under relevant national legislations and regulations are complied with. The minimum number of planting material to be supplied by the applicants or his/her nominee/assignee during August-September shall be 07 (seven) for each DUS Test Centre.

2. The planting materials supplied shall be healthy, not lacking in vigour or Nutrition as well as free from pests or diseases or any mechanical damage. The age of the plant(s) shall be minimum 03-04 months from the date of grafting(propagated through grafting) raised in the polythene bags (25 cm x 10 cm size) with potting mixture (2:2:1 v/v of loam soil, compost and fine sand).

3. The planting material(s) shall not have undergone any treatment (chemical/bio-physical or others) which would affect the expression of the characteristics of the variety, unless the Competent Authority allow or request for such treatment. If it has been treated, full details of the treatment must be given.

1. **Conduct of tests**

1. The minimum duration of the tests shall normally be at least two independent similar fruiting seasons in different years.

2. Tests shall be conducted at least at two places. If any essential characteristic of the candidate variety are not expressed for visual observation at these locations, the variety shall be considered for further examination at another appropriate test site or under special test protocol on expressed request by the applicant for which additional quantity of planting material shall be required.

3. The tests should be carried out under favourable conditions ensuring normal growth for the expression of the relevant characteristics of the variety and for the conduct of the tests. In particular, it is essential that the plants produce a satisfactory crop of fruit in each of the two growing cycles.

2. Test plot design

1. The design of the tests should be such that plants or parts of plants may be removed for measurement or observation without prejudice to the observations which must be made up to the end of the growing cycle. The additional test protocol for special purpose may be established by PPV & FRA. As a minimum, each test shall include five plants per location, planted at DUS test centre with a spacing of 8m x 8m.

2. The additional test protocol for special purpose may be established by PPV & FRA.

2. On-site DUS testing

The applicant or his/her nominee on his/her behalf shall submit a request to the Authority for conducting a reliable trial according to Test Guidelines and the instructions from Authority before on-site examination of the candidate variety.

The applicant or his/her nominee shall submit a request to the Authority for on-site examination prior to start of growing cycle as mentioned in Test Guidelines for site

examination of the candidate variety.

On-site testing may be conducted at the places specified by the applicant. The age of the trees at on-site shall be minimum 3 years.

As a minimum, 05 trees planted in uniform spacing (8x8m) should be available for inspection and examination for 'on site' DUS testing. The trees must be healthy and free from pest & disease and raised under standard management practices. For farmer's variety or landraces, the authority may notify suitable guidelines on the number of plant(s) and season(s), if any.

On-site examination shall be arranged during the fruiting season, when distinguishing characteristics of candidate variety can most easily be seen. The characteristics of the candidate variety can be examined and compared with those of the comparative varieties as per the Test guidelines.

The Expert Committee constituted by the PPV & FRA in consultation with the DUS Centre shall be authorized to inspect on-site testing and recording of the appropriate characters. Applicant shall supply the Expert Committee with summary of distinct characteristics supported by photographs.

The Expert Committee shall take notes and observations on distinctness and shall confirm preliminary data and/or summary of distinctness from applicant.

The Expert Committee shall submit examination report to the Authority.

Methods and observations

The characteristics described in the Table of characteristics (see section7) shall be used for the testing varieties and hybrid for their DUS.

For the assessment of Distinctiveness and Stability observation shall be made on 5 plants or parts taken from each of 5 plants. In the case of parts of plants, the number to be taken from each of the plants should be 2.

Fully mature leaves, not showing the sign of active growth, in the middle of tertiary branches should be selected for the observations on the leaf.

Observations on the mature fruit should be recorded at harvest maturity.

For assessment of all colour characteristics, the Royal Horticultural Society (RHS) colour chart shall be used.

1. Grouping of varieties

The candidate varieties for DUS testing shall be divided into groups to facilitate the assessment of Distinctiveness and Characteristics, which are known from experience not to vary, or to vary only slightly within a variety and which in their various states are fairly evenly distributed across all varieties in the collection are suitable for grouping purpose.

The following characteristics are to be used for grouping *Aonla* varieties:

a. Growth habit (Characteristic2)

b. Leaf: Shape (Characteristic 5)

c. Inflorescence colour (Characteristic 10)

d. Mature fruit: Shape (Characteristic 12)

e. Mature fruit: Colour (Characteristic 13)

f. Stone shape (Characteristic 18)

2. Characteristics and symbols

2. To assess Distinctiveness, Uniformity and Stability, the characteristics and their states as given in the Table of characteristics (Section VII) shall be used.

3. Notes (l to 9) shall be given for each state of expression for different characteristics for the purpose of electronic data processing.

Legend

Characteristics that shall be observed during every growing season on all varieties and shall always be included in the description of the variety, except when the state of expression of any of these characters is rendered impossible by a preceding phenological characteristic or by the environmental conditions of the testing region. Under such exceptional situation, adequate explanation shall be provided.

(+) See Explanation on the Table of characteristics in Section VIII. It is to be noted that for certain characteristics, the plant parts on which observations to be taken are given in the explanation or figure(s) for clarity and not the color variation.

5. Type of assessment of characteristics indicated in column

seven of Table of Characteristics are as follow:

MG: Measurement by single observation of a group of plants or part of plants. MS: Measurement by a single observation of individual plants or part of plants.

VG: Visual assessment by a single observation of a group of plants or parts of plants. VS: Visual assessment by observation of individual plant or part of plants.

5. A code number in the sixth column of Table of characteristics indicates the optimum stage for the observation of each characteristic during the growth and development of plant. The relevant growth stages corresponding to these code numbers are described below:

a) Observation on growth habit, shoot surface and leaf characters should be recorded three months after pruning, when canopy attains its characteristic shape. Fully mature leaves, not showing the sign of active growth, in the middle of tertiary branches should be selected for the observations on the leaf.

b) Observation on immature fruit should be recorded when fruit has not attained its full size and is predominantly green and quite hard in texture.

c) Observations on the mature fruit and stone should be recorded when fruit is ready for harvesting.

Table I: DUS characters of Aonla

Characteristics	State	Note	Example variety	Stage of observation	Type of assessment
2	3	4	5	6	7
Tree height	Dwarf	2	Narendra Aonla-6, Banarasi, Krishna, Chakaiya, Francis, Goma Aishwarya	A	VG
	Tall	1	Kanchan, Narendra Aonla -7, Anand-1, Anand-2 Narendra Aonla-10		
Growth Habit	Erect	3	NA-6,Chakaiya, Anand-1,Anand-2	A	VG
	Spreading	5	Narendra Aonla-10,NA-7, Francis, Goma Aishwarya		
	Drooping	7	Banarasi, Krishna, Kanchan,		
Foliage	Sparse	3	Banarasi,Krishna, Chakaiya, Kanchan, Anand-1, Anand-2, NA-6	A	VG
	Dense	5	Francis, Narendra Aonla-10,Narendra Aonla-7, Goma Aishwarya		
Leaf size	Small(<1.25 cm)	1	Narendra Aonla-7,Krishna, Francis, Anand-1, Anand-2,	A	MS
	Large(>1.35 cm)	5	Chakaiya, Narendra Aonla-10,Narendra Aonla-7		

Leaf shape	Elliptical	3	Narendra Aonla-7	A	VG
	Oblong	5	Chakaiya, Banarasi, Chakaiya, Narendra Aonla-10, Anand-1, Anand-2		
	Oval	7	Francis, Kanchan, Narendra Aonla-6, Goma Aishwarya		
Leaf apex	Acute	1	Narendra Aonla-6, Chakaiya, Kanchan	A	VG
	Obtuse	7	Banarasi, Krishna, Francis, Narendra Aonla-7, Narendra Aonla-10, Anand-1, Anand-2		
Leaf Surface	Non glabrous	9	Narendra Aonla-7, Banarasi, Krishna	A	VG
	Glabrous	1	NarendraAonla-6, Kanchan, Francis		
Trunk colour	Grey(197 A)	1	Chakaiya, Banarasi, Francis, Anand-1, Anand-2	a	RHS
	Whitish grey(199 B)	2	Narendra Aonla-7, Kanchan, Narendra Aonla-10,Goma Aishwarya,Krishna		
	Brownish grey(202 B)	3	Narendra Aonla-6		
Branchlet Colour	Deep red(181 A)	3	Banarsi	a	RHS
	Pinkish green(149A)	5	NarendraAonla-6		
	Yellowish	7	NarendraAonla-10		

	green(144 B)				
Inflorescence colour	Deep pink(47C)	3	Krishna, Banarasi, Narendra Aonla-10,NA-7	a	RHS
	Pinkish green(149 A)	5	NA-6, Chakaiya,Anand-2, Anand-1,		
	Yellowish green(147A)		Francis, Goma Aishwarya, Kanchan		
Fruit surface	Smooth	1	Krishna, Goma Aishwarya, NA-7,Anand-2, Anand-1		
	Rough	9	NarendraAonla-10,Francis,Kanchan		
Fruit Shape	Flattened Round	1	Chakaiya, Francis, Kanchan, NarendraAonla-10, Goma Aishwarya	a	VG
	Round	3	NarendraAonla-6		
	Triangular	5	Krishna, Banarasi		
	Oval	7	NarendraAonla-7, Anand-1, Anand-2		
Fruit colour	Greenish(146A)	1	Anand-1, Anand-2, Banarasi,	a	RHS
	Yellowish green ithinkish tinge(144A)	3	NarendraAonla-7		
	Light green(145A)	5	NarendraAonla-6, Krishna, Francis, Chakaiya, NarendraAonla-10		
Fruit Stalk	Thick	1	Narendra Aonla-	C	VG

			7,Banarasi,Krishna,NA-10		
	Thin	2	NA-6,Francis, Chakaiya,Kanchan,Anand-1		
Stem end	Flate	1	Krishna, NarendraAonla-7, NarendraAonla-10,	C	VG
	Depressed	2	Goma Aishwarya,Anand-2, Anand-1,Krishna		
Bearing tendency	Shy bearing		Banarasi, Krishna	C	VG
	Heavy bearing		Narendra Aonla-7,Anand-1.Goma Aishwarya		
Stone size	Small	3	Krishna, Kanchan, Chakaiya, Anand-1, Anand-2	C	MS
	Medium	5	NarendraAonla-10, Francis, Goma Aishwarya		
	Large	7	Chakaiya, Banarasi, Narendra Aonla -7		
Stone shape	Triangular	1	Krishna,Banra	a	VG
	Round	3	Kanchan ,Anand-1,Anand-2		
	Oval round	5	NA-7,Banarasi		
	Oval	7	NA-6, Francis,NA-10		
Seed colour	Light Brown(177C)	3	Narendra Aonla-6, Goma Aishwarya	C	VG
	Dark Brown(177A)	7	Narendra Aonla-7,Banarasi		
Harvest	Early	1	Narendra Aonla-10, Banarasi,	D	VG

Maturity			Krishna		
	Mid	5	NarendraAonla-7, Francis,Goma Aishwarya		
	Late	7	Chakaiya,NarendraAonla-6, Kanchan,		
Fruit Weight	Low 30-40 gm	5	Narendra Aonla-10, Chakaiya, Francis	C	MS
	Medium40-45gm	7	Banarasi,Goma Aaishwarya,		
	Very High >45gm	9	Krishna, Narendra Aonla-7		
Fruit Segment	Six	1	NarendraAonla-6, NarendraAonla-10,Chakaiya,Anand-1,Anand-2,Banarasi,Kanchan,Francis,Goma Aishwarya	C	MS
	Six to Eight	2	Krishna, Narendra Aonla-7		
Fruit Fibre (%)	Low fiber	3	Narendra Aonla-6, Krishna, Chakaiya, Goma Aishwarya,	C	VG
	High fiber	5	Kanchan, Francis, Anand-1, Anand-2		
Pulp (%)	Low	1	Kanchan,Anand-2,NA-6	C	MS
	High	3	Narendra Aonla-6, Banarasi, NA-10		
Total Phenol content(TAEg/ 100g)	Low<1	1	Krishna, Banarasi,NA-6,NA-7,Anand-1,Banarasi, Narendra Aonla-7	C	MS
	High>1	7	Kanchan, Anand-2, Anand-1, Francis, Goma		

			Aishwarya, Chakaiya,NA-10		
Vitamin C (mg/100g)	Low<400mg	1	Francis	C	MS
	Medium400-500mg	3	NA-4 NA-5 Chakaiya,		
	High>500mg	7	NA-10, Goma Aishwarya, Banarasi		

Characteristics 2: Different Growth habit of Aonla tree

Spreading

Erect

Drooping

Characteristic 5: Leaf Shape

Elliptical

Oblong

Oval

Characteristic 6: Leaf Apex

Acute

Obtuse

Characteristic 14: Inflorescence Color

Deep Pink

Pinkish Green

Characteristic 16: Fruit shape

Flattened round

Round

Triangular

Flattened oval

Characteristic 20: Fruit stem end

Prominent

Less-Prominent

Characteristic 24: Stone shape

Triangular

Round

Oval round

3.1 LOCATION OF THE EXPERIMENTAL SITE

a) The experimental site of ICAR-IIHR, Bengaluru

It falls under the Agro Climatic Zone No. 6, Southern Plateau and Hills, Latitude 13.0722° N, Longitude 77.5760° E, and Altitude 890 meters above mean sea level (MSL).

b) The experimental site of ANDUAT, Ayodhya, Uttar Pradesh

It falls under the Eastern Plain Agro-Climatic Zone (UP-9). This zone is characterized by a humid and damp climate, with an average annual rainfall of approximately 1067 mm and temperatures ranging from 32°C in summer to 16°C in winter. Latitude ~ 26.5°, Longitude ~ 83.5° E and Altitude ~ 80 to 100 meters above sea level (avg. elevation

3.2 AGRO-CLIMATIC CONDITIONS

a) ICAR-IIHR, Bengaluru

It is located in the Southern region at ICAR-IIHR, Bengaluru, which experiences a moderately warm climate and mild summer months. The site falls under a tropical region, with the mean maximum temperature varying between 28.5°C and 31.15°C and an average temperature of 29.49°C. The mean minimum temperature ranged from 17.3°C to 21.17°C, with an average temperature of 19.77°C. A total of 42.5 mm of rainfall was recorded during the observation period**.** Relative Humidity (RH) recorded for Morning (08:30 hrs.) was 87% while in Afternoon (14:30 hrs.) was 52%.

b) ANDUAT, Ayodhya

The site comes under a sub-tropical region comprising three distinct season's viz. summer, rainy, and winter. The summer season from March to June is characterized by temperatures ranging from 23˚C to 45˚C and hot, desiccating winds from March to June. The rainy season starts from July to September with an average annual rainfall of 125 cm, of which about 80% - 85% is concentrated from mid-June to the end of September. While winter season starts from November to March, with an average temperature range of 10˚C to 30˚C.

The weather data collected at the both universities and institute meteorological observatory during the experimentation period are detailed in Annexure- 1.1 and depicted in Annexure- 1.2

3.3 SOIL CHARACTERISTICS

i. ICAR-IIHR, Bengaluru soil

   a. The soils are sandy to red sandy loams and P^H range of 6.8 to 6.9

ii. ANDUAT, Ayodhya soil

   a. The soil in the region is predominantly sandy loam and the pH of 7.5 to 11.5.

3.4 EXPERIMENTAL CONDITIONS

The experiment was conducted using a factorial Randomized Complete Block Design (RCBD) with three replications during the 2024–2025.

a) Southern region ICAR-IIHR, Bengaluru orchard

The trees were planted in 2002 at 5m × 5m spacing and maintained in healthy condition.

b) Northern region ANDUAT, Ayodhya orchard

The trees were planted in 2013 at 8 m × 8 m spacing and maintained in healthy condition. Along with the Aonla tree, turmeric is also cultivated as mixed cropping.

3.5 VARIETIES

Table 1. The following List of Aonla varieties was used in the present study.

S. No	Varieties of ICAR-IIHR	Varieties of ANDUA&T
1	NA-4	NA-4
2	NA-5	NA-5
3	NA-6	NA-6
4	NA-7	NA-7
5	NA-10	NA-10
6	BSR-1	BSR-1
7	Chakaiya	Chakaiya

3.6 EXPERIMENT NO.1

3.6.1 To assess Aonla varieties for morphological parameters

3.7 OBSERVATIONS RECORDED

Periodical observations were recorded in the field for various morphological characters. All morphological observations were taken as per the descriptor for Aonla (Mahajan *et al*, 2002) and guidelines for DUS testing of PPV and FRA (Anonymous, 2016). The observations recorded and the methodology adopted for molecular analysis and units of measurement are presented hereunder.

3.7.1 TREE MORPHOLOGICAL CHARACTERS

3.7.1.1 Plant height (m)

It was measured by the wooden scale from ground level to the tip of the highest shoot.

3.7.1.2 Trunk girth (cm)

It was measured by measuring tape at 15 cm above the graft union in grafted/budded ones.

3.7.1.3 Trunk color

The color of the trunk was recorded using the RHS (Royal Horticultural Society) color charts for accurate color classification, whitish grey and wrownish grey.

3.7.1.4 Tree spread:

It was measured by measuring tape as the canopy diameter (average of East-West and North-South dimensions)

3.7.1.5 Shape of the tree

The shape of the tree was recorded at the 'pea stage' of fruit based on upright spreading and drooping.

3.7.1.6 Young shoot color

The color for the shoot was selected after a physical inspection, like light green, green, and dark green

3.7.1.7 Bearing tendency

The yield variation among varieties was assessed by evaluating the number of fruits per plant, classifying them into heavy-bearing or shy-bearing types accordingly.

3.7.1.8 Growth habit of the tree

The growth habit of the tree was recorded based on the erect, spread, drooping.

3.7.1.9 Branching pattern

Phyllanthoid branching pattern was recorded in Aonla tree.

3.7.1.10 Foliage Density

At the pea stage of fruit development, plants were evaluated and classified into sparse, medium, and dense categories based on branch density.

3.7.1.11 Foliage retention

It was observed based on the leaf drop in plants. deciduous**,** semi-deciduous, and evergreen.

3.8.1 Leaf characters

The leaf characteristics, including shape and size, were recorded based on visual and physical examination for morphological analysis.

3.8.1.1 Leaf length (cm)

The leaf length was measured with a ruler scale in centimeters and noted variation in leaf length among the samples.

3.8.1.2 Leaf width (cm)

Leaf width was measured in centimeters using a ruler scale to recorded variations in leaf width among the samples.

3.8.1.3 Leaflet Shape

The mature leaflets were classified based on their shapes, which included ovate, oblong, oblong-oval, oval, and elliptical forms.

3.8.1.4 Leaf Apex

The leaf apices observed on the mature leaflets were predominantly obtuse and acute in shape.

3.8.1.5 Leaf surface

The leaf surface varied in texture, ranging from smooth and glossy to rough.

3.9.1 Flower characters

Five indeterminate branches were identified in each direction on the selected plants, and all flowering parameters were documented from these tagged branches.

3.9.1.1 Date of start of flowering

The flowering start date was noted when approximately 5–10 percent of the flower's buds had bloomed.

3.9.1.2 Date of end of flowering

Flowering was considered complete when 5 percent of the buds were still open and 95 percent of the flower's buds had closed.

3.9.1.3 Inflorescence colour

The inflorescence exhibited a color variation ranging from deep pink to pinkish green.

3.9.1.4 Number of male flower / branchlet

The Male flowers on the determinate branches of the selected plants were counted.

3.9.1.5 Number of female flowers/ branchlet

Several female flowers of determinate branches on the selected branch were counted.

3.9.1.6 Sex ratio

It was determined by calculating the ratio of Female flowers to the total flower count.

3.9.1.7 Female flower position on branchlet

In Aonla crops, Female flowers are typically borne singly or in small clusters near the tips or middle of the branchlets.

3.10.1 FRUIT CHARACTERS

3.10.1.1 Fruit maturity group

Fruit maturity was assessed and grouped into early, mid, and late categories, depending on the duration from flowering to harvest maturity.

3.10.1.2 Extent of fruit drop

The fruit drop was categorized as low, medium, or high based on the percentage of fruits that fell off during development.

3.10.1.3 Fruit apex (stylar end)

The characteristics of the fruit apex (stylar end) were assessed through direct physical observation to evaluate its shape and condition. depressed, flat, and papillate.

3.10.1.4 Fruit base cavity at stem end

The fruit base cavity at the stem end was examined physically to determine its depth and structure, contributing to the overall fruit quality assessment. **a**bsent, shallow, and deep.

3.10.1.5 Fruit length (mm)

The length of ten randomly selected fruits was measured with Vernier calipers. The measurement of the length was made in the polar axis of the fruit, i.e., between ventral and dorsal, and the average was computed and expressed in millimeters.

3.10.1.6 Fruit width (mm)

The diameter of ten randomly selected fruits was measured with a Vernier caliper at the point of maximum width/girth in the direction perpendicular to the axis, the average was computed and expressed in millimeters.

3.10.1.7 Fruit weight (g)

The weights of ten randomly picked fruits were measured by an electric weighing balance, and the mean value was calculated in grams.

3.10.1.8 Fruit Shape

The fruit shape characteristics were recorded based on visual and physical examination to ensure accurate morphological classification. flattened round, flattened oblong, flattened triangular, oval round oval.

3.10.1.9 Fruit surface texture

This parameter, fruit surface texture, was examined through physical observation to categorize the fruits based on surface characteristics such as smoothness, roughness, or ribbing

3.10.1.10 Fruit segment

Fruit segments were physically observed and counted to assess variation under this parameter.

3.10.1.11 Segment ridges at the Stem end

Segment ridges at the stem end were examined through physical observation to assess their prominence and structural variation among the fruits, like absent**,** less prominent, and prominent.

3.10.1.12 Fruit stalk

The fruit stalk was evaluated through physical examination to record variations in its thickness and mode of attachment to the fruit, like as thick and thin.

3.10.1.13 fruit skin ground color

The ground color of the fruit skin was recorded using the RHS (Royal Horticultural Society) color charts for accurate color classification. yellowish, greenish yellow, greenish, and russet-green.

3.10.1.14 Fruit skin over color

The fruit skin over-color characteristic was assessed through direct physical observation. white streaked, green tinge, pink ting, and red ting.

3.10.1.15 Pulp Taste

Pulp taste was assessed based on sensory evaluation to determine its flavor profile, including sweetness, tanginess, and overall palatability. acrid**,** acidic, and medium sweet.

