# Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening

A Thesis Submitted

To

**Sikkim University**

in Partial Fulfilment of the Requirement for the

**Degree of Doctor of Philosophy**


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December, 2024

# Dedication

*I Dedicate this Thesis to…*

*My Beloved Father*

*Lt. Man Bir Limboo,*

*My Dada*

*Shri Sancha Man Limboo*

*&*

*To My Entire*

*"Nugu Muringla Family"*

# DECLARATION

I, Dewan Limboo, hereby declare that the research work embodied in the thesis titled **"Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening"** submitted to Sikkim University for the award of the degree of Doctor of Philosophy under the supervision of Dr. Manju Rana, Assistant Professor, Department of Horticulture, School of Life Sciences is my original research work and solely carried out by me. The thesis has not been submitted for any other degree or diploma in any other University/Institution.

Place: Gangtok

Date: 20.12.2024

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# CERTIFICATE

This is to certify that the thesis entitled **"Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening"** submitted to the Sikkim University, in partial fulfillment of the requirements of the award of the degree of **Doctor of Philosophy** in the **Department of Horticulture**, embodies the result of bonafide research work carried out by **Mr Dewan Limboo** under my guidance and supervision. No part of the thesis has been submitted for any other degree, diploma, associateship, or fellowship.

All the assistance and help received during the course of the investigation have been duly acknowledge by him.

I recommend this thesis be placed before the examiners for evaluation.

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This is to certify that a plagiarism check has been carried out for the following Ph.D. thesis with the help of **DrillBit Software** and the result is **8 % tolerance rate**, within the permissible limit **(below 10% tolerance rate)** as per the norms of Sikkim University.

**"Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening"**

Submitted by **Dewan Limboo** under the supervision of **Dr. Manju Rana, Assistant Professor, Department of Horticulture, Sikkim University, Gangtok, 737102, India.**

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**Vetted by Librarian**

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Date: **(Dewan Limboo)**

Place:

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# Abbreviations

| | |
|---|---|
| $^{0}C$ | Degree Celsius |
| ANOVA | Analysis of variance |
| AOAC | Association of Official Analytical Chemists |
| CD | Critical Difference |
| cm | Centimeter |
| CTAB | Cetyl trimethyl ammonium bromide |
| CV | Coefficient of variation |
| DNA | Deoxyribonucleic acid |
| DW | Distilled Water |
| EDTA | Ethylenediamine tetraacetic acid |
| FCR | Folin and Ciocalteu’s Reagents |
| G | Gram |
| GCV | Genotypic Coefficient of Variation |
| ICP-MS | Inductively Coupled Plasma Mass Spectrometry |
| Kg/ha | Kilogram per hectare |
| M | Meter |
| mg | Milligram |
| mm | Millimetre |
| NS | Non significant |

| | |
|---|---|
| OD | Optical density |
| PCA | Principal Component Analysis |
| PCR | Polymerase Chain Reaction |
| PCV | Phenotypic Coefficient of Variation |
| PIC | Polymorphic Information Content |
| PPM | Part per million |
| RAPD | Random Amplified Polymorphic DNA markers |
| RBD | Randomized Block Design |
| SD | Standard Deviation |
| SE | Standard Error |
| TAE | Tris-acetate-EDTA |
| TE | Tris-EDTA |
| $T_M$ | Melting temperature |
| viz., | Namely |
| µg | Microgram |
| µl | Microliter |

# Chapter 01

# Introduction

Sikkim is abundant in natural resources, which people have long utilized in diverse ways. The region's diverse climate supports a wide range of agricultural conditions, from subtropical climates in the lower valleys to alpine climates at higher altitudes. While the weather is generally cold and humid for most of the year, rainfall patterns vary across different regions. The state's unique biodiversity and climate, coupled with government support, present immense potential for sustainable and profitable agriculture and horticulture. These sectors are integral to Sikkim's economy, supporting the livelihoods of a significant portion of the state's population and contributing to its overall development. As a majority of Sikkim's people reside in rural areas, these sectors form the backbone of their economic activity. Nearly 60-70% of Sikkim's population relies on agriculture as their primary source of income, primarily engaged in small-scale, family-run farms (Kumar & Rai, 2021).

Sikkim is known for its diverse range of vegetables, many of which possess significant nutritional and medicinal value but remain underutilized on a larger scale. These indigenous vegetables have been a staple in the local diet for generations, thriving in the state's diverse agro-climatic conditions (Sarkar et al., 2024). With their unique flavours and high nutritional content, these underutilized vegetables hold immense potential for large-scale production, both for domestic consumption and export. By promoting these crops, Sikkim can diversify its agricultural output, bolster food security, and contribute to its economy.

Underutilized vegetables are locally grown and consumed crops that remain underexploited or overlooked in mainstream agriculture and markets or global food systems. These crops are crucial in ensuring food security, enhancing nutrition and supporting sustainable agriculture.

Often highly adapted to local conditions, underutilized vegetables are resilient and can thrive in marginal environments. This makes them crucial for sustainable farming, especially in regions affected by climate change. Characterizing these crops helps us understand their traits, enabling farmers to optimize cultivation practices, promote biodiversity, and make informed decisions about their role in building resilient agricultural systems.

Furthermore, it reveals the economic potential of underutilized vegetables, opening doors to niche markets and value-added products. By promoting these crops, one can also preserve traditional knowledge and cultural heritage.

*Cyclanthera pedata* (L.) Schrad. (Stuffing cucumber) is one such underexploited and lesser-known vegetable from Sikkim, belonging to the Cucurbitaceae family, with a chromosome number of 2n= 2x= 32 (Whitaker, 1933). The young fruits of stuffing cucumber are consumed both raw and cooked, offering a flavor reminiscent of cucumbers, though they lack their crisp texture. Older fruits are typically cooked and can be stuffed, while the seeds are used to prepare pickles in Sikkim. This crop is known by various names across different regions, including achoccha, quishiu, caiba, caiga, or kaiwa in Mexico and Bolivia. In Brazil, it is referred to as pepino-do-ar, pepino-de-porco, pepino-de-comer, chuchu-paulista, and taiuiá-de-comer or maxixe do reino. In Argentina, it is called Achocha (in Quechua) or Achokcho (Klein et al., 1989). In Sikkim, it is called as *Chuchae karala* or *Tukrukay*.

*C. pedata* a native of South America is now cultivated in most of South and Central America, in particular in the Andes (Klein et al., 1989). It has spread to several islands of the Caribbean and Asia, where it is typically located in the Northern part of India and its neighboring country viz. Nepal, and Bhutan (Zuccolo et al., 2023). The most important producers of stuffing cucumber are Peru, Bolivia, Colombia, and Mexico.

*C. pedata* is an herbaceous vine grown for its edible mature fruit which is predominantly used as a vegetable. By nature, *C. pedata* is an annual plant, producing stems that can be of length from 4.5 to 12 meters. The stems scramble over the ground, clambering into surrounding vegetation and supporting themselves using long tendrils. It is a plant of the tropics by origin, but it is commonly found at elevations up to 3,000 meters. It can be cultivated in the subtropics and temperate zones that have a long, warm season of at least 4 months. It requires a warm, sunny, and sheltered position in rich well-drained soil. The first harvest of fruit can take place within 3 months after planting and can be continued for several months. Young and immature fruits can be used as salads and mature fruits can be eaten after cooking.

*C. pedata* fruits are reported to contain water (94 g/100g), protein (0.7 g), K (152 mg), fat (0.1 g), carbohydrate (44 g), fiber (0.7 g), Ca (13 mg), P (20 mg), Fe (0.8 mg), Mg (8.4 mg) thiamin (0.05 mg), riboflavin (0.04 mg), niacin (0.29 mg), ascorbic acid (14 mg) (Oliveira et al., 2014). Given its nutritional richness, *C. pedeta* can be strongly recommended as a nutritional supplement considering its high content of potassium, calcium, phosphorous, and magnesium (Oliveira et al., 2014). It has been used for a very long time as a strong fat absorber, fruit can reduce body fat, as well as cellulite, and it increases good cholesterol (HDL) while reducing bad LDL cholesterol, thus attributing medicinal properties such as anti-inflammatory, hypo-glycemic and hypo-cholesterolemic (Ranilla et al., 2010; Monigatti et al., 2013). It represents an example of a plant valued for its medicinal properties (Rivas et al., 2013). The fruit is also rich in phytochemicals (Zuccolo et al., 2023) and phenolic compounds, flavonoids, coumarins, tannins, and terpenes compounds (Macchia et al., 2009).

For crop improvement programs, assessing the nature and extent of genetic variability, heritability, and genetic advancement in the initial parental material is crucial. The success of selection depends significantly on the level of variability present within the breeding population. Therefore, understanding the genetic diversity within a crop species' gene pool is essential for initiating an effective breeding strategy. Heritability, a quantitative measure that indicates the relationship between genotypic and phenotypic variance, serves as a valuable index for predicting the transmission of traits from parents to offspring (Falconer, 1981). It represents the proportion of phenotypic variance attributable to genetic differences and is expressed as a percentage. A high percentage suggests that a trait is highly heritable, whereas a lower percentage indicates that environmental factors primarily shape the trait's phenotypic expression.

To date, the degree of genetic diversity in *C. pedata* has not been assessed at a morphological and genetic level. Being a cross-pollinated plant, the study of genetic markers associated with stuffing cucumber is necessary to understand its genetic diversity. Molecular markers have enormous potential to quantify genetic diversity by detecting polymorphisms of particular crops at the DNA level as compared to other approaches. The identification of the variants within the species and determination of the level of variation is gaining importance due to documentation, registration, and protection of landraces and other plant genetic resources of commercial importance. At present, very few or no molecular genetic/genomic resources are publicly available for *C. pedata*.

In Sikkim, *C. pedata* is found enormously in all the districts, and there is visible diversity among the available population, that could offer resources for crop improvement in breeding aspects. Considering its importance in the local economy and nutritional status, it is necessary to identify the variants of *C. pedata*. This shows great diversity in its morphological traits, particularly in fruit shape and size, number of seeds per fruit, presence, and absence of spines on the fruit, fruit colour, etc.

Keeping in view the foregoing facts, it is envisaged to undertake to study entitled “Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening” with the following objectives:

1. To collect stuffing cucumber genotypes based on farmers' perceptions of desirable horticultural traits in selected regions of Sikkim.
2. To assess the genetic diversity on the basis of morphological characters and DNA marker-based genotyping in stuffing cucumber.
3. To assess the biochemical composition of the collected genotypes of stuffing cucumber.

# Chapter 2

# Review of Literature

*Cyclanthera pedata* is a valuable local vegetable crop in Sikkim, yet research on its genetic diversity remains limited both in India and globally. This chapter reviews the relevant literature on key aspects of the current study, focusing on the crop's resilience to local climate conditions, the importance of genetic biodiversity, and the integration of farmers' perceptions. The literature review is organized under the following headings:

2.1 Farmers' perception

2.2 Genetic diversity

2.3 Molecular diversity

2.4 Biochemical composition

## 2.1 Farmers' perception

Farmers' perceptions of horticultural traits in *C. pedata* remain undocumented; however, insights from similar studies offer valuable context. For example, a study conducted in Chiang Mai explored farmers' perceptions of practices, constraints, and adaptability in organic vegetable farming, focusing on factors influencing sustainable livelihoods (Pinthukas, 2015). Using semi-structured questionnaires, the study interviewed 108 farmers and found that factors such as age, education, household labor, income, and extension visits significantly shaped farmers' perceptions. Younger, better-educated farmers with improved access to resources and training showed more positive perceptions and adaptability. Similarly, a study on cocoyam farming in Ethiopia highlighted a wide range of farmer perceptions (Wada et al., 2017). Farmers, aged 20-83, had cultivated cocoyam for varying periods, with 74% growing it for over 10 years. The crop was valued for its edibility, versatility, and resilience.

Mengistu et al. (2019) examined farmers' perceptions of preferred traits in sorghum, involving 165 respondents and 180 focus group discussions. The research revealed that farmers prioritized anthracnose resistance and bird tolerance as essential traits in sorghum varieties. Similarly, Hevia et al. (2021) found that 92.7% of farmers recognized the importance of pollinators for crop production, with farmers displaying moderate knowledge of pollinators, scoring an average of 6.1 out of 10.

Suvi et al. (2021) assessed farmers’ perceptions of variety and breeding priorities in rice. Farmers used primarily saved seeds and preferred traits such as high yield, disease resistance, drought tolerance, early maturity, and local adaptability with high market value. Kosgei et al. (2021) reported that farmers in Kenya preferred desirable traits in chickpea, such as early maturity, pest resistance, and good taste, with variations in ranking depending on the location.

Chataika et al. (2022) investigated the horticultural trait preferences of smallholder farmers in Malawi and Namibia, focusing on spider plant cultivation. In the present study, female-headed households were more widespread, with higher literacy levels found in Namibia. Malawian farmers prioritized drought tolerance and high-yielding plants, while Namibian farmers emphasized drought tolerance and pest resistance. In Rwanda, sweet potato production was dominated by female farmers (58.4%), with male farmers representing 41.6%. Most farmers (67.3%) had completed primary school. Sweet potato was highly valued for food security and income generation, with farmers preferring cultivars that offered high yield, early maturity, and drought resistance (Placide et al., 2015).

Mutari et al. (2021) on Navy bean indicated that the majority of respondents were aged 41–50 years (28%), with women comprising the majority (57.4%). Farmers expressed a strong preference for cultivars that are drought-tolerant, heat-resistant, high-yielding, and resistant to pests and diseases. Efisue et al. (2008) reported farmers in irrigated ecologies favored high-yielding, long-duration varieties of Rice in Sikasso Region of Mali. While traditional framers preferred grain quality and plant height over yield.

McElhinny et al. (2007) reported farmers' preferences prioritized yield, earliness, and plant color in quinoa.  These will help to understand the importance of integrating farmers' diverse perspectives, particularly those of women, into quinoa breeding programs. Consumer preferences for specific ALVs (African leaf vegetables) were based on nutritional benefits, with mixed effects of market development on biodiversity (Irungu et al., 2011).

As reported by Sharma et al. (2017), farmers' perception of farming systems in Sikkim is driven by organic farming. Farmers recognize biophysical constraints such as pest incidence, inadequate irrigation, and weed problems, as major challenges, alongside socio-economic issues like high seed costs, lack of quality or improved seeds, labor scarcity, and low market value. Education plays a significant role in agricultural productivity as reported by (Eric et al., 2014).

Paltasingh and Goyari. (2018) reported the impact level of education significantly, which influences the adoption of modern paddy varieties. Early planting season significantly increases the cucumber yield (Nwofia et al., 2015).

## 2.2 Genetic diversity

### 2.2.1 Morphological characterization

Valcarcel et al. (2018) study focused on the morphological evaluation of 206 cucumber accessions. 26 descriptors were used, including eight plant traits and 18 fruit traits Notable differences were observed among the collected genotypes. Harshitha & Shyamalamma (2021) conducted a study focusing on the morphological characterization of 30 local cucumber genotypes, highlighting variations in fruit, seed, and plant traits. The variability among genotypes suggests significant potential for improving fruit quality traits.

Pandey et al. (2013) evaluated the genetic diversity of 44 cucumber genotypes focusing on morphological traits, significant variability in days to 50% female flowering (37–46 days), number of fruits per plant (1.4–6.0), fruit weight (0.04–0.552 kg), and root length (14.25–32.8 cm) was observed. As reported by Grumet et al. (2023) cucumber fruits, consumed at an immature stage, exhibit significant morphological diversity regarding fruiting traits.

Jadid et al. (2015) conducted a morphological characterization of three cucumber varieties available in the Indonesian market, namely Natanz CU 531, Semi CU 699, and Image Baby. They observed variation in their vegetative and fruiting traits. Similarly, Diouf et al. (2023) evaluated the agro-morphological characteristics of four cucumber varieties, including green and white *Cucumis sativus* and bitter and non-bitter *Cucumis metuliferus*, reporting significant variation in growth parameters among the varieties.

Cebeci et al. (2020) analyzed the morphological diversity of 90 Turkish cucumber genotypes using 20 UPOV descriptors. Their findings highlighted considerable variability in fruit traits, with lengths ranging from 6.5 to 32.5 cm, diameters between 25 and 52 mm, and weights spanning 28 to 625 g, indicating significant diversity among the genotypes. Similarly, Mladenović et al. (2012) evaluated a diverse bottle gourd germplasm collection to conserve genetic resources and study variability. They identified substantial intraspecific variation in plant, fruit, and seed morphology; however, this variation has decreased over time due to selective breeding for ornamental purposes, which has prioritized specific fruit shapes and sizes.

Rambabu et al. (2017) conducted morphological characterization using NBPGR minimal descriptors, revealing notable diversity, particularly in fruit shape and color, with notable differences observed across all vegetative and fruiting traits. Similarly, Kalyanrao et al. (2016) evaluated genetic diversity and relatedness among 15 commercial bottle gourd genotypes, reporting substantial morphological diversity within the genotypes.

Duhan et al. (2017) reported significant variability across 31 bottle gourd genotypes evaluated for 10 morphological traits, encompassing both fruit and leaf characteristics. Similarly, Kandasamy et al. (2019) emphasized that the success of a breeding program relies on selecting superior genotypes with desirable traits. Their study revealed substantial variation among 20 genotypes for key traits, including days to flowering, vine length, fruit characteristics, and yield.

Mallikarjuna et al. (2023) assessed genetic diversity in 96 genotypes and found significant variation in morphological traits. Notably, gynoecious lines DBGS-21-06 and DBGS-48-00 exhibited early maturity and high yield. Similarly, Mehta et al. (2024) evaluated 27 bitter gourd genotypes, observing considerable variability in seven morphological traits, such as leaf shape, fruit neck, and fruit shape at the blossom and peduncle ends. The genotypes IC 85624, HK 113, IC 85610, and IC 85605 demonstrated superior performance in terms of fruit yield and growth characteristics.

Reddy et al. (2017) assessed 35 muskmelon (*Cucumis melo* L.) germplasm lines to explore the relationships among 18 quantitative traits related to growth, earliness, and yield. The lines RNMM-31, RNMM-32, RNMM-3, and RNMM-12 were identified as promising candidates for fruit production and quality. The study found that fruit yield exhibited a positive correlation with traits such as vine length, number of primary branches per vine, fruit length, fruit diameter, average fruit weight, number of fruits per vine, fruit cavity length, fruit cavity width, rind thickness, and seed yield. However, it showed a negative correlation with traits such as the node number of the first pistillate flower, days to last fruit harvest, and pulp thickness. Traits like the number of primary branches per vine, fruit length, fruit cavity length, and fruit cavity width exhibited positive correlations with fruit yield, but their direct effects on yield were either negative or negligible. Therefore, it is important to consider indirect causal factors in the selection process.

### 2.2.2 Correlation analysis

Afangideh & Uyoh (2007) reported fruit number per plant and vine length were positively associated with yield of cucumber and phenotypic differences were greater than the genotypic coefficients of variation. High variation was observed for yield associated traits for cucumber at both genotypic and phenotypic levels (Ullah et al., 2012). Positive correlations between fruit yield and traits like fruit size, number of fruits, and plant height were reported by Ahmed et al. (2022) in cucumber genotypes.

Higher genetic and phenotypic variations were observed than environmental variation for most traits in cucumber genotypes, where, phenotypic correlations showed that plant yield exhibited a strong positive correlation with fruit (Al-Jaf et al., 2024). Analysis of correlation showed that traits like number of female flowers, fruit diameter, and number of fruits per vine exhibited positive significant correlations with fruit yield (Shet et al., 2018) and the phenotypic coefficient of variability was higher than the genotypic.

In this study, traits such as the node at first female flower appearance, vine length, number of fruits per plant, fruit length, fruit diameter, seed cavity breadth, fruit flesh thickness, and the mean fruit weight were found to have significant positive correlations with yield per plant at both phenotypic and genotypic levels. This suggests that enhancing these traits could result in a higher yield in snap melon. Conversely, days to first harvest (DAS) showed a significant negative correlation with yield per plant at both the genotypic and phenotypic levels, indicating that improvements in this trait would likely lead to a reduction in yield (Pasha et al., 2019).

A field study was conducted to assess variability, heritability, and genetic advance for yield and related traits in 38 advanced cucumbers (*Cucumis sativus* L.) lines. The highest values for both the Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were found in traits such as node at first female flower appearance, node at first male flower appearance, yield per plant, seed cavity breadth, average fruit weight, and number of fruits per plant. Additionally, traits like nodes per vine, node at first female flower appearance, and days to first female flower opening exhibited high genetic advance over the mean and heritability, indicating their potential for improvement through selection (Veena et al., 2012).