3.10.1.16 Pulp texture

The texture of the pulp was examined by knife cutting, focusing on its firmness, ease of cutting, and overall, like soft, medium, and hard.

3.11.1 Seed characters

The seed characteristics of Aonla were assessed by evaluating attributes such as seed size, shape, color, and texture to determine their quality.

3.11.1.1 Stone color

The stone color was recorded through visual observation and compared using the RHS (Royal Horticultural Society) color charts to ensure accurate classification. light brown and dark brown.

3.11.1.1 Stone shape

The Stone shape was assessed based on its morphological characteristics, outline, and uniformity to classify it accurately. Round, oval-round, and triangular.

3.11.1.3 Stone size

The size of the stone was visually assessed and recorded small and large.

3.11.1.4 Seed length (mm)

The length of ten randomly selected fruits was precisely measured using Vernier calipers along the polar axis, extending from the apex to the stem. The average length was then calculated and recorded in millimeters for accurate assessment.

3.11.1.5 Seed width (mm)

The width of ten randomly selected seeds was measured at their widest point, perpendicular to the axis, using a Vernier caliper. The average width was then computed and expressed in millimeters.

3.11.1.6 Seed weight (g)

The weight of a seed from each fruit was measured in grams using an electronic weighing balance for ten randomly selected fruits, and the average weight was calculated to determine the seed weight per fruit."

3.11.1.7 Pulp %

Pulp percentage was computed by dividing the pulp weight by the total fruit weight and multiplying by 100 to obtain the value in percentage.

3.11.1.8 Pulp-to-stone ratio

Fruit pulp and seeds were weighed separately for ten randomly selected fruits, and their average weights were recorded in grams. The ratio of their average weights was recorded as a pulp-to-seed ratio. The ratio of fruit pulp to seed was recorded as given below.

$$\text{Pulp to seed ratio} = \frac{\text{Weight of pulp (g)}}{\text{Weight of seed (g)}}$$

3.11.1.9 Harvesting Maturity

The harvesting maturity of the fruits was assessed by evaluating physiological traits such as size, color change, and ripeness to determine the ideal harvest time.

3.11.1.10 Estimated Fruit yield per tree (kg)

Fruits are harvested when they have reached their full maturity. The weight of the fruit was recorded in kilograms every time it was harvested. The total yield was calculated by adding the values obtained in different harvests of the year and expressed in kilograms per tree per year.

3.12 Molecular Characterization

3.12.1 To generate DNA profiles of Aonla varieties using polymorphic ISSR/SRAP markers

Young leaves were used for the DNA extraction.

3.12.1.1 Reagents required

i. **Extraction Buffer:** Prepared by mixing 100 mL autoclaved Tris base (pH 8.0), 40 mL autoclaved 0.5 M EDTA (pH 8.0), and 280 mL 5 M NaCl. Added 20 g PVP and 40 g CTAB, made up to 1,000 mL with distilled water, adjusted pH to 8.0, mixed well, and autoclaved at 121°C for 15–20 min.

ii. **Chloroform: Isoamyl Alcohol (24:1):** Prepared by mixing 24 parts of chloroform with 1-part isoamyl alcohol. Mixed well and stored in a sealed amber bottle at room temperature.

iii. **NaCl (5 M, Autoclaved):** Made by dissolving 292.2 g NaCl in water to 1 L (based on MW 58.44 g/mol). Autoclaved at 121°C for 15–20 min.

iv. **TE Buffer (pH 8.0):** Prepared with 13.2 g Tris base + 7.44 g Na_2EDTA in 1 L of water. PH adjusted to 8.0, then autoclaved at 121°C for 15–20 min.

v. **Ammonium Acetate (7.5 M, pH 7.7):** Prepared by dissolving the required amount of ammonium acetate in distilled water to make 7.5 M solution, adjusted pH to 7.7, then autoclaving for sterility.

vi. **Wash Solution (70% Ethanol):** Prepared by mixing 70 mL of absolute ethanol with 30 mL of distilled water. Stored at room temperature and used for washing DNA pellets.

vii. **PVP** (polyvinylpyrrolidone)

viii. **TAE Buffer:** Prepared by dissolving 242 g Tris base in 800 mL distilled water, adding 57 mL glacial acetic acid and appropriate 0.5 M EDTA (pH 8.0), adjusting to 1 L, pH 8.0, and autoclaving at 121°C for 15–20 min.

ix. **Bromophenol Blue (6X):** Prepared by dissolving 0.25 g bromophenol blue in 50 mL glycerol (50%) + 50 mL distilled water. Mixed until fully dissolved to form a homogeneous solution.

x. **Ethidium Bromide (0.5 mg/mL):** Made by dissolving 50 mg EtBr in 100 mL distilled water. Mixed thoroughly, stored in a dark, labeled container at room temp or 4°C. Handled with PPE due to mutagenicity.

Xi PCR reagents such

a) 10X Buffer with $MgCl_2$ (TAKARA)

b) dNTP's (2.5 mM TAKARA)

c) Taq Polymerase(5 U/µL TAKARA)

Xii. ISSR primers (10 UBC set)

3.12.1.2 DNA Extraction protocol: Genomic DNA was extracted using the modified CTAB method.

i. Grind 0.2 g of leaf tissue without liquid nitrogen. Add 20 mg PVP and 1 ml CTAB buffer pre-heated and one drop betamarcaptoethenol and 250 µl NaOH 0.1M at the grinding time

ii. Incubate the tube for 1 hr. at 65°C, shake intermittently every 15 minutes, and cool to RT.

iii. Add 1 ml of chloroform: Isoamyl alcohol (24:1) and mix gently by inverting tubes 25 times to form an emulsion.

iv. Spin at 12000 rpm for 15 min and transfer the aqueous phase to a new centrifuge tube using cut tips.

v. Transfer the clear aqueous phase to fresh centrifuge tubes. Add 30 µl of 5M NaCl and 1 1ml cold isopropanol, mix gently, and keep overnight at 20°C.

vi. Centrifuge at 10000 rpm for 15 min. Pour off the supernatant, wash the pellet with 1 ml of cold ethanol 70%, and centrifuge as above for 15 min.

vii. Repeat washing twice.

viii. Drain out the supernatant. Remove ethanol without completely drying the DNA by leaving tubes uncovered at 37°C for 1 to 2 hours at RT.

ix. Suspend the pellet by adding 30µl of TE buffer and pool using cut tips.

3.12.1.3 DNA Quantification using spectrophotometer and gel electrophoresis

i. DNA concentration in the sample can be estimated by recording the absorbance at 260nm in UV/visual spectrophotometer.
ii. Firstly, check the blank with TE buffer and take 1µl of DNA sample in the pipette and place a drop at the Nano pore.
iii. Measure the absorbance of the solution at 260nm and 280nm.
iv. Calculate the ratio of A260/A280
v. A good DNA preparation exhibits the following spectral properties.
vi. A260/A280 1.7 to 2.0 0.D. units.
vii. A280/A260-0.5 O.D. units.
viii. Calculate the DNA concentration using the relationship for double-stranded DNA.
ix. I.O.D. at 260 nm gives 50µg/ml
x. Total quantity of DNA (µg/ml) = $\frac{\text{OD at 260 nm x 50 x dilution factor}}{10000}$

xi. Dilution factor = $\frac{\text{Volume made}}{\text{Volume of the aliquot}}$

3.12.1.4 Agarose gel electrophoresis

3.12.1.5 Casting of agarose gel

Prepare 0.8% agarose solution in 10X TAE buffer for 2 mL/100 mL distilled water and 0.8g agarose/100ml of distilled water. Heat it to dissolve it completely, cool to 40°C and add ethidium bromide solution (0.5 µg/ml). Pour it into the cast and insert the comb. When the gel is set, remove the comb and keep it in the gel electrophoresis tank.

3.12.1.5 Running the gel

Fill the electrophoresis tank with 0.5X TBE buffer, then place the gel and load the DNA sample. Apply an 82V current for 1.5 to 2 hours. Afterward, remove the gel and visualize the DNA under UV light. Near to the well a single band indicates the DNA is intact.

3.12.1.6 Dilution of stock DNA

3.12.1.7 Based on the OD values, dilutions were made to 84ng/µl

$$C_1 V_1 = C2 \ V_2$$

Where,

C_1 Stock conc, V_1 volume made-up, C_2 Working conc, V_2 volume Require

3.12.1.8 DNA Fingerprinting of varieties using ISSR (Inter Simple Sequence Repeats) marker technique

3.12.1.9 Standardization of the PCR Protocol (Polymerase Chain Reaction):

The PCR reaction conditions were optimized to produce clear, consistent, and reproducible fingerprint profiles. Amplification was performed in a 13.5 µL reaction mixture with the following components:

3.12.1.10 PCR Mixture Volume for ISSR Protocol

PCR Reagents	**Volume**
a) 10 X Buffer	2.0 µl
b) DNTP (2.5 mM)	2.µl
c) Primer (10 pmols)	2.5 µl
d) Taq DNA Polymerase (5 U/µL)	0.50 µl
e) Template DNA (84 ng/µl)	2.50 µ
f) Millqi water	4 µl
g) Total mixture volume	**13.50 µl**

3.12.1.11 PCR was performed in an Eppendorf Nexus Gradient Master Cycler Thermal Cycler (Hamburg, Germany) with the following profile

a) **Thermal profile**

PCR was carried out in an Eppendorf Nexus Gradient Master Cycler.

a.	Initial denaturation	94°C for 4 min
b.	Denaturation	94°C for 45 sec
c.	Primer annealing	"C for 45 sec
d.	Primer extension	72°C for 1 min
e.	Cycles	40 Cycles
f.	Final extension	72°C for 8 min

For better results to avoid contamination in the PCR reaction, autoclaved all reagents and also tips, tubes, pipettes, were used.

3.12.1.13 screening of primers

Initially, 90 primers were screened against diluted genomic DNA from 3 samples for their ability to amplify DNA fragments. Out of 90 primers screened**, 10 primers** gave good amplification at different annealing temperatures. The remaining primers given resulted in either no amplification or faint bands on a highly smeared background.

3.12.1.14 Standardization of annealing temperature for different ISSR primers

The annealing temperature played a critical role in achieving efficient amplification. Its optimal value varied depending on the base composition of the primers. To standardize the annealing temperature, the melting temperature (Tm) of each primer was used as a reference.

3.12.1.15 Gel electrophoresis:

PCR products were analyzed using agarose gel electrophoresis. For ISSR analysis, 1.5% agarose gels (1.5g agarose per 100 ml of 2 ml, 1X TAE buffer/100 ml distilled water) were prepared and run using a horizontal electrophoresis system and visualized with the UVITEC Cambridge Gel Doc system.

To prepare the gel, agarose was weighed and added to 2 mL of 1X TAE buffer in a conical flask, mixed thoroughly, and heated in a microwave until proper boiling of agarose solution with intermittent shaking until fully melted and clear. The gel-casting tray was cleaned with water and ethanol. Once the agarose cooled to around 50–55 °C, 2 µl of ethidium bromide (10 mg/ml) was added, mixed well, and poured into the tray. Combs were inserted, and the gel was left to solidify at room temperature for 20–30 minutes, ensuring no air bubbles formed.

After solidification, the gel was placed in a tank containing 1X TAE buffer. PCR products were mixed with 1/6th volume of loading dye (50% glycerol and 0.25% bromophenol blue) before loading into the wells. A 100 bp DNA ladder was included for each primer set to estimate fragment sizes. Electrophoresis was conducted at 50–55 V for 3.5 to 4 hours. DNA bands were then visualized under UV light and photographed using the UVITEC Cambridge system. The resulting gel images were saved for analysis and record-keeping.

3.13 To assess genetic relationships of varieties using molecular markers

3.13.1 Data analysis

The amplified fragments were analyzed based on their presence or absence. Only strong, clear, and intense fragments were scored, as faint or diffuse bands are prone to low reproducibility. A 100 bp DNA ladder marker was used to estimate band sizes, with

unambiguous bands scored. Markers were assigned a value of '1' for presence and '0' for absence across the genotypes. This binary data matrix was then subjected to further analysis.

3.13.2 Procedure of cluster formation

a) Data Preparation

a. The input data should be formatted correctly, either as raw data files (.var) or as dissimilarity matrices (.dis). Import the data using the **File > Import** option in DARwin. Ensure that the data is clean, with no missing values or inconsistencies.

b) Computation of Dissimilarity Matrix

a. Navigate to **Dissimilarity > Compute**. Select the appropriate data type (e.g., Single data, Allelic data, or Sequence data). Choose the suitable dissimilarity measure (such as Euclidean, Jaccard, etc.) based on the nature of your data. Click on **Compute** to generate the dissimilarity matrix.

c) Cluster Analysis

a. Tree Construction Go to Tree > Construct. Select the desired clustering method such as UPGMA, Neighbor-Joining, or Minimum Spanning Tree. Configure the required parameters and then click Construct to generate the cluster tree based on the dissimilarity matrix.

d) **Tree Visualization**

a. To visualize the resulting cluster tree, navigate to **Tree > Draw**. This allows graphical representation of the dendrogram or tree structure. Customize the view by modifying node labels, branch lengths, and other graphical elements.

b.

e) **Exporting Results**

a. Save the dissimilarity matrix and the cluster tree using **File > Export**. You can export results in various formats for documentation or further statistical analysis.

f) **Interpretation**

Analyze the generated cluster tree to understand the genetic or morphological relationships among the genotypes. Clusters formed will represent groups with minimum dissimilarity, useful in genetic diversity and grouping studies.

3.13.3 Cluster analysis

The agglomerative method of clustering using UPGMA (Unweighted Pair Group Method with arithmetic averages) was adopted to develop a dendrogram. This calculates the congruence between assays of values, typically, densitometric assays.

Chapter IV

RESULTS AND DISCUSSION

The present study was carried out to determine the "**Impact of Climate on Morphological Characteristics, Yield and Molecular Profiling of Aonla Varieties"** maintained in the Division of fruit crops, Indian Institute of Horticultural Research, Bangalore, and ANDUA&T Kumarganj Ayodhya (U.P.) during the season 2024-25. The results of the study are as follows.

The present investigations comprised the following experiments;

I. To assess Aonla varieties for morphological parameters
II. To generate DNA profiles of Aonla varieties using polymorphic ISSR/SRAP markers
III. To assess genetic relationships of varieties using morphological and molecular markers

The data collected was statistically analyzed, and the results obtained were discussed under the following heads.

Morphological characters

Genetic variability

Molecular characterization

4.1 TREE MORPHOLOGICAL CHARACTERS

4.1.1 Growth habit of the tree

The varieties under study exhibited differences in growth habit, ranging from spreading to erect (Table no. 4.1). A spreading growth habit was observed in NA-7, NA-10, BSR-2, NA-4, and NA-5 (drooping), whereas an erect growth habit was recorded in NA-6 and Chakaiya.

Similar results were reported by Singh *et al*. (2022) and Kumar *et al.* (2021) during the evaluation of the same Aonla varieties. Singh *et al.* (2021) the tree shape of the studied genotypes has categorized in three groups, viz. spreading (CHES 1, Chakaiya, Krishna, NA 6, NA 7, NA 10 and BSR 1); drooping (NA 20) and upright type (G 1, S 1 and S 2).

4.1.2 Young Shoot color

The varieties displayed variation in shoot color in (Table N.4.1). The varieties revealed differences in young shoot color (Table 2). It was observed that light green young shoots were observed in NA-5, NA-6, NA-7, and Chakaiya, while green was detected in NA-4, dark green in BSR-1, and light green with a pinkish tint in NA-10. Observations were recorded as per the descriptor for Aonla (Mahajan *et al*. 2002).

These findings are in agreement with the results reported by Singh *et al. (*2022) during their evaluation of Aonla varieties.

4.1.3 Foliage density of the tree

The variation in foliage among different Aonla genotypes is presented in (Table N.4.1) Most of the varieties, namely NA-10, NA-6, NA-4, NA-5, and Chakaiya, exhibited sparse foliage, whereas NA-7, BSR-1 and NA-10 recorded dense foliage. This character was observed consistently under both North and South conditions across all seven varieties.

Similar observations have been reported by Singh *et al.* (2022) and Singh *et al.* (2021). A similar finding was observed by Kumar *et al.* (2016), the foliage in Francis and NA 7 was sparse, whereas Chakaiya, Goma Aishwarya, and Anand 2 had dense foliage.

4.1.4 Trunk color

The varieties differed in trunk color, as presented in (Table No.4.1), with the majority of varieties showing white grey (199 B) trunk color in NA-7, NA-5, NA-10, and NA-4. Brownish grey (202 B) trunk color was recorded in NA-6 and BSR-1, whereas Chakaiya exhibited a grey (197 A) trunk color.

A similar finding was observed by Kumar *et al* (2016) the trunk color (grey and whitish green

4.1.5 Foliage retention

The Aonla plants show both type of foliage retention i.e. deciduous and semi-deciduous nature (Table No.4.1). NA-4, NA-5, NA-10 and BSR-1 displayed deciduous nature, while NA-6, NA-4 and Chakaiya showing semi-deciduous nature.

Table 4.1: Influence of Climate on Morphological Characters and Bearing Habits of Selected Aonla Cultivars in North and South regions.

Varieties	Growth habit	Young Shoot color	Foliage Density	Trunk color	Foliage retention	Bearing tendency
NA-4	Drooping	Green	Sparse	Whitish Grey (199 B)	Semi deciduous	Shy bearing
NA-5	Spreading	Light green	Sparse	W G (199 B)	Deciduous	Heavy bearing
NA-6	Spreading	Light Green	Sparse	Brownish Grey (202 B)	Semi deciduous	Heavy bearing
NA-7	Spreading	Light green	Dense	W G (199 B)	Deciduous	Heavy bearing
NA-10	Spreading	Light green with pinkish	Dense	W G (199 B)	Deciduous	Shy
Chakaiya	Erect	Light green	Sparse	Grey (197A)	Semi deciduous	Heavy Bearing
BSR-1	Spreading	Dark green	Dense	Brownish Grey (202 B)	Deciduous	Heavy bearing

4.1.6 Bearing tendency

The bearing tendency varied among the different varieties. (Table No.4.1) Varieties NA-4, NA-5, NA-7, BSR-1, and Chakaiya exhibited a heavy bearing tendency, indicating their potential for high fruit production. In contrast, varieties NA-6 and NA-10 were categorized as shy bearers, reflecting a comparatively lower fruiting capacity.

4.2. Leaf Characters

4.2.1 Leaf Shape

The leaf shape of the Aonla varieties exhibited variation (Table N. 4.2), ranging from oval to oblong forms. Most of the varieties, including NA-5, NA-6, NA-10, BSR-1, and Chakaiya, noted an oblong leaf shape, while NA-4 showed an oval leaf shape, and NA-7 exhibited an elliptical leaf shape.

These findings are consistent with the observations reported by Singh *et al.* (2021) and Singh *et al.* (2022) during their evaluation of Aonla varieties

4.2.2 Leaf apex

The varieties presented variation in leaf apex morphology (Table N.4.2). The leaf apex was predominantly acute, as observed in NA-4, NA-6, NA-10, BSR-1, and Chakaiya, whereas an obtuse apex was recorded in NA-5 and NA-7.

These observations are consistent with the findings reported by Singh *et al.* (2021) and Singh *et al.* (2022), who observed similar patterns during the evaluation of Aonla genotypes. Similar findings, Kumar *et al.* (2016), the leaf apex was mainly of two kinds, i.e., acute and obtuse. All the varieties had an obtuse leaf apex, excluding Chakaiya.

4.2.3 Leaf surface

The varieties differed in leaf surface character was found to be glabrous in the varieties NA-4, NA-5, NA-7, NA-10, and Chakaiya, while the varieties NA-6 and BSR-1 exhibited a non-glabrous leaf surface in (Table N.4.2). Similar observation taken by Dra. Fitotecnia. (2016).

4.2.4 Leaf size

The leaf size also displayed variation in different varieties in (Table N.4.2). Large-sized leaves were recorded in NA-10 and Chakaiya, whereas other varieties were shown to be small. Similar observation taken by Dra Fitotecnia (2016).

Table No.4.2 Influence of climate on Morphological Diversity in Leaf Traits among Aonla Cultivars exhibited same characters in both regions

Varieties	Leaf Shape	Leaf apex	Leaf surface	Leaf size
NA-4	Oval	Acute	Glabrous	Small
NA-5	Oblong	Obtuse	Glabrous	Small
NA-6	Oblong	Acute	Non-Glabrous	Small
NA-7	Elliptical	Obtuse	Glabrous	Small
NA-10	Oblong	Acute	Glabrous	Large
Chakaiya	Oblong	Acute	Glabrous	Large
BSR-1	Oblong	Acute	Non-Glabrous	Small

4.3 Flower characters

4.3.1 The nature of the flowering branch let

In varieties NA4 and NA-10, flowering is expressed on all branches (**Table No.4.3**). In NA-5, NA6, NA-7, and Chakaiya, flowering was displayed on tertiary branches. In BSR-1, flowering was also observed on all branches.

4.3.2 Female flower Position on the branch

Variation was displayed among the Aonla Varieties for the position of female flowers on the branchlet (Table no.4.3). In most varieties, including NA-4, NA-5, BSR-1, and Chakaiya, the female flowers were located at the middle portion of the branchlet**.** However, in NA-7, the position was also recorded in the middle portion. It varies the upper branchlet positioning of female flowers was characteristic of NA-6 and NA-10 (Table No.4.3).

Table No.4.3: Influence of climate on Variability in Flowering Behavior and Floral Morphology in Aon

Varieties	**Nature of the flowering branchlet**	**Female flower Position on the branch**	**Inflorescence color**	**Date of start of flowering (South)**	**Date of start of flowering (North)**	**Date of the end of flowering (South)**	**Date of the end of flowering (North)**
NA4	All branches	The middle of the branch let	Pinkish Green (149 A)	10/2/2025	20/3/2025	1/3/2025	12/4/2025
NA-5	Tertiary branches	The middle of the branch let	Deep pink(47C)	10/2/2025	20/3/2025	4/3/2025	15/4/2025
NA6	Tertiary branches	The upper portion of the branch lets	Pinkish green(149 A)	25/01/2026	5/4/2025	2/3/2025	22/4/2025
NA-7	Tertiary branches	Middle of the	Deep pink(47C)	10/2/2025	10/4/2025	22/2/2025	2/5/2025
NA-10	All branches	The upper portion of the branch lets	Deep pink(47C)	10/2/2025	12/3/2025	2/3/2025	4/4/2025
BSR-1	All branches	The middle of the branch let	Pinkish Green (149G)	15/02/2025	7/4/2025	6/3/2025	22/4/2025
Chakaiya	Tertiary branches	The middle of the branch let	Pinkish Green (149G)	25/01/2025	16/3/2025	20/02/2025	13/4/2025

4.3.3 Inflorescence color

The inflorescence color in NA4 and NA6 was pinkish green (149 A) (Table N.4.3). . In NA-5, NA-7, and NA-10, it was deep pink (47C). In Chakaiya and BSR-1, the inflorescence color was pinkish green (149 A) (Table No.4.3). Observations were taken as per the descriptor for Aonla Mahajan *et al* (2002).

The similar findings were also reported by Kumar *et al.* (2016), the inflorescence color was yellowish-green in Francis, Pinkish green in Chakaiya, green to light pink in NA 7, Goma Aishwarya, and light green to pinkish in Anan.

4.3.4 Date of start of flowering

The flowering in Aonla plants varied in the north and south conditions due to different climate impacts (Table No.4.3). The higher leaf width was recorded in Chakaiya (0.35) in Bangalore region and NA-6 (0.39) in Ayodhya regions, and the minimum leaf width was recorded in NA-4 in Bangalore regions and Ayodhya regions NA-6 & NA-7 was recorded. The mean was higher in Ayodhya regions (0.35) compared to Ayodhya regions (0.32).

Similar findings observed by Aulakh *et al.* (2013) flowering in all the Aonla cultivars occurred in the month of April.

4.3.5 Date of the end of flowering

The flowering will be drops in March 1, 2025, in NA4, on March 4, 2025, in NA-5, on March 2, 2025, in NA6 and NA-10, on February 22, 2025, in NA-7, on March 6, 2025, in BSR-1, and on February 20, 2025, in Chakaiya. (Table No.4.3).

4.4 Fruit character

4.4.1 Fruit Surface

The surface of the fruit varied among the varieties (Table No.4.4). Most varieties, including NA4**,** NA-5**,** NA6**,** NA-7, and Chakaiya**,** expressed a smooth surface**.** However, NA-10 and BSR-1 had a rough texture**.**

Table No.4.4 Influence of Climate on Diversity in Fruit Structure and Quality Attributes of Aonla Cultivars

Varieties	Fruit Surface	Fruit shape	Fruit Taste	Fruit color (South)	Fruit color (North)	Fruit Stem end	Fruit stalk
NA4	Smooth	Round	Acrid	Y G (2 B)	G Y (145) Pale Green	Prominent	Thick
NA-5	Smooth	Triangular	Acidic	YG (3C)	G Y (150 C) Lime tint	Prominent	Thick
NA6	Smooth	Flattened round	Acrid	Y G (7B)	YG (7 B)	Less prominent	Thick
NA-7	Smooth	Round	Acidic	YG (7 B)	YG (150 D) Medium green	Prominent	Thick
NA-10	Rough	Flattened round	Acrid	Y G (9 A)	G Y (145A) Medium Green	Prominent	Thick
BSR-1	Rough	Oval	Acrid	G Y (D)	G Y (145 C) pale Green	Less prominent	Thin
Chakaiya	Smooth	Triangular	Acidic	Y G (4 B)	G Y (145 A) Very Pale	Prominent	Thick

Note- Y G (2 B) Yellowish, Green, GY (D) Greenish Yellow

4.4.2 Fruit shape

The variation was observed in fruit shape among the different varieties (Table No.4.4). Round fruits were observed in NA4 and NA-7. Triangular fruits were recorded in NA-5 and Chakaiya. Flattened round fruits were characteristic of NA6 and NA-10. Oval-round (CHES 1, Chakaiya, NA 6, NA 10, G 1, and BSR 1), triangular (Krishna), oval (NA 7), and round (NA 20).Shaped fruits were noted in BSR-1 Similar findings, Kumar *et al*. (2012), fruit shape was observed as flattened

4.4.3 Fruit Stem end

The varieties NA-4, NA-5, NA-7, NA-10, and Chakaiya exhibited a prominent stem end, indicating a more distinct and elevated fruit shoulder near the pedicel attachment (Table No.4.4). In contrast, the varieties NA-6 and BSR-1 showed a less prominent stem end, where the transition between the stem and the fruit surface was more gradual and flatter.

These differences in stem end morphology can be useful in varietal characterization and may also influence market acceptance, especially where appearance is a key quality attribute. The results are summarized in Table 4.4.

4.4.4 Fruit Taste

Acrid taste was noted in NA4, NA6, NA-10, and BSR-1(Table No.4.4**)**. Acidic taste was dominant in NA-5, NA-7, and Chakaiya.

Comparable results were reported by Kumar *et al.* (2016). The astringency level was high in Chakaiya, medium to high in Anand 2, medium in Francis and Goma Aishwarya, and lowest in NA 7.

4.4.5 Fruit color

The fruit color showed considerable diversity exhibiting a Yellow Green 2B (YG 2B) shade. NA-5 showed a Yellow Green 3C (YG 3C) shade.NA6 was characterized by a mix between Yellow Green 6 and 7. NA-7 displayed a Yellow Green 7B (YG 7B) tone. NA-10 exhibited a Yellow Green 9A (YG 9A) shade. BSR-1 showed a Green Yellow Dark (GYD) tone. Chakaiya had a Yellow Green 4B (YG 4B) shade (Table no.4.4). Similar findings were also observed by Kumar *et al*. (2016). The fruit color was light green in Francis and Chakaiya; yellowish-green in NA 7, Goma Aishwarya, and Anand 2. The flesh color of all tested varieties was whitish-green.

4.4.6 Fruit stalk

Most of the varieties including NA-4, NA-5, NA-6, NA-7, NA-10, and Chakaiya were found to possess thick fruit stalks, indicating a strong fruit attachment to the branches (Table N.4.4). This trait may help reduce premature fruit drop and is generally advantageous under conditions of wind or mechanical stress. In contrast, the variety BSR-1 exhibited a thin fruit stalk, which may facilitate easier detachment during harvesting but might be more prone to fruit drop under adverse environmental conditions (Table N.4.4).

Such variation in stalk thickness highlights the genetic diversity among Aonla varieties and provides valuable information for selecting suitable varieties for cultivation.

4.4.7 Fruit drop

Significant variation was observed among the Aonla varieties for fruit drop (Table N.4.5). Fewer fruit drop was recorded in NA-4, NA-5, NA-6, NA-10, BSR-1, and Chakaiya, whereas heavy fruit drop was noted in NA-7, due to weak branch nature plants. These findings suggest genotypic influence on fruit retention capacity and some external and internal factors like weather, rainfall temperature, humidity, and nutrient deficiency in soil. Similar findings observed by Mishra et al. (2017).

4.4.8 Fruit maturity group

The varieties exhibited distinct differences in fruit maturity periods (Table N.4.5). Early maturity was observed in NA-5, NA-7, NA-10, and Chakaiya, while NA-4 and NA-6 were categorized under mid-maturity groups. BSR-1 was the only variety classified as late maturing, indicating its prolonged fruit development phase. These results are comparable with previous studies by Singh *et al.* (2021). Time of fruit maturity was recorded early (i.e., before 15 November) in cultivar Krishna and NA 10; mid (i.e., 15 November to 15th December) in CHES 1, NA 6, NA 7, NA 20, G 1 and S 2, and late (i.e., after 15th December) in S 1, Chakaiya and BSR 1.

A similar trend was noted in the study by Kumar *et al.* (2016). The time of fruit maturity was observed during the last week of October in Francis and NA 7, the first week of November in Goma Aishwarya, the second week of November in Chakaiya, and the last week of November in Anand 2. Days of maturity in different varieties were 207-220 days.

4.4.9 Fruit-based cavity at the stem end

Considerable variability was recorded for fruit-based cavity characteristics at the stem end (Table N.4.5). A shallow cavity was predominant in NA-4, NA-5, NA-7, NA-6, whereas a deep cavity was observed in Chakaiaya, BSR-1, and NA-10. This trait is important from a fruit quality perspective, especially regarding processing suitability.

Similar findings were reported by Kumar *et al.* (2021) fruit base (cavity at stem end) was observed as absent (CHES 1), shallow (Chakaiya, NA 6, NA 20, G 1), deep (Krishna), flat (NA 7, NA 10, BSR 1).

4.4.10 Fruit apex (stylar end)

Differences were evident among the varieties in fruit apex morphology (Table N.4.5). A flat stylar end was observed in NA-4, NA-5, NA-7, BSR-1, and Chakaiya, while a papillate apex was recorded in NA-6 and NA-10. Such morphological traits may influence market preferences and fruit handling characteristics. Similar findings by Kumar *et al.* (2021) fruit apex was observed as flat (CHES 1, Chakaiya, Krishna, NA 7, NA 10, NA 20), papillate (NA 6, BSR 1), and depressed (G 1).

A similar trend was noted in the study by Kumar *et al.* (2016). Styler's end was levelled in Francis and Chakaiya; less prominent in NA 7, Goma Aishwarya, and Anand 2.

4.4.11 Number of Segments

Generally, in Aonla fruits 6 segments was observed in most of the varieties. Similar observation was taken by Singh *et al.* (2022).

4.4.12 Fruit skin ground color

Variation in fruit skin ground color was discernible (Table N.4.5). Greenish ground color was recorded in NA-4, NA-5, NA-7, and BSR-1, whereas yellowish ground color was observed in NA-6 and Chakaiya. NA-10 exhibited a yellowish-green ground color, indicating a transitional pigmentation. Similar observations were taken by Mahajan *et al*. (2002).

4.4.13 Fruit skin over color

Differences in fruit skin over color were also apparent (Table N.4.5). A green tinge over color was observed in NA-4, NA-6, and NA-7, whereas white streaks were recorded in NA-5. Pink tinges were found in NA-10 and BSR-1, while Chakaiya exhibited a white tinge. These over-color characteristics contribute to the external appearance and consumer acceptance of fruits. Similar observations were taken by Mahajan *et al*. (2002).

4.4.14 Pulp texture

The varieties showed notable variation in pulp texture (Table N.4.5). A soft pulp texture was characteristic of NA-4, NA-5, NA-6, and NA-7, whereas a hard pulp texture was recorded in NA-10, BSR-1, and Chakaiya. Pulp texture plays a crucial role in determining the suitability of fruits for processing and table purposes.

Table No.4.5: Influence of climate on Fruit Maturation and Quality Attributes of Aonla *(Emblica officinalis)* Varieties

Varieties	Fruit drop	Fruit maturity group	Fruit-based cavity at the stem end	Fruit apex (stylarend)	Fruit skin ground color	Fruit skin over color	Number of Segments	Pulp texture
NA-4	Less	Mid	shallow	Flat	Greenish	Green tinge	6	Soft
NA-5	Less	Early	Shallow	Flat	Greenish yellow	White streaked	6	Softs
NA-6	Less	Mid	Shallow	Papillate	Yellowish	Green tinge	6	soft
NA-7	Heavy drop	Early	Shallow	Flat	Greenish yellow	Green tinge	6	soft
NA-10	Less	Early	Deep	Papillate	Yellowish green	Pink tinge	6	Hard
BSR-1	Less	Late	Deep	Flat	Greenish	Pink tinge	6	Hard
Chakaiya	Less	Early	Deep	Flat	Yellowish	White ting	6	Hard

4.5 Seed characters

4.5.1 Stone size

The assessment of stone size across different varieties revealed distinct variations (Table No.4.6). Varieties such as NA-4, NA-6, and Chakaiya were categorized under the large stone size group, indicating a tendency towards heavier and larger seeds. In contrast, varieties such as NA-5, NA-7, NA-10, and BSR-1 exhibited medium stone size, suggesting relatively moderate seed development. Similar observations were taken by Mahajan *et al*. (2002).

4.5.2 Stone shape

The evaluation of stone shape among the different varieties revealed noticeable variations (Table No.4.6). Most varieties exhibited a round stone shape, including NA-4, NA-7, NA-10, BSR-1, and Chakaiya. However, varieties NA-5 and NA-6 were characterized by triangular stone shapes. This diversity in stone morphology could be associated with the genetic variability among the cultivars studied. Similar observations were taken by Mahajan *et al*. (2002).

4.5.3 Stone color

The assessment of stone color across the studied varieties revealed two distinct categories (Table No.4.6). Varieties NA-4, NA-5, NA-6, and NA-7 exhibited a light brown color. In contrast, varieties NA-10, BSR-1, and Chakaiya were characterized by dark brown stones. The variation in stone pigmentation may be attributed to inherent genetic differences among the genotypes. Similar observations were taken by Mahajan *et al*. (2002).

4.5.4 Harvesting Maturity

The harvest maturity period varied between the southern and northern regions (Table No.4.6). In the southern region, most varieties mature between September 2, 2024**,** and March 18, 2025. Specifically, varieties such as NA-5, NA-6, and NA-7 attained harvesting maturity around September 14–15, 2024, whereas NA-4 matured slightly earlier on September 11, 2024. BSR-1 showed the latest maturity and reached harvest readiness by March 18, 2025. In contrast, in the northern region, harvesting maturity was generally delayed compared with in that the south. Maturity ranged from November 26, 2024 (Chakaiya) to January 6, 2025 (NA-4). The

varieties NA-5, NA-6, and NA-7 matured in December 2024, whereas BSR-1 was harvested slightly earlier on January 5, 2025.

Similar parameters taken by Aulakh *et al.* (2013), fruits of Kanchan were the earliest to mature (15th-19th Nov.) followed by Amrit (19th-22nd Nov.), Neelam (21st-22nd Nov.), Krishna (23rd-25th Nov.), and Balwant (26th-28th Nov.). Fruits of Chakaiya matured at the last (21st-23rd Dec.) and took 3 to 4 weeks more than the other cultivars to mature.

Similarly observed by Singh *et al*. (2021) time of fruit maturity was recorded early (i.e., before 15 November) in cultivar Krishna and NA 10; mid (i.e. 15 November to 15th December) in CHES 1, NA 6, NA 7, NA 20, G 1 and S 2, and late (i.e., after 15th December) in S 1, Chakaiya and BSR 1.

Table No.4.6 Influence of climate on Variability in Stone Morphology and Harvesting Time across Aonla Cultivars.

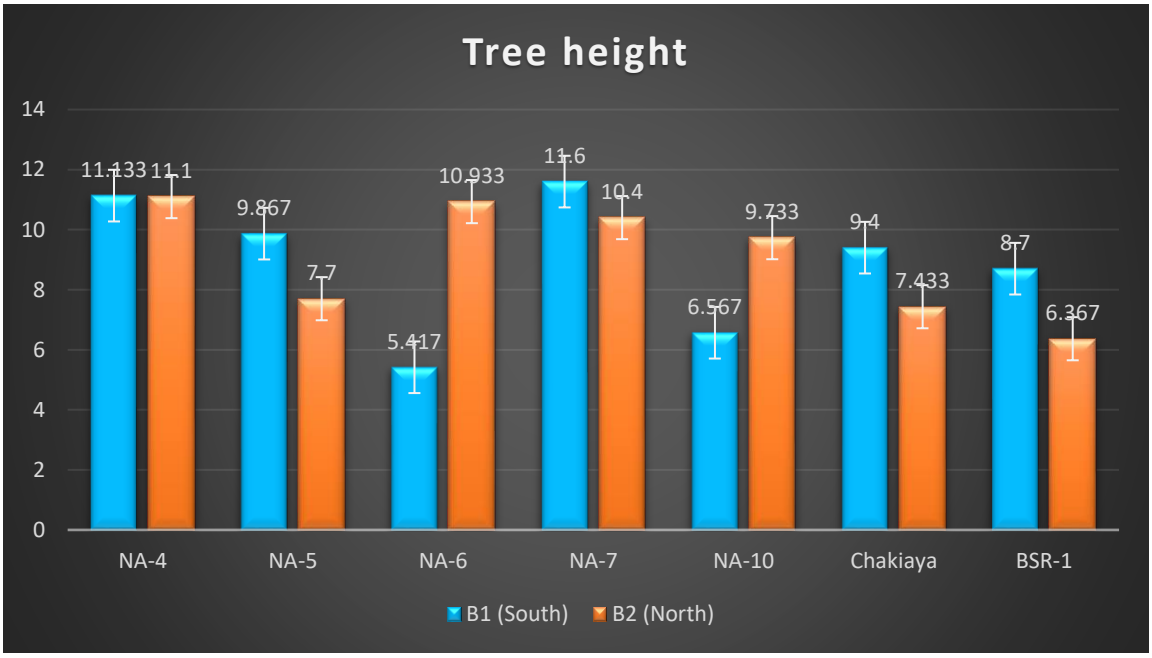
Varieties	Stone size	Stone shape	Stone color (South)	Stone color (North)	Harvesting Maturity (South)	Harvesting Maturity North
NA-4	Large	Round	Light brown	Dark Brown	9/11/2024	01/06/2025
NA-5	Medium	Triangular	Light brown	Light Brown	9/14/2024	12/20/2024
NA6	Large	Triangular	Light brown	Light Brown	9/14/2024	12/18/2024
NA-7	Medium	Round	Light brown	Light Brown	9/14/2024	12/12/2024
NA-10	Medium	round	Dark brown	Light Brown	9/15/2024	12//12/2024
BSR-1	Medium	Round	Dark brown	Dark Brown	18/3/2025	01/05/2025
Chakaiya	Large	round	Dark brown	Dark Brown	9/2/2024	11/26/2024

Table No.4.7 Influence of North and South Agro-climates on plant height of Aonla Tree

Tree Height (m)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	11.133	11.10	11.11
NA-5	9.867	7.700	8.783
NA-6	5.417	10.933	8.175
NA-7	11.600	10.400	11.00
NA-10	6.567	9.733	8.150
Chakiaya	9.400	7.433	8.417
BSR-1	8.700	6.367	7.533
Mean B	8.955	9.095	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	2.254	1.091	0.771
Locations (B)	N/A	0.583	0.412
Factor(A X B)	3.188	1.542	1.091

Fig N.4.1. Influence of North and South Agro-climates on plant height of Aonla.



4.6 Quantitative Parameters

4.6.1 Tree height (m)

The tree height analysis showed significant difference among varieties at both locations (South and North). The variety NA-4 recorded maximum height (11.11 m) followed by NA-7 (Table N. 4.8). In the southern region (Bangalore), tree height ranged from 5.417 m (NA6) to 11.600 m (NA-7). In contrast, in the northern region (Ayodhya), tree height varied from 6.367 m (BSR-1) to 11.100 m (NA4). The interaction between varieties and location was significant and the maximum height was recorded in NA-7 (11.6 m) variety under southern conditions, followed by NA-4 11.13. (Table No.4.7**)**

The locations were different non-significantly for this character however the southern location recorded maximum height and northern location minimum height. The trees recorded more height in southern location might be due to higher availability of day hours in southern region which results in higher production of photosynthesis.

The present findings also corroborated with the findings of earlier researchers like as Kumar et al (2021) also noted that the maximum plant height (6.70 m) was recorded in Gujrat-1, followed by NA 6 with a plant height (6.32 m, and Krishna's Plant height was found minimum in Chakaiya (4.90 m). Similar results were found by Singh *et al.* (2021) average plant height among the studied genotypes varied from 4.83 m to 6.62 m; the genotypes Chakaiya and BSR 1 were found to be comparatively less vigorous among the selected genotypes. The less vigorous/ dwarf genotypes are considered suitable material for high-density planting.

4.6.2 Trunk girth @ base (m)

The trunk girth analysis showed significant difference among varieties at both locations (South and North). The variety NA-5 recorded maximum trunk girth (1.08 m) followed by NA-4 (Table N. 4.8). In the southern region (Bangalore), trunk girth ranged from 0.48 m (Chakaiya) to 1.08 m (NA-5). In contrast, in the northern region (Ayodhya), trunk girth varied from 0.59 m (Chakaiya) to 0.88 m (NA-5). The interaction between varieties and location was significant and the maximum trunk girth recorded in NA-5 (0.88 m) variety under southern conditions followed by NA-4 (0.87 m).(Table No.4.8)

The locations were differ non-significantly for this character however, the southern location recorded maximum trunk girth. The trees recorded more trunk girth in the southern

location might be due to higher availability of day hours in the southern region, which results in higher production of photosynthesis, and due to favorable temperature for vegetative growth.

A similar result observed by Kumar *et al.* (2021) stem girth (95.37 cm) were recorded in Gujrat-1, followed by NA-6, Krishna, and stem girth (46.45 cm) in CHES 1. Similar findings, Singh *et al* (2021) Average stem girth among the studied genotypes. CHES 1, BSR 1, Krishna, NA 6, NA 7, NA 10, NA 20, Chakaiya and G 1, varied from 46.40 cm to 95.33 cm.

Trunk girth @ base (m)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	0.87	0.86	0.86
NA-5	1.08	0.67	0.88
NA-6	0.69	0.69	0.69
NA-7	0.85	0.64	0.75
NA-10	0.70	0.64	0.67
Chakiaya	0.48	0.70	0.59
BSR-1	0.57	0.78	0.68
Mean B	0.751	0.712	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	0.184	0.089	0.063
Locations (B)	N/A	0.048	0.034
Factor(A X B)	0.260	0.126	0.089

Table No.4.8 Influence of North and South Agro-climates on Trunk Girth at the base of Aonla.

4.6.3 Trunk Girth at First Branch (m)

The analysis of trunk girth at the first branch exhibited significant differences among the varieties and locations. The variety Chakaiya recorded the maximum average trunk girth (1.27 m), followed by NA-5 (1.04 m**)** and NA-10 (0.95 m**)** (Table No. 4.9). The minimum trunk girth was observed in BSR-1 (0.76 m) and NA-6 (0.77 m)**.** Under southern conditions, trunk girth ranged from 0.39 m (NA-6) to 1.35 m (Chakaiya), whereas in the northern region, it ranged from 0.88 m (NA-7) to 1.28 m (NA-5). The interaction between varieties and locations was

found to be significant, with Chakaiya under southern location recording the highest girth (1.35 m)**,** while NA-6 in South showed the lowest (0.39 m).(Table No.4.9)

The mean trunk girth was higher in the northern region (1.09 m) compared to the southern region (0.80 m)**.** This difference between locations was statistically significant**.** Suggesting that regional agro-climatic conditions influenced stem thickening. Cooler temperatures and moderate growth rates in the North likely allowed for more girth accumulation as compared to the rapid vertical growth in the South.