Deepa et al. (2018) demonstrated significant positive correlations between fruit yield per vine and various fruiting traits in cucumbers at both genotypic and phenotypic levels. Similarly, Kumar et al. (2011) found strong positive associations of cucumber yield with traits such as fruits per plant, fruit length, fruit breadth, average fruit weight, and harvest duration.

Husna et al. (2011) reported phenotypic variances exceeded genotypic variances, indicating a major genetic contribution to phenotypic variance in bottle gourd genotypes. Considerable variability was observed among bottle gourd genotypes for all traits, with the highest GCV (34.84%) and PCV (35.14%) recorded for the sex ratio (Mandal et al., 2015). Panigrahi et al. (2018) evaluated morphological traits of 37 bottle gourd genotypes and identified a strong positive between fruit yield per vine and hectare at both genotypic and phenotypic levels. Husna et al. (2014) reported that phenotypic variances were higher than genotypic variances in bottle gourd genotypes, where 31 bottle gourd genotypes were undertaken for the study.

Sharma and Sengupta (2013) conducted experiments to assess the performance of 16 bottle gourd genotypes. A high genotypic coefficient of variation was reported for fruit weight (39.48%), with phenotypic coefficients exceeding genotypic values for all traits. Kumar et al. (2007) reported phenotypic variance and the phenotypic coefficient of variation exceeded the corresponding genotypic variance and coefficient of variation for all the traits. A study was done by Chouhan et al. (2020) to evaluate 25 bottle gourd genotypes. Moderate GCV and PCV were recorded for fruiting traits. Fruit yield showed significant positive correlations with traits like fruits per vine, fruit length, and vine length.

Singh et al. (2012) evaluated fourteen genotypes of bottle gourd, where, high genotypic and phenotypic coefficients of variation were observed and yield per plant. showed a positive correlation with the number of fruits per plant. As stated by Varalakshmi et al. (2018) phenotypic variation exceeded genotypic variation in the investigation of ten yield-contributing traits in bottle gourd.

### 2.2.3 Clustering using Mahalanobis $D^2$

Ene et al. (2016) classified bottle gourd genotypes into five clusters, with Cluster F showing the best performance for most traits. Similarly, Quamruzzaman et al. (2020) evaluated 25 bottle gourd germplasms and identified five distinct clusters, reporting a maximum intra-cluster distance of 2.25 and inter-cluster distance of 12.24. In another study, Shubha et al. (2019) performed hierarchical cluster analysis on 127 bottle gourd germplasms driven by quantitative traits, grouping the genotypes into three clusters.

Visen et al. (2015) identified five clusters among bottle gourd genotypes using $D^2$ cluster analysis, with cluster V containing the highest number of genotypes (10). Similarly, a genetic diversity study by Chetariya and Vaddoria (2017) on 50 bottle gourd genotypes revealed 13 clusters, recommending genotypes from cluster XII for crossing to enhance genetic variability.

Damor et al. (2017) conducted a genetic diversity study on 40 bottle gourd genotypes using Mahalanobis $D^2$ and grouped them into seven clusters, recommending crossing between clusters II and IV to enhance genetic variability and yield. Similarly, Abhishek et al. (2020) evaluated 52 bottle gourd genotypes, grouping them into three clusters, with the maximum inter-cluster distance observed between clusters II and III (3.234).

In the study by Garnepudi et al. (2020) on 18 cucumber genotypes, cluster analysis grouped the genotypes into two clusters, with 17 genotypes placed in Cluster II and one in Cluster I. Similarly, Hasan et al. (2015) evaluated the genetic diversity in commercial cucumber genotypes, where seven genotypes were divided into three clusters, with the highest inter-cluster distance (4.078) observed between clusters I and II.

Pal et al. (2017) conducted a study on genetic divergence analysis using Mahalanobis $D^2$, which grouped the genotypes into five clusters, with Cluster III containing the most genotypes (15). The maximum average inter-cluster distance (10.755) relationship was observed between Cluster II and Cluster. Similarly, Kumawat et al. (2020) performed a genetic divergence analysis on twenty-one cucumber genotypes using Mahalanobis $D^2$, resulting in four clusters. Cluster III again had the highest number of genotypes, and the average intra-cluster distance was 163.11, while the maximum average inter-cluster distance (1439.432) was observed between Cluster IV and Cluster V.

## 2.3 Molecular diversity

Ntuli et al. (2015) examined the genetic diversity in pumpkin landraces using 10 SSR markers and revealed that the SSR markers are highly polymorphic. Yunli et al. (2020) conducted a study on the genetic diversity of 64 *Cucurbita pepo* accessions that were analyzed with SSR markers. Dar et al. (2017) assess the genetic differences and marker attributes of 104 cucumber genotypes using 23 SSR markers, yielding 67 alleles with an average of 2.91 per locus, and polymorphic information content ranging from 0.158 to 0.495. Primers such as SSR65, SSR61, and SSR60 possess the highest values for allele frequency and polymorphic information content.

Kong et al. (2014) evaluated the genetic diversity of 25 *Cucurbita* rootstock cultivars and 10 inbred lines using 80 SSR markers. 94 polymorphic alleles were identified, averaging 2.29 alleles per locus, with polymorphism information content values ranging from 0.16 to 0.60. Likewise, Lv et al. (2012) have reported 23 highly polymorphic SSR markers, while assessing 3,342 cucumber accessions from Chinese, Dutch, and U.S.

Mujaju et al. (2010) evaluated the genetic variability using 9 SSR primers for 10 watermelon accessions and observed that dendrograms and multidimensional scaling (MDS) revealed two primary clusters. Nyabera et al. (2021) investigated the genetic diversity in 80 pumpkin fruits using 5 SSR primers and revealed that SSR loci exhibited polymorphism, resulting in a total of 33 alleles and an average PIC value of 0.534.

Innark et al. (2013) assessed the genetic diversity in 38 cucumber accessions based upon 20 SSR primers, the study revealed 36 polymorphic loci with average polymorphism information content (PIC) was 0.33, with a range from 0 to 0.62. Alhariri et al. (2021) analyzed the genetic diversity of 51 bitter gourd accessions using 30 SSR primers, which exhibited polymorphism and proved to be highly informative. The primers had an average polymorphic information content (PIC) of 0.429. The number of alleles per locus ranged from 2 to 5, with an average of 3.3 alleles per locus.

Hu et al. (2011) assessed genetic diversity among 29 cucumber (*Cucumis sativus* L.) accessions using 13 genomic SSR markers and 16 EST-SSR markers. The genomic SSR markers revealed an average of 4.46 alleles per locus with a mean polymorphic information content (PIC) of 0.664, while the EST-SSR markers identified a mean of 3.38 alleles per locus and a PIC of 0.397. In another study, Normohamadi et al. (2017) performed a genetic diversity analysis of 20 cucumber genotypes using 9 SSR primers. Their study indicated substantial diversity, with Jaccard similarity coefficient values ranging from 0.51 to 0.92. The average PIC was 0.69, with the highest value of 0.8 recorded for the primer SSR13251.

A standardized DNA amplification protocol for *Cyclanthera pedata* using ISSR markers was designed by Kumar et al. (2023). In a separate study, Kumar et al. (2020) assessed the genetic diversity of 78 cucumber accessions using 60 SSR markers. SSR analysis identified 171 alleles with an average of 2.85 alleles per locus, and the polymorphism information content (PIC) ranged from 0.05 to 0.59, with a mean value of 0.36.

In a study by Pandey et al. (2018), 21 EST-SSR markers were employed to evaluate polymorphism among 40 cucumber genotypes. The number of alleles identified ranged from 2 to 6, with an average of 3.05. The polymorphism information content (PIC) varied between 0.002 and 0.989, with a mean of 0.308. The effective number of alleles (Ne) ranged from 1.079 to 1.753, while the expected heterozygosity (He) and unbiased expected heterozygosity (uHe) ranged from 0.074 to 0.428 and 0.074 to 0.434, respectively. In another study by Kaur et al. (2023), 59 SSR markers revealed polymorphism across cucumber genotypes, identifying a sum of 252 alleles, with an average of 4.27 alleles per locus. The PIC values ranged from 0.34 to 0.84, with a mean of 0.62. The major allele frequency varied from 0.21 to 0.75, and the heterozygosity values ranged from 0.43 to 0.89.

In a study by Yunli et al. (2020), 35 polymorphic markers were employed to evaluate fingerprint 64 accessions, revealing 145 distinct amplification products. Each marker pair amplified between 2 and 5 fragments, with an average of 4.12 bands per marker. The diversity index of the 64 accessions, calculated using Shannon's information index, ranged from 0.2 to 3.8, with an average of 0.89. The marker H39 exhibited the highest diversity index, while P18 had the lowest. In a separate study by Coskun et al. (2022), genetic characterization of 47 pumpkin genotypes was conducted using 16 SSR markers. The similarity coefficients between genotypes ranged from 0.68 to 1.0. Three genotypes were identified to cluster separately from the others, demonstrating that SSR markers are an effective tool for assessing genetic diversity and differentiation among genotypes.

In a study by Sidhu and Pathak (2020), Forty primers were tested across 36 genotypes, and 11 primers showed polymorphism. The mean number of polymorphic bands per primer was 2.1. The dendrogram analysis showed that the accessions were classified into three main clusters, with various sub-clusters within them. In another study by Bhutia et al. (2017), genetic diversity was assessed in 47 Luffa genotypes using 17 EST-SSR primers, which generated 34 alleles, with 1-2 loci per primer. The genotypes were largely divided into two clusters: Cluster I included genotypes such as DRG-98, Utkal Tripti, DRG-6, Sel-102, Pusa Nasdar, Pusa Nutan, Arka Sujat, DRG-73, DRG-61, DRG-42, and DRG-50, while Cluster II contained the remaining 36 genotypes. The EST-SSR primer C90830_G3 showed the highest informativeness, with a PIC value of 0.3750.

In a study by Kwon et al. (2010) genetic characterization of 49 watermelon varieties was carried out using 30 SSR markers developed from both melon and watermelon. A total of 121 polymorphic amplified fragments were obtained, with a mean polymorphism information content (PIC) of 0.502, ranging from 0.223 to 0.800. In a separate study by Mashilo et al. (2016), 31 dessert watermelon and 34 citron watermelon landraces from South Africa were genotyped using 10 polymorphic SSR markers. The number of alleles detected per marker ranged from 2 to 23, with an average of 13.5 alleles. In total, 135 putative alleles were amplified across the sampled watermelon populations. The number of effective alleles varied from 1.99 to 10.88, with a mean of 5.83. The mean observed and expected heterozygosity were 0.50 and 0.79, respectively, while the mean PIC was 0.79, suggesting a substantial degree of genetic diversity.

In research by Shi et al. (2023), the genetic diversity of 21 chayote accessions was assessed using six microsatellite markers. The results indicated low genetic heterozygosity globally, with observed heterozygosity (Ho) of 0.286 and expected heterozygosity (He) of 0.408. The accessions were grouped into three distinct genetic clusters. In another investigation by Cheng et al. (2024), 48 primer pairs were used to successfully amplify bands, with 42 showing polymorphism. These polymorphic primers identified a total of 153 alleles, averaging 3.64 alleles per locus. The polymorphism information content (PIC) ranged from 0.03 to 0.78, with a mean of 0.41, suggesting a high level of genetic variability.

Xu et al. (2011) used fourteen SSR primers to evaluate 44 Chinese bottle gourd accessions, identifying 51 alleles with an average of 3.64 alleles per locus. The polymorphism information content (PIC) ranged from 0.11 to 0.72, with an average of 0.4. Similarly, Hu et al. (2010) used twenty-one EST-SSR markers to amplify 29 cucumber accessions from various ecotypes in China, detecting 73 alleles with sizes ranging from 110 to 489 bp. Each marker revealed 2 to 5 alleles, with a mean of 3.48. The PIC values for cucumber ranged from 0.185 to 0.642, with a mean of 0.374.

## 2.4 Biochemical composition

Fernandes et al. (2005) examined the biochemical and nutritional aspects of *Cyclanthera pedata*, emphasizing its mineral requirements and their influence on dry matter production. Across two cultivation cycles, deficiencies in macronutrients, particularly phosphorus, calcium, magnesium, and sulfur, were found to greatly reduce dry matter yield, whereas the absence of micronutrients had a relatively minor impact.

Rivas et al. (2013) examined the phytochemical, nutritional, and bioactive properties of *C. pedata*, highlighting its potential as a functional food. The fruit is low-calorie, with balanced macronutrients, and its flour is rich in potassium (7400 mg/100 g) and low in sodium (77 mg/100 g), supporting cardiovascular health. It contains high levels of free phenolic compounds (670 mg GAE/100 g) and ascorbic acid (123 mg/100 g), contributing to strong antioxidant activity, as shown by ABTS and linoleic acid-β-carotene methods. The fruit also exhibits significant anti-inflammatory effects, inhibiting lipoxygenase (LOX) with an IC50 value of 40 µg GAE/ml. Both fresh and processed forms of the fruit provide similar nutritional and bioactive benefits, making it a promising dietary supplement for health promotion. Its rich phytochemical content and antioxidant and anti-inflammatory properties highlight its potential in functional food development. Similarly, Oliveira et al. (2013) carried out a study to determine the mineral composition of *C. pedata*. The evaluation of 29 samples using inductively coupled plasma optical emission spectrometry revealed the following average concentrations (in mg per 100 g): 0.91 for sodium, 152 for potassium, 19.4 for phosphorus, 11.9 for calcium, 8.4 for magnesium, 0.074 for manganese, 0.21 for iron, 0.013 for copper, 0.13 for zinc, and 0.015 for vanadium. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were employed to assess the data.

Orsini (2018) investigates the phenolic compound profiles of *C. pedata* fruits and leaves, highlighting their potential as bioactive food supplements. The study identifies a range of glycosylated flavonoids, particularly those with anti-hyperglycemic properties, using advanced chromatographic techniques like HPLC and HPTLC. HPTLC facilitated efficient screening, while HPLC provided detailed analysis, revealing ten glycosylated flavonoids, including one novel compound. The research also examines how pedo-climatic conditions and plant growth stages influence phenolic content, with leaves showing variability and fruits maintaining stable levels. A simplified hydrolysis method was developed for phenolic characterization, optimizing sample and solvent use. The study underscores the therapeutic potential of caigua, particularly for nutraceutical applications, emphasizing the significance of growth conditions in maximizing bioactive compound concentration.

Valadares et al. (2020) highlighted the nutritional potential of *C. pedata* as a valuable food source, with its fruits comprising 12.9% crude protein, 3.9% ether extract, 20.3% crude fiber, and 10.7% ash, along with high moisture content (94.4%) and moderate dry matter (5.6%). The study demonstrated that while nitrogen treatments, such as urea and green manures, significantly boosted fruit productivity and nutrient accumulation (e.g., nitrogen and potassium), they did not alter the bromatological properties of the fruits. These results emphasize *C. pedata* role in human nutrition and medicinal use, providing essential nutrients and dietary protein.

Nieto et al. (2023) introduce an innovative method to enhance the agronomic and physiological characteristics of *C. pedata* by combining cytokinin and compost. The research evaluated the effects of two levels of cytokinin (100 and 150 ml of Anthesis Plus per 200 L of water) and organic compost applied at 10 tons/ ha on *C. pedata* cultivation. Specifically, the treatment increased plant length by 85.0%, diameter by 46.9%, number of fruits per plant by 81.8%, and both dry and fresh fruit weights by 80.6% and 83.2%, respectively. Physiological parameters, such as chlorophyll content, quantum yield, photosynthetically active radiation, fluorescence yield, and electron transport reaction, showed significant enhancements of up to 68.8%, 66.4%, 79.2%, 51.1%, and 74.0%, respectively.

Zuccolo et al. (2023) analyzed "Ciuenlai," an Italian variety of *C. pedata* from the Camonica Valley, highlighting its agronomic, ecological, phytochemical, and nutritional qualities. The fruits contain phenolic compounds significantly at higher levels of cafeoylquinic acid (0.64% vs. 0.15%), enhancing their therapeutic value.

# Chapter 03

# Materials and Methods

The study entitled "Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening" was conducted at the Department of Horticulture, Sikkim University, 6$^{th}$ mile Samdur, Tadong, Gangtok. The details of materials used and procedures followed during the investigation are described in the present chapter.

## 3.1 Experimental materials

To collect the seeds of the *C. pedata* genotypes for the present experiment a survey was carried out. Farmers from all over Sikkim were contacted and randomly 100 farmers (respondents), those who had been growing *C. pedata* for at least past five years were interviewed with a structured questionnaire. Details of the structured questionnaire used for the survey and farmers interviewed are given in Annexures I and II. Based on the survey, fifty-one genotypes were collected from different parts of Sikkim which were used for the present work. The collected *C. pedata* was given code SCP and numbered as per the area it was collected. SCP01, SCP02, SCP03, SCP04, SCP05, SCP06, SCP07, and SCP08 were collected from Pakyong districts, SCP09, SCP10, SCP11, SCP12, SCP13, SCP14, SCP15, and SCP16 were collected from Gangtok districts. SCP26, SCP27, SCP28, SCP29, SCP30, SCP31, SCP32, SCP33 were collected from Gyalshing district and SCP34, SCP35, SCP36, SCP36, SCP37, SCP38, SCP39, SCP40, SCP41, SCP42 were collected from Soreng district.

SCP52, SCP53, SCP54, SCP55, SCP56, SCP57, SCP58, SCP59, SCP60, SCP61, SCP62 were collected from Namchi district and SCP72, SCP73, SCP74, SCP75, SCP76, SCP77 and SCP78 were collected from Mangan district. These genotypes were maintained in the field of the experiment for further study. The details of the site of the collection are given in Annexure III.

**Plate 1: Experimental field view for morphological evaluation of *C. pedata***

## 3.2 Location of the experiment site

For the morphological evaluation of the collected 51 genotypes of *C. pedate*, a field experiment was set up in South Kartok, Pakyong districts, Sikkim. The experimentation site was located at an elevation of 1100 meters above sea level (MSL) with coordinates of 27º18’22” N latitude and 88º35’20” E longitude. The field experiment was carried out during the summer growing season of Cucurbitaceae crops (month of April to December) for two seasons of the years 2020 and 202.

## 3.3 Evaluation of fifty-one genotypes of *C. pedata* for quantitative and qualitative morphological parameters

The assessment of vegetative and fruiting characteristics of collected *C. pedata* genotypes was carried out in Randomized Block Design (RBD) with five replications. Seeds were directly sown in April and maintained the distance of 3×3 m plant to plant and row to row.

### 3.3.1. Observations Recorded

The observations were recorded based on descriptors of other related Cucurbitaceae crops like bottle gourd, cucumber, and bitter gourd (plantauthority.gov.in). The data were recorded from 5 plants per replication of the collected genotype at the vegetative, fruiting, and harvesting stages. These traits included the following characters.

#### *3.3.1.1. Vine length (m)*

The vine length was measured from the base of the stem at ground level to the tip of the main shoot after uprooting the whole plant by using a centimeter scale at the time of harvest. The data were taken in centimeters (m).

#### *3.3.1.2. No. of primary branches*

The branches emerging from the main stem were counted at harvest and recorded as the number of primary branches per plant.

#### *3.3.1.3. Internodal length (cm)*

Internodal length was determined by measuring the two consecutive nodes' distance, starting after leaving 2-3 nodes from the base of the stem.

#### *3.3.1.4. Days to 50% flowering*

Time taken (in days) from sowing to 50% of the plants reached flowering was calculated and recorded as ‘days to 50% flowering’.

#### *3.3.1.5. Node no. to first staminate flower*

The node number of the first staminate flower was recorded by counting the nodes from the base of the stem to the node where the flower first appeared.

#### *3.3.1.6. Node no. to first pistillate flower*

The node number of the first pistillate flower was taken by counting the nodes from the base of the stem to the node where the flower first appeared.

***3.3.1.7. Peduncle length (cm)***

To measure peduncle length, the peduncle was identified, and measure the distance from the base of the fruit to the tip of the peduncle, recording the length in centimeters (cm).

***3.3.1.8. Fruit length (cm)***

Selected fruit was measured by vernier calliper from the base to the tip of the fruit, recording the length in centimeters (cm).

***3.3.1.9. Fruit girth (cm)***

A vernier calliper was used to measure the girth of the fruit by carefully measuring its widest part, recording the girth in centimeters (cm).

***3.3.1.10. No. seeds per plant***

To get the seed number per fruit, seeds were extracted and counted from the fruit of each collected genotype.

***3.3.1.11. 100 seed weight (g)***

True 100 seeds were taken and weighed by using a digital balance.

***3.3.1.12. Average fruit weight (g)***

The average fruit weight was obtained by weighing randomly selected fruits from several plants, summing the weights, and dividing by the same number of fruits.

### *3.3.1.13. No. of fruit per plant*

To calculate the total number of fruits per plant, fruits were counted during each harvesting and summed up for the total.

### *3.3.1.14. Petiole length (cm)*

The petiole length was measured by selecting a leaf, identifying the petiole, and using a ruler to measure its length from the stem attachment to the tip, measurement recorded in centimeters (cm).

### *3.3.1.15. Yield/plant (kg)*

Yield per plant in kg was recorded as the average yield obtained from each plant across all replications.

### *3.3.1.16. Qualitative parameters*

The evaluation for qualitative parameters of the collected genotypes was conducted for the characters outlined in the table below.

**Table 3.1 Qualitative characterization of *C. pedata* genotypes**

| Sl. No. | Characteristics | States | Types of assessment |
|---|---|---|---|
| 1. | Stem shape | Round/ Angular | Visual Assessment |
| 2. | Stem pubescence | Present/Absence | Visual Assessment |
| 3. | Leaf colour | Light Green/ Green/Dark Green/ Greenish Violet/ Violet | RHS Colour Chart |
| 4. | Leaf blade | Entire/Serrated/Multifold | Visual Assessment |
| 5. | Flower colour | White/Yellowish White/ Greenish White/ Pale Violet/ Light Violet/ Bluish Violet/ Others | Visual Assessment |
| 6. | Fruit colour | Yellow/ Light green/Dark Green/ Violet Green | RHS Colour Chart |
| 7. | Fruit Shape | Elongated/ Curved/ Pointed/ Oval/ Round | Visual Assessment |
| 8. | Spine | Present/ Absence | Visual Assessment |
| 9. | Seed colour | Black/ Yellow/ Brown | Visual Assessment |
| 10. | No. of leaf lobes | 2,3,5,7 | Visual Assessment |

*RHS Colour Chart: Royal Horticultural Society colour chart was used for indexing colour

### 3.3.2 Statistical Analysis

Morphological data collected was evaluated and analysed for interpretation of results with the given statistical analysis. INDOSTAT software was utilized for these analyses.

#### *3.3.2.1 Analysis of Mean Performance*

It was calculated by using the following formula:

$$\text{Mean } (X) = \sum X / N$$

Where, $\sum X$= Summation of N variables, N=Number of total observations

#### *3.3.2.2 Analysis of Variance*

For the interpretation of valid conclusions, Randomized Block Design (RBD) data was analyzed using the method suggested by Panse and Sukhatme (1985). The calculated F-value was compared to the critical F-value at the 5% significance level. Subsequently, the critical difference was calculated for pairwise comparisons. Each character was analyzed separately using the same statistical method.

#### *3.3.2.3 Range*

Range is the difference between the highest and the lowest value of each

$$\text{Range} = X_H - X_L$$

Where, $X_H$= Highest value, $X_L$ = Lowest value

### *3.3.2.4 Estimation of Variability*

Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) for different characters were estimated as per the method suggested by Burton and DeVane (1953). The formula is as follows:

$$\text{GCV} = \frac{\text{Genotypic standard deviation}}{Mean} \times 100$$

$$\text{PCV} = \frac{\text{Phenotypic standard deviation}}{Mean} \times 100$$

### *3.3.2.5 Character Association Analysis*

#### *3.3.2.5.1 Correlation*

To measure the relationship among various characters at genotypic (g), and phenotypic (p) levels coefficient of correlation was used. The correlation coefficient was estimated as per the method suggested by (Al-Jibouri et al., 1958)

**Genotypic correlation coefficients (rg)**

$$\text{rg} = \frac{cov.XY\ (g)}{\sqrt{var.Xg.var.Yg}}$$

**Phenotypic correlation coefficient (rp)**

$$\text{rp} = \frac{cov.XY\ (p)}{\sqrt{var.Xp.var.Yp}}$$

Where, cov.XY(g) and cov.XY(p) is genotypic and phenotypic covariance between characters X and Y, respectively.

#### *3.3.2.5.2 Path Analysis*

Path coefficient was analyzed according to Dewey and Lu (1959). Yield per plant was assumed to be the dependent variable (effect) which is influenced by 15 characters (independent variable). Path coefficients were estimated by solving the following simultaneous equation indicating the basic relationship between correlation and path coefficient. The equations used are as follows:

$$r_{1y} = P_{1y} + r_{12}P_{2y} + \text{------------------} \; r_{1p} \, P_{py}$$

$$r_{2y} = r_{21}P_{1y} + r_{2p\ p}p_{y}$$

$$r_{py} = r_{p1}P_{1y} + rP_{2}P_{2y} + \text{-----------} \; P_{py}$$

Where, $P_{1y}$... $P_{py}$ is direct effects of characters 1,2,.. P, and $r_1y_1$, $r_1y_1$---$r_{py}$ denote correlation coefficient between independent and dependent characters "y".

Residual effect was calculatedusing the following formula.

$$\mathrm{I} = P^2R_y + \sum P_{1y}\, R_{1y}$$

Or

$$P_{RY} = \sqrt{1 - P1y\, r1y - (P2y.r2y) - (Ppxrpy)}$$

Where, $P_{RY}$ is the residual effect and y stands for seed yield.

#### *3.3.2.5.3 D² statistics*

$D^2$ statistics described the multivariate analysis of genetic divergence using Mahalanobis's $D^2$ statistics (Rao, 1952). Transformation of actual means of various characters (X1‟S) to uncorrelated varieties (YI's) was carried out by using pivotal condensation as the common dispersion matrix calculated on a computer. This made the $D^2$ value a simple sum of squares of difference in transformed values of various characters. The distance between any two populations is defined as.

$$D^2P = b1d1 + b2d2 + \ldots + bpdp$$

Where, X1, X2, X3….., Xp as a multiple measurements available on each individual

d1d2….., dp as x, X1-1 – X1-2, X2-1 – X2-2 ….. Xp-1 – Xp-2, respectively, being the difference in the mean of two populations.

In terms of variance and co-variance, the $D^2$ value is obtained as follows.

$$D^2P = Wij\ (Xi\text{-}1 - Xi\text{-}2)\ (Xj\text{-}1 - Xj\text{-}2)$$

Where, Wij = is the inverse estimated variance, covariance matrix

## 3.4 Assessment of the genetic diversity of *C. pedata* genotypes by using SSR marker

The seeds of 51 genotypes *C. pedata* were sown in pro trays and raised with proper care. DNA extraction was done from one week old seedlings having tender leaves to evaluate genetic diversity.

### 3.4.1 DNA extraction of *C. pedata*

DNA from the young leaves of each plant sample was extracted using the CATB (Cetyl Trimethyl Ammonium Bromide) protocol as described by Doyle (1991) with required modification. The following procedures were adopted for the DNA extraction:

- Fine powder of plant sample weighing 110-115 mg was prepared by using liquid nitrogen in a mortar and pestle.
- A pinch of Polyvinylpyrrolidone (PVP) and 30 µl of β-Mercaptoethanol were added to the sample, followed by 1 ml of DNA isolation buffer.
- The homogenate was then divided into two Eppendorf tubes, with 750 µl of the sample placed in each tube.
- The tubes were incubated in a water bath at 65ºC for 45 minutes, gently inverting the sample at 10-minute intervals, and subsequently allowed to cool to room temperature.
- After cooling, 750 µl of chloroform: isoamyl alcohol in a ratio of 24:1 was added to each tube, and was gently mixed by inverting for 10 minutes before centrifuging for 5 minutes at 10,000 rpm.
- The upper phase containing nucleic acid was transferred to new Eppendorf tubes, where an additional 750 µl of chloroform: isoamyl alcohol (24:1) was added.
- The mixture was centrifuged again at 10,000 rpm for 5 minutes, and the upper aqueous phase was collected.
- The chilled isopropanol (750 µl) was added to the Eppendorf tubes and the sample was centrifuged at 10,000 rpm for 5 minutes to precipitate the DNA.
- The supernatant was discarded, and the DNA pellet was washed with 500 µl of 70% ethanol, followed by another centrifugation for 5 minutes.
- After discarding the supernatant, the DNA pellet was dried in a dry bath for 30-40 minutes.

- Finally, 15 µl of TE buffer (Tris and EDTA in a 10:1 ratio) was added to rehydrate the DNA pellet.

The DNA quality was examined by gel electrophoresis followed by visualization of the DNA band in the gel documentation system.

### 3.4.2 DNA quantification

The extracted DNA was quantified by using Thermo Scientific Nanodrop 2000 C Spectrophotometer.

### 3.4.3 DNA Sample Dilution

After the quantification of the DNA, the sample was diluted for further PCR amplification process. The formula for diluting a DNA solution is based on the general dilution equation: $C_1V_1=C_2V_2$

Where, $C_1$ is the initial concentration of the DNA (stock concentration),

$V_1$ is the volume of the DNA stock solution to be diluted,

$C_2$ is the final concentration of the DNA (desired concentration),

$V_2$ is the final volume of the diluted solution.

To calculate how much of the stock DNA solution was needed, the formula was rearranged as:

$$V_1= C_2V_2/C_1$$

This equation gave the volume of the stock solution required. The appropriate amount of diluent (e.g., water or buffer) was then added to achieve the final volume $V_2$.

### 3.4.4 SSR (Simple sequence repeats) based PCR (Polymerase chain reaction) amplification

The components of PCR (Polymerase chain reaction) mixture are given in Table No 2. PCR was performed on a Bio-Rad thermal cycler (C1000 Touch™). Amplified PCR products were visualized using gel electrophoresis on a 1% agarose gel with 1X TBE buffer. Each gel included a 100 bp DNA ladder in the first well to determine the allele bands in the image derived from the gel documentation system.

**Table 3.2 Components of PCR mixture**

| Components | Quantity used/tube |
|---|---|
| DNA template | 1 µl |
| Master mix | 12.5 µl |
| Forward primer | 1 µl |
| Reverse primer | 1 µl |
| Nuclease free water | 9.5 Nuclease free water |
| Total Volume | 25 µl/tube |

The following steps were performed during PCR amplification:

**Denaturation**: The DNA mixture was heated to 94ºC for 4 minutes, followed by 36 cycles at 94ºC for 1 minute each.

**Annealing**: The annealing temperature, ranging between 55-63ºC depending on the specific primer, was set for the primer binding process.

**Extension**: The temperature for the extension step was set to 72°C, maintained initially for 1 minute, and held at the same temperature for a final 5 minutes.

### 3.4.5 Details of SSRs

Primers were selected based on other cucurbit crops such as cucumber, bottle gourd, bitter gourd, and pumpkin, and genetic diversity was evaluated using 30 pairs of SSR (Simple Sequence Repeats) primers. The following table provides the details of the primers.

**Table 3.3 Detail of SSR primers used for the experiment**

| Sl. No. | Primer code | Primer sequence (5'- 3') |
|---|---|---|
| 1. | LSR011 | F: TTCGCCTCAGTCCATCTAGTTT,<br>R: ATGTCGTACCTTTTCCCCTTTT |
| 2. | LSR015 | F: CTTACCTTCACAAAACCCCATC,<br>R: ACTCTGTTTCGACTCTGCTTCC |
| 3. | LSR030 | F: GGAGACAAAACCAACAACGAA,<br>R: GAAAATGCAGACAAAGAAAGCC |
| 4. | LSR040 | F: TTCCCATCCAGACCAAACCTATC,<br>R: CAAAGGCCATAGACAAACACAA |
| 5. | LSR047 | F: CAATAGAGTAGGGTGGGGCATA,<br>R: TAAAATAGTGGGAGAGCAAGGG |
| 6. | LSR063 | F: AAGAGAGGGGCAGGAAGTAAAT,<br>R: AGAAAACACACAGTACGCCTCC |
| 7. | LSR074 | F: TATCAACTCCAGAAGACGGGTT,<br>R: GGTAAACGTAGGGATACAAAGAAG |
| 8. | LSR077 | F: GACAGATCCTTCTGGGACTTTT,<br>R: TTCTGCAATAGAGTACGTTGGC |
| 9. | LSR108 | F: AGCTCTGGGAAGAGGAAGAGTA,<br>R: GCAGACAGAAGAAGAAAGTTAGAGA |
| 10. | LSR109 | F: TGGGGTAGAAATTGAAGAGGAG,<br>R: TTGGATCAGCTTGGGTTTTACT |
| 11. | LSR112 | F: TCTCTATATGTCTAATTCCTCGCC<br>R: CAAATTCACAGTTGTTGTCACG |
| 12. | MC08_106502 | F: TGCCAAGCAAATACCACAAA,<br>R: TGAAGAAGCAACAGCAGCAG |
| 13. | MC04_54893 | F: GAAAGCGAGAACGAAC<br>R: TGCCATCGGTACTCATCAAA |
| 14. | MC10_152791 | F: TTGCAGCTTCCTTTCTGGTT<br>R: AGGAAACACATCTTGGGCTG |

| | | |
|---|---|---|
| 15. | MC07_95456 | F: TTCTTGAGAGACGGTGGCT<br>R: GATACAAAGAAACGGTGGCG |
| 16. | MC06_73242 | F: AGCGAAGCATTTATCGAG<br>R: CGAAACGCACTATTCCCATT |
| 17. | EC11 | F: TCTTCGCAGTCACCATTTC<br>R: CCTTCCTCTGTTTCTGTTCC |
| 18. | EC49 | F: CGTGTTTTCTCAGATTTCCCA<br>R: CACTTCCCTTCATCAACCCCA |
| 19. | EC34 | F: GATCCCCATCATAATCACCC<br>R: CAAAGGGCTACAATAACAAAC |
| 20. | EC27 | F: GTTGGAAGGCACACAAAGTC<br>R: CGAGATGATTGGAGGATGATG |
| 21. | SSR18362 | F: CAAGTGGACAAATATGAGCCAA<br>R: TTCGTTTCGCAGAGTGATTG |
| 22. | SSR12810 | F: TTCCCAAAAACAAATCTTGG<br>R: TTTTGGAGAGAAAAGGTTGGA |
| 23. | SSR05737 | F: TTGCCTTCGTAAGCAAAA<br>R: GAAGTAAATGGGTTGGACGC |
| 24. | SSR15477 | F: CTGCCATTTCTGGGTTTGAT<br>R: AATTCTTCTGGGAATGGCCT |

### 3.4.6 Statistical Analysis

Polymorphism Information Content (PIC) for each primer pair was calculated with PowerMarker v3.23. The population structure of the *C. pedata* genotypes was analyzed using the model-based software STRUCTURE v2.3.4, applying a Bayesian approach to infer the genetic composition.

## 3.5 Analysis of biochemical properties of the fruits

The observations were recorded from the fruits of five plants (chosen randomly) from each replication of 51 genotypes for the biochemical parameters.

### 3.5.1 Estimation of fresh weight, dry weight, and moisture percent

The freshly harvested fruit samples were weighed by using a digital weighing balance to determine their fresh weight. The fresh weight was recorded in grams (g).

Weighed samples were kept in the hot air oven at 100-105ºC till they reached constant dried weight (g). The moisture content in percentage was calculated by the following formulae:

$$\text{Moisture content (\%)} = \frac{\text{weight of the fresh sample- weight of dry matter}}{\text{weight of fresh sample}} \times 100$$

### 3.5.2 Estimation of Total Carbohydrate Content

Total carbohydrate content (mg/100 g) was estimated as per the method given by Tamboli et al. (2020).

**Sample preparation:** 1 g dried powdered sample was mixed with 10 ml distilled water and kept in the rotary for 24 hours at 120 rpm. After 24 hours, the sample was filtered and the filtrate was utilised for the estimation of total carbohydrate content.

**Reagents required:** Phenol Solution, Concentrated Sulphuric Acid

**Standard preparation:** 100 mg glucose was mixed with 5 ml 2.4 N HCl and allowed to boil in a water bath for 3 hours. Then the sample was taken out and cooled to room temperature. Afterward, 1 M Sodium carbonate was added to the solution until the effervescence ceased. Then the solution was diluted to a final volume of 100 ml using distilled water. From the solution, serial solution of concentrations 0.2, 0.4, 0.6, 0.8, and 1 ml was taken and the volume was made up to 1 ml using distilled water. One test tube was placed blank using only 1 ml of distilled water. 1 ml phenol and 5 ml conc. Sulphuric acid were added to each test tube and shaken well to mix properly for 10 minutes. Afterward, the samples were kept in a water bath for 25-30◦C for 20 minutes. In a hot acidic medium, glucose gets dehydrated to hydroxymethylfurfural which makes the samples turn into green colour products in the presence of phenol. Then the absorbance was taken at 490 nm.

### 3.5.3 TSS (Total soluble solids)

Total soluble solids of the fruits were determined using the help of hand refractometer and it was expressed in (ºB) degree brix.

### 3.5.4 Estimation of Protein

The protein estimation was done by Lowry's method (Lowry et al., 1951) by using UV/VIS Spectrophotometer, Perkin Elmer, Lamda 35 UV/VIS spectrometer.

**Reagents used:**

- Reagent A- 2.0% $Na_2Co_3$ mixed in 0.1N NaOH (20 g of sodium carbonate and 4 g of sodium hydroxide in distilled water and volume was made up to 1litre).
- Reagent B- 1% $CuSO_4.5H_2O$ in 2% sodium potassium tartarate (1 g of copper sulphate was dissolved in 100 ml distilled water and 2 g of sodium potassium tartarate is dissolved in 100 ml distilled water then the working solution of reagent B was prepared fresh by mixing the solution of 1% CuSo4.5H2O in 2% sodium potassium tartarate).
- Reagent C- Alkaline Copper solution: prepared by mixing 50 ml of reagent A and 1ml of reagent B prior to use.
- Reagent D- Folin Ciocalteu Reagent
- Protein Stock Solution- BSA mg/ml (100 mg of Bovine serum albumin was weighed accurately and disallowed 100 ml water).
- Working standard- Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. 1 ml of this solution contain 200 µg protein.

**Procedure:**

A. Extraction of fruit sample: Fresh fruit sample (0.5 g) was weighed and ground well with mortar and pestle in 5 ml of phosphate buffer. It was centrifuged for 20 minutes at 5000 rpm and supernatant was collected for protein estimation.

B. Preparation of standard curve and estimation of protein:

- From the BSA solution 0.1, 0.2, 0.4, 0.6, 0.8 was pipette out and 1 ml of working standard and poured into series of test tubes.
- Sample extract (0.5 ml) was pipetted out in other set of test tubes.
- Volume made up to 1 ml in all the test tubes with distilled water. A tube with 1 ml of water served as a blank.
- Reagent C (5 ml) was added in each tube and was mixed well and waited for 10 minutes.
- FCR (Reagent D) at the rate of 0.5 ml was added in each tube and mixed well and then incubated at room temperature for 30 minutes and a reading was taken at 660 nm.
- Plot a standard curve of absorbance versus protein concentration for the standard solutions.
- Protein was estimated in the sample by interpolating its absorbance value on the standard curve and the amount of protein was expressed in percentage.

### 3.5.5 Ascorbic Acid

Determination of ascorbic acid content in the fruits was carried out by following 2,6 dichlorophenol indophenols dye method described by AOAC (2000). The amount of ascorbic acid content in fruits is expressed as mg/100g.

### 3.5.6 Elemental Analysis

Estimation of essential minerals and nutrients like P, K, Fe, Na, Ca, Mg, Zn, and Mn was done through ICP-MS. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a highly sensitive analytical technique used to detect and quantify trace elements and isotopes in various sample types. It is capable of analyzing elements at parts-per-billion (ppb) to parts-per-trillion (ppt) levels.

**Sample Preparation:**

**Digestion:** Dried samples were grounded and digested using acids (such as nitric acid and perchloric acid in 9:3 ratio) to convert them into a liquid form.

**Microwave Digestion:** This method was often employed for tough samples. The sample was subjected to heat and pressure in a microwave system with acid to ensure complete dissolution.

**Filtration:** After digestion and dilution, the sample was filtered to remove particulates that could have interfered with the instrument.

Prepared samples were introduced to ICP-MS for estimation of essential minerals present in *C. pedata*

### 3.5.7 Statistical Analysis

Data was analyzed by ANOVA for a Randomized Block Design (RBD). The mean value of five replicates for each observation with their SE value is presented in tabular form. The mean value will be compared by the least significant difference (LSD) at a 5% probability level (p=0.05). Further data were also subjected to Duncan's multiple range test for comparisons between means.

# Chapter 04

# Result and Discussion

This study focused on gaining insight into the variations, diversity, and inter-relationships among the qualitative, quantitative, and biochemical traits of *C. pedata*. The research entitled "Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening" was conducted at the Department of Horticulture, Sikkim University. This chapter presents the results obtained from the statistical analysis of the data gathered throughout the investigation.