The effect of varieties**,** locations**,** and their interaction (Treatment × Location) was all statistically significant**.**

Trunk girth @ first branch (m)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	0.97	0.95	0.96
NA-5	0.79	1.28	1.047
NA-6	0.39	1.16	0.773
NA-7	0.90	0.88	0.893
NA-10	0.64	1.26	0.955
Chakiaya	1.35	1.18	1.267
BSR-1	0.55	0.96	0.757
Mean B	0.80	1.09	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	0.31	0.15	0.106
Locations (B)	0.16	0.08	0.056
Factor(A X B)	0.44	0.21	0.149

Table No.4.9 Influence of North and South Agro-climates on Trunk Girth at the first Branching of Aonla.

4.6.4 Canopy Spread (East–West Direction) (m)

The canopy spread measured in the East–West direction showed significant variation among different varieties and between the two locations (South and North). The maximum mean canopy spread was recorded in BSR-1 (8.37 m)**,** followed by NA-7 (8.12 m) and Chakaiya (6.82 m). The minimum mean canopy spread was observed in NA-10 (4.78 m**)** (Table No. 4.10). In the southern location (Bangalore**)**, the canopy spread ranged from 2.30 m (NA-10) to 6.60 m

(NA-7), while in the northern location (Ayodhya**)**, it varied from 7.27 m (NA-10) to 10.53 m (BSR-1). The average canopy spread across locations was higher in the northern region (8.57 m) than in the southern region (4.94 m)**.** (Table no.4.10)

The effect of varieties (A**)** and locations (B) was statistically significant. However, the interaction effect (A × B**)** was statistically non-significant, indicating that the response of varieties for canopy spread remained consistent across both locations. The larger canopy spread in the northern region might be attributed to slower but more lateral vegetative growth under relatively moderate temperatures and better light interception angles, leading to a broader horizontal expansion of the canopy.

Kumar et al. (2021) observed the maximum plant spread EW (7.15 m) and stem girth (95.37 cm) were recorded in Gujrat-1; followed by NA 6 and Krishna with plant spread EW (6.58 m) Minimum plant spread EW (4.47 m) was found in NA 7, whereas; stem girth (46.45 cm) in CHES 1.

Canopy (EW) (m)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	4.70	7.77	6.23
NA-5	5.23	8.03	6.63
NA-6	3.93	8.70	6.32
NA-7	6.60	9.63	8.12
NA-10	2.30	7.27	4.78
Chakiaya	5.60	8.03	6.82
BSR-1	6.20	10.53	8.37
Mean B	4.94	8.57	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	1.29	0.63	0.44
Locations (B)	0.69	0.33	0.24
Factor(A X B)	N/A	0.89	0.63

Table No.4.10 Influence of North and South Agro-climates on Canopy (EW) of Aonla.

4.6.5 Canopy Spread (North–South Direction) (m)

The canopy spread in the North–South direction showed significant variation among different varieties and across locations. The highest average canopy spread was observed in Chakaiya (8.25 m), followed by NA-6 (7.67 m) and NA-7 (7.35 m) (Table No. 4.11). The lowest mean spread was recorded in NA-10 (5.52 m**)** and NA-4 (6.52 m)**.** Under southern conditions,

canopy spread ranged from 2.90 m (NA-10) to 7.87 m (Chakiya), whereas in the northern region**,** it ranged from 7.03 m (BSR-1) to 10.40 m (NA-6 and NA-7). On average, the northern location

Showed greater canopy spread (8.976 m) as compared to the southern location (4.990 m)**.** (Table No.4.11)

The effect of varieties (A) and locations (B) was statistically significant. However, the interaction between varieties and locations (A × B) was non-significant, suggesting that the varietal performance pattern remained relatively stable across locations for this parameter.The greater canopy spread in the northern region might be due to a combination of factors such as cooler climate, better soil moisture retention, and slower internodal elongation, which favors horizontal canopy development.

Same result observed by Singh *et al* (2021) average plant spread (N-S) among the studied genotypes varied from 46.40 cm to 95.33 cm, respectively. The genotypes Chakaiya and BSR 1 were found to be comparatively less vigorous amongst selected genotypes. The less vigorous/ dwarf genotypes are considered suitable material for high-density planting.

Kumar *et al.* (2021) observed the maximum plant spread EW (7.15 m) and SW (7.53 m) were recorded in Gujrat-1; followed by NA 6 and Krishna with plant spread EW (6.58 m) and plant spread NS (6.46) Minimum plant spread EW (4.47 m) and NS (4.44 m) was found in NA 7, whereas; stem girth (46.45 cm) in CHES 1**.**

Canopy (NS) (m)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	3.633	9.400	6.517
NA-5	4.500	8.833	6.667
NA-6	4.933	10.400	7.667
NA-7	4.300	10.400	7.350
NA-10	2.900	8.133	5.517
Chakiaya	7.867	8.633	8.250
BSR-1	6.800	7.033	6.917
Mean B	4.990	8.976	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	1.293	0.625	0.442
Locations (B)	0.691	0.334	0.236
Factor(A X B)	N/A	0.885	0.625

Table No.4.11 Influence of North and South Agro-climates on Canopy (NS) of Aonla.

4.6.6 Leaf Length (cm)

The analysis of leaf length among the varieties revealed statistically significant differences due to varietal effects, while the effect of locations and the interaction between varieties and locations was non-significant. Among all the varieties, Chakaiya recorded the highest mean leaf length (1.91 m), followed by NA-10 (1.89 m) and NA-5 (1.67 m). The shortest leaves were observed in NA-4 and NA-7 (1.45 m each) (Table No. 4.12). In terms of location-wise performance, the leaf length ranged from 1.47 m to 1.88 m in the southern region and from 1.43 m to 1.97 m in the northern region. However, the mean leaf length remained equal in both regions (1.63 m), indicating that environmental influence was minimal for this trait. (Table No 4.12)

Statistical analysis showed that the varietal effect was significant, while the location effect and interaction between variety and location were non-significant. This suggests that leaf length is primarily governed by genetic factors rather than environmental conditions.

Hence, varieties such as Chakaiya and NA-10 can be considered superior in terms of leaf length, which may contribute positively to photosynthetic activity and canopy spread. Similar observation taken by Dra. Fitotecnia. (2016).

Leaf Length (cm)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	1.47	1.43	1.45
NA-5	1.67	1.67	1.67
NA-6	1.47	1.50	1.48
NA-7	1.47	1.43	1.45
NA-10	1.88	1.90	1.89
Chakiaya	1.86	1.97	1.91
BSR-1	1.57	1.53	1.55
Mean B	1.63	1.63	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	0.08	0.04	0.03
Locations (B)	N/A	0.02	0.01
Factor(A X B)	N/A	0.05	0.04

Table No.4.12 Influence of North and South Agro-climates on Leaf length of Aonla.

4.6.7 Leaf Width (cm)

The data on leaf width showed minor variation among the varieties and between the two locations. The highest average leaf width was recorded in NA-5, NA-6, and Chakiya (0.36 m each), followed by NA-4 (0.33 m). The lowest mean value was observed in NA-7 (0.30 m) and NA-10 (0.31 m) (Table No. 4.13). At the southern location, the leaf width ranged from 0.30 m (NA-4 and NA-7) to 0.35 m (Chakiya), whereas at the northern location, it varied from 0.31 m (NA-10 and NA-7) to 0.39 m (NA-6). On average, the northern region recorded slightly wider leaves (0.35 m) compared to the southern region (0.32 m).(Table No 4.13)

Statistical analysis revealed that the effect of locations was significant. However, the varietal effect and the interaction between variety and location were non-significant, indicating that environmental conditions, especially regional differences, had more influence on leaf width than genetic variation.

These findings suggest that leaf width is relatively stable among varieties but can be slightly influenced by growing conditions, with northern conditions favoring marginally wider leaves.). Similar observation taken by Dra. Fitotecnia (2016).

Leaf Width (cm)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	0.30	0.36	0.33
NA-5	0.34	0.38	0.36
NA-6	0.32	0.39	0.36
NA-7	0.30	0.31	0.30
NA-10	0.32	0.31	0.31
Chakiaya	0.35	0.37	0.36
BSR-1	0.31	0.33	0.32
Mean B	0.32	0.35	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	N/A	0.02	0.02
Locations (B)	0.03	0.01	0.01
Factor (A X B)	N/A	0.03	0.02

Table No. 4.13 Influence of North and South Agro-climates on Leaf Width of Aonla

4.6.8 Fruit Weight (g)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	33.547	24.200	28.873
NA-5	36.217	25.453	30.835
NA-6	31.553	33.840	32.697
NA-7	39.047	34.257	36.652
NA-10	31.433	27.767	29.600
Chakiaya	36.643	29.787	33.215
BSR-1	16.689	9.813	13.251
Mean B	32.161	26.445	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	8.080	3.909	2.764
Locations (B)	4.319	2.090	1.478
Factor (A X B)	N/A	5.529	3.909

Table No.4.14 Influence of North and South Agro-climates on Fruit Weight of Aonla.

4.6.8 Fruit Weight (g)

The fruit weight exhibited highly significant differences among varieties as well as between locations. The maximum mean fruit weight was recorded in variety NA-7 (36.65 g), followed by Chakaiya (33.22 g), NA-6 (32.70 g), and NA-5 (30.84 g). The minimum fruit weight was observed in BSR-1 (13.25 g) (Table No. 4.14). In the southern region (Bangalore), fruit weight ranged from 16.69 g (BSR-1) to 39.05 g (NA-7), whereas in the northern region (Ayodhya), it ranged from 9.81 g (BSR-1) to 34.26 g (NA-7). On average, fruits were heavier in the southern location (32.16 g) as compared to the northern location (26.45 g), indicating a significant influence of environmental factors. (Table No 4.14)

Statistical analysis showed that the effect of varieties (A) and locations (B) was significant. The interaction between varieties and locations (A × B) was non-significant, indicating that varietal performance was consistent across both regions for fruit weight.The higher fruit weight in the southern region may be attributed to favorable climatic conditions such as longer growing periods, higher temperature ranges, or better soil fertility, which could support better fruit development. A similar result was observed by Kumar *et al.* (2021). Maximum fruit weight (55.43 g) was recorded in NA 20, and minimum (5.89 g) in BSR 1. Similar findings were observed by Singh *et al.* (2021) the maximum.

Fruit Length (mm)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	33.83	30.41	32.12
NA-5	35.73	30.35	33.04
NA-6	32.53	33.06	32.80
NA-7	37.20	32.42	34.81
NA-10	32.90	32.00	32.45
Chakiaya	33.57	32.24	32.91
BSR-1	26.10	22.06	24.08
Mean B	33.12	30.37	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	3.30	1.59	1.13
Locations (B)	1.76	0.85	0.60
Factor(A X B)	N/A	2.26	1.59

Table No.4.15. Influence of North and South Agro-climates on Fruit Length of Aonla.

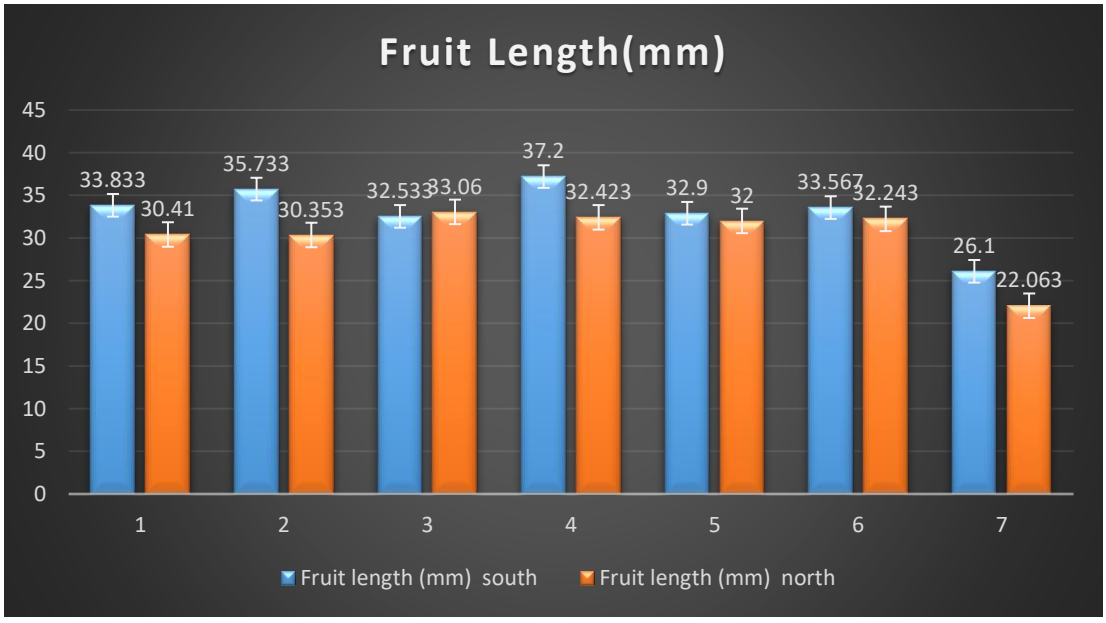


Fig.No.4.2 Graph showing fruit length parameters of Aonla varieties of two different locations in India.

4.6.9 Fruit Length (mm)

The data on fruit length showed significant variation among the varieties and between the two locations. The highest mean fruit length was recorded in NA-7 (34.81 mm), followed by NA-5 (33.04 mm), Chakaiya (32.91 mm), and NA-6 (32.80 mm). The lowest fruit length was observed in BSR-1 (24.08 mm) (Table No. 4.15). In the southern region (Bangalore), fruit length ranged from 26.10 mm (BSR-1) to 37.20 mm (NA-7), whereas in the northern region (Ayodhya), it varied from 22.06 mm (BSR-1) to 33.06 mm (NA-6). On average, the southern location recorded longer fruits (33.12 mm) compared to the northern location (30.37 mm). (Table no.4.15)

Statistical analysis revealed that the effect of varieties and locations was significant. However, the interaction effect between varieties and locations was found to be non-significant, indicating that the varietal trend in fruit length remained stable across both environments. The slightly longer fruits observed in the southern region may be due to favorable climatic conditions, possibly including higher temperature and longer photoperiod, which could enhance vegetative and reproductive growth in fruit crops.

Similar findings, Kumar *et al.* (2021). Maximum fruit length (3.33 cm) was recorded in NA 20 and fruit breadth (3.70 cm) in Chakaiya, whereas minimum fruit length (1.87 cm) and breadth (2.28 cm) were recorded in BSR 1.

Also observed by Singh *et al.* (2021), the fruit length (3.30 cm) was measured higher in NA 7 and NA 20, which was statistically at par with Chakaiya (3.28 cm), however, the minimum fruit length (1.90 cm) was measured in BSR 1.

Fruit Width (mm)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	38.13	33.65	35.89
NA-5	39.53	33.67	36.60
NA-6	36.87	37.37	37.12
NA-7	39.77	36.67	38.22
NA-10	36.83	35.90	36.37
Chakiaya	37.30	35.31	36.31
BSR-1	29.70	26.30	28.00
Mean B	36.88	34.12	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	3.67	1.78	1.26
Locations (B)	1.96	0.95	0.67
Factor(A X B)	N/A	2.51	1.78

Table No.4.16 Influence of North and South Agro-climates on Fruit Width of Aonla.

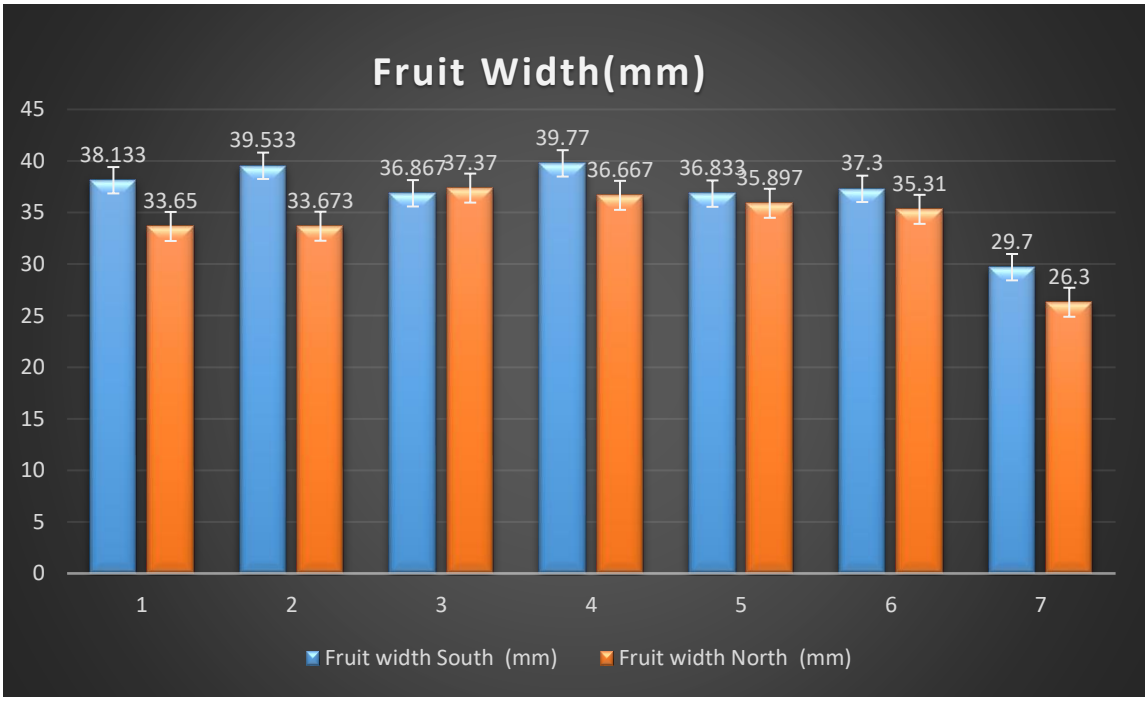


Fig.No.4.3 Graph showing fruit width parameters of Aonla varieties of two different locations in India.

4.6.10 Fruit Width (mm)

The analysis of fruit width among the tested varieties revealed significant variation due to both varietal and locational effects. The highest mean fruit width was recorded in NA-7 (38.22 mm), followed by NA-6 (37.12 mm), NA-10 (36.37 mm), and Chakiya (36.31 mm). The lowest fruit width was observed in BSR-1 (28.00 mm) (Table No. 4.16). At the southern location (Bangalore), fruit width ranged from 29.70 mm (BSR-1) to 39.77 mm (NA-7), while in the northern location (Ayodhya), it ranged from 26.30 mm (BSR-1) to 37.37 mm (NA-6). The average fruit width was higher in the southern region (36.88 mm) than in the northern region (34.12 mm). (Table no 4.16)

Statistical analysis showed that the effect of varieties and locations was significant. The interaction between varieties and locations was found to be non-significant, indicating that the varietal performance for fruit width was consistent across both regions. The larger fruit width observed in the southern region may be attributed to more favorable growing conditions that support better fruit development. Overall, NA-7 and NA-6 stood out as superior varieties in terms of fruit girth, which may influence market preference and yield potential.

Similar findings, Singh *et al.* (2021) fruit breadth was measured as maximum (3.70 cm) in Chakaiya while minimum (2.30 cm) in BSR 1.

4.6.11 Total Number of Flowers

The total number of flowers per plant showed significant variation among the varieties and between the two locations. The highest mean flower count was observed in NA-7 (661.83), closely followed by NA-6 (658.83), while the lowest was recorded in NA-4 (410.00) (Table No. 4.17). Other varieties like NA-5 (518.33), NA-10 (539.67), and Chakaiya (516.00) also showed moderate flower production. In the southern location (Bangalore), the number of flowers ranged from 370.67 (NA-4) to 615.67 (NA-6), whereas in the northern location (Ayodhya), it varied from 449.33 (NA-4) to 736.67 (NA-7). The overall average number of flowers was higher in the northern region (585.86) compared to the southern region (489.67). (Table No 17). Statistical analysis showed that the effects of both varieties and locations were significant. However, the interaction between varieties and locations was found to be non-significant,

indicating that varietal response for flower production remained stable across both environments. The greater flower production in the northern region may be due to more favorable temperature fluctuations, extended floral initiation periods, or better pollination conditions. Varieties such as NA-6 and NA-7 consistently produced a high number of flowers and may be considered promising for yield potential.

Similar results were found by Aulakh *et al.* (2013), the highest flower density was recorded in cultivar Balwant (750 flowers per m), and the minimum in Krishna (227.70 flowers per m).

Number of Total flowers

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	370.67	449.33	410.00
NA-5	466.00	570.67	518.33
NA-6	615.67	702.00	658.83
NA-7	587.00	736.67	661.83
NA-10	525.67	553.67	539.67
Chakiaya	463.33	568.67	516.00
BSR-1	399.33	520.00	459.67
Mean B	489.67	585.86	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	76.61	37.07	26.21
Locations (B)	40.95	19.81	14.01
Factor (A X B)	N/A	52.42	37.07

Table No.4.17 Influence of North and South Agro-climates on Total flowers of Aonla.