## 4.1 Farmers' perception of desirable horticulture traits of *C. pedata*

Understanding farmers' perception of *C. pedata* desirable traits is crucial for improving varieties and sustainably promoting its cultivation. Farmers usually focus on characteristics like fruit size, shape, taste, texture, and plant yield, as these traits are key factors in meeting market demand and consumer preferences. They also emphasize pest and disease resistance and plants' ability to adapt to local climatic conditions and tolerate various environmental stresses such as drought, which is particularly suitable for regions like Sikkim, where the farming system is entirely organic and relies more on sustainable practices without any uses of synthetic chemicals. Incorporating these insights into breeding programs helps researchers in the development of varieties that are both economically viable and culturally relevant.


**Plate 2: Farmers participitated survey**

### 4.1.1 Socio-economic information of farmers (respondents)

The socio-economic information of the farmers (respondents), who participated in the survey is summarized in Table 4.1. As presented in the table, the cultivation of *C. pedata* in Sikkim was dominantly done by women farmers (57%). Their active participation in farming emphasizes the critical role women play in agriculture, which can influence decision-making processes, crop management practices, and the overall production and productivity of farming systems. Women farmers' dominance compared to men farmers involved in farming is quite common (Mc Elhinny et al., 2007; Efisue et al., 2008). Among the farmers surveyed, 71% were married, whereas 24% were unmarried and a small proportion i.e. 5%, were found widowed. This finding highlights the diverse marital statuses within the farming community. Most of these farmers were in the age group of 30-39 years old and married (74%), followed by more than 50 years old (31%), the age group of 20-29 (19%), and 40-49 (16%). The majority of respondents were unemployed (55%) and sustained their livelihood through the means of agriculture. However, a combined 34% of service people and students were also engaged in crop production. 11% of service retired personnel were also active in farming. For family size, data reveals that 52% of respondents had an average of 5 family members, indicating a relatively moderate family size, while 26% and 22% of respondents had an average family number of 2 and more than 7, respectively. The size of the family plays a critical role in shaping the economic situation and well-being of households. Education plays a vital role in enhancing agricultural productivity (Eric et al., 2014). About 13% of respondents were not able to read and write. This can be related to the work reported by Irungu et al., (2011), Mwaura et al., (2013), and Sharma et al., (2017). 19% of the farmers had attained graduation and above-level education which shows they can apply their education to use the modern farm technologies in the improvement of the crop and its yield. Whereas, 32% of respondents were found to have a primary level of education and 22% have achieved high school education. The differences in education suggest that some farmers might be able to adopt new technologies. This result highlights that there is a pressing need for training programs for the target group of people to improve their knowledge in the agriculture field. The minimum threshold level of education plays a significant role in the adapting of modern varieties, which positively affects the farm production and productivity of those who adopt them (Paltasingh & Goyari, 2018). The data indicated that the majority of children (63%) were receiving education in school, with 1 to 2 children per household enrolled. This was followed by households with more than 2 children attending school (22%), while 15% of households had no children receiving education.

The data revealed that on average 1.5 family members from 75% of the respondents were involved in the cultivation of *C. pedata*. This was followed by households with 3 to 5 members (23%) and those with more than 5 members (2%). Regarding farming experience, 46% of respondents had more than 10 years of experience, 32% had less than 10 years, and 22% had less than 5 years of experience. The majority of households belong to a small number of families, involved in *C. pedata* farming, indicating that cultivation of this crop doesn’t require much input and labour.

**Table 4.1 Socio-economic information of farmers (respondents)**

| Variables | Categories | Percentage (%) | Mean | SD |
|---|---|---|---|---|
| Gender | Men | 43 | 50 | 9.90 |
| | Women | 57 | | |
| Age (years) | 20-29 | 19 | 25 | 8.83 |
| | 30-39 | 34 | | |
| | 40-49 | 16 | | |
| | More than 50 | 31 | | |
| Marital Status | Married | 71 | 33.33 | 33.98 |
| | Unmarried | 24 | | |
| | Widowed | 5 | | |
| Employment status | Employees | 17 | 25.00 | 20.20 |
| | Retired | 11 | | |
| | Unemployed | 55 | | |
| | Students | 17 | | |
| Household size | 1-3 | 26 | 33.33 | 16.29 |
| | 4-6 | 52 | | |
| | More than 7 | 22 | | |
| Education | Can't read and write | 13 | 20.00 | 7.65 |
| | Primary | 32 | | |
| | Secondary | 22 | | |
| | High Secondary | 14 | | |
| | Graduation and above | 19 | | |
| Children in Education | No children in school | 15 | 33.33 | 25.93 |
| | 1-2 | 63 | | |
| | More than two | 22 | | |
| Family members engaged in farming | 1-2 | 75 | 33.33 | 37.58 |
| | 3-5 | 23 | | |
| | More than 5 | 02 | | |
| Experience in farming (years) | Less than 5 | 22 | 33.33 | 12.06 |
| | Less than 10 | 32 | | |
| | More than 10 | 46 | | |

### 4.1.2 Framers' perception of cultivation aspects and purpose of cultivation of *C. pedata*

Farmers were asked about their perception of *C. pedata* cultivation aspects such as time of planting, method of sowing, type of support required, and purpose of growing. The information collected is presented in Table 4.2. As per the information provided by the farmers, the majority of them generally sow *C. pedata* seeds between April to May (52%), and March to April (27%). 21% of farmers sow the seeds during September and October. Farmers' perceptions regarding the planting time of *C. pedata* play a vital role in regulating the productive crop. Most farmers prefer to plant *C. pedata* during the period from April to May and March to April. This timing aligns with the general planting season for other cucurbitaceous crops in the region, as it coincides with favorable environmental conditions. Early or seasonal planting can ensure a healthy plant and is directly associated with high yield of the plant and fetch more market return. The results are consistent with Nwofia et al., (2010), where early planting season significantly affected the fresh yield of cucumber and had higher yield. Similarly, highest number of bottle gourds when planted in May (Soubeih, 2016). Farmers in Sikkim also prefer late planting of *C. pedata* during September and October, especially in the regions with warmer areas of the state. The warmer temperatures in these regions favour the growth and development of crops, making late planting a viable option.

85% of respondents practice direct sowing of seeds in the main field. However, 15% of farmers do not store seeds; instead, they transplant germinated seeds or seedlings found growing in other fields into the main field. Direct sowing ensures the seeds germinate and exhibit roots directly in the main field, which could minimize the risk of transplant stress and promote better adaptation to the soil and environmental conditions of the field.

Since the *C. pedata* is a vine structure and needs support for its growth, 67% of farmers make traditional trellis of big-sized branches of trees as support to the plants. Live trees were also used as support for the crop as perceived by 33 % of respondents. This may be due to the easily assessable and cost-effective supporting material and also provides a sturdy framework to the *C. pedata*.

The economical part of the crop is fruits, and they were mainly grown for marketing (54%) for the local markets, self-consumption (32%), and fodder (9%). Along with this, some local communities of Sikkim also use this crop for medicinal purposes (5%), as evidenced by the work done by Renila et al., (2010) and Gonzales et al., (1995).

### 4.1.3 Farmers' perception of horticultural traits of *C. pedata.*

The farmers' perception of the horticultural traits of *C. pedata* are important indicators of its suitability and performance in agriculture (Table 4.3). Interviewed farmers perceived these traits based on their experience, knowledge regarding the crop, local climatic conditions of Sikkim, and the specific requirements of the *C. pedata*. As shown in Table 4.3, 78% of farmers preferred the long vine length (more than 7 m) followed by short height (less than 3 m) (21%) and medium height (3-7 m) (1%). The reason for their choices was given by the respondents that the long vine bears more fruits resulting in more income and this may also be since longer vine can produce more no. of primary branches which is directly proportional to higher yield.

**Table 4.2 Framers' perception of cultivation aspects and purpose of cultivation of *C. pedate***

| Variables | | Percentage (%) | Mean | SD |
|---|---|---|---|---|
| Planting time | Mar- April | 27 | 33.3 | 16.4 |
| | Apr-May | 52 | | |
| | Sept- October | 21 | | |
| Method of cultivation | Direct sowing | 85 | 50 | 49.5 |
| | Transplanting | 15 | | |
| Types of support | Traditional trellis | 67 | 50 | 24.0 |
| | Other (live tree, etc.) | 33 | | |
| Purpose of cultivation | Self-consumption | 32 | 25 | 22.7 |
| | Fodder | 09 | | |
| | Medicinal use | 05 | | |
| | Marketing | 54 | | |

However, shorter vine length may be preferred due to the limited available area and the ease of managing and harvesting the fruits.

Farmers preferred the maximum number of branches per plant (more than 20) (68%), followed by least (less than 10) (20%) and moderate (10-20) (12%). No. of primary branches is a key trait that contributes to the productivity of *C. pedata*. This preference indicates that farmers focus on enhancing the total production and productivity of plants.

Data revealed that the majority of farmers (70%) preferred short internodal length (less than 5 cm), followed by medium (5-8 cm) and long (8 cm) (15% each). Short internodal length produces more internode within a given vine length and increases the potential site for flower and fruit production, ultimately enhancing the productivity of *C. pedata*. Medium and long internodes preferred by farmers may be due to ease in plant management with adequate spacing for airflow and light penetration. Less crowding of plants leads to minimizing pest and disease infestation.

86% of respondents preferred spineless fruit, followed by least spines (10%) and many spines (4%). As per the respondent, the spineless fruits taste better, enhancing their suitability for culinary purposes. It is also easy to handle the fruit, which can influence the market demand and acceptability. The strong preference for spineless fruits suggests that breeding programs should prioritize this trait to enhance consumer satisfaction and marketability. Data revealed from Table 4.3 that the short fruit length (5-10 cm) (69%) got more preference among the farmers compared to long (15-20 cm) (16%) and medium fruit (10-15 cm) (15%). Short fruits are easier to harvest, transport, and store, which can also reduce post-harvest losses. Plants producing short fruits may give a higher yield/per plant and breeding efforts should focus on developing genotypes with short fruit length while ensuring other desirable traits, such as taste, yield, and resistance to pests and diseases.

56% of respondents preferred a moderate number of seeds per fruit (15-20) followed by the least (5-10) (28%) number of seeds per fruit and 16% preferred highest no. of seeds (25-30). As per the respondents, moderate no. of seeds per fruit is better because it can balance between culinary and reproductive utility. The preference for a moderate number of seeds suggests that both culinary and reproductive traits are crucial for *C. pedata*.

*C. pedata* is eaten mainly in the green or immature stage, so, a high number of seeds cannot be useful during cooking and less no. of seeds can be problematic in the next season for reproduction. Its seeds are also utilized to make pickles in some parts of Sikkim which may be the reason to choose a moderate number of seeds per fruit.

As revealed from the study dominant preference among the farmers for fruit with mild taste (93%) with only a small percentage favoring sweet (5%) and bitter (2%) tastes. A mild taste allows the fruit to blend with a variety of dishes and cooking, making it a highly adaptable vegetable and can also be served as a good substitute. The preference for mild taste among farmers, indicates that this character should be a primary focus in breeding programs for *C. pedata*. Selecting genotypes with mild flavor can ensure the crop remains versatile and widely accepted.

By 96 %, soft flesh is considered as ideal choice of fruit. Regarding the harvesting period, early harvesting (86%) of fruits perceived by farmers is listed as the ideal character of plant for cultivation, however, medium (5%) and late (6%) harvesting of fruits were also included as their preferences about the crop.

**Table 4.3 Farmers' perception of horticultural traits of *C. petada.***

| Characters | | Percentage | Mean | SD |
|---|---|---|---|---|
| | Long (more than7) | 78 | | |
| Plant height (m) | Medium (3-7) | 1 | 33.33 | 23.1 |
| | Small (less than 3) | 21 | | |
| | Highest (more than 20) | 68 | | |
| No. of primary branches | Moderate (10-20) | 12 | 33.33 | 17.5 |
| | Least (less than 10) | 20 | | |
| | Long (more than 8) | 15 | | |
| Internodal length (cm) | Medium (5-8) | 15 | 33.33 | 18.3 |
| | Short (less than 5) | 70 | | |
| | Many | 4 | | |
| Fruit spines | Least | 10 | 33.33 | 26.4 |
| | Spineless | 86 | | |
| | Long (15-20) | 16 | | |
| Fruit length (cm) | Medium (10-15) | 15 | 33.33 | 17.8 |
| | Short (5-10) | 69 | | |
| | Highest (150) | 86 | | |
| No. of fruit per plant | Moderate (90-100) | 9 | 33.33 | 26.4 |
| | Least (70-90) | 5 | | |
| | Highest | 16 | | |
| No. of seed per fruit | Medium | 56 | 33.33 | 27.4 |
| | Least | 28 | | |
| Flesh texture | Medium soft | 4 | 50.0 | 46.0 |
| | Soft | 96 | | |
| | Mild | 93 | | |
| Fruit taste | Bitter | 2 | 33.33 | 29.8 |
| | Sweet | 5 | | |
| | Early | 86 | | |
| Duration for Maturity | Medium | 5 | 32.3 | 26.8 |
| | Late | 6 | | |

Early harvesting of fruits enables farmers to bring their produce to market sooner, which could be directly related to higher demand and better market prices. Shorter growing periods are also linked to early harvesting, which can facilitate farmers with the flexibility to grow many crops within a single season. Late harvesting could be beneficial in niche markets or specific uses where fully mature fruits are required, such as seed production or processing.

## 4.2 Morphological characterization of *C. pedata* genotypes

### 4.2.1 Analysis of variance

The mean data of fifteen traits for two seasons and pooled data were analyzed using ANOVA for RBD (Randomized Block Design). The mean sum of squares attributed to genotypes was highly significant, suggesting that the genotypes included in the study exhibited a considerable amount of variability. To understand the morphological diversity among the collected genotypes, pooled mean data has been discussed in the current chapter. ANOVA table for the pooled mean analysis is presented in Annexure V. Season-wise mean tables are given in Annexure VI for reference.

### 4.2.2 Mean performance of fifty-one *C. pedata* genotypes for morphological characterization

The mean performance of the collected genotypes, along with the range, standard error of the mean, and critical difference values at 5% for pooled analysis is shown in Table 4.4. the results reveal significant variability among the genotypes for all the traits studied.

#### *4.2.2.1. Vine length (m)*

The vine length varied significantly across all *C. pedata* genotypes (Table 4.4), with the longest vine length recorded in genotype SCP27, reaching 11.91 m, while the shortest vine length was recorded in genotype SCP01 (4.01 m). This substantial variation in vine length highlights the diversity present and significantly varying among the genotypes studied. The mean vine length across all observations was 7.80 m. The significant variation demonstrates the rich genetic base for *C. pedata* population, which can be exploited or used for breeding purposes. Longer vine lengths such as those in SCP27, may have evolved to climb and compete for light in natural environments, whereas shorter vines like SCP01 could be better adapted to limited resources. Longer vines can be ideal for traditional trellis-based systems and also maximize the photosynthetic efficiency but they may require more intense management and resources for cultivation. Longer vine can produce more internodes and primary branches which is directly related to the character yield/plant. Shorter vines are more suitable for small landholder farmers, where trellising is not feasible. Short vines may also facilitate in easy management and harvesting of *C. pedata* fruits. A considerable variation in plant height of bottle gourd which ranges from 281 cm to 322 cm (Mladenovic et al., 2012). Authors like Sivaraj and Pandravada, (2005) also reported the genetic variation in bottle gourd.

#### *4.2.2.2 No. of primary branches*

Among all the genotypes studied, maximum number of primary branches was recorded in SCP36 (19.60) and minimum in SCP09 (9.0). Primary branch numbers play a critical role in assessing the plant's overall productivity, growth pattern, and yield potential. The average mean value of this character was 14.75 (Table 4.4). The no. of primary branches is primarily governed by the genetic makeup of the plant. Genotype like SCP36, which recorded the highest number of primary branches may possess alleles that promote enhanced branching. A higher number of primary branches can lead to higher biomass and yield/plant. The variation in primary branches suggests that this trait can serve as an important selection criterion for breeding programs. Afangideh and Uyoh, (2007) and Ene et al. (2016) also observed significant differences observed among the cucumber genotypes for all traits including no. of primary branches.

#### *4.2.2.3 Internodal length*

Table 4.4 clearly shows that the maximum internodal length was observed in genotype SCP01 (12.73 cm) and minimum in SCP06 (7.64 cm). The overall mean for this trait was 10.50 cm. Longer internodal trait could be significant in agricultural contexts, where taller and elongated plants are desired, such as in crops grown for their climbing ability or for maximizing. While shorter internodal length could be valuable to influence other vegetative and reproductive characteristics, such as plant height, branching patterns, or yield potential. The variation in internodal length can be attributed largely to genetic factors but environmental conditions, regulation of plant hormones, and developmental stages also play significant roles.

#### *4.2.2.4 Days to 50% flowering*

Table 4.4 shows that days to 50% flowering were found non-significant. While, SCP01 was the earliest to reach 50% flowering, taking 85.30 days, followed by SCP31 at 88.70 days and SCP42 took the maximum number of days (214.70 days) to produce 50% flowering. The mean of this trait across all observations was 108.06 days. The variations in the number of days taken to reach 50% flowering could be due to developmental stages of plants vary between genotypes. Some plants initiate flowering earlier due to internal physiological factors, which could regulate flowering time. Genotypes like SCP01 might have more efficient flowering initiation mechanisms compared to other genotypes that flower later. Early flowering is directly associated with the early fruiting of the plants. Early yield is influenced by factors like plant vigor, branching, and fruiting capacity and does not solely depend on early flowering and it appears that early flowering does not necessarily result in a higher yield. Similar variation in days to 50% flowering among bitter gourd and cucumber genotypes was reported by Rathod et al. (2008) and Ukachukwu et al. (2019), respectively.

#### *4.2.2.5 Node no. to first staminate flower*

Table 4.4 shows that the first staminate flower appeared in the genotype of SCP02 (at 4.50$^{th}$ node), followed by SCP07 (at 4.90$^{th}$ node) and SCP27 produced the 1$^{st}$ staminate flower last (at 10.10$^{th}$ node). The overall mean for this character was 6.45$^{th}$ node. The emergence of the first staminate flower varied significantly among the genotypes of *C. pedata*. The variation may be attributed to genetic differences among the genotypes. Early flowering at a lower node indicates rapid reproductive development, which could be beneficial for the early initiation of fruit. Likewise, later flowering might be associated with longer vegetative growth, resulting in late fruiting. The variation in node position of the first staminate flower of *C. pedata* can be used in hybridization to select genotypes that meet specific reproductive requirements and can be better suited to areas with shorter growing seasons.

#### *4.2.2.6 Node no. to first pistillate flower*

Table 4.4 reveals the node number to the first pistillate flower is crucial for achieving early yields, maximizing productivity, and guiding breeding and selection for high-performing, adaptable varieties. The appearance of the first pistillate flower was observed in the genotype SCP53 (at 3.90$^{th}$ node), followed by SCP62 (at 4.30$^{th}$ node) and SCP27 produced the 1$^{st}$ pistillate flower last (at 10.20$^{th}$ node). The average mean value of this character was 6.15$^{th}$ node. The variation in node no. to the first pistillate flower is found significant which is directly impacting the early yield and overall plant production and productivity. Genotypes like SCP53 and SCP62, which produced the first pistillate flower at early nodes (3.90$^{th}$ and 4.30$^{th}$ node, respectively), are likely to achieve early yields. These early flowering genotypes can be selected for cropping systems where early market availability is important. Genotypes with longer vegetative phases may delay flowering. SCP27, with its later appearance of the first pistillate flower, may exhibit more vigorous vegetative growth, resulting in delayed reproduction but those genotypes can be considered for regions where larger fruit production or longer growing seasons are prioritized.

#### *4.2.2.7 Peduncle length (cm)*

The maximum peduncle length was found in genotype SCP41 (2.65 cm), subsequently, the genotype was followed by SCP33 (2.40 cm), and the minimum length was observed in SCP32 (0.89 cm) as presented in Table 4.4. The overall mean value for this trait was 1.46 cm. The differences in peduncle length are primarily driven by the genetic makeup of each genotype. Longer peduncles could be selected for breeding programs to improve flower position or to facilitate easier harvesting of fruits. The variation in peduncle length among the genotypes indicates the diversity among the genotypes, which is important for flower positioning, pollination efficiency, and ultimately yield potential.

#### *4.2.2.8. Fruit length (cm)*

Data shown in Table 4.4 revealed that the maximum fruit length was observed in SCP33 (18.56 cm), followed by SCP12 (14.63 cm), SCP11 (12.34 cm), and minimum in SCP03 (5.75 cm). The mean for this trait was 8.75 cm. The observed variation in fruit length among the genotypes is found to be significant. Genotypes like SCP33 could be valuable in breeding programs focused on producing large-fruited varieties for such markets. Smaller fruits as seen in SCP03 (5.75 cm), may have a niche market in certain culinary uses or preferred plants with higher fruit sets. The variation in fruit size for all genotypes provides opportunities for breeding programs to select the desired fruit size based on consumer preference. On the other hand, small fruited genotypes like SCP03 could be utilized for breeding varieties suited for high-density cultivation. Significant variation in *C. pedate* fruit length was also reported by Macchia et al. (2009). Comparable variation was also noted in the fruit length of cucumber genotypes ranging from (18.93 to 11.36 cm) long reported by Mainali & Jyakhwa (2023) and Raza et al., (2020).