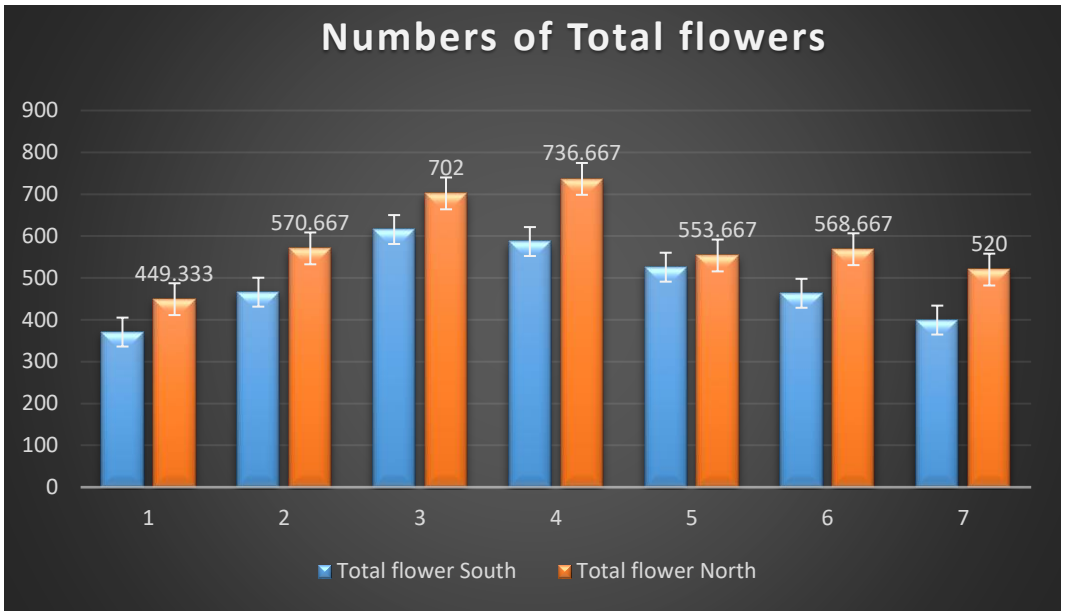


Fig.No.4.4 Graph showing total flowers present in Aonla varieties of two different locations in India

4.6.12 Number of Male /Branch

The number of male plants per variety exhibited significant variation across both the locations and among the different varieties. The highest mean number of male plants was recorded in variety NA-7 (656.83), followed closely by NA-6 (651.17) and NA-10 (533.33). The lowest count was found in NA-4 (404.50). Other varieties such as NA-5 (512.00), Chakaiya (507.83), and BSR-1 (456.67) showed moderate numbers of male plants. In the southern location (Bangalore), the number of male plants ranged from 365.67 (NA-4) to 607.67 (NA-6), while in the northern location (Ayodhya), it ranged from 443.33 (NA-4) to 731.33 (NA-7). The average number of male plants was significantly higher in the northern region (578.24) compared to the southern region (485.29) (Table No.4.18).

Statistical analysis revealed that the effects of both varieties and locations were significant. However, the interaction between varieties and locations was found to be non-significant, indicating a consistent varietal performance across both environments with respect to male plant production. The higher number of male plants observed in the northern location could be attributed to favorable environmental conditions such as photoperiod, temperature range, or soil fertility. Notably, NA-6 and NA-7 emerged as promising genotypes due to their consistently high number of male plants across both locations, and may be considered ideal for

enhancing fruit set and yield potential in dioecious species. These findings suggested that the male/branchlet is influenced by both environmental and genetic factors. Similar results were found by Aulakh *et al.* (2013).

Number of male/Branch

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	365.67	443.33	404.50
NA-5	460.33	563.67	512.00
NA-6	607.67	694.67	651.17
NA-7	582.33	731.33	656.83
NA-10	520.33	546.33	533.33
Chakaiya	464.00	551.67	507.83
BSR-1	396.67	516.67	456.67
Mean B	485.29	578.24	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	76.69	37.11	26.24
Locations (B)	40.99	19.83	14.02
Factor (A X B)	N/A	52.47	37.11

Table No.4.18 Influence of North and South Agro-climates on Number of Male Flowers of Aonla.

4.6.13 Number of Female/Branch

The number of female plants varied significantly among the different varieties and between the two locations (Table No.4.18). The highest mean number of female plants was observed in variety NA-6 (7.67), followed by NA-5 and NA-10 (6.33 each). The lowest was found in BSR-1 (3.00), while other varieties such as Chakaiya (4.17), NA-7 (5.00), and NA-4 (5.50) showed intermediate values. In the southern location (Bangalore), the number of female plants ranged from 2.67 (BSR-1) to 8.00 (NA-6), whereas in the northern location (Ayodhya), it ranged from 3.33 (BSR-1) to 7.33 (NA-6 and NA-10). On average, the northern region (5.91) had a higher number of female plants compared to the southern region (4.95). (Table No.4.19)

Statistical analysis revealed that both varietal and locational effects were significant. The interaction effect between varieties and locations was non-significant. This suggests that the varietal performance for female plant numbers remained relatively stable across both environments. The increased number of female plants in the northern region might be attributed to environmental conditions such as photoperiod, soil moisture, or nutrient availability, which favor female expression. Among the tested genotypes, NA-6 emerged as the most promising variety due to its consistent and highest female plant count across both regions. These findings suggested the number of females varied among varieties and locations. Similar results were found by Aulakh *et al.* (2013).

Number of Female/Branch

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	5.000	6.000	5.500
NA-5	5.667	7.000	6.333
NA-6	8.000	7.333	7.667
NA-7	4.667	5.333	5.000
NA-10	5.333	7.333	6.333
Chakaiya	3.333	5.000	4.167
BSR-1	2.667	3.333	3.000
Mean B	4.952	5.905	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	1.448	0.701	0.495
Locations (B)	0.774	0.374	0.265
Factor(A X B)	N/A	0.991	0.701

Table No.4.19 Influence of North and South Agro-climates on Female flowers of Aonla.

Number of male /female

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	73.86	73.89	73.87
NA-5	81.47	81.11	81.29
NA-6	101.89	96.22	99.06
NA-7	132.14	139.67	135.90
NA-10	98.58	74.68	86.63
Chakiaya	151.83	118.72	135.28
BSR-1	163.33	167.50	165.42
Mean B	114.73	107.40	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	32.50	15.72	11.12
Locations (B)	N/A	8.41	5.94
Factor (A X B)	N/A	22.24	15.72

Table No.4.20 Influence of North and South Agro-climates on Sex ratio of Aonla.

4.6.14 Number of Male/Female Ratio

The male to female plant ratio showed notable differences among varieties but relatively minor variation between the two locations (Table No.4.20). The highest mean ratio was recorded in BSR-1 (165.42), followed by Chakaiya (135.28) and NA-7 (135.90), indicating a strong dominance of male plants in these varieties. The lowest ratio was observed in NA-4 (73.87), suggesting a more balanced male-to-female distribution. At the southern location (Bangalore), the ratio ranged from 73.86 (NA-4) to 163.33 (BSR-1), while at the northern location (Ayodhya), it ranged from 73.89 (NA-4) to 167.50 (BSR-1). The average male/female ratio was slightly higher in the southern region (114.73) compared to the northern region (107.40), although the difference was not statistically significant.

Statistical analysis revealed a significant effect of varietal differences. However, the effect of location and the interaction between variety and location were found to be non-

significant, as no critical differences were observed for these factors, although their standard errors were recorded.

These findings indicate that the male to female plant ratio is primarily influenced by genetic variation among varieties rather than environmental conditions. Varieties like BSR-1 and Chakaiya showed an excessively high male/female ratio, which may be undesirable for fruit production in dioecious crops, where a balanced sex ratio is critical. Conversely, NA-4 showed a nearly balanced ratio and may be better suited for enhancing fruit yield.

4.6.15 Estimated Yield per Plant (kg)

The estimated yield per plant exhibited considerable variation among the varieties, while the differences between locations were relatively small (Table No.4.21). The highest mean yield was recorded in variety NA-4 (81.50 kg), followed by NA-5 and NA-7 (both 75.67 kg), indicating good yield potential. On the other hand, BSR-1 recorded the lowest mean yield (52.67 kg), mainly due to a significant drop in yield at the northern location. At the southern location (Bangalore), yield values ranged from 60.00 kg (NA-10) to 77.00 kg (NA-4), whereas at the northern location (Ayodhya), they varied from 36.00 kg (BSR-1) to 86.00 kg (NA-4). The average yield across all varieties was nearly identical in both regions, with 69.24 kg in the south and 69.14 kg in the north, indicating a minimal location effect.

Statistical analysis revealed that the effect of varieties was significant. However, the effect of location was non-significant, suggesting that environmental differences between the two sites did not strongly influence overall yield performance. The interaction effect (A × B) was found to be significant, with a critical difference of 14.45, indicating that the performance of some varieties varied between locations.

Notably, variety BSR-1 showed a marked decline in yield in the northern location (36.00 kg) compared to the southern location (69.33 kg), whereas NA-4 showed consistent and high performance across both locations. Such genotypic stability makes NA-4 a promising variety for yield enhancement under varying environmental conditions.

Estimated Yields/plant (kg)

	Locations		
Varieties	**B1 (South)**	**B2 (North)**	**Mean A**
NA-4	77.00	86.00	81.50
NA-5	73.33	78.00	75.67
NA-6	66.33	74.67	70.50
NA-7	71.00	80.33	75.67
NA-10	60.00	61.67	60.83
Chakiaya	67.67	67.33	67.50
BSR-1	69.33	36.00	52.67
Mean B	69.24	69.14	
	C.D.	**SE(d)**	**SE(m)**
Varieties (A)	10.22	4.94	3.50
Locations (B)	N/A	2.64	1.87
Factor (A X B)	14.45	6.99	4.94

Table No.4.21 Influence of North and South Agro-climates on Yields of Aonla.

4.7 STANDARDIZATION OF DNA EXTRACTION PROTOCOL

DNA isolation was standardized using young leaf following the CTAB protocol. DNA was extracted from 21 Aonla varieties maintained in the division of fruit crops at IIHR, Hessaraghatta, and 21 Aonla varieties ANDU&T Kumargunj Ayodhya. DNA was quantified using a spectrophotometer and gel electrophoresis. DNA quality and yield were checked using a spectrophotometer and agarose gel electrophoresis. DNA quality was good, and the absorbance values ranged from 1.7 to 2.0. The DNA isolation protocol was good, resulting in good-quality DNA.

4.8 OPTIMIZATION OF PCR PROTOCOL FOR IISR MARKER ANALYSIS

4.8.1 PCR protocol

Optimization was done for different components of PCR such as

a) Template DNA concentration

b) Primer concentration

c) DNTP concentration

d) Taq DNA polymerase concentration

e) Primer annealing temperature

List of Nano reading of DNA of selected varieties of Aonla.

Sample number	Varieties	A260/A280	DNA yield (ng/µl)
1	NA-4	1.7	2427
2	NA-5	1.7	2830
3	NA-6	1.8	2054
4	NA-7	1.8	1790
5	NA-10	1.8	2130
6	BSR-1	1.8	2240
7	Chakaiya	1.7	1854

4.8.2 The PCR protocol is as follows

Components	**Concentration** / µl
Template DNA (80/µl)	2.5 µl
Primer (10 Pm)	2.5 µl
DNTP's (2.5 mM TAKARA)	2 µl
Taq Polymerase (5 U/µL TAKARA)	0.50 µl
10X Buffer with $MgCl_2$ (TAKARA)	2 µl
Milliq Water	4 µl
Total mixture	13.50 µl

4.8.3 PCR Thermal Profile

a)	Initial denaturation	94°C for 4 min
b)	Denaturation	94°C for 45 sec
c)	Primer annealing	"C for 45 sec
d)	Primer extension	72°C for 1 min
e)	Cycles	40 Cycles
f)	Final extension	72°C for 8 min

4.9 ISSR FINGERPRINTING

Of the 90 primers that were tested, the 10 ISSR primer produced reproducible and consistent bands. Primers 855, 856, 857, 852, 823, 834, 908, 888, 865, 844 produce polymorphic markers.

Table No.4.22 List of ISSR primers with sequence used in analysis

Sr. N.	primer	Sequence of the primers (5'-3')
1	852	TCT CTC TCT CTC TCT CRA
2	855	ACA CAC ACA CAC ACA CYT
3	856	ACA CAC ACA CAC ACA CYA
4	857	ACA CAC ACA CAC ACA CYG
5	823	TCT CTC TCT CTC TCT CC
6	888	BDB CAC ACA CAC ACA CA
7	908	GCC GCC GCC GCC GCC
8	834	AGA GAG AGA GAG AGA GYT
9	865	CCG CCG CCG CCG CCG CCG
10	844	CTC TCT CTC TCT CTC TRC

Table No.4.23: Amplification products produced by different primers for Aonla varieties

Sr. N.	ISSR	No. of monomorphic bands	No. of polymorphic bands	Total bands	Polymorphism %	Size of the band
1.	UBC-855	1	11	12	91.66	100-1400bp
2.	UBC-856	5	4	9	44.44	200-1300bp
3.	UBC-857	4	3	7	42.85	200-1300bp
4.	UBC-852	6	4	10	40	200-1300bp
5.	UBC-823	3	6	9	66.66	200-1000bp
6.	UBC-834	3	9	12	75	200-1300bp
7.	UBC-908	3	7	10	70	300-1300bp
8.	UBC-888	0	10	10	100	300-1300bp
9.	UBC-865	2	6	8	75	300-1200bp
10.	UBC-844	2	5	7	71.42	300-1000bp
Total	10	29	65	94	67.70%	100-1400bp

A majority of the ISSR primers tested exhibited monomorphic banding patterns, although some primers produced distinct polymorphic bands, with a few being specific to certain genotypes. These findings demonstrate the utility of ISSR markers in revealing genetic variability among different Aonla *(Emblica officinalis)* cultivars.

The ISSR primer UBC-855 generated a total of nine amplification products ranging in size from 100 to 1400 base pairs (bp). A 200 bp fragment was consistently present across all cultivars, indicating its conserved nature. Additional bands at 900 bp, 800 bp, 600 bp, 400 bp, 300 bp, and 100 bp were also commonly detected in most genotypes. However, BSR-1 displayed a distinct banding pattern, lacking several of these commonly shared fragments. While bands between 100 and 400 bp were observed in BSR-1, these were not unique, as they were also present in other cultivars.

Primer UBC-856 also produced nine bands, including five monomorphic and four polymorphic bands of 200 bp, 400 bp, 600 bp, and 900 bp, which were shared across all cultivars. Notably, a 1300 bp fragment was absent in NA-4 and NA-5, though it appeared in the remaining genotypes. Meanwhile, 1000 bp and 500 bp bands were universally present in all accessions except for BSR-1, further highlighting its unique genetic profile.

For primer UBC-857, a total of seven bands were amplified, of which four were monomorphic and three polymorphic. A 1300 bp fragment was observed in all varieties except NA-4 and Chakaiya, suggesting the absence of this locus in these two cultivars. A 1000 bp band was detected exclusively in NA-10, indicating possible unique allele presence. The 800 bp band was found in most cultivars but was absent in NA-4 and Chakaiya, suggesting partial differentiation among these accessions.

Primer UBC-852 generated mostly monomorphic bands, with four polymorphic bands observed exclusively in genotype BSR-1. At 900 bp, bands were present only in NA-5, Chakaiya, and BSR-1. Primer UBC-823 amplified a total of nine bands, including six polymorphic and three monomorphic bands. A 900 bp band was common in NA-5, NA-6, NA-7, and NA-10, but absent in NA-4 and Chakaiya. The 800 bp band was found across all varieties except NA-7 and BSR-1. At 700 bp, bands were observed only in NA-10 and Chakaiya. Bands

at 600 bp and 500 bp were commonly found across all varieties, except in NA-4 and BSR-1, where the 600 bp band was absent, and in NA-4 and NA-7, where the 500 bp band was missing.

Primer UBC-834 produced a total of 12 bands, out of which nine were polymorphic and three monomorphic. Chakaiya exhibited only a single band at 1300 bp. At 1200 bp and 1000 bp, bands were present in NA-5, NA-6, NA-10, and Chakaiya. A band at 900 bp was observed in NA-5, NA-6, NA-7, NA-10, and Chakaiya. At 700 bp, three bands were found in NA-5, NA-6, and NA-10. No bands were observed at 500 bp and 300 bp in NA-4 and BSR-1, while these bands were present in all other varieties. At 400 bp, bands were detected in NA-4, NA-5, NA-7, and Chakaiya.

Primer UBC-908 generated 10 bands in total, including seven polymorphic and three monomorphic bands. Bands at 1100 bp, 1200 bp, and 1300 bp were consistent across all genotypes except Chakaiya, where the 1300 bp band was absent. Bands at 1000 bp and 900 bp were found in all genotypes except BSR-1 (absent at 1000 bp) and NA-7 (absent at 900 bp). At 800 bp, bands were present only in NA-4 and Chakaiya. The 700 bp band was detected in all varieties except NA-5 and BSR-1. At 500 bp, two bands were recorded in NA-4 and NA-5, but were absent in the other varieties.

ISSR primer UBC-888 generated a total of 13 loci ranging from 100 bp to 1300 bp across seven varieties. 11 loci showed polymorphism, while two loci (200 bp and 100 bp) bands were absent in all varieties. This primer's high degree of polymorphism indicates its effectiveness in assessing genetic variability. The maximum number of bands was observed in genotypes NA-5, NA-7, NA-10, Chakaiya, and BSR-1, which amplified at most loci (especially from 1300 bp to 400 bp), whereas NA-6 consistently exhibited the lowest number of bands, amplifying only at 300 bp. This displayed significant genetic divergence of NA-6 from the other genotypes. The polymorphic bands included loci at 1300 bp, 1200 bp, 1100 bp, and 300 bp, among others. The 1100 bp locus, for instance, was specifically absent in NA-4, NA-6, and NA-7 but present in NA-5, Chakaiya, and BSR-1, indicating its potential as a genotype-specific marker. Similarly, the unique amplification at 300 bp in NA-6 (and absence in all others) supports its distinct identity within the genetic pool. The percentage of polymorphic bands (PPB) for primer UBC-888 was calculated to be 100 % polymorphism.

Primer UBS-865 generated total 8 bands, which is range from 300 bp to 1200 bp. A single band was present in NA-5 and NA-10 at 1200 bp, 1000 bp. At 900 bp and 800 bp presents only 2 bands in na-5 and NA-10, while absents in all varieties. A similar bands was presents in all varieties at 700 bp and 600 bp except in NA-6, AN-7 and, a bands was absents in NA-7 at 600 bp.

Primers UBC-844 produced a total of 7 bands, 5 of which was polymorphic and 2 of which bands was monomorphic. The band's size is ranged from the 200bp to 1000 bp. Most of the bands was present at 400 bp only absent in NA-10. The 1100 bp, 800 bp and 500bp present same bands in NA-7, NA-10 and Chakaiya whereas absents in others varieties while at 500 bp also present in BSR-1.

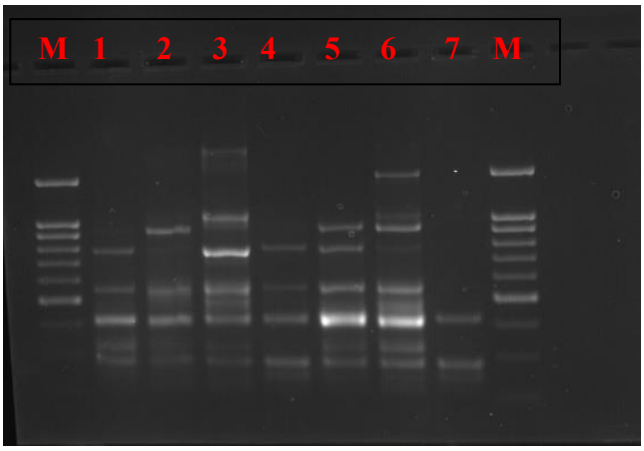


Plate1: ISSR Profile of Aonla varieties using UBC 855 primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

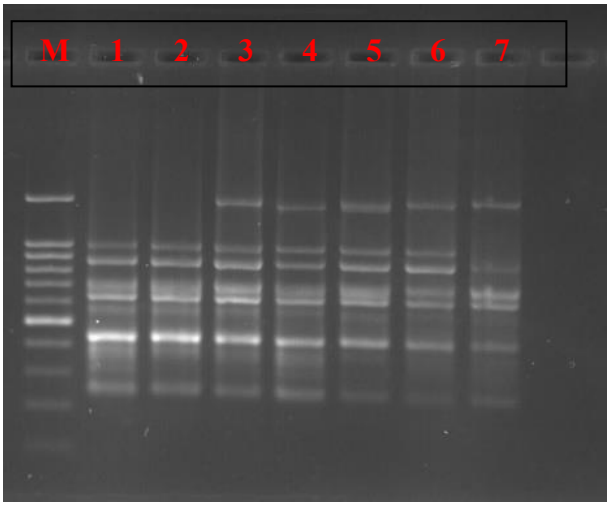


Plate 2: ISSR Profile of Aonla varieties using UBC 856 primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

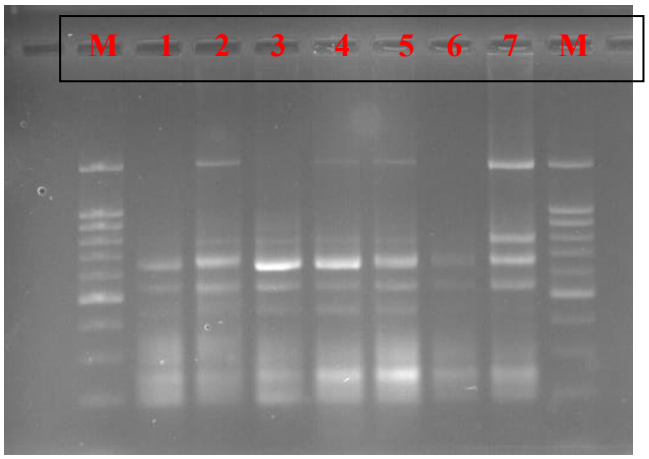


Plate 3: ISSR Profile of Aonla varieties using UBC 857primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

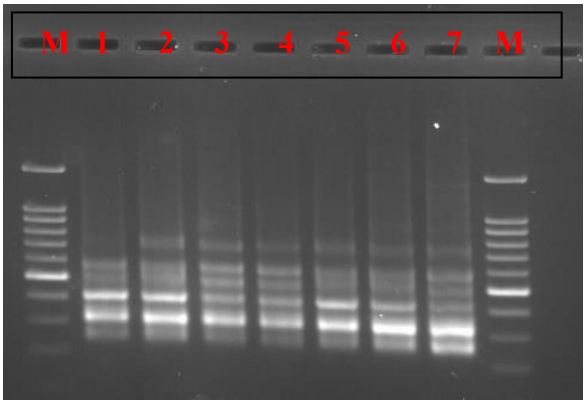


Plate 4: ISSR Profile of Aonla varieties using UBC 852primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