#### *4.2.2.9 Fruit girth (cm)*

Data shown in Table 4.4 revealed that the maximum fruit girth was observed in SCP75 (5.12 cm), followed by SCP74 (5.04 cm), and minimum in SCP01 (2.14 cm). The grand mean of this trait was (3.35 cm). Variability in the case of fruit girth may be due to genetic makeup, inherent character, and genetic vigour of various genotypes. The variation in fruit girth among genotypes provides a valuable trait for breeding programs. Breeders can select genotypes like SCP75 for developing larger-fruited varieties or SCP01 for compact-fruited varieties. Hossain et al. (2010) and Khan et al. (2015) also reported similar findings in cucumber genotypes.

#### *4.2.10. No. of seeds/fruit*

As indicated in Table 4.4 the highest number of seeds per fruit was found in SCP78 (13.2), followed by SCP27 (12.6) while minimum number of seeds was observed in genotype SCP72 (7.3). The grand mean for this trait was 10.4. Genotypes SCP78 and SCP27, with higher seed counts, could be advantageous for seed production programs, ensuring a higher yield of seeds per fruit. These genotypes could be ideal for commercial propagation and genotypes that produce big sized fruit also tend to produce more no. of seeds per fruit. No. of seed is often associated with traits like fruit size, shape, and market preferences. Understanding these attributions can help breeders to develop genotypes with an optimal balance of seed. A similar kind of result for seed numbers per fruit was reported to Macchia et al. (2009).

#### *4.2.2.11 100 seed weight (g)*

Table 4.4 clearly shows that the genotype with the highest value for the 100-seed weight (g) was found in genotype SCP74 (10.8 g), subsequently in SCP11 (9.9 g) and minimum in SCP04 (4.6 g). The grand mean for 100 seed weight was 7.1 g. The variation in 100 seed weight among the genotypes may be due to genetic differences or in genetic makeup. Genotypes with higher seed weights could be used to develop varieties for improved germination and vigor, while genotypes with lower seed weight can be used for small fruited types of fruit for marketing purposes. This trait can be a vital selection criterion for hybridization and other breeding programs which focus on seed quality, vigor, and adaptability. Similar results were reported by Priyadarshini et al. (2021) and Ezin et al. (2022), where significant variation was observed in the seeds of cucumber and pumpkin genotypes, respectively.

#### *4.2.2.12 Average fruit weight (g)*

Data given in Table 4.4 shows that the maximum fruit weight was obtained in genotype SCP74 (51.2 g) followed by SCP27 (47.6 g) and minimum in SCP61 (8.7 g). The grand mean of this trait was 22.2 g. The significant variation in average fruit weight may be due to genetic variability. Larger fruit weight is associated with higher fruit girth and length, suggesting these genotypes also possess traits that enhance overall fruit size. Breeders can utilize this variability among genotypes of *C. pedata* to develop new varieties.

#### *4.2.2.13 No. of fruit per plant*

Table 4.4 shows that the number of fruits per plant is a crucial yield attribute, and it varied significantly across all genotypes. The maximum no. of fruits was obtained in genotype SCP36 (414.7), followed by SCP39 (404.7) while SCP78 obtained the minimum no. of fruits per plant (47.1). The mean value for this trait was 177.7. Considerable variation in the number of fruits per plant was noted among genotypes, highlights the genetic diversity within the population of crops. Genotypes with higher fruit counts may contribute significantly to the overall yield of the plants, which could make them ideal genotypes for cultivation, prioritizing productivity. The results observed in fruiting characteristics such as fruit length, fruit girth, and average fruit weight suggest that the number of fruits per plant is closely associated with these traits. Plants bearing smaller to medium sized fruits were found to produce a higher number of fruits compared to those with larger sized fruits. The average for all the genotypes was 177.7 no. of fruit per plant highlighting the moderate fruiting capacity across the genotypes. A wide phenotypic variability was observed in the case of trait no. of fruit per plant in cucumber genotypes (Pandey et al., 2018). Significant variation among bottle gourd landraces in the trait no. of fruit per plants was reported by Mashilo et al. (2016).

#### *4.2.2.14 Petiole length (cm)*

Table 4.4 shows the highest petiole length was recorded in SCP40 (1.22 cm) and the lowest in SCP01 (0.50 cm). The average mean value for this trait was 0.78 cm. The significant differences in petiole length among genotypes were observed and it may be due to the influence of genetic factors. Petiole length is very essential for supporting the leaf and positioning for an optimum amount of photosynthesis. Variations could have resulted from different growth habits or physiological requirements of the genotypes. Genotypes that bear longer petiole lengths like SCP40 might have an advantage in intercropping or in dense planting systems where leaf positioning is essential to maximize light interception. Shorter petiole genotypes, such as SCP01, may be effective in compact growth systems or areas with high wind pressure, where shorter plants are less prone to lodging.

#### *4.2.2.15 Yield per plant (kg)*

It was apparent from Table 4.4 that the maximum yield per plant was observed in SCP39 (8.41 kg), followed by SCP40 (7.97 kg), and minimum in SCP72 (0.99 kg). Average mean value for this trait was 3.31 kg. The significant variation in terms of yield per plant among genotypes can be associated with genetic diversity. SCP39 and SCP40, which produce maximum yield/plant may possess superior genetic traits that enhance their potential yield, such as better fruiting efficiency, higher photosynthetic rates, and stronger reproductive growth. Genotypes that give higher yields may possess suitable combinations like medium size fruits, ideal fruit number per plant, optimum fruit length and size. High-yielding genotypes of *C. pedata* are vital resources in terms breeding programs. SCP39 and SCP40 could serve as parental lines for developing new yield potential verities. Significant variation in yield among different bitter gourd genotypes was reported by Dey et al., (2006). Similar variations in yield were also observed in cucumber genotypes in the studies conducted by Kumar et al. (2008); Yadav et al. (2009), Innark et al. (2013), and Kumar et al. (2013).


**Plate 3: Variability in fruit shape, size, length and spine characteristics among genotypes of *C. pedata* at full maturity**

**Table 4.4 Mean performance of fifty-one *C. pedata* genotypes for morphological characterization (Pooled analysis)**

| Genotypes | VL (m) | NPB | IL (cm) | D50%F | NFSF | NFPF | PL (cm) | FL (cm) | FG (cm) | NSPF | 100SW (g) | AFW (g) | NFPP | PtL (cm) | Y/P (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP01** | 4.01 | 12.30 | 12.73 | 85.30 | 6.40 | 6.00 | 0.94 | 6.01 | 2.14 | 7.9 | 6.3 | 8.9 | 178.6 | 0.50 | 1.59 |
| **SCP02** | 6.87 | 13.00 | 11.44 | 89.00 | 4.50 | 6.70 | 1.84 | 12.22 | 4.17 | 12.5 | 7.9 | 34.9 | 84.6 | 0.71 | 3.04 |
| **SCP03** | 4.90 | 10.50 | 11.17 | 99.60 | 5.60 | 6.10 | 1.07 | 5.75 | 2.65 | 7.7 | 6.9 | 10.3 | 184.0 | 0.73 | 1.92 |
| **SCP04** | 6.42 | 14.00 | 10.37 | 115.70 | 7.20 | 6.20 | 1.04 | 7.19 | 3.00 | 10.7 | 4.6 | 13.9 | 230.4 | 0.77 | 4.22 |
| **SCP05** | 8.43 | 12.90 | 9.52 | 109.20 | 7.90 | 7.90 | 2.20 | 11.76 | 4.13 | 12.3 | 8.2 | 46.0 | 98.7 | 0.87 | 4.77 |
| **SCP06** | 6.74 | 13.90 | 7.64 | 115.20 | 5.60 | 5.40 | 1.07 | 6.87 | 2.58 | 8.2 | 5.4 | 10.8 | 227.5 | 0.55 | 2.62 |
| **SCP07** | 11.20 | 13.10 | 12.34 | 95.20 | 4.90 | 4.70 | 1.08 | 6.53 | 2.67 | 10.1 | 6.4 | 11.5 | 204.7 | 0.85 | 2.40 |
| **SCP08** | 7.36 | 9.00 | 10.45 | 111.70 | 6.90 | 7.00 | 1.57 | 8.08 | 2.89 | 11.4 | 7.8 | 13.6 | 149.6 | 0.72 | 2.03 |
| **SCP09** | 9.41 | 10.40 | 9.87 | 107.30 | 6.10 | 5.70 | 1.31 | 9.68 | 2.84 | 9.9 | 7.4 | 16.4 | 155.0 | 0.83 | 2.63 |
| **SCP10** | 7.82 | 16.30 | 9.96 | 108.30 | 5.10 | 4.80 | 2.52 | 11.89 | 4.05 | 10.2 | 7.2 | 20.3 | 97.7 | 0.66 | 1.97 |
| **SCP11** | 8.29 | 17.00 | 9.74 | 107.40 | 6.50 | 6.10 | 1.84 | 12.34 | 4.57 | 10.6 | 9.9 | 37.8 | 102.6 | 0.82 | 3.69 |
| **SCP12** | 7.03 | 14.70 | 11.14 | 111.50 | 6.10 | 5.40 | 2.31 | 14.63 | 4.28 | 10.2 | 9.0 | 33.1 | 127.4 | 0.91 | 4.27 |
| **SCP13** | 8.16 | 16.70 | 11.75 | 100.20 | 5.70 | 5.50 | 1.27 | 7.77 | 3.11 | 11.2 | 6.2 | 15.8 | 321.2 | 0.89 | 5.17 |
| **SCP14** | 8.93 | 11.30 | 8.59 | 119.70 | 5.40 | 5.00 | 1.80 | 11.91 | 4.70 | 11.5 | 7.4 | 44.6 | 60.7 | 0.76 | 2.61 |
| **SCP15** | 8.07 | 12.30 | 12.09 | 99.40 | 5.70 | 6.00 | 1.19 | 6.17 | 2.96 | 9.4 | 6.0 | 14.4 | 134.1 | 0.65 | 1.85 |
| **SCP16** | 7.25 | 12.50 | 10.40 | 101.60 | 6.00 | 6.20 | 1.22 | 7.45 | 2.62 | 9.5 | 7.4 | 14.7 | 203.3 | 0.76 | 3.04 |
| **SCP26** | 7.85 | 17.20 | 9.76 | 101.70 | 6.50 | 5.20 | 1.08 | 7.22 | 2.88 | 9.2 | 6.1 | 16.2 | 178.8 | 0.67 | 2.82 |
| **SCP27** | 11.91 | 16.40 | 10.69 | 102.80 | 10.10 | 10.20 | 1.13 | 9.64 | 4.16 | 12.6 | 9.4 | 47.6 | 49.5 | 0.73 | 2.32 |
| **SCP28** | 8.30 | 14.80 | 9.56 | 89.20 | 5.40 | 4.50 | 1.02 | 6.43 | 2.55 | 9.9 | 5.6 | 11.7 | 154.2 | 0.70 | 1.85 |
| **SCP29** | 10.11 | 15.00 | 12.05 | 103.40 | 6.30 | 6.10 | 1.26 | 7.06 | 3.07 | 10.4 | 6.8 | 22.6 | 347.8 | 0.75 | 7.72 |
| **SCP30** | 9.57 | 13.50 | 11.72 | 101.90 | 6.50 | 6.50 | 1.77 | 8.55 | 2.94 | 11.1 | 6.1 | 20.1 | 357.2 | 0.91 | 6.96 |
| **SCP31** | 8.68 | 12.90 | 11.23 | 88.70 | 5.40 | 5.00 | 1.20 | 6.42 | 2.66 | 10.2 | 6.3 | 12.1 | 394.9 | 0.67 | 4.85 |
| **SCP32** | 5.51 | 11.00 | 10.81 | 96.60 | 5.90 | 5.00 | 0.89 | 6.97 | 2.50 | 9.3 | 6.0 | 13.2 | 217.7 | 0.68 | 3.09 |
| **SCP33** | 10.05 | 16.70 | 11.32 | 96.90 | 5.20 | 5.00 | 2.40 | 18.56 | 2.61 | 10.3 | 7.7 | 27.1 | 174.8 | 0.92 | 4.67 |
| **SCP34** | 7.06 | 16.40 | 11.26 | 114.20 | 5.60 | 5.20 | 0.90 | 6.16 | 2.63 | 10.1 | 6.1 | 15.3 | 220.1 | 0.73 | 3.43 |
| **SCP35** | 6.40 | 16.20 | 8.24 | 100.90 | 6.30 | 5.00 | 0.99 | 5.90 | 2.64 | 9.5 | 5.8 | 12.2 | 282.4 | 0.64 | 3.54 |
| **SCP36** | 7.56 | 19.60 | 10.09 | 110.90 | 6.30 | 5.80 | 1.10 | 6.74 | 2.59 | 9.3 | 5.8 | 17.4 | 414.7 | 0.81 | 7.38 |
| **SCP37** | 5.53 | 19.00 | 9.85 | 109.70 | 6.10 | 5.30 | 1.13 | 7.35 | 3.15 | 10.1 | 7.4 | 15.2 | 360.2 | 0.82 | 5.41 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP38** | 7.45 | 17.10 | 10.29 | 89.10 | 5.30 | 5.40 | 1.24 | 7.05 | 2.90 | 9.3 | 6.5 | 13.0 | 316.3 | 0.80 | 3.99 |
| **SCP39** | 7.94 | 18.70 | 12.36 | 100.60 | 6.30 | 6.00 | 1.29 | 7.69 | 3.29 | 12.2 | 7.6 | 20.9 | 404.7 | 0.96 | 8.41 |
| **SCP40** | 6.91 | 17.40 | 10.77 | 117.60 | 6.40 | 5.10 | 1.64 | 8.89 | 3.74 | 10.8 | 7.6 | 34.1 | 219.9 | 1.22 | 7.97 |
| **SCP41** | 7.56 | 14.30 | 10.18 | 116.20 | 6.00 | 4.90 | 2.65 | 11.20 | 3.72 | 11.1 | 8.5 | 29.5 | 131.1 | 1.17 | 4.48 |
| **SCP42** | 7.19 | 15.40 | 8.39 | 214.70 | 8.10 | 7.80 | 1.73 | 10.75 | 4.05 | 11.4 | 8.8 | 30.6 | 118.3 | 0.75 | 3.66 |
| **SCP52** | 6.84 | 12.80 | 9.40 | 108.20 | 5.50 | 5.60 | 1.32 | 9.28 | 3.70 | 11.7 | 7.8 | 25.9 | 87.3 | 0.69 | 2.25 |
| **SCP53** | 6.50 | 16.10 | 10.62 | 99.80 | 5.00 | 3.90 | 1.25 | 7.11 | 3.12 | 11.6 | 5.7 | 14.1 | 147.5 | 0.75 | 2.03 |
| **SCP54** | 10.01 | 15.60 | 10.91 | 100.30 | 6.30 | 5.70 | 1.29 | 7.44 | 3.13 | 9.1 | 6.2 | 18.6 | 162.4 | 0.70 | 2.99 |
| **SCP55** | 6.89 | 14.90 | 9.43 | 98.80 | 5.80 | 6.00 | 1.28 | 7.71 | 2.96 | 10.5 | 5.3 | 8.7 | 130.1 | 0.73 | 1.15 |
| **SCP56** | 8.16 | 14.10 | 10.47 | 118.00 | 6.90 | 6.80 | 1.85 | 10.54 | 4.29 | 11.5 | 8.5 | 34.0 | 121.4 | 0.97 | 4.10 |
| **SCP57** | 7.07 | 15.30 | 11.00 | 105.30 | 7.30 | 6.40 | 1.26 | 7.12 | 2.94 | 9.7 | 5.6 | 9.7 | 126.6 | 0.70 | 1.25 |
| **SCP58** | 9.07 | 15.20 | 10.55 | 111.80 | 6.50 | 5.90 | 1.21 | 6.07 | 2.74 | 9.2 | 5.6 | 13.0 | 116.7 | 0.76 | 1.52 |
| **SCP59** | 7.99 | 17.40 | 8.06 | 116.70 | 9.30 | 8.50 | 1.52 | 8.36 | 3.98 | 11.6 | 9.6 | 28.5 | 68.4 | 0.76 | 1.98 |
| **SCP60** | 8.31 | 17.90 | 10.24 | 96.20 | 5.70 | 5.70 | 1.40 | 7.75 | 2.91 | 10.7 | 6.4 | 13.4 | 165.4 | 0.72 | 2.35 |
| **SCP61** | 6.70 | 17.30 | 11.31 | 98.60 | 6.20 | 5.30 | 1.27 | 7.33 | 3.12 | 9.5 | 5.6 | 8.7 | 399.2 | 0.75 | 3.45 |
| **SCP62** | 7.58 | 11.60 | 9.82 | 100.60 | 5.20 | 4.30 | 1.05 | 6.14 | 2.59 | 10.0 | 6.2 | 12.1 | 183.5 | 0.68 | 2.20 |
| **SCP72** | 5.71 | 11.50 | 9.92 | 108.20 | 5.60 | 5.90 | 1.32 | 8.17 | 2.29 | 7.3 | 5.8 | 19.2 | 51.8 | 0.62 | 0.99 |
| **SCP73** | 7.79 | 15.00 | 10.29 | 158.50 | 5.90 | 5.70 | 1.08 | 7.22 | 2.96 | 8.9 | 7.0 | 9.9 | 134.7 | 0.64 | 1.29 |
| **SCP74** | 8.71 | 14.30 | 11.60 | 114.20 | 8.10 | 8.50 | 2.13 | 11.54 | 5.04 | 11.2 | 10.8 | 51.2 | 49.6 | 0.91 | 2.56 |
| **SCP75** | 7.70 | 10.70 | 9.90 | 110.50 | 9.40 | 9.70 | 1.95 | 11.76 | 5.12 | 12.8 | 9.1 | 41.0 | 56.9 | 0.74 | 2.24 |
| **SCP76** | 6.56 | 15.00 | 10.59 | 115.60 | 9.90 | 9.10 | 1.88 | 11.10 | 4.56 | 10.8 | 9.3 | 38.7 | 53.1 | 0.78 | 2.00 |
| **SCP77** | 9.13 | 12.70 | 11.82 | 112.10 | 8.10 | 8.30 | 1.75 | 11.40 | 4.88 | 11.7 | 9.7 | 37.3 | 57.3 | 0.87 | 2.19 |
| **SCP78** | 10.81 | 10.50 | 11.81 | 106.20 | 9.10 | 9.70 | 1.91 | 11.31 | 4.89 | 13.2 | 8.3 | 40.7 | 47.1 | 0.91 | 1.92 |
| **Mean** | 7.80 | 14.50 | 10.50 | 108.06 | 6.45 | 6.15 | 1.46 | 8.75 | 3.35 | 10.4 | 7.1 | 22.2 | 177.7 | 0.78 | 3.31 |
| **SE** | 0.83 | 0.74 | 0.66 | 17.24 | 0.66 | 0.64 | 0.11 | 1.32 | 0.13 | 0.3 | 0.2 | 1.9 | 26.9 | 0.06 | 0.61 |
| **CD 5%** | 2.30 | 2.06 | 1.84 | - | 1.84 | 1.78 | 0.31 | 3.67 | 0.37 | 0.9 | 0.7 | 5.2 | 74.7 | 0.17 | 1.69 |
| **Range Lowest** | 4.01 | 9.00 | 7.64 | 85.30 | 4.50 | 3.90 | 0.89 | 5.75 | 2.14 | 7.3 | 4.6 | 8.7 | 47.1 | 0.50 | 0.99 |
| **Range Highest** | 11.91 | 19.60 | 12.73 | 214.70 | 10.10 | 10.20 | 2.65 | 18.56 | 5.12 | 13.2 | 10.8 | 51.2 | 414.7 | 1.22 | 8.41 |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

### 4.2.3 Phenotypical correlation matrix of 15 quantitative traits

The correlation among different traits is primarily attributed to genetic factors like linkage, where genes are located close to each other on the same chromosome are inherited, and pleiotropy, where a single gene could influence multiple trait (Anwar et al., 2009). According to Ali et al. (2009), environment plays a vital role in phenotypic correlations, where environment can influence the expression and interaction of traits. Assessment of correlation matrix for the pair of characters in the *C. pedata* are presented in Table 4.5. Results show that the highest significant positive correlation for fruit yield per plant was recorded with no. of fruit per plant (p=0.742), no. of primary branches (p=0.237), peduncle length (p=0.1457), no. of seed per plant (p=0.137), average fruit weight (p=0.189), and petiole length (p=0.286) in phenotypical level. Thus, these traits can be efficient for selecting characters for *C. pedata* improvement. The number of fruits per plant showed the strongest correlation, which could be a key trait for yield improvement. Traits such as the number of primary branches and average fruit weight also directly contribute to higher yields and this could be because a higher number of branches and longer petioles enhance photosynthesis, while an increased number of seeds and fruits directly boosts yield. Additionally, longer peduncles support larger or more fruits, contributing to greater overall production. These results are in partial agreement with those of Ogbonna and Chukwudi (2016). Kumar et al. (2008) suggested that for the selection of genotypes aimed at achieving higher fruit yield per plant, it is recommended to focus on key traits. Current findings are in conformation with Deepa et al. (2018) and Choudhary et al. (2004) in cucumber for average fruit weight and number of fruit/plants.

Traits like days taken to 50% flowering (p= -0.0297) show a negative correlation with that to yield/plant, followed by node no. to staminate flower (p= -0.0116) and node no. to first pistillate flower (p= -0.0156), suggesting that earlier flowering and lower node positions for flower may be marginally favorable for higher yields.

Traits like vine length (p=0.0379), internodal length (p=0.0865), fruit length (p=0.0633), fruit girth (p=0.0423), and 100 seed weight (p=0.0055) show no correlation with yield/plant. This lack of association suggests that these traits do not influence yield but they might contribute to other agronomic traits.

Among other traits, trait vine length had a strong positive correlation with no. of seed per fruit (p= 0.21) and average fruit weight (p= 0.20). Node no. to first pistillate flower (p=0.094) had a positive correlation with that to vine length, followed by peduncle length (p=0.088), fruit girth (p=0.12), and 100 seed weight (p=0.13), suggesting that longer vines may enhance reproductive parts of plants such fruit size. Whereas, no. of fruit per plant (p= -0.0560) showed a negative correlation with that to vine length. And no. of primary branches (p=0.082), internodal length (p=0.005), days taken to 50% flowering (p=0.0001), node no. to first staminate flower (p=0.072), fruit length (p=0.085) and petiole length doesn't show any correlation with to vine length. These findings suggest that while vine length is associated with certain yield-related traits, its direct role in overall fruit yield per plant is limited.

The trait no. of primary branches exhibited a strong positive correlation with no. of fruit per plant (p= 0.277) and petiole length (p= 0.1230). Whereas, internodal length (p= -0.039), days taken to 50% flowering (p= -0.0575), node no. to first pistillate flower (p= -0.077), fruit girth (p= -0.0268), no. of seeds per fruit (p= -

0.0094), 100 seed weight (p= -0.0362) and average fruit weight (p= -0.0672) showed a negative correlation with that to no. of primary branches. Node no. to first staminate flower (p=0.018), peduncle length (p=0.0050) and fruit length (0.0083) exhibited no correlation with that to no. of primary branches. The findings underline the role of primary branches in promoting yield through increased fruit count but also emphasize the potential compromises with other traits.

Internodal length showed a strong positive correlation with no. of fruit per plant (p= 0.138), petiole length (p= 0.393), and fruit length (p=0.1022). Node to first staminate flower (p= -0.0306), fruit girth (p= -0.0194), 100 seed weight (p= -0.0127), and average fruit weight (p= -0.0135) showed a negative correlation with that to internodal length. Whereas, days taken to 50% flowering (p= -0.107) had a significantly negative correlation with that to internodal length and node no. first pistillate flower (p=0.0321), peduncle length (p=0.0664), and no of seeds per fruit (p=0.0167) did not show any correlation with internodal length. This concludes that while internodal length positively affects plant structure and fruit placement, it may come at the cost of fruit size and seed quality.

Days taken to 50% flowering show significant positive correlation with node no. to staminate flower (p=0.090) and node no. first pistillate flower (p=0.0911). No. of fruit per plant (p= -0.0772) showed a negative correlation with that to days taken to 50% flowering. And peduncle length (p=0.0136), fruit length (p=0.0046), fruit girth (p=0.0598), no. of seeds per fruit (p=0.0478), 100 seed weight (p=0.0668), average fruit weight (p=0.0449) and petiole length (p=0.0189) showed no correlation with that to days taken to 50% flowering. Node to first staminate flower had a significant positive correlation with node no. to first pistillate (p=0.824), peduncle length (p=0.1312), fruit girth (p=0.28), no. of seed per fruit (p=0.2484), 100 seed weight (p=0.34) and average fruit weight (p=0.368). No of fruit per plant (p= -0.171) showed a significantly negative correlation with node to first staminate flower. Fruit length (p=0.0823) and petiole length (p=0.0532) showed no correlation with that to node no. to the first staminate flower. Similarly, node no. to first pistillate flower had a significant positive correlation with peduncle length (p=0.17), fruit length (p=0.1221), fruit girth (p=0.333), no. of seeds per fruit (p=0.3065), 100 seed weight (p=0.38) and average fruit weight (p= 0.413). No of fruit per plant (p= -0.203) showed a significant negative correlation and petiole length (p=0.0215) showed no correlation with that to node no. to pistillate flower. The results suggest several potential trade-offs in the plants' reproductive traits. Such as the number of fruits per plant appears to be negatively correlated with traits associated with fruit size, seed characteristics, and plant structure. This indicates that resources allocated toward producing more fruit may limit the resources available for the development of larger or higher-quality fruits. Similarly, early flowering traits (node to first staminate or pistillate flowers) often correlate with better fruit and seed characteristics but may come at the cost of reducing the total number of fruits produced.

The fruit parameters like peduncle length had a strong positive correlation with fruit length (p= 0.513), fruit girth (p= 0.592), no. of seeds per fruit (p= 0.3387), 100 seed weight (p= 0.472), average fruit weight (p= 0.5498) and petiole length (p= 0.393). Whereas, no. of fruit per plant (p= -0.212) showed a significant negative correlation with peduncle length. Fruit length had a significant positive correlation with fruit girth (p= 0.373), no. of seeds per fruit (p= 0.227), 100 seed weight (p= 0.333), average fruit weight (p= 0.387), and petiole length (p= 0.0.219). While no. of fruit per plant (p= -0.177) showed a significant negative correlation. Fruit girth length had a strong positive correlation with no. of seeds per fruit (p= 0.539), 100 seed weight (p= 0.648), average fruit weight (p= 0.753), and petiole length (p= 0.238). Similarly, no. of fruit per plant (p= -0.344) showed a significant negative correlation with fruit girth. No. of seed per plant had a strong positive correlation with 100 seed weight (p= 0.426), average fruit weight (p= 0.52), and petiole length (p= 0.204). A significant negative correlation was shown by no. of fruit per plant (p= -0.177) with no. of seeds per fruit. 100 seed weight had a significant positive correlation with average fruit weight (p= 0.691) and petiole length (p= 0.213). Average fruit weight had a significant positive correlation with petiole length (p= 0.269). The trait, no. of fruit per plant was found to have a strong negative correlation with node no. to first staminate flower 100 seed weight (p = -0.368) and average fruit weight (p = -0.368). The selection of larger fruits may result in fewer fruits per plant. Fruit girth also had strong positive correlations with seed number, seed weight, average fruit weight, and petiole length, while showing a negative correlation with the number of fruits per plant, emphasizing on inverse relation between fruit size and number. The number of seeds per fruit was positively correlated with 100 seed weight, average fruit weight, and petiole length, but negatively correlated with the number of fruits per plant, suggesting that more seeds per fruit could reduce the overall fruit count. Average fruit weight was positively correlated with petiole length and negatively correlated with the number of fruits per plant, highlighting an inverse relation between fruit size and fruit number.

**Table 4.5 Estimation of phenotypical correlation matrix for 15 quantitative traits in *C. pedata***

| | VL | NPB | IL | D50%F | NFSF | NFPF | PL | FL | FG | NSPF | 100SW | AFW | NFPP | PtL | Y/P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VL** | **1.0000** | 0.082 | 0.005 | 0.0001 | 0.072 | 0.094* | 0.088* | 0.085 | 0.12* | 0.21*** | 0.13** | 0.2*** | -0.0560 | 0.0756 | 0.0379 |
| **NPB** | | **1.0000** | -0.039 | -0.0575 | 0.018 | -0.077 | 0.0050 | 0.0083 | -0.0268 | -0.0094 | -0.0362 | -0.0672 | 0.277*** | 0.1230** | 0.237*** |
| **IL** | | | **1.0000** | -0.107* | -0.0306 | 0.0321 | 0.0664 | 0.1022* | -0.0194 | 0.0167 | -0.0127 | -0.0135 | 0.138** | 0.393*** | 0.0865 |
| **D50% F** | | | | **1.0000** | 0.090* | 0.0911* | 0.0136 | 0.0046 | 0.0598 | 0.0478 | 0.0668 | 0.0449 | -0.0772 | 0.0189 | -0.0297 |
| **NFSF** | | | | | **1.0000** | 0.824*** | 0.1312** | 0.0823 | 0.28*** | 0.2484*** | 0.34*** | 0.368*** | -0.171*** | 0.0532 | -0.0116 |
| **NFPF** | | | | | | **1.0000** | 0.17*** | 0.1221** | 0.333*** | 0.3065*** | 0.38*** | 0.413*** | -0.203*** | 0.0215 | -0.0156 |
| **PL** | | | | | | | **1.0000** | 0.513*** | 0.592*** | 0.3387*** | 0.472*** | 0.5498*** | -0.212*** | 0.393*** | 0.1457** |
| **FL** | | | | | | | | **1.0000** | 0.373*** | 0.227*** | 0.333*** | 0.387*** | -0.177*** | 0.219*** | 0.0633 |
| **FG** | | | | | | | | | **1.0000** | 0.539*** | 0.648*** | 0.753*** | -0.344*** | 0.238*** | 0.0423 |
| **NSPF** | | | | | | | | | | **1.0000** | 0.426*** | 0.52*** | -0.177*** | 0.204*** | 0.137** |
| **100S W** | | | | | | | | | | | **1.0000** | 0.691*** | -0.368*** | 0.213*** | 0.0055 |
| **AFW** | | | | | | | | | | | | **1.0000** | -0.37*** | 0.269*** | 0.189*** |
| **NFPP** | | | | | | | | | | | | | **1.0000** | 0.1016* | 0.742*** |
| **PtL** | | | | | | | | | | | | | | **1.0000** | 0.286*** |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

### 4.2.4 Genotypical correlation matrix for 15 quantitative traits

A correlation study was done to understand the essence of relationships among the economically important traits that can be further utilized in breeding programs. Assessment of the genotypical correlation matrix for the 15 traits in the *C.pedata* are presented in Table 4.6. Results revealed that fruit yield per plant had a positively strong correlation with vine length (g=0.1448), no. of primary branches (g=0.4825), internodal length (g=0.2747), peduncle length (g=0.1004), no. of seeds per fruit (g=0.2013), no. of fruits per fruit (g=0.6826) and petiole length (g=0.6684) at genotypic level. These findings suggest that breeding strategies focusing on increasing the number of fruits per plant and improving petiole length, alongside other vegetative traits like the number of branches, could be operative for enhancing overall yield.

Node no. to first staminate flower (g= -0.128) and node no. to first pistillate flower (g= -0.201) showed a significantly negative correlation with yield per plant, suggesting that earlier flowering, especially the appearance of staminate and pistillate flowers, may not always be associated with higher fruit yields. This implies that plants with a higher node number to the first flower may allocate resources differently, possibly prioritizing vegetative growth over reproductive output, thus limiting fruit production. Fruit girth had a negative correlation with yield per plant and days taken to 50% flowering (g=0.0214), fruit length (g=0.0665), 100 seed weight (g=0.0203) and average fruit weight (g=0.0952) showed no correlation with yield/plant.

The trait vine length had a significant positive correlation with internodal length (g=0.3084), node number to first staminate flower (g=0.3113), node number to first pistillate flower (g=0.3650), peduncle length (g=0.2908), fruit length (g=0.3945), fruit girth (g=0.3472), no. of seeds per fruit (g=0.5243), 100 seed weight (g=0.3068).

average fruit weight (g=0.4463) and petiole length (g=0.4036)**.** Findings are parallel with those of Choudhary et al. (2004), Deepa et al., (2018), and Ahmed et al. (2022) who reported that vine length has a positive correlation with fruit weight.

Days taken to 50% flowering (g= -0.1058) and no. of fruit per plant (g= -0.1443) exhibited a significantly negative correlation with vine length. However, no. of primary branches (g=0.0066) doesn't show any correlation with vine length. No. of primary branches exhibited a significant correlation with days taken to 50% flowering (g=0.2114), no. of fruit per plant (g=0.4337) and petiole length (g=0.2331). Internodal length (g= -0.1020) and node no. to first pistillate flower (g= -0.2250) had a significantly negative correlation with no. of primary branches. Whereas, node no. to first staminate flower (g= -0.0445), peduncle length (g= -0.0691), fruit length (g= -0.0687), fruit girth (g= -0.0672), no. of seeds per fruit (g= -0.0117), 100 seed weight (g= -0.0610) and average fruit weight (g= -0.0824) showed a negative correlation with no. of primary branches suggest that longer vines may delay flowering and result in fewer fruits per plant. This indicate that plants with longer vines may prioritize vegetative growth over early reproductive development, leading to a delay in flowering and reduced fruit set. This highlights a potential trade-off where increasing vine length may come at the expense of faster flowering and fruit production. Flowering and reproductive traits, such as days to 50% flowering and node to first staminate and pistillate flowers, are positively correlated with fruiting characteristics because early and well-positioned flowers lead to more fruit set. This results in a longer fruiting period and better resource allocation, ultimately enhancing yield potential. High correlation among flowering and fruiting characters are the essential criteria for selecting high yield genotypes in stuffing cucumber. These results are in concurrence with Dalamu & Behera (2013), Khan et al. (2016) and Upadhyay et al. (2019).

The trait internodal length displayed a significant positive correlation with no. of fruit per plant (g=0.2595) and petiole length (g=0.1369). Days taken to 50% flowering (g= -1.0492) recorded a significant negative correlation with internodal length. Whereas, node no. to first staminate flower (g= -0.0955), peduncle length (g= -0.0329), fruit length (g= -0.0917), fruit girth (g= -0.0794), 100 seed weight (g= -0.0021) and average fruit weight (g= -0.0469) had a negative correlation with internodal length, suggests that internodal length plays a complex role in shaping plant architecture and reproductive characteristics. While it positively influences number of fruits per plant and petiole length, it may also delay flowering and negatively impact fruit size and weight. Node no. to first pistillate flower (g=0.0523) and no. of seeds per fruit (g=0.0486) had shown no correlation with internodal length.

The trait days taken to 50% flowering displayed a significant positive correlation with node no. to first staminate flower (g=0.7945), node no. to first pistillate flower (g=0.5885), peduncle length (g=0.5021), fruit length (g=0.5565), fruit girth (g=0.7301), no. of seeds per fruit (g=0.3079), 100 seed weight (g=0.7438), average fruit weight (g=0.5814) and petiole length (g=0.2551). However, no. of fruit per plant (g= -0.5541) had significant negative correlation with days taken to 50% flowering.

The trait node no. to first staminate flower had a significant positive correlation with node no. to first pistillate flower (g=0.9433), peduncle length (g=0.2663), fruit length (g=0.3147), fruit girth (g=0.6976), no. of seeds per fruit (g=0.5430), 100 seed weight (g=0.6732), average fruit weight (g=0.6713) and petiole length (g=0.1894). No. of fruit per plant (g= -0.4542) showed the significantly negative correlation with node. No. to first pistillate flower. The analysis highlights a complex interplay between flowering traits, fruit production, and vegetative growth. Days taken to 50% flowering negatively correlated with number of fruits per plant, indicating that all flowers are not necessarily developed into fruits. In contrast, the node number to first staminate flower and node number to first pistillate flower are positively associated with several important fruit traits, suggesting that flowering at higher nodes may contribute to larger fruits with better seed development.

The trait node no. to first pistillate flower exhibited significant positive correlation with peduncle length (g=0.3292), fruit length (g=0.4174), fruit girth (g=0.7350), no. of seeds per fruit (g=0.5976), 100 seed weight (g=0.7234), average fruit weight (g=0.7262) and petiole length (g=0.1162). No. of fruit per plant (g=-0.5387) showed significant negative correlation with node no. to first pistillate flower. The trait peduncle length had a significant positive correlation with fruit length (g=1.0010), fruit girth (g=0.6993), no. of seeds per fruit (g=0.5174), 100 seed weight (g=0.6689), average fruit weight (g=0.7027) and petiole length (g=0.6042). No. of fruit per plant (g= -0.4614) had a significant negative correlation with peduncle length.

The character fruit length exhibited significant positive correlation with fruit girth (g=0.7691), no. of seeds per fruit (g=0.5910), 100 seed weight (g=0.7899), average fruit weight (g=0.8547) and petiole length (g=0.5404). No. of fruit per plant (g= -0.5678) had a significant negative correlation with fruit length. The trait fruit girth had a significant positive correlation with no. of fruit per plant (g=7566), 100 seed weight (g=0.8402), average fruit weight (g=0.9099) and petiole length (g=0.4532). No. of fruit per plant (g= -0.5806) had a significant negative correlation with fruit girth.

The trait no. of seeds per fruit showed significant positive correlation with 100 seed weight (g=0.6004), average fruit weight (g=0.7063) and petiole length (g=0.5462). Whereas, no. of fruit per plant (g= -0.2849) shows significant negative correlation with no. of seeds per fruit. 100 seed weight exhibited a significant positive with average fruit weight (g=0.8509) and petiole length (g=0.4864). No. of fruit per plant (g= -0.5227) had a significant negative correlation with 100 seed weight.

The trait average fruit weight shows a significant positive correlation with petiole length (g=0.5227) and negative correlation no. of fruit per plant (g= -0.5671). The trait no. of fruit per fruit had no correlation with petiole length (g=0.0466). The genotypic correlation coefficients were generally higher than the phenotypic correlation coefficients. This difference may have cause due to the impact of environmental factors, which tend to weaken and modify the natural associations between the various characters, leading to a reduction in the observable correlations at the phenotypic level. Consequently, the genotypic correlations more accurately reflect the inherent genetic relationships between the traits, unaffected by the environmental fluctuations. Similar results were seen in Khan et al. (2015) in cucumber, the research additionally reported that the GCV was greater than the PCV for the characters examined in cucumber.

**Table 4.6 Estimation of genotypic correlation matrix of 15 quantitative traits in *C. pedata***

| | VL | NPB | IL | D50%F | NFSF | NFPF | PL | FL | FG | NSPF | 100SW | AFW | NFPP | PtL | Y/P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VL** | **1.0000** | 0.0066 | 0.3084*** | -0.1058* | 0.3113*** | 0.3650*** | 0.2908*** | 0.3945*** | 0.3472*** | 0.5243*** | 0.3068*** | 0.4463*** | -0.1443* | 0.4036*** | 0.1448** |
| **NPB** | | **1.0000** | -0.1020* | 0.2114*** | -0.0445 | -0.2250* | -0.0691 | -0.0687 | -0.0672 | -0.0117 | -0.0610 | -0.0824 | 0.4337*** | 0.2331*** | 0.4825*** |
| **IL** | | | **1.0000** | -1.0492* | -0.0955 | 0.0523 | -0.0329 | -0.0917 | -0.0794 | 0.0486 | -0.0021 | -0.0469 | 0.2595*** | 0.1369** | 0.2747*** |
| **D50%F** | | | | **1.0000** | 0.7945*** | 0.5885*** | 0.5021*** | 0.5565*** | 0.7301*** | 0.3079*** | 0.7438*** | 0.5814*** | -0.5541** | 0.2551*** | 0.0214 |
| **NFSF** | | | | | **1.0000** | 0.9433*** | 0.2663*** | 0.3174*** | 0.6976*** | 0.5430*** | 0.6732*** | 0.6713*** | -0.4542** | 0.1894** | -0.128* |
| **NFPF** | | | | | | **1.0000** | 0.3292*** | 0.4174*** | 0.7350*** | 0.5976*** | 0.7234*** | 0.7262*** | -0.5387** | 0.1162* | -0.20** |
| **PL** | | | | | | | **1.0000** | 1.0010*** | 0.6993*** | 0.5174*** | 0.6689*** | 0.7027*** | -0.4614** | 0.6042*** | 0.1004* |
| **FL** | | | | | | | | **1.0000** | 0.7691*** | 0.5910*** | 0.7899*** | 0.8547*** | -0.5678** | 0.5404*** | 0.0665 |
| **FG** | | | | | | | | | **1.0000** | 0.7566*** | 0.8402*** | 0.9099*** | -0.5806** | 0.4532*** | -0.037 |
| **NSPF** | | | | | | | | | | **1.0000** | 0.6004*** | 0.7063*** | -0.2849** | 0.5462*** | 0.2013** |
| **100SW** | | | | | | | | | | | **1.0000** | 0.8509*** | -0.5227** | 0.4864*** | 0.0203 |
| **AFW** | | | | | | | | | | | | **1.0000** | -0.5671** | 0.5227** | 0.0952 |
| **NFPP** | | | | | | | | | | | | | **1.0000** | 0.0466 | 0.6826*** |
| **PtL** | | | | | | | | | | | | | | **1.0000** | 0.6684*** |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

### 4.2.5 Direct and indirect effect of traits on yield per plant at phenotypic level

Path coefficient analysis enables a detailed assessment of both direct and indirect effects of traits, providing a clearer understanding of their relative importance in determining yield. Path coefficient analysis is being increasingly used in plant breeding to enhance selection efficiency by accurately identifying traits that have the greatest impact on yield and its components, helping breeders make more informed and effective decisions (Toebe & Filho 2013).