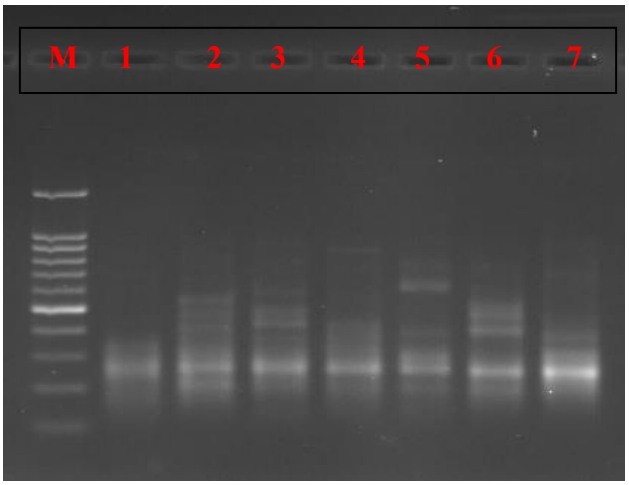


Plate 5: ISSR Profile of Aonla varieties using UBC 823 primers,M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

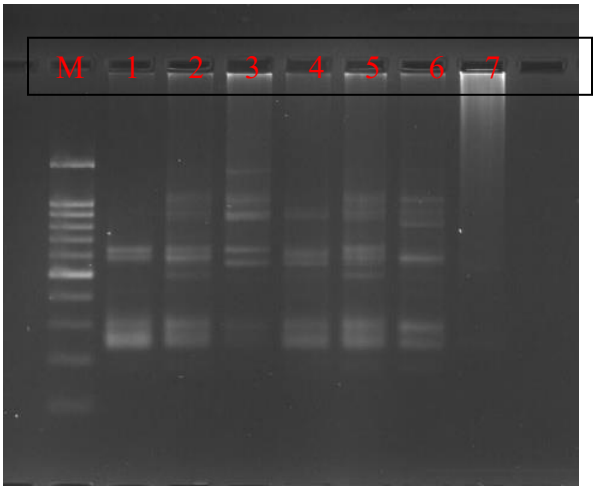


Plate 6: ISSR Profile of Aonla varieties using UBC 834primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

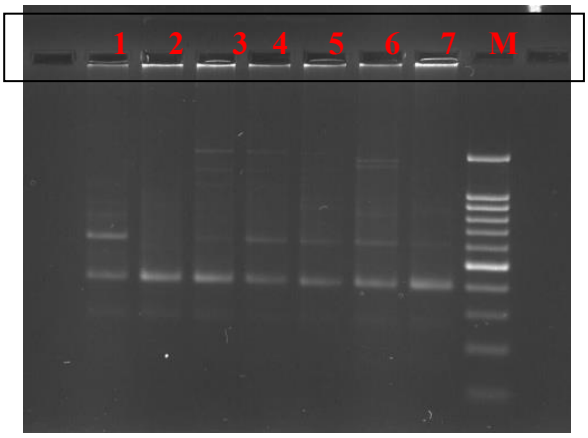


Plate 7: ISSR Profile of Aonla varieties using UBC 908 primers,M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

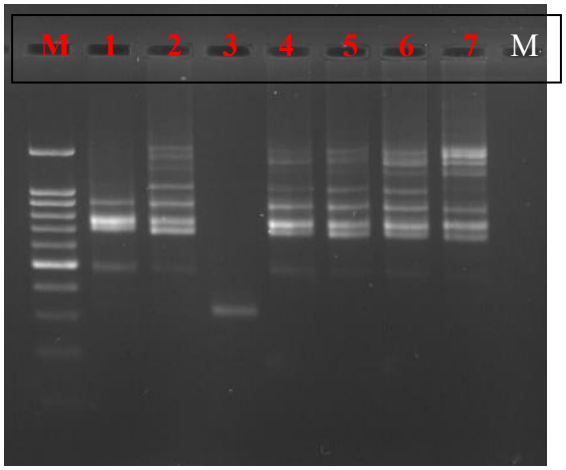


Plate 8: ISSR Profile of Aonla varieties using UBC 888 primers,M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

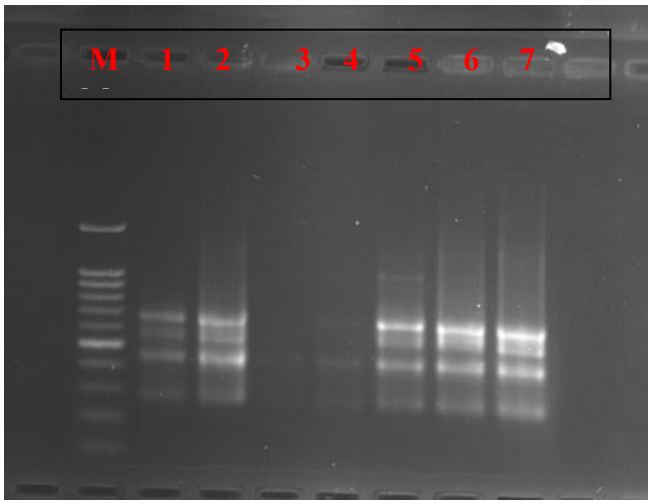


Plate 9: ISSR Profile of Aonla varieties using UBC 865 primers,M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

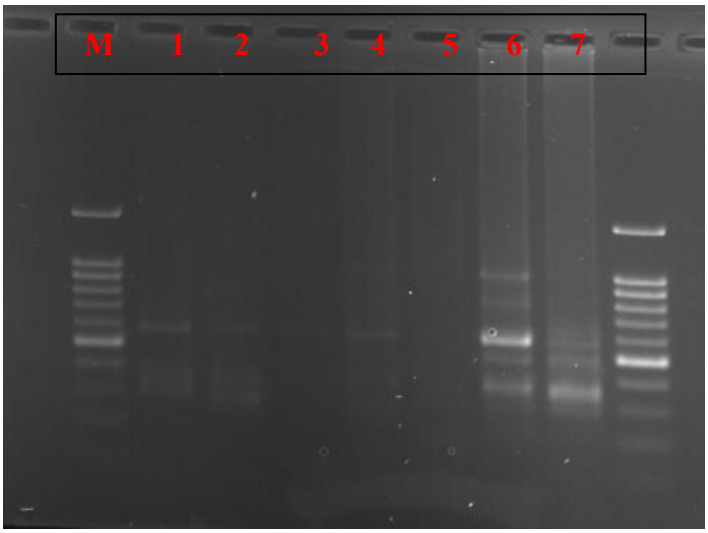


Plate 10: ISSR Profile of Aonla varieties using UBC 844 primers, M-medium, 1-NA-4, 2-NA-5, 3-NA-6, 4-NA-7, 5-NA-10, 6-Chakaiya, 7-BSR-1.

4.10 Genetic relationship analysis using a dendrogram based on ISSR Analysis

A dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to assess the genetic relationships among seven Aonla (*Emblica officinalis*) varieties based on ISSR marker data. The genotypes included were NA-4, NA-5, NA-6, NA-7, Chakaiya, NA-10, and BSR-1.

The dendrogram displayed the 7 varieties of Aonla in two major clusters. The first major cluster exhibited 6 varieties, which were further divided into two sub-clusters. Sub-cluster I-A displayed Chakaiya, NA-10, NA-5, presented close genetic affinity, while sub-cluster I-B included NA-7, NA-6, and NA-4, which were grouped together based on their moderate genetic similarity. Cluster II contained only the genotype 'BSR-1', which appeared on a distinct branch, showing the greatest genetic distance from the other genotypes. BSR-1' is the most genetically distinct and may carry unique alleles or characteristics. The scale at the bottom of the dendrogram (ranging from 0.0 to 0.1) represents genetic dissimilarity; greater distances between branches reflect higher genetic variation. A similar investigation on the genetic diversity of Aonla by RAPD markers was studied by Singh *et al* (2014). They observed distinct differences among the varieties. Dinh HaTran *et al.* (2025) reported genetic diversity of peach (*Prunus persica*) accessions collected in northern Vietnam using ISSR Markers. They found that there was clear variation between varieties.

Overall, this clustering pattern highlights the presence of considerable genetic diversity among the studied Aonla cultivars, which can be utilized in selection and hybridization programs to enhance crop improvement efforts.

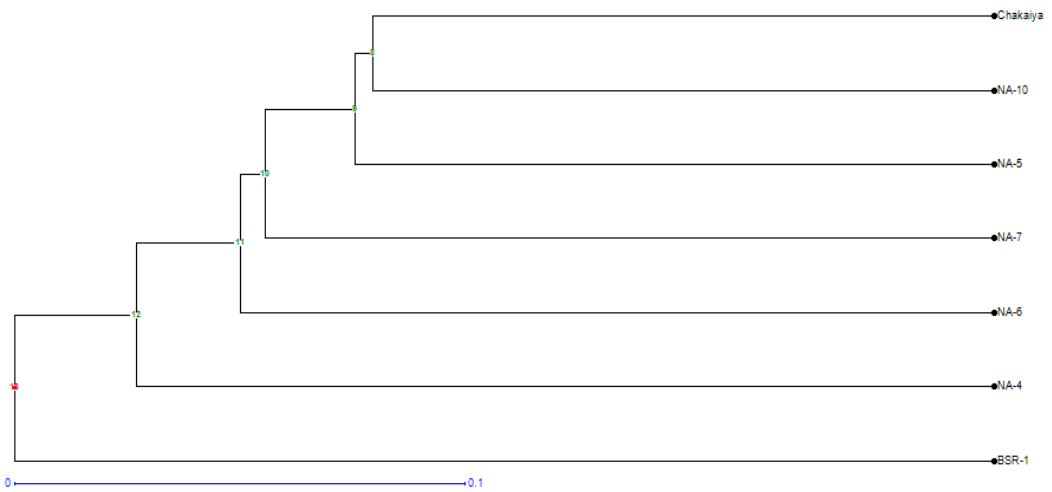


Plate No. 11: Dendrogram displaying inter-relationships among 7 varieties of Aonla using 10 ISSR primers.

DNA fingerprinting is an extensively utilized approach for detecting multiple highly polymorphic loci within the genome simultaneously. It serves as an essential tool in diverse fields of plant science, including genotype identification, population genetics, taxonomy, and disease detection. In the current study, a PCR-based DNA fingerprinting technique was adopted, which involved amplifying specific DNA regions using oligonucleotide primers and thermostable DNA polymerase in a thermal cycler. Among the available molecular marker techniques, Inter-Simple Sequence Repeat (ISSR) markers were selected.

ISSRs are dominant multilocus markers that target microsatellite regions and do not necessitate prior knowledge of the genome. These markers enable direct amplification of genomic DNA by anchoring primers to simple sequence repeats. Ratnaparkhe *et al.* (1998) initially showcased the usefulness of ISSRs in tagging genes and associating markers with traits of interest. Additionally, Arens *et al.* (1995) and Broun and Tanksley (1996) reported that SSRs often aggregate in certain genomic regions rather than being randomly distributed, which adds value to their application in genetic studies.

Compared to SSRs, RFLPs, and RAPDs, ISSR markers offer broader genomic coverage, ease of use, cost-effectiveness, and the capacity to process numerous samples efficiently. Unlike RFLPs, ISSRs do not rely on radioactive probes or large DNA quantities. Furthermore, their reproducibility surpasses that of RAPDs due to stringent PCR conditions, which include using longer primers (16 to 25 bp) and higher annealing temperatures (45–60°C).

Though the ISSR method shares similarities with RAPD, its use of microsatellite-based primers ensures greater specificity. Simon and Muehlhauser (1997) demonstrated that ISSR amplification can deliver multi-locus data and noted that the detection of markers near target genes depends on the distribution and density of microsatellites within the genome.

The effectiveness of ISSRs in marker identification is influenced by genome size, the number of primers used, and sequence divergence near the targeted regions. These markers are particularly useful in generating sequence-tagged sites, increasing marker density, and identifying molecular markers. Despite their benefits, ISSRs also present certain challenges, such as difficulty in confirming homology of bands with similar size, lengthy optimization procedures, and ambiguity about the exact genetic basis of polymorphic bands. Nonetheless, ISSR is now widely recognized as an efficient and reliable technique for genetic profiling in numerous plant species.

Kijas *et al.* (1997) indicated that although microsatellite marker development is underway in several species including citrus, the progress is relatively limited. Moriguchi *et al*. (1998) stated that most co-dominant markers being developed are sequence-based, but in citrus, the availability of such data remains limited despite advances in genome sequencing. While RFLPs don't need prior sequence data, they are labor-intensive, time-consuming, and may involve radioactive materials.

Since RAPDs are mainly dominant markers already employed in population mapping, a system capable of detecting co-dominant loci without prior sequence data was required. To address this, Cai *et al*. (1994) proposed ISSRs as a new approach for identifying polymorphisms useful in genetic mapping. This method provided a simpler way to enhance genetic linkage maps, particularly in crops like citrus.

ISSR analysis has shown great promise in crops including citrus and Gladiolus. In the present study on Gladiolus cultivars, out of 90 ISSR primers tested, ten exhibited high polymorphisms, highlighting their efficiency in revealing genetic diversity. The ISSR-PCR products were reproducible and distinct, and bands were selected based on clarity, intensity, and consistency. All ten primers demonstrated 100% polymorphism, producing 94 distinct bands.

Zietkiewicz *et al.,* (1994) found that (CA) n primers, with or without 5′ or 3′ anchors, could amplify multiple loci, corresponding to microsatellite regions of various lengths and positions in the genome. One key advantage of ISSR is its simplicity—primers can be sourced from published studies or designed from existing sequence databases. These primers need not be locus-specific, as they bind to any region containing complementary microsatellite motifs.

According to Wu *et al.,* (1994), ISSRs can be considered co-dominant due to their microsatellite origin, which allows for co-dominant inheritance when analyzed as sequence-tagged sites or random amplified microsatellite polymorphisms.

Each of the ten primers used in the study was assigned a specific annealing temperature ranging from 48°C to 64°C to ensure optimal amplification and consistent band patterns. These conditions might vary for different plant species. In Aonla, the complete polymorphism observed across all bands confirmed high genetic variability, reinforcing the effectiveness of ISSR markers in varietal differentiation.

The polymorphism rate (67.70%) obtained in this study was higher than those reported for RFLPs and RAPDs by Nagaoka and Ogihara (1997), suggesting the superior efficiency of ISSRs in genetic diversity analysis. The findings also exhibited a Mendelian inheritance pattern for ISSR loci and showed that these markers are well distributed across the wheat genome. Their use in selective genotyping enhances their value for gene tagging and genetic mapping. Due to their longer primers, higher annealing temperatures, simplicity, rapid data generation, and predictable inheritance, ISSRs are emerging as highly efficient tools for plant genome analysis. In this investigation, ISSR markers proved highly effective in assessing genetic diversity among Aonla genotypes. Cluster analysis using ISSR data enabled the development of dendrograms to visualize genetic relationships among seven Aonla varieties. The results revealed complete

polymorphism and considerable genetic variability, especially among genotypes such as BSR-1, NA-4, NA-6, NA-5, NA-7, NA-10, and Chakaiya. The observed clustering patterns accurately reflected the genetic relationships among the varieties, confirming that ISSRs are dependable tools for identifying, characterizing, and distinguishing Aonla cultivars, and they hold potential for similar applications in other plant species.

(A) Aonla trees from ICAR-IIHR region

(B) Aonla trees from ANDUA&T region

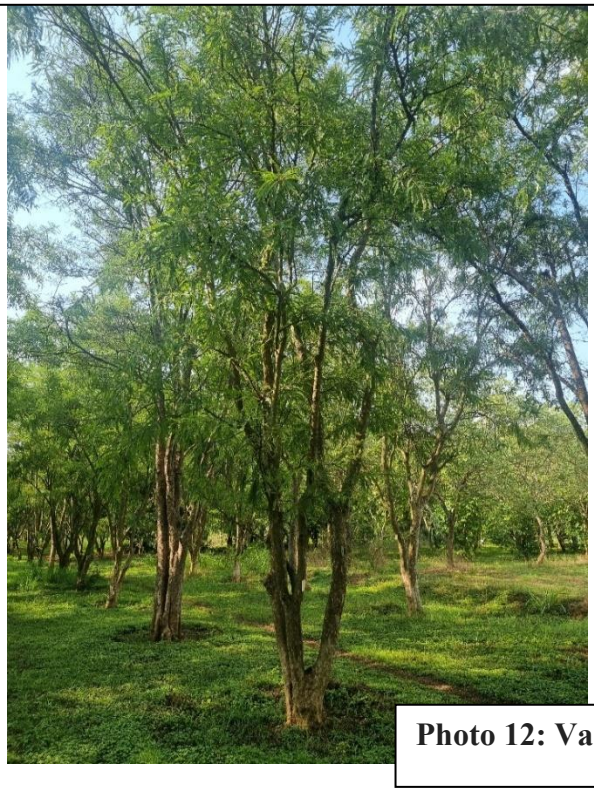


Photo 12: Var. NA-4(Kanchan)

Photo 13: Var. NA-5(Krishna)

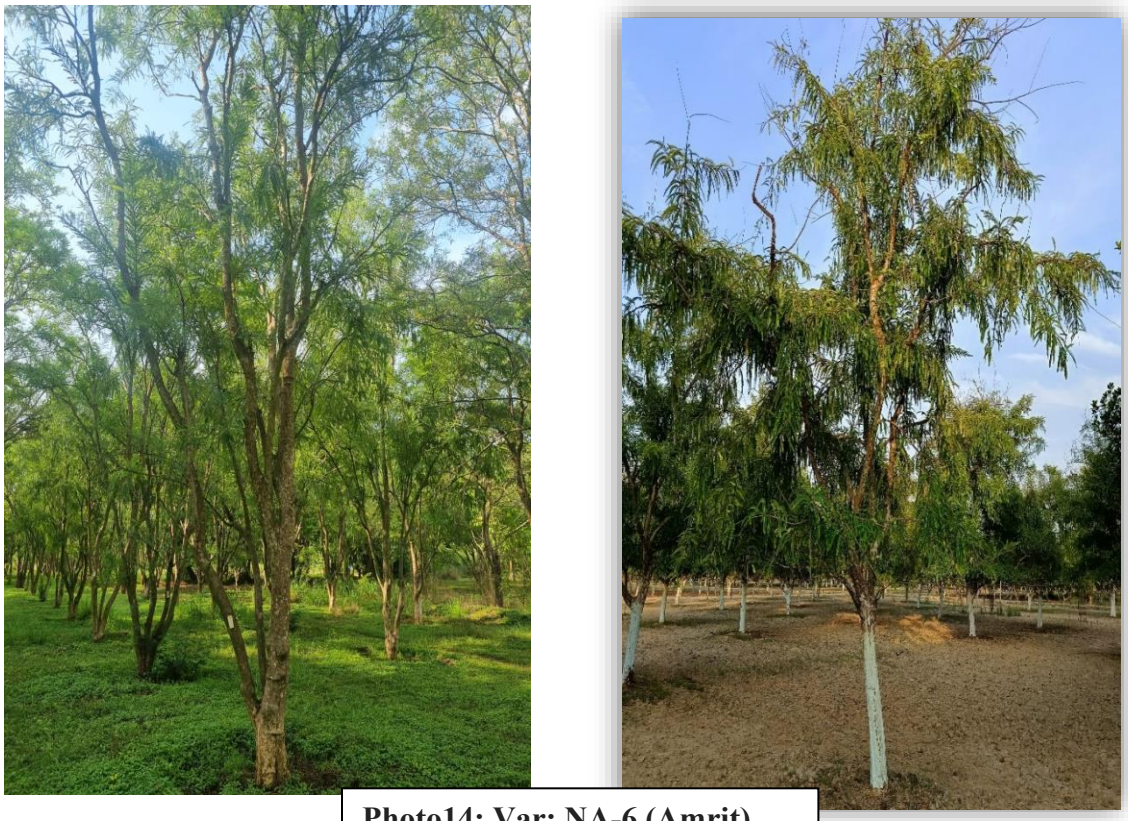


Photo14: Var; NA-6 (Amrit)

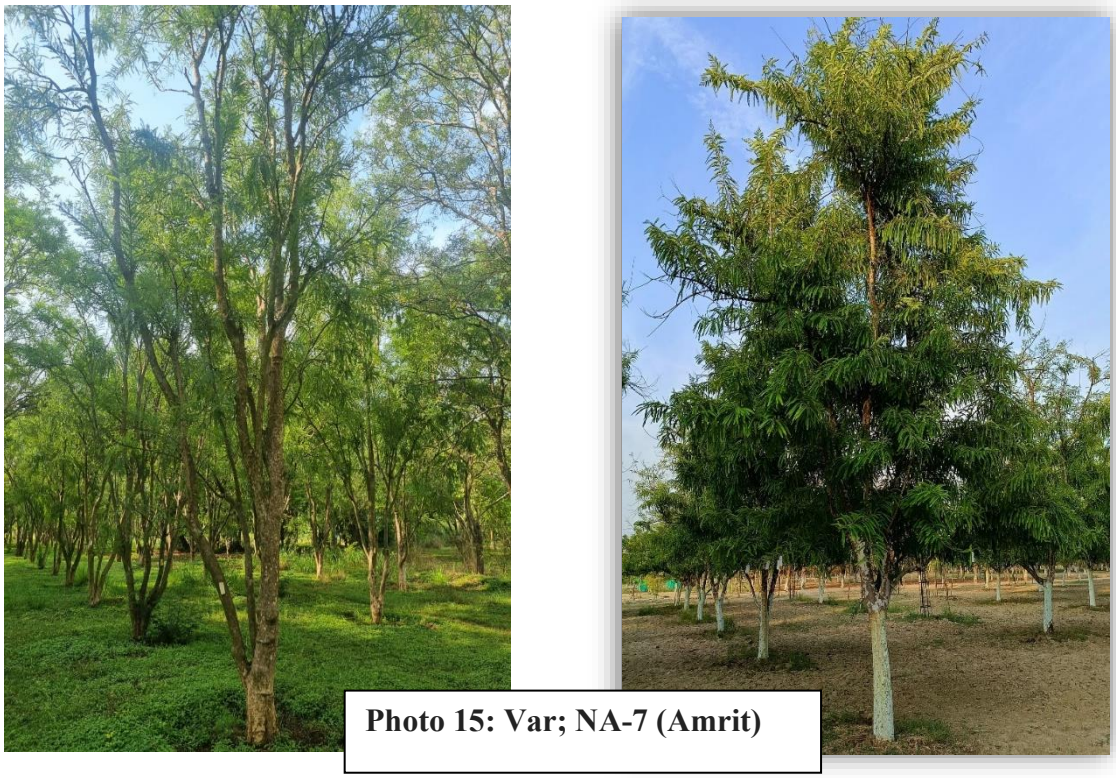


Photo 15: Var; NA-7 (Amrit)