Table 4.7. revealed the traits that have maximum direct effect on yield/plant of *C. pedata* was no. of fruit per plant (0.9156), followed by average fruit weight (0.5759), peduncle length (0.0845), no. of seed per fruit (0.0638), petiole length (0.0588), days to 50% flowering (0.0185), no. of primary branches (0.0103), fruit length (0.0076). However, fruit girth (-0.1474), internodal length (-0.0653), node no to first staminate flower (-0.0500), 100 seed weight (-0.0256), vine length (-0.0147), and node no. to first pistillate flower (-0.0007) exerted a negative direct effect on yield. Whereas, the values of direct effect and indirect effect contribution of different traits toward yield revealed the maximum positive indirect effect of fruit girth (0.4335), 100 seed weight (0.3980), peduncle length (0.3166), no. of seeds per fruit (0.2992), no. of primary branches (0.2540), node no. to first pistillate flower (0.2380), fruit length (0.2226), node no. to first staminate flower (0.2117), petiole length (0.1549), internodal length (0.1267), vine length (0.1006), no. of fruit per plant (0.0507), average fruit weight (0.0465) and days to 50% flowering (0.0258). The result reveals a clear distinction between traits with direct and indirect effects on yield, highlighting the complexity of breeding for yield in *C. pedata*.

Number of fruits per plant and average fruit weight are the primary direct contributors to yield, underscoring their importance in breeding programs aimed at improving yield potential. However, other traits like fruit girth, 100 seed weight, and peduncle length exert substantial indirect effects, suggesting that optimizing these traits could further enhance yield through their influence on fruit development and plant structure.

Traits with negative direct effects, such as fruit girth, internodal length, and node number to first staminate flower, should be carefully considered in breeding programs. While they may have indirect positive effects, their direct negative impact on yield advocates that these traits should be managed to avoid compromising yield potential. Selecting these traits simultaneously could boost genetic improvements in fruit yield for stuffing cucumber breeding. The results align with those of Srivastava & Srivastava (1976) and Mashilo et al. (2016), who also reported that the fruit number per plant with other fruiting characteristics, has the greatest and most positive direct effect on bottle gourd yield.

**4.2.6. Direct and indirect effect of traits on yield per plant at genotypic level**

The genotypic path coefficient analysis (Table 4.8) displays that the no. of fruit per plant (0.7700) had a maximum positive direct effect on yield/plant, followed by average fruit weight (0.5700), no. to first pistillate flower (0.3821), peduncle length (0.2011) and no. of primary branches (0.1586). 100 seed weight had a moderate direct effect on the yield/plant and internodal length showed maximum negative effects (-0.3020), followed by days to 50% flowering (-0.2981), fruit girth (-0.2312), fruit length (-0.2117), no. of node to first staminate flower (-0.1816), vine length (-0.1149) and no. of seed per fruit (-0.0923). Similar results regarding yield per plant were reported by Pal et al. (2017) in cucumber and Aishwarya et al. (2024) in bitter gourd.

Whereas, the maximum indirect positive effect was shown by average fruit weight (0.5186) via fruit girth followed by node no. to first pistillate flower (0.3605) via node no. to first staminate flower, petiole length (0.3467) via peduncle length, no of fruit per plant (0.3339) via no. of primary branches and days to 50% flowering (0.3128) via internodal length.

The path coefficient analysis provides an understanding of the complex relationships between different traits and yield in *C. pedata*. The number of fruits per plant and average fruit weight emerge as the most influential direct contributors to yield which emphasizes that these traits may lead to significant yield improvements. Moreover, optimizing traits like node number to the first pistillate flower, peduncle length, and number of primary branches could indirectly enhance yield by supporting better flower initiation, fruit development, and plant structure.

The negative direct effects of traits like internodal length, days to 50% flowering, and fruit girth suggest that excessive vegetative growth or delayed flowering might limit reproductive success, and these traits should be carefully managed in breeding programs to avoid negative impacts on yield.

The analysis reveals that the residual effects for both phenotypic coefficient (0.4288) and genotypic coefficient (0.3520) are relatively low, suggesting that a significant proportion of the variation in yield per plant can be attributed to the traits included in the path analysis. Specifically, 81.61% and 87.61% of the total phenotypic and genotypic variation, respectively, were accounted for by the selected traits. These results indicate that the traits included in the analysis are powerful predictors of yield, and much of the variation in yield can be explained by genetic and phenotypic factors considered in the study. Further, the model provides a solid framework for understanding the genetic and phenotypic relationships driving yield in *C. pedata*.

**Table 4.7 Path matrix analysis showing direct and indirect effect on yield/plant of *C. pedata* at phenotypic level**

| | VL | NPB | IL | D50%F | NFSF | NFPF | PL | FL | FG | NSPF | 100SW | AFW | NFPP | PtL | Y/P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VL** | **-0.02** | -0.012 | -0.001 | 0.001 | -0.001 | -0.0014 | -0.0013 | -0.0012 | -0.0016 | -0.0030 | -0.0018 | -0.0026 | 0.0008 | -0.0011 | 0.04 |
| **NPB** | 0.0008 | **0.0103** | -0.001 | -0.001 | 0.002 | -0.001 | 0.0001 | 0.0001 | -0.0003 | -0.0001 | -0.0004 | -0.0007 | 0.0029 | 0.0013 | 0.24 |
| **IL** | -0.003 | 0.0025 | **-0.07** | 0.007 | 0.002 | -0.0021 | -0.0043 | -0.0067 | 0.0013 | -0.0011 | 0.0008 | 0.0009 | -0.009 | -0.0257 | 0.09 |
| **D50%F** | 0.0000 | -0.0011 | -0.002 | **0.019** | 0.002 | 0.0017 | 0.0003 | 0.0001 | 0.0011 | 0.0009 | 0.0012 | 0.0008 | -0.001 | 0.0003 | -0.03 |
| **NFSF** | -0.004 | -0.0009 | 0.002 | -0.005 | **-0.050** | -0.0412 | -0.0066 | -0.0041 | -0.0139 | -0.0124 | -0.0168 | -0.0184 | 0.0086 | -0.0027 | -0.01 |
| **NFPF** | -0.001 | 0.0001 | 0.000 | -0.001 | -0.006 | **-0.0007** | -0.0001 | -0.0001 | -0.0002 | -0.0002 | -0.0003 | -0.0003 | 0.0001 | 0.0000 | -0.02 |
| **PL** | 0.0074 | 0.0004 | 0.006 | 0.001 | 0.011 | 0.0141 | **0.0845** | 0.0433 | 0.0500 | 0.0286 | 0.0399 | 0.0465 | -0.018 | 0.0332 | 0.15 |
| **FL** | 0.0006 | 0.0001 | 0.008 | 0.000 | 0.006 | 0.009 | 0.0039 | **0.0076** | 0.0028 | 0.0017 | 0.0025 | 0.0029 | -0.001 | 0.0017 | 0.06 |
| **FG** | -0.016 | 0.0040 | 0.003 | -0.009 | -0.041 | -0.0491 | -0.0872 | -0.0549 | **-0.1474** | -0.0794 | -0.0955 | -0.1109 | 0.0507 | -0.0351 | 0.04 |
| **NSPF** | 0.0131 | -0.0006 | 0.001 | 0.003 | 0.016 | 0.0195 | 0.0216 | 0.0145 | 0.0344 | **0.0638** | 0.0271 | 0.0331 | -0.011 | 0.0130 | 0.14 |
| **100SW** | -0.003 | 0.0009 | 0.003 | -0.002 | -0.009 | -0.0097 | -0.0121 | -0.0085 | -0.0166 | -0.0109 | **-0.0256** | -0.0177 | 0.0094 | -0.0054 | 0.01 |
| **AFW** | 0.1006 | -0.0387 | -0.008 | 0.026 | 0.212 | 0.2380 | 0.3166 | 0.2226 | 0.4335 | 0.2992 | 0.3980 | **0.5759** | -0.212 | 0.1549 | 0.19 |
| **NFPP** | -0.051 | 0.2540 | 0.127 | -0.071 | -0.157 | -0.1862 | -0.1928 | -0.1623 | -0.3149 | -0.1616 | -0.3365 | -0.3364 | **0.9156** | 0.0930 | 0.74 |
| **PtL** | 0.0044 | 0.0072 | 0.023 | 0.001 | 0.003 | 0.0013 | 0.0231 | 0.0129 | 0.0140 | 0.0120 | 0.0125 | 0.0158 | 0.0060 | **0.0588** | 0.29 |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

**Table 4.8 Path matrix analysis showing direct and indirect effect on yield/plant of *C. pedata* at genotypic level**

| | **VL** | **NPB** | **IL** | **D50% F** | **NFSF** | **NFPF** | **PL** | **FL** | **FG** | **NSPF** | **100SW** | **AFW** | **NFPP** | **PtL** | **Y/P** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **VL** | **-0.115** | -0.008 | -0.0355 | 0.0122 | -0.0358 | -0.0419 | -0.0334 | -0.0453 | -0.0399 | -0.0603 | -0.0353 | -0.051 | 0.0166 | -0.046 | 0.1448 |
| **NPB** | 0.001 | **0.159** | -0.0162 | 0.0335 | -0.0071 | -0.036 | -0.0110 | -0.0109 | -0.0107 | -0.0019 | -0.0097 | -0.013 | 0.0688 | 0.037 | 0.4825 |
| **IL** | -0.093 | 0.031 | **-0.302** | 0.3168 | 0.0288 | -0.0158 | 0.0099 | 0.0277 | 0.0240 | -0.0147 | 0.0006 | 0.0142 | -0.0784 | -0.041 | 0.2747 |
| **D50%F** | 0.032 | -0.063 | 0.3128 | **-0.298** | -0.2369 | -0.1754 | -0.1497 | -0.1659 | -0.2176 | -0.0918 | -0.2217 | -0.173 | 0.1652 | -0.076 | 0.0214 |
| **NFSF** | -0.056 | 0.008 | 0.0173 | -0.1443 | **-0.1816** | -0.1713 | -0.0483 | -0.0576 | -0.1267 | -0.0986 | -0.1222 | -0.121 | 0.0825 | -0.034 | -0.119 |
| **NFPF** | 0.140 | -0.086 | 0.0200 | 0.2249 | 0.3605 | **0.3821** | 0.1258 | 0.1595 | 0.2808 | 0.2284 | 0.2764 | 0.2775 | -0.2058 | 0.044 | -0.201 |
| **PL** | 0.059 | -0.014 | -0.0066 | 0.1010 | 0.0535 | 0.0662 | **0.2011** | 0.2013 | 0.1406 | 0.1040 | 0.1345 | 0.1413 | -0.0928 | 0.122 | 0.1004 |
| **FL** | -0.084 | 0.015 | 0.0194 | -0.1178 | -0.0672 | -0.09 | -0.2119 | **-0.2117** | -0.1628 | -0.1251 | -0.1672 | -0.181 | 0.1202 | -0.114 | 0.0665 |
| **FG** | -0.080 | 0.016 | 0.0184 | -0.1688 | -0.1613 | -0.1699 | -0.1617 | -0.1778 | **-0.2312** | -0.1749 | -0.1943 | -0.210 | 0.1342 | -0.105 | -0.037 |
| **NSPF** | -0.048 | 0.001 | -0.0045 | -0.028 | -0.0501 | -0.0551 | -0.0477 | -0.0545 | -0.0698 | **-0.0923** | -0.0554 | -0.065 | 0.0263 | -0.050 | 0.2013 |
| **100SW** | 0.016 | -0.003 | -0.0001 | 0.0394 | 0.0357 | 0.0384 | 0.0355 | 0.0419 | 0.0445 | 0.0318 | **0.0530** | 0.0451 | -0.0277 | 0.026 | 0.0203 |
| **AFW** | 0.254 | -0.047 | -0.0267 | 0.3314 | 0.3826 | 0.4139 | 0.4005 | 0.4871 | 0.5186 | 0.4026 | 0.4850 | **0.5700** | -0.3232 | 0.298 | 0.0952 |
| **NFPP** | -0.111 | 0.334 | 0.1998 | -0.4267 | -0.3497 | -0.4148 | -0.3553 | -0.4372 | -0.4471 | -0.2194 | -0.4025 | -0.437 | **0.7700** | 0.036 | 0.6826 |
| **PtL** | 0.232 | 0.134 | 0.0786 | 0.1464 | 0.1087 | 0.067 | 0.3467 | 0.3101 | 0.2600 | 0.3134 | 0.2790 | 0.2999 | 0.0267 | **0.574** | 0.6684 |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

### 4.2.7 Qualitative traits analysis of fifty-one genotypes of *C. pedata*

#### *4.2.7.1 Fruit shape*

As presented in Table 4.9, variability was observed for the fruit shape in the collected genotypes. Out of 51 genotypes, 90% of fruit shapes were found to be elongated with pointed tips, 5% elongated with curved tips, 3% oval with pointed tips, and 2% round with pointed tips. The variation in qualitative traits of *C. pedata* reflects the genetic diversity of the plant and its adaptation to various environmental conditions. These traits are often controlled by the genes which provide valuable information about genotypes for identification and different breeding approaches. The observed variation in fruit shape is associated with genetic diversity, while elongated fruit shapes, particularly those with pointed tips were found predominant case of fruit shapes, which indicates a potential selection benchmark. Similar results were found in a study on the bitter gourd and cucumber genotypes for qualitative traits showing great diversity by Looregipoor et al. (2023), and Shah et al. (2017), respectively.

#### *4.2.7.2 Fruit spines*

Similarly, for the trait presence of fruit spines, variation was recorded in the collected genotypes. As presented in Table 4.9, dense spines were found in 44% of the genotypes, followed by moderate spines in 42%, less spines in 12%, and spineless in 2% genotypes. The significant variability among fruit spines suggests a potential adaptive role in deterring pests in the case of dense spines fruited genotypes, whereas less spined and spineless genotypes, may fulfill the specific farmers or market preferences and also easier in handling fruits. Variation in fruit spines may be due to the genetic makeup of genotypes found in Sikkim and the cause of environmental adaptations.

### *4.2.7.3 Fruit colour*

Table 4.9 indicates that the most common fruit color observed was medium green, accounting for 49%, followed by deep green at 42%, and light green at 9%. Medium green was the most dominant fruit color found in the fruit of *C. pedata*, which may indicate the consumer preference and better adaptability of these genotypes to prevailing conditions. Deep green fruits were found in the least genotypes but also contribute to the overall genetic variability. Qualitative traits are visually assessable which forms a distinct phenotypic category, making them valuable for crop breeding and genetic analysis (Xu et al., 2011). As reported by Morimoto et al. (2005), Achigan-Dako et al. (2008) and Mashilo et al. (2017) the bottle gourd displays significant diversity in terms of fruit qualitative traits which includes variations in fruit shape, skin color, and skin texture.

### *4.2.7.4 Leaf colour*

As evident from Table 4.9, there was a noticeable variation in leaf color among the genotypes. Medium green was the most prevalent, observed in 57% of the genotypes, followed by deep green at 29%, and light green at 14%. The dominance of medium green leaf color in 57% of genotypes suggests a balance between efficient photosynthesis and adaptability under typical growth conditions. The variations in leaf colour highlight the importance of color as a qualitative marker for selecting genotypes.

**Table 4.9 Qualitative traits of fifty-one genotypes of *C. pedata***

| Sl. No. | Characteristics | Percentage (%) |
|---|---|---|
| 1 | **Fruit shape** | |
| | Elongated with pointed tips | 90 |
| | Elongated with curved tips | 5 |
| | Oval with pointed tips | 3 |
| | Round with pointed tips | 2 |
| 2 | **Fruit spine** | |
| | Dense spines | 44 |
| | Moderate spines | 42 |
| | Less spines | 12 |
| | Spineless | 2 |
| 3 | **Fruit colour** | |
| | Medium green | 49 |
| | Deep green | 42 |
| | Light green | 9 |
| 4 | **Leaf colour** | |
| | Medium green | 57 |
| | Deep green | 29 |
| | Light green | 14 |
| 5 | **No. of leaf lobes** | |
| | Five lobes present | 100 |
| 6 | **Stem shape** | |
| | Angular | 100 |
| 7 | **Stem pubescence** | |
| | Absence | 100 |
| 8 | **Leaf shape** | |
| | Compound (Palmate) | 100 |
| 9 | **Leaf blade** | |
| | Serrated | 100 |
| 10 | **Flower colour** | |
| | Yellowish white | 100 |
| 11 | **Seed colour** | |
| | Black | 100 |