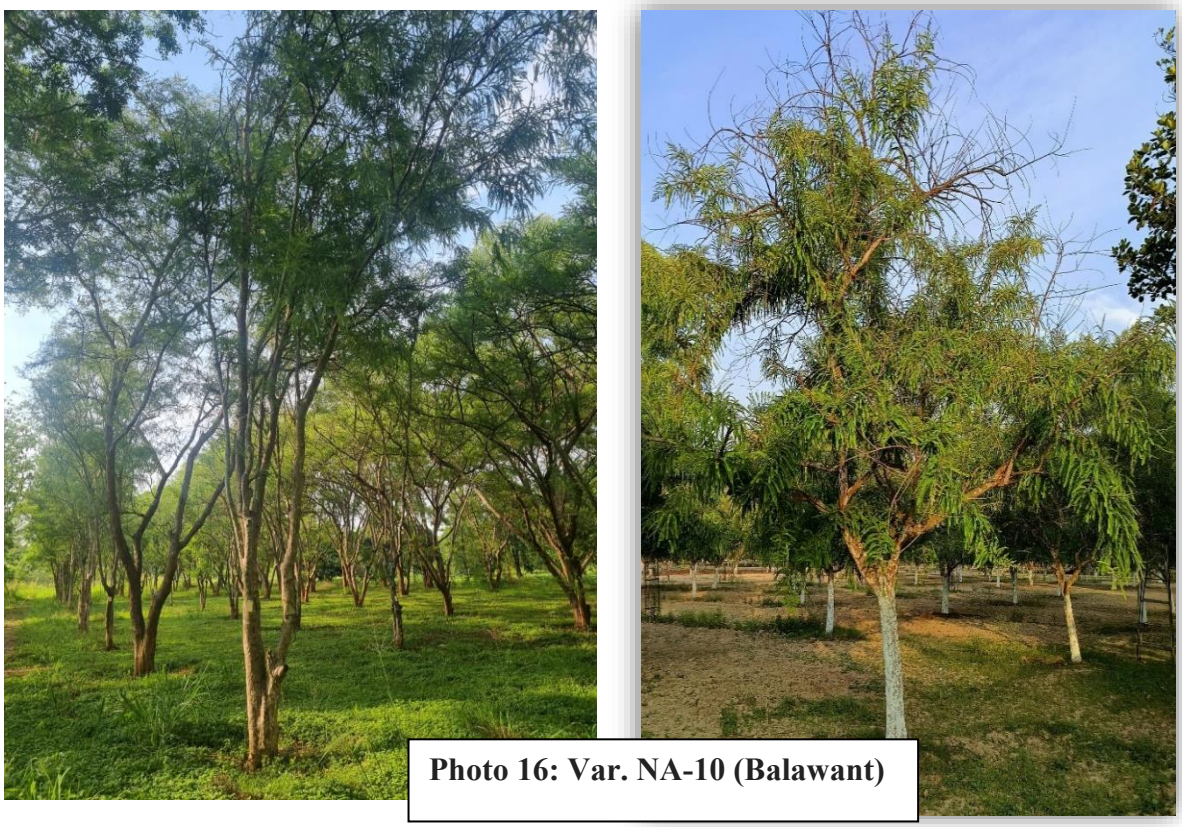


Photo 16: Var. NA-10 (Balawant)

Photo 17: Var. Chakaiya

Photo 18: Var. BSR-1 (Bhavanishagar)

Plate 12: Different varieties of Aonla tree undertaken for this experiment from two different regions of India (A) trees from ICAR-IIHR, Bangalore and (B) trees from ANDUA&T, Ayodhya

Photo 19: Flowers from ICAR-IIHR

Photo 20: Flowers from ANDUA&T, Ayodhya

Photo 21: Flowers from ICAR-IIHR

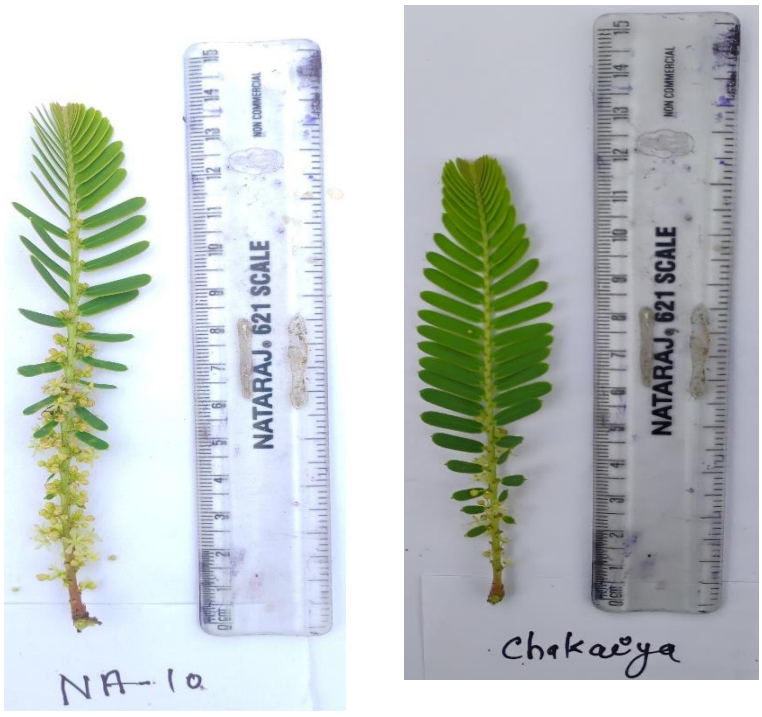


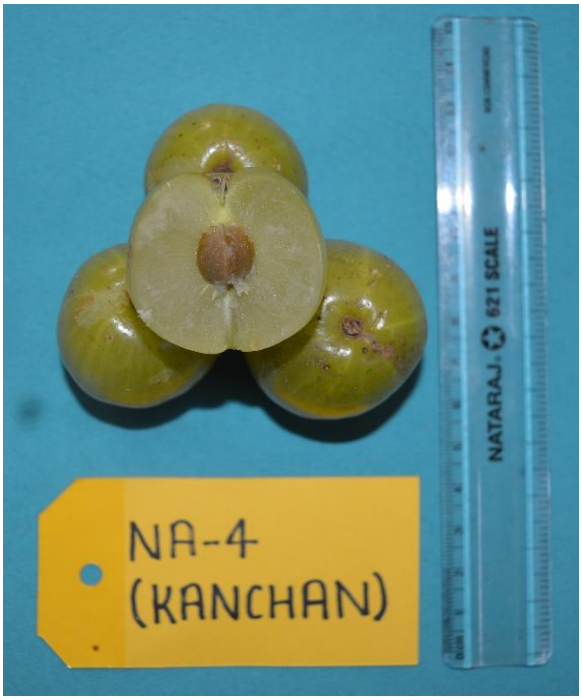
Photo 22: Flowers from ANDUA&T, Ayodhya

Photo 23: Flowers from ICAR-IIHR

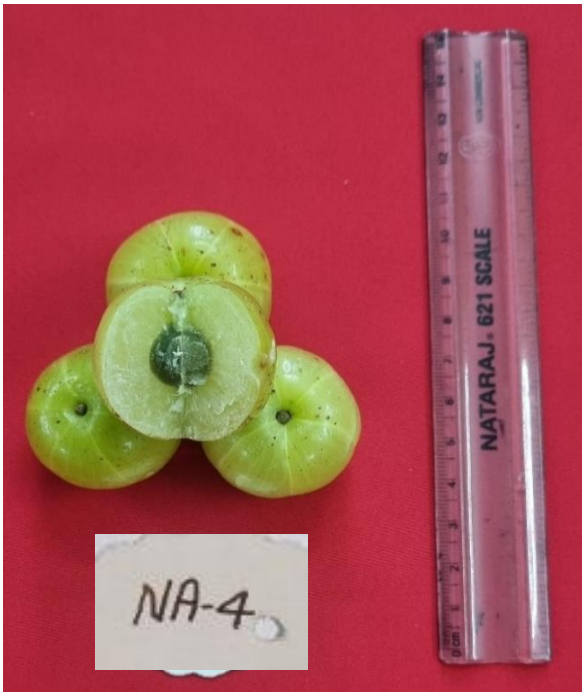
Photo 24: Flowers from ANDUA&T, Ayodhya

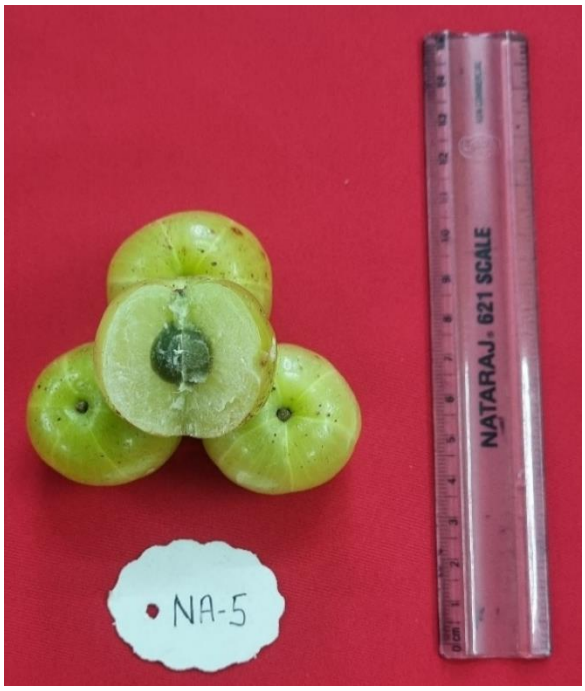
Plate 13: Differences of the flowers undertaken for this experiment from two different regions in India.

(A)- Fruits from ICAR-IIHR region



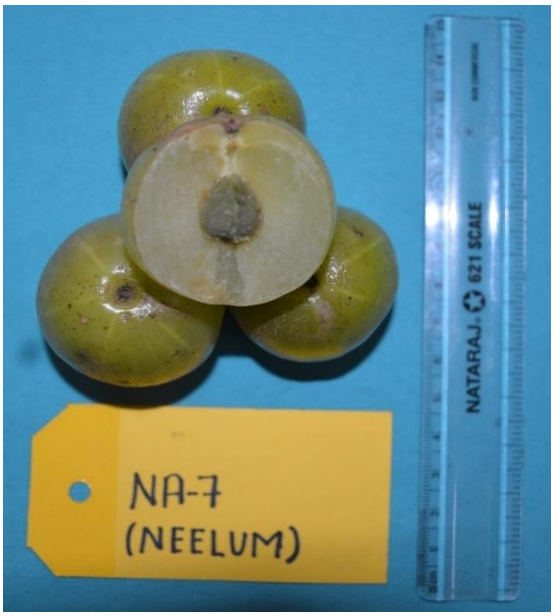
(A)- Fruits from ANDUA&T, Ayodhya






NA-6
(AMRIT)
NATARAJ. 621 SCALE


NA-6
NATARAJ. 621 SCALE


NA-7
(NEELUM)
NATARAJ. 621 SCALE


NA-7
NATARAJ. 621 SCALE


NA-10
NATARAJ. 621 SCALE


NA-10
NATARAJ. 621 SCALE


CHAKAIYA
NATARAJ. 621 SCALE


CHAKAIYA
NATARAJ. 621 SCALE

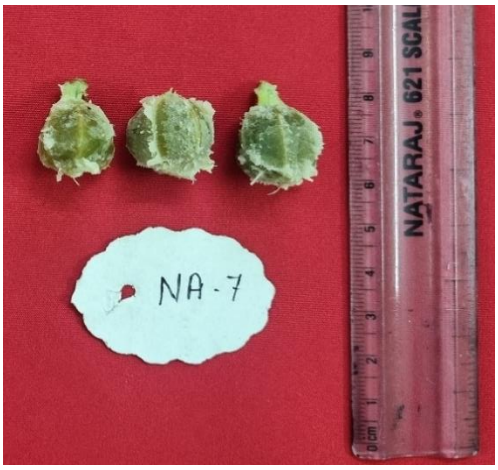
photo 25: Fruits from ICAR-IIHR & ANDUA&T Ayodhya

Plat 14: Differences of Aonla fruits undertaken for this experiment from two different region in India.

(A)- Seeds from ICAR-IIHR region

(A)- Seeds from ANDUA&T, Ayodhya regions


NA-7
NATARAJ 621 SCALE


NA-7
NATARAJ 621 SCAL


NA-10
NATARAJ 621 SCA


NA-10
NATARAJ


CHAKAIYA
NATARAJ 621 SCAL


CHAKAIYA
NATARAJ 621 SC

Photo 26: Seeds from ICAR-IIHR & ANDUA&T Ayodhya

Plat 15: Differences of Aonla seeds undertaken for this experiment from two different region in India.

Chapter- V

SUMMARY AND CONCLUSION

The present investigation entitled **"Impact of climate on morphological characteristics, yields and molecular profiling's of Aonla varieties"** was conducted at the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore, from 2024-2025 and ANDUA&T Kumargunj Ayodhya with 7 varieties of Aonla to document information on morphological characteristics and Molecular works.

6.1 Morphological Parameters

Trees exhibited greater height in the southern location compared to the northern area, except for NA6, which showed better growth in the north (10.933 m) than in the south (5.417 m). Among the varieties, NA-7 recorded the highest tree height (11.600 m) under Bangalore conditions, while NA-4 showed the highest in Ayodhya (11.100 m).

The Assessment of seven Aonla varieties exhibited significant genotypic variations across morphological, reproductive, and fruit quality traits under both climate conditions (Bangalore and Ayodhya). Traits such as fruit drop, maturity group, fruit apex morphology, leaf traits, and bearing tendency display clear differentiation among the varieties. Varieties like NA-4, NA-5, NA-7, BSR-1, and Chakaiya were identified as promising due to their desirable traits, including less fruit drop, heavy bearing, and favorable pulp texture. These findings provide valuable information for selection in Aonla improvement programs and for cultivation under assorted agro-climatic conditions. It is advisable to seek further verification across different seasons to enhance the reliability of these observations.

Further morphological evaluations highlighted considerable variation among the varieties in fruit surface texture. A smooth surface was noted in NA-4, NA-5, NA-6, NA-7, and Chakaiya, while NA-10 and BSR-1 displayed a rough texture, which may affect consumer preference and postharvest handling.

Some differences were also noted in fruit shape, with round fruits recorded in NA-4 and NA-7, triangular forms in NA-5 and Chakaiya, flattened round shapes in NA-6 and NA-10, and oval-shaped fruits in BSR-1.

Generally fruit taste, acrid flavor was predominant in NA-4, NA-6, NA-10, and BSR-1, while acidic taste was prevalent in NA-5, NA-7, and Chakaiya. Taste is a key sensory trait and determines the fruit's taste quality for fresh consumption and processing. So it is suggested that farmers should grow NA-5, NA-7, and chakaiya for better taste and quality.

A broad spectrum of fruit color variation was documented, as characterized by Royal Horticultural Society (RHS) color codes. NA-4 showed Yellow Green 2B (YG 2B), NA-5 had Yellow Green 3C (YG 3C), NA-6 displayed a blend of Yellow Green 6 and 7, NA-7 exhibited Yellow Green 7B (YG 7B), NA-10 showed Yellow Green 9A (YG 9A), BSR-1 displayed a Green Yellow Dark (GYD) tone, and Chakaiya presented Yellow Green 4B (YG 4B). These variations of color significantly contribute to the visual attraction to people and the market classification of the fruits. For better fruit color and quality, it is suggested that farmers should grow NA-4, NA-5, and chakaiya. These are attractive to the consumers.

The highest canopy spread in the north was recorded in NA-6 and NA-7 (10.400 meters), whereas the minimum was observed in BSR-1 (7.033 meters). Based on the land area, farmers can use large canopy and small canopy varieties.

Variation in fruit stem end prominence was also clear, with NA-4, NA-5, NA-7, NA-10, and Chakaiya exhibiting a more prominent stem end, while NA-6 and BSR-1 had less pronounced ends. This trait may influence both aesthetics and stem detachment character.

The fruit stalk thickness was mostly consistent across varieties, with all varieties showing a thick stalk, except BSR-1, which had a thin stalk. This trait may affect mechanical harvesting and fruit detachment.

A marked difference was noted in fruit drop, with varieties such as NA-4, NA-5, NA-6, NA-10, BSR-1, and Chakaiya displayed lower fruit drop, indicating superior fruit retention

potential, while NA-7 exhibited the highest fruit drop, which may adversely affect its yield potential.

Based on fruit maturity group, the varieties were categorized as early (NA-5, NA-7, NA-10, and Chakaiya), mid-season (NA-4 and NA-6), and late (BSR-1). Such variation suggests the feasibility of extending the harvesting period and ensuring a continuous supply of fruits for the market and processing units. Farmers should grow early fruiting varieties like NA-5, NA-7, and NA-10 because early fruits give high values.

Significant variability was also recorded in the fruit base cavity at the stem end, where shallow cavities were observed in NA-4, NA-5, NA-6, and NA-7, while deep cavities were noted in Chakaiya, NA-10, and BSR-1. This morphological trait is important in determining the suitability of fruits for processing purposes. The fruit apex (stylar end) morphology also varied, with most genotypes (NA-4, NA-5, NA-7, BSR-1, and Chakaiya) exhibiting a flat apex and others (NA-6 and NA-10) showing a papillate apex, which may influence consumer preferences and packaging behavior.

Considerable differences were evident in fruit skin ground color, with greenish shades in NA-4, NA-5, NA-7, and BSR-1, yellowish hues in NA-6 and Chakaiya, and a transitional yellowish-green in NA-10. Similarly, the over color ranged from green tinges (NA-4, NA-6, NA-7) to white streaks (NA-5), pink tinges (NA-10 and BSR-1), and white tinge (Chakaiya). These characteristics are critical in determining fruit appeal and marketability.

Variation in pulp texture was also pronounced, with soft pulp recorded in NA-4, NA-5, NA-6, and NA-7, making them more suitable for table use, while hard pulp was observed in NA-10, BSR-1, and Chakaiya, indicating better processing quality.

Overall, the observed diversity among the varieties highlights their potential for utilization in specific horticultural purposes. These findings can serve as a valuable resource for selecting desirable traits in future breeding programs, as well as for identifying suitable cultivars for fresh consumption or processing industries.

Significant variation was observed among Aonla genotypes for key fruit traits. NA-7 consistently recorded the highest fruit length (39.047 mm), width, weight (32.161 g), and pulp weight (33.545 g), with superior performance under Bangalore conditions. Fruit length, width, and weight were significantly influenced by genotype and environment, while their interactions were non-significant, indicating stable performance across locations.

Pulp percentage showed no significant variation, with NA-6 having the highest value (96.190%), suggesting genetic control and minimal environmental influence. In contrast, the number of fruits per kg showed significant genotype × environment interaction, with BSR-1 recording the highest (73.667), influenced by its smaller fruit size and site-specific effects. These findings suggest that fruit size and weight traits are largely genotype-dependent but responsive to environmental conditions, while pulp percentage remains relatively stable.

Seeds grown under Bangalore conditions showed a higher average weight (1.427 g) than those from Ayodhya (1.207 g). Among all the treatments, the Chakaiya variety exhibited the maximum mean seed weight of 1.490 g. Chakaiya has higher weight and width was less, so pulp recovery is high in this variety, so it is suggested that farmers grow it for better pulp and higher yield in NA-4.

Although Ayodhya exhibited a slightly greater mean seed width (10.595 mm) compared to Bangalore (10.338 mm), the difference was not statistically meaningful. The variety NA-7 had the highest seed width among the treatments. These findings suggest that seed width is a relatively stable trait, unaffected by the environment. Farmers can grow Aonla in both regions, seed width is not affected by the environment, and so seed width is not a problem in any region.

A) Morphological Changes due to Climates

Environmental factors such as temperature, rainfall, humidity, and sunlight play a critical role in shaping the morphological characteristics of Aonla trees. High temperatures can cause accelerated growth, influencing tree height, canopy size, and fruit size. On the other hand, excessive heat can stress the plant, resulting in stunted growth or reduced leaf area. Rainfall patterns are equally important, as both drought and excessive water can alter the tree's

morphology, including leaf size, fruit shape, and skin texture. The nutrient availability and soil conditions, influenced by climate, also affect the plant's root development, flowering patterns, and fruit setting.

B) Climate's Impact on Yield

Aonla fruiting is directly related to climatic conditions. Optimum temperature ranges between 25°C to 35°C, along with adequate water availability, promote heavy fruit set and better development. Deviations from these conditions, such as prolonged droughts, early frosts, or unseasonal rains, can drastically reduce yield. Moreover, extreme weather events, such as cyclones or heat waves, can damage plants or cause premature fruit drop. The timing of flowering and fruiting, influenced by temperature and photoperiod, also affects the quantity and quality of the harvested Aonla.

Climate exerts a profound impact on the morphological, yield, and molecular characteristics of aonla varieties. By understanding how climate interacts with the plant at different levels, from growth patterns to genetic responses, farmers can adopt more effective cultivation practices and breeding strategies to enhance aonla productivity, quality, and resilience in the face of climate change.

6.2 Molecular markers

Molecular markers have become vital tools in assessing genetic diversity and supporting plant breeding. The present study aimed to evaluate the genetic variability among seven aonla (*Emblica officinalis* Gaertn.) varieties through ISSR marker analysis. Leaf samples were collected from two distinct locations—ICAR-IIHR, Bangalore, and ANDUA&T, Ayodhya. Using a set of ten ISSR primers (855, 856, 857, 852, 834, 823, 908, 888, 865, and 844), polymorphic bands were successfully amplified, indicating notable genetic variation. The ISSR markers proved effective in distinguishing the varieties at the DNA level. These results confirm the reliability of ISSR markers for diversity analysis and the identification of genetically distinct germplasm.

6.2.1 Molecular Characterization

Climate stress can also induce genetic and molecular changes in Aonla varieties. Molecular markers, such as DNA fingerprinting, are used to assess genetic diversity among different Aonla varieties. Research has shown that environmental stress factors like temperature and water availability can trigger changes in gene expression, leading to adaptations in growth and fruiting patterns. These molecular adaptations may involve the upregulation of stress-responsive genes, which help the plant cope with adverse conditions. Understanding these molecular responses is crucial for developing climate-resilient Aonla varieties through breeding programs.

CONCLUSION

In plants, the genetic material plays a crucial role in determining traits such as yield potential, disease resistance, fruit quality, and adaptability. It serves as the fundamental basis for crop improvement, conservation of biodiversity, and the development of new cultivars through traditional breeding and biotechnological approaches.

In conclusion, genetic material forms the foundation of all biological inheritance and is vital for the continuity of life. In plants, it governs key traits such as growth, productivity, resistance to biotic and abiotic stresses, and adaptability to various environments. Understanding and utilizing genetic material through conservation and breeding programs not only helps in the development of improved crop varieties but also ensures long-term sustainability and food security. Hence, the study and management of genetic material is of paramount importance in modern agriculture and plant science.

Future Line of this research Work

1. Genotypes showing high genetic divergence under varying climatic conditions should be explored further for hybridization programs to develop climate-resilient varieties.

2. Trait-linked molecular markers identified under specific environmental conditions can be converted into SCAR markers for climate-adaptive Marker-Assisted Selection (MAS).

3. Climate-responsive molecular profiling can support the characterization and conservation of elite varieties, aiding in variety protection strategies.

4. DNA fingerprinting of genotypes across diverse agro-climatic zones will assist in conserving biodiversity and ensuring protection under intellectual property rights (IPR) regimes.

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APPENDICES

Extraction Buffer (1000 ml 4% CTAB)

0.5M EDTA	40 ml
5M NaCl	280 ml
PVP powder	20 grams
Distilled water	500 ml
CTAB	40 g

50X TAE BUFFER (1000 ml)

Tris base	242 g
0.5 M EDTA	100 ml
Glacial Acetic acid	57 ml

TE BUFFER (PH-8)

Tris Base	13.2 g
Na2EDTA	7.4 g

LOADING DYE

Bromophenol blue	0.25 g
Glycerol	50 ml (50% con.)
Distilled water	50 ml

Ethidium bromide

10mg

ANNEXURE 1.1

ICAR-IIHR Meteorological data:

Month	**Rainfall (mm)**	**Temperature (°C)**		**Relative humidity (%)**		**Evaporation (mm)**	**Mean wind speed (km h^{-1})**
		Maximum	Minimum	08.30 hrs	13.30 hrs		
July	58.2	28.3	21.7	89.1	71.0	4.5	8.0
August	225.9	29.0	21.6	90.5	70.9	4.6	4.5
September	17.9	29.6	20.5	88.3	63.0	5.1	5.1
October	391.0	28.6	20.8	92.6	71.9	3.6	2.0
November	57.9	27.4	18.4	90.5	66.8	3.5	2.9
December	48.1	26.6	17.7	92.3	69.9	2.9	2.8

ANDUA&T Meteorological data:

Month	**Rainfall (mm)**	**Temperature (°C)**		**Relative Humidity (%)**	**Total Evaporation (mm/Day)**	**Mean wind speed (km h^{-1})**
		Maximum	Minimum			
July	11	31.6	26.6	78.8	5.6	4.8
August	13	32.5	25.1	78.4	5.1	2.1
September	11	32.4	24.7	77.1	4.5	4.0
October	--	32.6	21.4	72.7	4.42	4.1
November	--	27.8	13.1	62.1	--	1.3
December	--	20.8	11.0	70.8	--	3.1

ANNEXURE 1.2

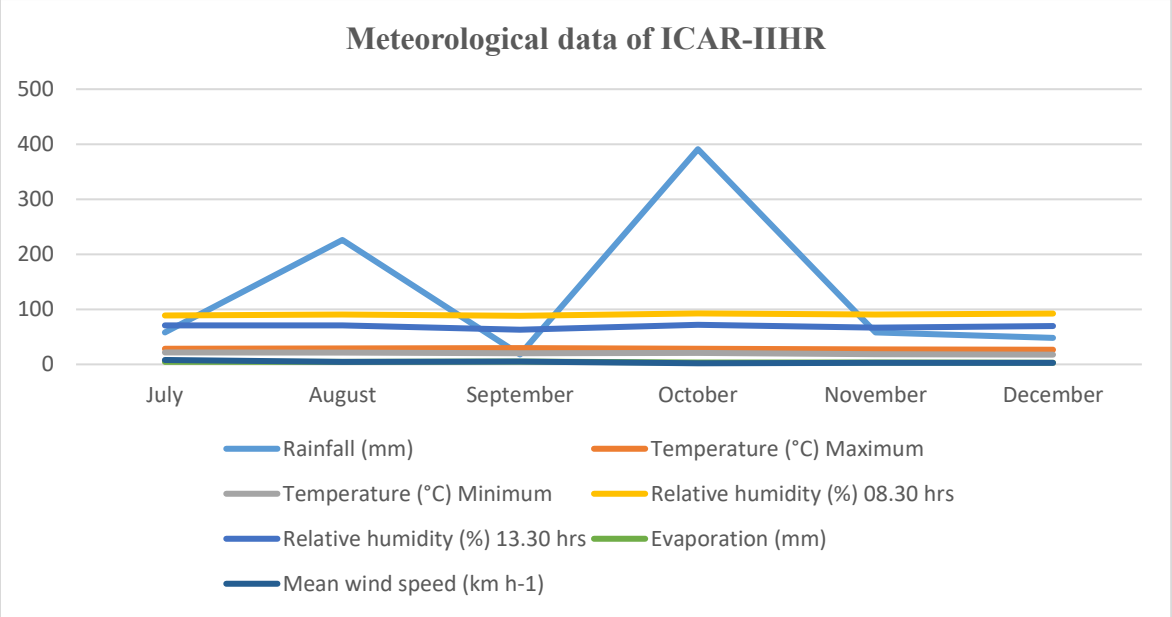

Fig: 1.1 Meteorological data of ICAR-IIHR, Bangalore

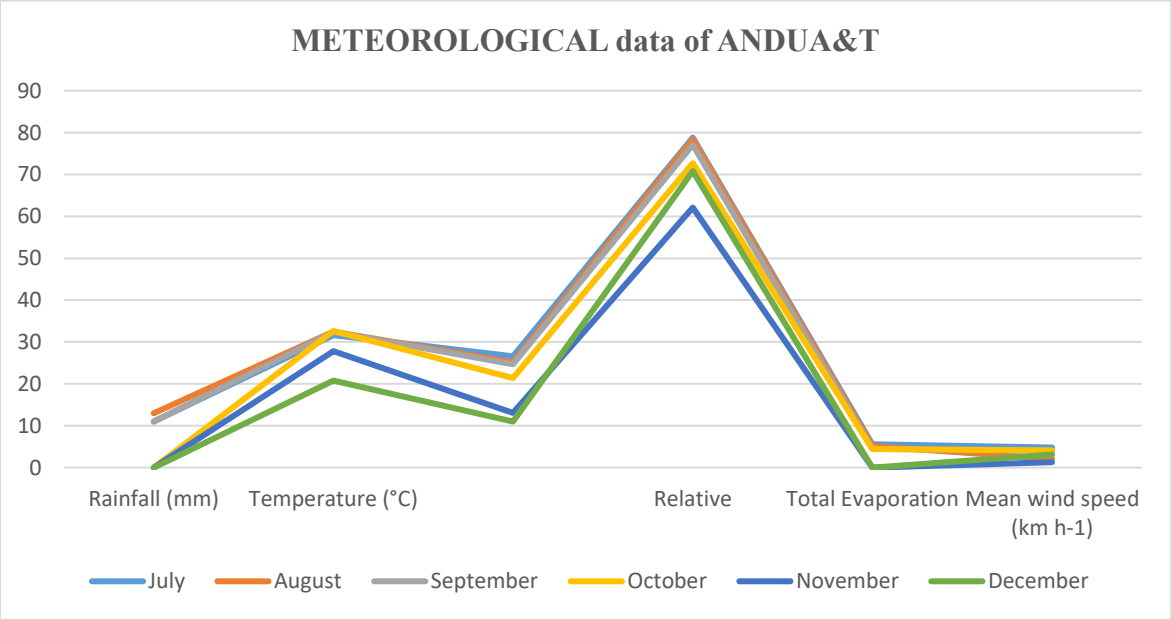

Fig: 1.2 Meteorological data of ANDUA&T, Ayodhya

Curriculum Vitae

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Examination/Degree	Year of passing	Institute	Board/University	% / OGPA
M.Sc.(Horti) Fruit Science	2025	Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, UP-224229	Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, UP-224229	7.9 OGPA
B.Sc. (Ag) Hons.	2023	Tilak Dhari P.G. College, Jaunpur	Veer Bahadur Singh Purvanchal University	7.5OGPA
Intermediate.	2015	T.D. Inter College Jaunpur	UP BOARD	74.9%
High-School	2013	Kishan Adarsh Rastriy Inter College Pratapgunj Jaunpur	UP BOARD	67.6%

APPENDICES

Extraction Buffer (1000 ml 4% CTAB)

0.5M EDTA	40 ml
5M NaCl	280 ml
PVP powder	20 grams
Distilled water	500 ml
CTAB	40 g

50X TAE BUFFER (1000 ml)

Tris base	242 g
0.5 M EDTA	100 ml
Glacial Acetic acid	57 ml

TE BUFFER (PH-8)

Tris Base	13.2 g
Na2EDTA	7.4 g

LOADING DYE

Bromophenol blue	0.25 g
Glycerol	50 ml (50% con.)
Distilled water	50 ml

Ethidium bromide

10mg

ANNEXURE 1.1

ICAR-IIHR Meteorological data:

Month	**Rainfall (mm)**	**Temperature (°C)**		**Relative humidity (%)**		**Evaporation (mm)**	**Mean wind speed (km h^{-1})**
		Maximum	Minimum	08.30 hrs	13.30 hrs		
July	58.2	28.3	21.7	89.1	71.0	4.5	8.0
August	225.9	29.0	21.6	90.5	70.9	4.6	4.5
September	17.9	29.6	20.5	88.3	63.0	5.1	5.1
October	391.0	28.6	20.8	92.6	71.9	3.6	2.0
November	57.9	27.4	18.4	90.5	66.8	3.5	2.9
December	48.1	26.6	17.7	92.3	69.9	2.9	2.8

ANDUA&T Meteorological data:

Month	**Rainfall (mm)**	**Temperature (°C)**		**Relative Humidity (%)**	**Total Evaporation (mm/Day)**	**Mean wind speed (km h^{-1})**
		Maximum	Minimum			
July	11	31.6	26.6	78.8	5.6	4.8
August	13	32.5	25.1	78.4	5.1	2.1
September	11	32.4	24.7	77.1	4.5	4.0
October	--	32.6	21.4	72.7	4.42	4.1
November	--	27.8	13.1	62.1	--	1.3
December	--	20.8	11.0	70.8	--	3.1

ANNEXURE 1.2

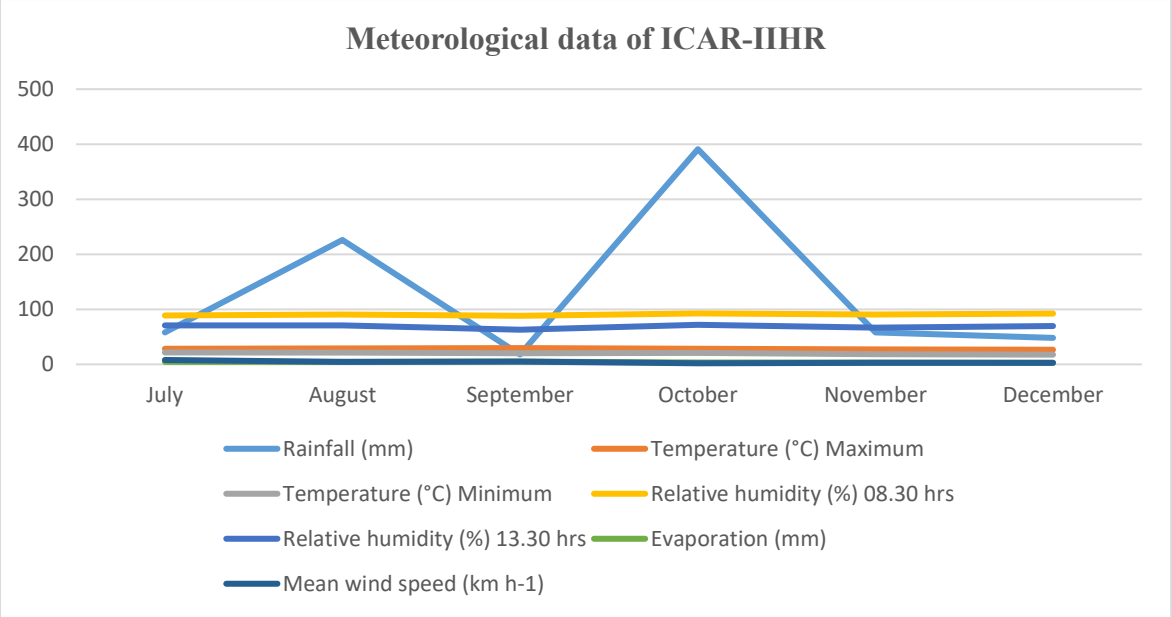

Fig: 1.1 Meteorological data of ICAR-IIHR, Bangalore

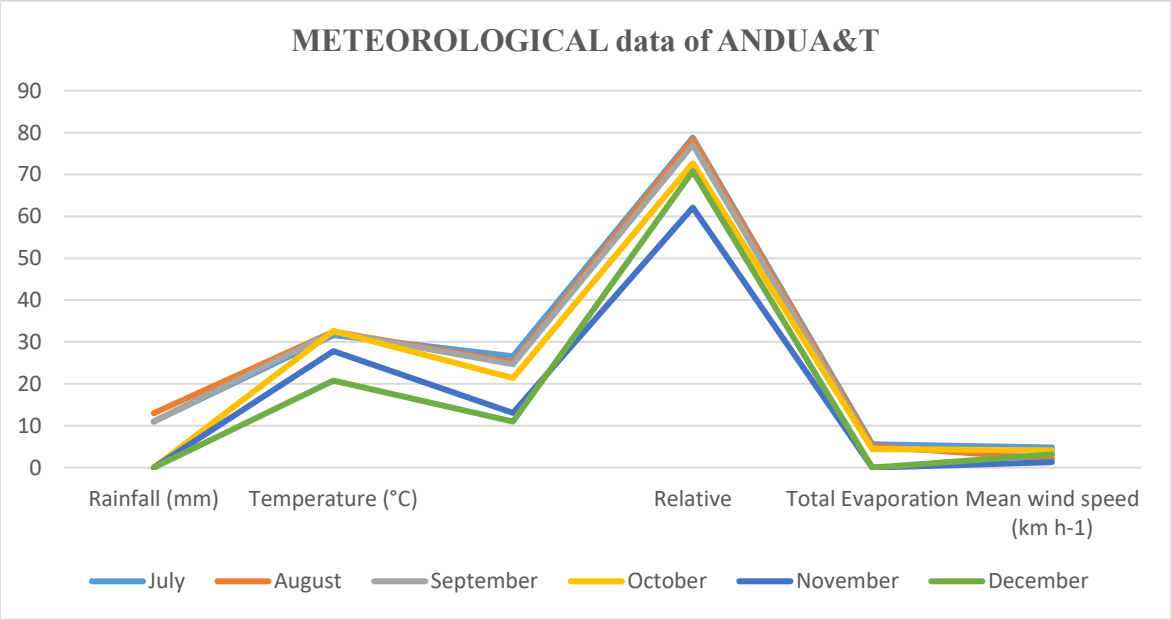

Fig: 1.2 Meteorological data of ANDUA&T, Ayodhya

Name	: Satyam Maurya	**ID No.**	: H-14732/23
Semester	: IV	**Degree**	: M.Sc. (Hort.) Fruit Science.
Year of admission	:2023-24	**Department:**	Fruit Science.
Major	: Fruit Science		
Minor	: Post-Harvest Management		
Thesis Title	**: Impact of Climate on Morphological Characteristics, Yields, and Molecularprofiling of Aonla varieties**		
Advisor	: Dr. Atul Yadav		

ABSTRACT

Aonla (*Emblica officinalis* Gaertn.), commonly known as Indian gooseberry, is a highly valued fruit crop known for its therapeutic properties and high vitamin-C content. The present study was conducted to assess the morphological traits and genetic diversity among seven Aonla varieties (NA-4, NA-5, NA-6, NA-7, NA-10, Chakaiya, and BSR-1) developed by A. N.D.U.A. &T., Faizabad, grown under two distinct agro-climatic regions- Bangalore (tropical) and Ayodhya (sub-tropical). Morphological traits revealed variability in tree habit, foliage density, trunk color, and canopy spread, flowering behavior, fruit & seed traits, and yield. Among the varieties, NA-7 exhibited superior performance in terms of fruit weight, pulp weight, and overall yield. DNA fingerprinting was conducted using ISSR markers, where ten markers (UBC-852, UBC-855, UBC-856, UBC-857, UBC-823, UBC-888, UBC-908, UBC-834, UBC-865, and UBC-844) displayed a high percentage of polymorphism. Dendrogram analysis using UPGMA clustering grouped the varieties into two major clusters, with BSR-1 forming a genetically distinct group. Dendrogram analysis using UPGMA clustering grouped and displayed the 7 varieties of Aonla in two major clusters. The first major cluster exhibited 6 varieties, which were further divided into two sub-clusters, Sub-cluster I-A displayed Chakaiya, NA-10, NA-5, presented close genetic affinity, while sub-cluster I-B included NA-7, NA-6, and NA-4, which were grouped based on their moderate genetic similarity. Cluster II contained only the 'BSR-1' variety, which appeared on a distinct branch, showing the greatest genetic distance from the other varieties. BSR-1' is the most genetically distinct and may carry unique alleles or characteristics. The integration of morphological and molecular tools proved effective in identifying diverse and promising varieties. This study highlights the potential of using ISSR markers in combination with morphological parameters for genotype discrimination, conservation, and breeding program applications in Aonla. The findings also emphasize the need for region-specific variety selection and the potential of BSR-1 and NA-7 in future hybridization programs for the development of climate-resilient varieties.

Keywords

Emblica officinalis, DNA fingerprinting, varieties evaluation, genetic diversity analysis, ISSR markers, fruit quality & yields.

26/08/25

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Advisor

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प्रवेश वर्ष : 2023-24 विभाग : फल विज्ञान
मुख्य विषय : फल विज्ञान गौण विषय : पोस्ट हार्वेस्ट मैनेजमेंट
शोध शीर्षक : जलवायु का आँवला की किस्मों की आकारिकी, उपज एवं आणविक गुणधर्मों पर प्रभाव का विश्लेषण
शोध निर्देशक : डॉ. अतुल यादव

सारांश

आंवला (*Emblica officinalis* Gaertn.), जिसे सामान्यतः भारत में आँवला या गूजबेरी के नाम से जाना जाता है, एक अत्यधिक महत्वपूर्ण फलवाली फसल है जो अपनी औषधीय गुणों एवं प्रचुर मात्रा में विटामिन-C की उपस्थिति के कारण विशेष प्रतिष्ठा रखती है। वर्तमान शोध का उद्देश्य सात विशिष्ट आँवला किस्मों (NA-4, NA-5, NA-6, NA-7, NA-10, चकैया और BSR-1), जो कि आचार्य नरेंद्र देव कृषि एवं प्रौद्योगिक विश्वविद्यालय, अयोध्या, उत्तर प्रदेश द्वारा विकसित की गई हैं, की आकारिकी लक्षणों तथा आनुवंशिक विविधता का दो भिन्न कृषि-जलवायु क्षेत्रों - बेंगलुरु (उष्णकटिबंधीय) और अयोध्या (उप-उष्णकटिबंधीय) में मूल्यांकन करना है।आकारिकीय विश्लेषण में वृक्ष की बनावट, पत्तियों का घनत्व, तने का रंग, छत्र का विस्तार, पुष्पन विशेषताएं, फल एवं बीज लक्षण तथा उपज संबंधित घटकों में पर्याप्त विविधता पाई गई। इन सभी में NA-7 किस्म ने फल भार, गूदा भार एवं कुल उत्पादन के आधार पर सर्वोत्तम प्रदर्शन किया। आणविक स्तर पर विविधता का मूल्यांकन ISSR (Inter Simple Sequence Repeat) मार्करों के माध्यम से किया गया, जिसमें दस मार्कर (UBC-852, UBC-855, UBC-856, UBC-857, UBC-823, UBC-888, UBC-908, UBC-834, UBC-865, एवं UBC-844) ने उच्च स्तर की बहुरूपता (पॉलीमॉरफिज्म) का प्रदर्शन किया।UPGMA क्लस्टर विश्लेषण द्वारा बनाए गए डेंड्रोग्राम में सातों किस्मों को दो प्रमुख समूहों में वर्गीकृत किया गया। पहला समूह छह किस्मों का था, जिसे दो उप-समूहों में विभाजित किया गया। उप-समूह I-A में चकैया, NA-10 एवं NA-5 सम्मिलित थीं, जिनमें आपसी आनुवंशिक निकटता पाई गई, जबकि उप-समूह I-B में NA-7, NA-6 एवं NA-4 थीं, जो मध्यम आनुवंशिक समानता प्रदर्शित करती हैं। द्वितीय प्रमुख समूह में केवल BSR-1 किस्म सम्मिलित थी, जो एक पृथक शाखा पर स्थित होकर अधिकतम आनुवंशिक दूरी को प्रदर्शित करती है, जिससे यह स्पष्ट होता है कि BSR-1 आनुवंशिक रूप से अन्य सभी किस्मों से विशिष्ट एवं संभावित रूप से अद्वितीय गुणधर्मों वाली किस्म है।यह अध्ययन इस निष्कर्ष पर पहुंचता है कि आकारिकी एवं आणविक दोनों प्रकार के उपकरणों का समन्वित प्रयोग आँवला की किस्मों की पहचान, विविधता निर्धारण एवं संभावनाशील जर्मप्लाज्म चयन हेतु अत्यंत प्रभावी है। साथ ही, यह अनुसंधान क्षेत्र-विशेष के अनुसार

उपयुक्त किस्म चयन की आवश्यकता एवं BSR-1 तथा NA-7 जैसी किस्मों को भावी संकरण कार्यक्रमों में जलवायु-सहिष्णु किस्मों के विकास हेतु प्राथमिकता देने की संस्तुति करता है

कीवर्ड्स: *Emblica officinalis*, डीएनए फिंगरप्रिंटिंग, किस्म मूल्यांकन, आनुवंशिक विविधता, ISSR मार्कर, फल गुणवत्ता, उपज।

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