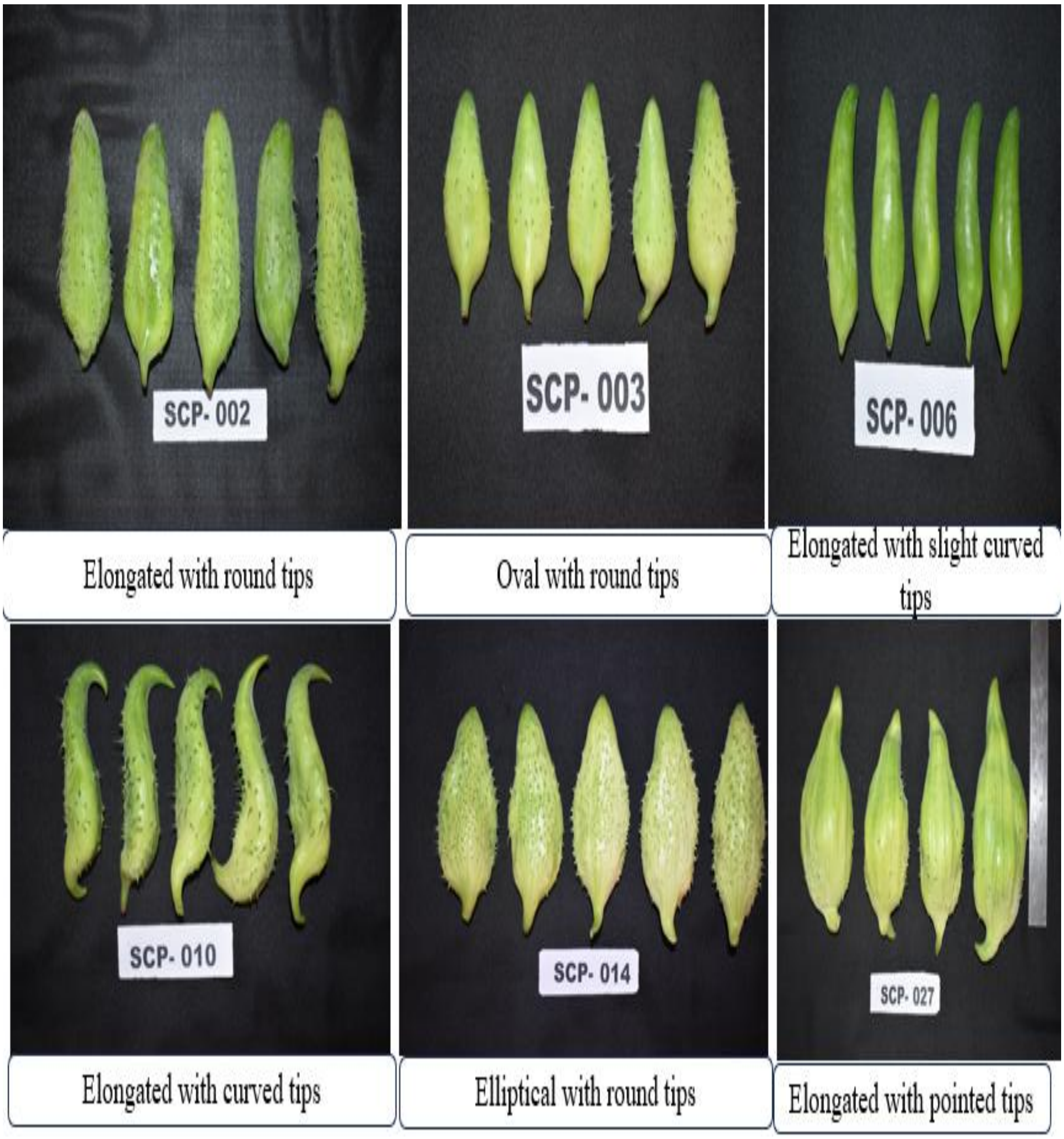


**Plate 4: Observed variation in fruit shape in *C. pedata* genotypes**

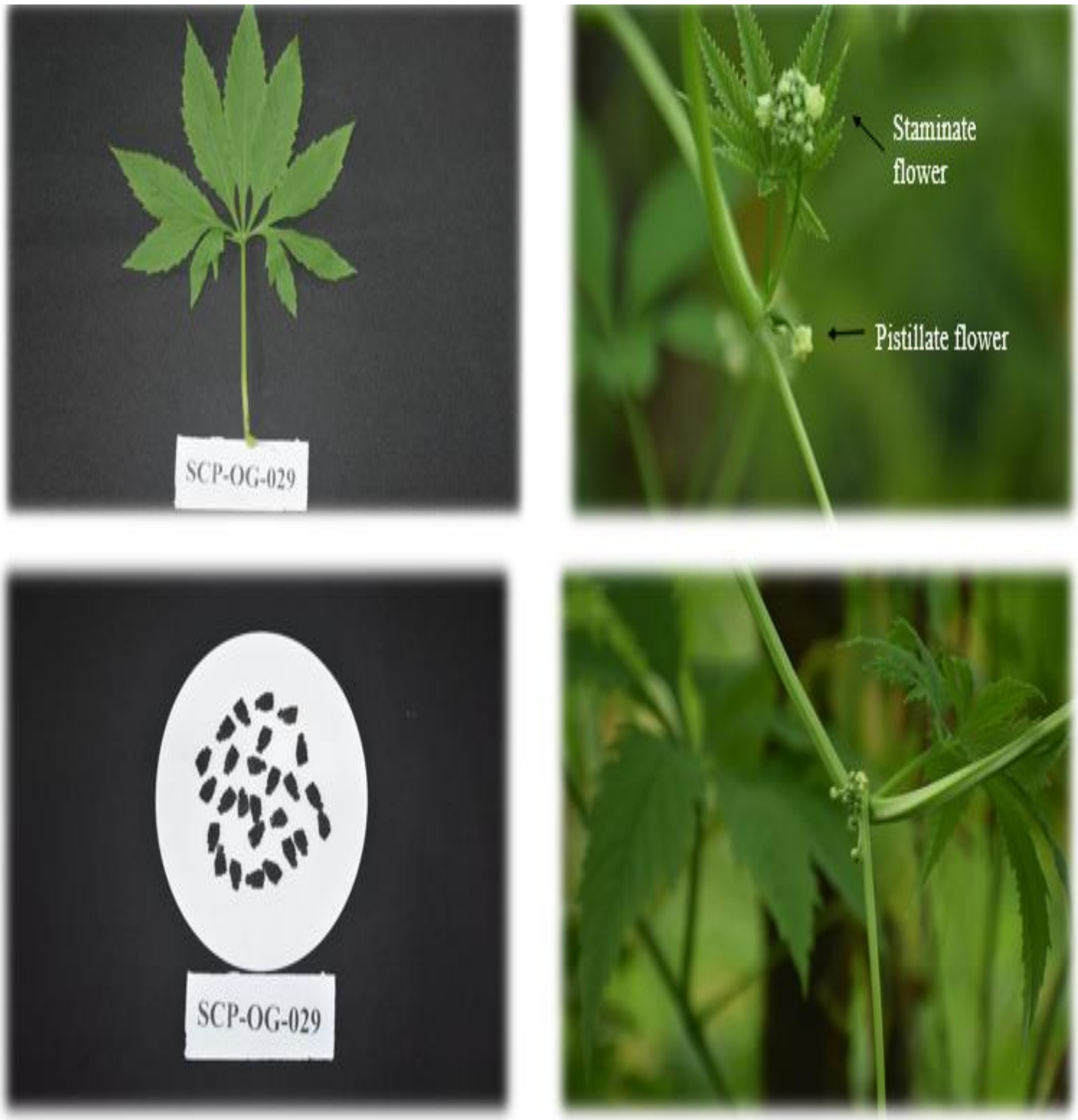


**Plate 5: Qualitative morphological characterization of *C. pedata* genotypes.**
**A: Leaf shape and lobes, B: Staminate and Pistillate flower position,**
**C: Seed colour, D: Stem shape**

#### *4.2.7.5 No. of leaf lobes*

The data presented in Table 4.9 indicates that *C. pedata* consistently has five leaf lobes, showing no variation, with a 100% occurrence rate in all collected genotypes.

#### *4.2.7.6 Stem shape*

The data regarding stem shape is depicted in Table 4.9 showed no variation and found to be 100% angular stem shape in all collected genotypes.

#### *4.2.7.7 Stem pubescence*

Data given in Table 4.9 indicates a 100% absence of stem pubescence among all the collected genotypes of *C.pedata*.

#### *4.2.7.8 Leaf shape*

As presented in Table 4.9, the leaf shape is consistently 100% palmately compound, with no observed differences among the collected genotypes.

#### *4.2.7.9 Leaf blade*

Similar to the leaf shape, there was no variation observed in the leaf blade. As given in Table 4.9, the leaf blade of all collected genotypes was found serrated with no variation.

#### *4.2.7.10. Flower colour*

As indicated in Table 4.9, flower color of the *C. pedata* was consistently observed to be yellowish-white across all genotypes. There were no differences observed, resulting in a 100% uniform occurrence of this specific flower color.

#### *4.2.7.11 Seed colour*

Seed color was found consistently black in all the genotypes, with a 100% occurrence rate and no observed differences (Table 4.9).

The observation in several qualitative traits such as stem shape, stem pubescence, leaf shape, leaf blade, flower colour, and seed colour among the collected *C. pedata* genotypes was found uniform. Highlights the highly conserved nature of these traits within the species. These traits possess strong genetic control and have no or minimum effect of environmental factors or natural selection. These uniform traits serve as reliable markers for species identification, confirming the genetic stability of *C. pedata* across diverse genotypes. Kalyanrao et al. (2016) portrayed a wide diversity in qualitative characteristics of bottle gourd which consist of early plant vigour, leaf size, fruit shape, fruit skin colour, and fruit pubescence whereas, stem shape, leaf margin, leaf shape, flower colour, and fruit taste doesn't show any variations.

### 4.2.8 Estimation of genetic divergence using Tocher method of $D^2$

#### *4.2.8.1 Grouping of genotypes into clusters by Tocher method*

The studies of genetic divergence among the fifty-one genotypes of *C. pedata* were carried out by using Tocher method of $D^2$ statistics described by Rao (1952). Based on this method, fifty-one genotypes were grouped into 8 clusters (Table 4.10).

Cluster I had the highest number of genotypes (30) followed by Cluster II (15), whereas Cluster III, IV, V, VI, VII, and VIII had only one genotype. A huge level of genetic diversity was observed across the studied population. This distribution gives valuable insights into the structure and diversity of the population. Cluster I and II contained the highest number of genotypes, which indicates that these genotypes are genetically similar to each other, and possess shared traits that place them within the same cluster. Despite genetic similarity among genotypes, there is still a great amount of variation within the cluster, showing a broad genetic base for further breeding programs. The existence of only one genotype in each of the Clusters III, IV, V, VI, VII, and VIII is particularly very interesting. These clusters constitute genotypes that are distinct within the checked genotypes and exhibit unique combinations of traits. These genotypes can be considered exotic genotypes, possessing valuable traits that could be useful for further breeding programs, particularly for introducing new characteristics or integration of new traits. These results are in accord with results found by Prasad et al. (2001), and Ahirwar et al. (2017) in cucumber and Osawaru et al. (2013) in okra.

#### *4.2.8.2 The estimates of intra and inter-cluster distances represented by the Tocher method*

The estimates of intra and inter-cluster distances represented by Tocher method values are given in Table 4.11. Maximum intra-cluster distance was recorded in Cluster II (1.83) and I (1.34) revealing great genetic diversity in these clusters. In Clusters III, IV, V, VI, VII, and VIII the intra-cluster distance was 0, which indicates no diversity within the same cluster, as it contained only a single genotype. Cluster II recorded the highest intra-cluster distance (1.83), followed by Cluster I (1.34). These results indicate that Cluster II possesses the highest genetic variation among its genotypes, which could provide a broader genetic base for selecting traits such as yield, disease resistance, or environmental adaptability. Likewise, the moderate diversity in Cluster I obtained, makes it another important source for the selection of new varieties. The genotypes with no intra-cluster distance are genetically distinct and represent unique combinations of traits, making them important resources for introducing novel genes.

The inter-cluster values of the eight clusters revealed that maximum inter-cluster distance was observed between Cluster II and V (7.02), followed by Cluster III to V (6.20), Cluster I to II (6.17), Cluster II to VIII (5.72), Cluster II to III (5.58), Cluster V to VII (5.56), Cluster IV to V (4.81), Cluster III to VI (4.61) and Cluster I to IV (4.53). While the lowest inter-cluster distance (1.70) was ascertained amongst clusters IV & VI. The inter-cluster distance was observed more than to intra-cluster distance demonstrating a considerable amount of genetic diversity amongst all genotypes under investigation. The high inter-cluster distance was observed between Cluster II to V and Cluster III to V, indicating maximal genetic divergence among genotypes in these clusters.

This huge genetic divergence between the clusters makes them ideal for hybridization, as crossing such diverse genotypes is likely to result in heterosis and the development of superior progeny. These results are in line with the results observed by Quamruzzaman et al. (2011) and Sarma et al. (2022) in sponge gourd.

**Table 4.10 Average of intra- and inter- clusters values for eight clusters**

| Cluster | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| **I** | **1.34** | 6.17 | 2.66 | 4.53 | 2.34 | 3.31 | 4.46 | 3.73 |
| **II** | | **1.83** | 5.58 | 3.48 | 7.02 | 3.72 | 4.20 | 5.72 |
| **III** | | | **0.00** | 4.19 | 6.20 | 4.61 | 2.34 | 4.27 |
| **IV** | | | | **0.00** | 4.81 | 1.70 | 2.20 | 2.20 |
| **V** | | | | | **0.00** | 3.44 | 5.56 | 3.30 |
| **VI** | | | | | | **0.00** | 4.20 | 2.16 |
| **VII** | | | | | | | **0.00** | 4.33 |
| **VIII** | | | | | | | | **0.00** |

**Table 4.11 Clustering pattern of fifty-one genotypes of *C. pedata* based on Tocher method**

| Cluster | No. of genotypes | Name of the genotypes |
|---|---|---|
| **I** | 30 | SCP01, SCP03, SCP04, SCP06, SCP07, SCP08, SCP09, SCP13, SCP15, SCP16, SCP26, SCP28, SCP29, SCP30, SCP31, SCP32, SCP34, SCP35, SCP36, SCP37, SCP38, SCP53, SCP54, SCP55, SCP57, SCP58, SCP60, SCP61, SCP62, SCP73 |
| **II** | 15 | SCP02, SCP05, SCP11, SCP12, SCP14, SCP27, SCP42, SCP52, SCP56, SCP59, SCP74, SCP75, SCP76, SCP77, SCP78 |
| **III** | 1 | SCP39 |
| **IX** | 1 | SCP41 |
| **V** | 1 | SCP72 |
| **VI** | 1 | SCP10 |
| **VII** | 1 | SCP40 |
| **VII** | 1 | SCP33 |

## 4.3 Marker attributes and genetic diversity analysis of *C. pedata* genotypes

Simple Sequence Repeats (SSRs) are commonly used as molecular markers to know the genetic diversity in the plant because it has several key advantages. They are highly informative and can reveal variations based on genetics due to their repetitive DNA sequences, which tend to differ in length between individuals (Gupta et al., 1996). Molecular markers serve as a powerful method for genetic variability assessment and offer an efficient approach to linking phenotypic traits with genotypic differences (Varshney et al., 2005). SSRs are a type of DNA marker characterized by short tandem repeat sequences (2–6 base pairs) that are widely distributed across the genome (Venkateswarlu et al., 2006). They are locus-specific, co-dominant, and highly polymorphic, making them an excellent choice for genetic mapping and comparative studies of genetic maps. Their ubiquity and distinct characteristics have made SSRs valuable tools in many applications, as reported by Grando et al. (2003), Kenis and Keulemans (2005), and Yamanaka et al. (2005). Moreover, they are codominant, meaning they can distinguish between both homozygous and heterozygous genotypes.

SSR markers were used to analyze the genetic variability among the collected fifty-one genotypes of *C. pedata*. The goal was to assess the level of genetic diversity and to examine the differences between the genotypes (Table 4.12). 24 SSR markers were used, out of that 21 markers showed polymorphism and in 3 markers monomorphic profiles were observed. Among the polymorphic markers, 19 markers had a polymorphic information content (PIC) value of more than 0.765 among them marker EC49 generated the highest PIC value i.e. 0.969. Whereas, marker

MC04_54893 showed the lowest PIC value i.e. 0.576. All markers showed a high level of polymorphism which was represented by PIC values greater than 0.5. The average mean for the PIC was 0.84. Based on the PIC values, all 21 markers were found to be highly polymorphic and were determined as highly informative markers, with all of them sowing values more than 0.5.

Markers like LSR015, EC49, SSR18362, and other showed a range of 1.43 (LSR015) to 2.0 for the number of different alleles ($N_a$). The average mean value for no. of different alleles was 1.85. The effective number of alleles (Ne) ranged from 1.18 to 1.89 with an average mean value of 1.43. The average values of Shannon's information index were I= 0.41 and Nei's gene diversity (h = 0.27). The markers like LSR108, LSR112, and EC11 showed a 100% polymorphism, while marker LSR015 showed the lowest polymorphism. The average PPL (Percentage of Polymorphic Loci) was 84.87%. The number of polymorphic loci ranged from 22 (LSR015) to 51 (LSR108, LSR112, MC04_54893, and EC11) with an average value of 43.29.

The use of SSR markers in this experiment proved that these microsatellite markers are effective in analyzing the genetic variability among the 51 *C. pedata* genotypes. The findings indicate a high level of genetic diversity, as evidenced by the polymorphic information content (PIC) values, allele diversity, and other genetic parameters. Out of 24 SSR markers used, 21 exhibited polymorphisms, which could be used in SSRs to distinguish genetic variability in *C. pedata* genotypes. Notably, marker like EC49 generated the highest PIC value (0.969) shows its effectiveness in exploiting genetic diversity, whereas the least informative marker was MC04_54893 (0.576) but still surpassed the threshold level of 0.5, signifying significant polymorphism.  Markers with high PIC values and polymorphic loci, such as EC49, LSR108, and LSR112, emerge as particularly useful for further such studies. Similar markers were used by Hu et al. (2010) in cucumber, Mashilo et al. (2016) in bottle gourd, and Cui et al. (2017) in bitter gourd.

### 4.12 Marker Attributes and Genetic Diversity Analysis of *C. pedata* genotypes

| Markers | Primer sequence 5'- 3' | $N_a$ | $N_e$ | H | I | PPL% | PL | PIC |
|---|---|---|---|---|---|---|---|---|
| **LSR015** | F: CTTACCTTCACAAAACCCCATC,<br>R: ACTCTGTTTCGACTCTGCTTCC | 1.43 | 1.31 | 0.18 | 0.26 | 43.14 | 22 | 0.775 |
| **LSR040** | F: TTCCCATCCAGACCAAACCTATC,<br>R: CAAAGGCCATAGACAAACACAA | 1.60 | 1.43 | 0.25 | 0.37 | 60.78 | 31 | 0.869 |
| **LSR047** | F: CAATAGAGTAGGGTGGGGCATA,<br>R: TAAAATAGTGGGAGAGCAAGGG | 1.87 | 1.32 | 0.20 | 0.34 | 86.27 | 44 | 0.952 |
| **LSR063** | F: AAGAGAGGGGCAGGAAGTAAAT,<br>R: AGAAAACACACAGTACGCCTCC | 1.97 | 1.34 | 0.23 | 0.38 | 96.08 | 49 | 0.937 |
| **LSR074** | F: TATCAACTCCAGAAGACGGGTT,<br>R: GGTAAACGTAGGGATACAAAGAAG | 1.90 | 1.32 | 0.22 | 0.35 | 90.20 | 46 | 0.940 |
| **LSR077** | F: GACAGATCCTTCTGGGACTTTT,<br>R: TTCTGCAATAGAGTACGTTGGC | 1.97 | 1.18 | 0.14 | 0.26 | 96.08 | 49 | 0.961 |
| **LSR108** | F: AGCTCTGGGAAGAGGAAGAGTA,<br>R: GCAGACAGAAGAAGAAAGTTAGAGA | 2.0 | 1.23 | 0.18 | 0.32 | 100 | 51 | 0.936 |
| **LSR109** | F: TGGGGTAGAAATTGAAGAGGAG,<br>R: TTGGATCAGCTTGGGTTTTACT | 1.98 | 1.33 | 0.22 | 0.37 | 98.04 | 50 | 0.925 |
| **LSR112** | F: TCTCTATATGTCTAATTCCTCGCC<br>R: CAAATTCACAGTTGTTGTCACG | 2.0 | 1.50 | 0.32 | 0.49 | 100 | 51 | 0.848 |
| **MC08_106502** | F: TGCCAAGCAAATACCACAAA,<br>R: TGAAGAAGCAACAGCAGCAG | 1.85 | 1.32 | 0.22 | 0.36 | 84.31 | 43 | 0.885 |
| **MC04_54893** | F: GAAAGCGAGAACGAAC<br>R: TGCCATCGGTACTCATCAAA | 2.0 | 1.89 | 0.47 | 0.66 | 100 | 51 | 0.576 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **MC10_152791** | F: TTGCAGCTTCCTTTCTGGTT<br>R: AGGAAACACATCTTGGGCTG | 1.90 | 1.59 | 0.35 | 0.52 | 90.20 | 46 | 0.790 |
| **MC07_95456** | F: TTCTTGAGAGACGGTGGCT<br>R: GATACAAAGAAACGGTGGCG | 1.94 | 1.37 | 0.26 | 0.42 | 94.12 | 48 | 0.904 |
| **MC06_73242** | F: AGCGAAGCATTTATCGAG<br>R: CGAAACGCACTATTCCCATT | 1.81 | 1.60 | 0.33 | 0.48 | 80.39 | 41 | 0.768 |
| **EC11** | F: TCTTCGCAGTCACCATTTC<br>R: CCTTCCTCTGTTTCTGTTCC | 2.0 | 1.56 | 0.35 | 0.53 | 100 | 51 | 0.766 |
| **EC49** | F: CGTGTTTTCTCAGATTTCCCA<br>R: CACTTCCCTTCATCAACCCCA | 1.51 | 1.26 | 0.17 | 0.26 | 50.98 | 26 | 0.969 |
| **EC34** | F: GATCCCCATCATAATCACCC<br>R: CAAAGGGCTACAATAACAAAC | 1.92 | 1.43 | 0.29 | 0.45 | 92.16 | 47 | 0.765 |
| **EC27** | F: GTTGGAAGGCACACAAAGTC<br>R: CGAGATGATTGGAGGATGATG | 1.86 | 1.52 | 0.31 | 0.46 | 86.27 | 44 | 0.861 |
| **SSR18362** | F: CAAGTGGACAAATATGAGCCAA<br>R: TTCGTTTCGCAGAGTGATTG | 1.57 | 1.38 | 0.22 | 0.32 | 56.86 | 29 | 0.815 |
| **SSR12810** | F: TTCCCAAAAACAAATCTTGG<br>R: TTTTGGAGAGAAAAGGTTGGA | 1.98 | 1.68 | 0.39 | 0.57 | 98.04 | 50 | 0.799 |
| **SSR15477** | F: CTGCCATTTCTGGGTTTGAT<br>R: AATTCTTCTGGGAATGGCCT | 1.78 | 1.56 | 0.33 | 0.47 | 78.43 | 40 | 0.639 |
| **Mean** | | 1.85 | 1.43 | 0.27 | 0.41 | 84.87 | 43.29 | 0.84 |
| **SE** | | 0.04 | 0.04 | 0.02 | 0.02 | 3.79 | 1.93 | 0.02 |

Note: (Na) Number of Alleles, (Ne) No. effective alleles, (h) Heterozygosity, (I) Shannon's information index, (PPL) Percentage of polymorphic loci, (PL) No. of polymorphic loci, (PIC) Polymorphism information content

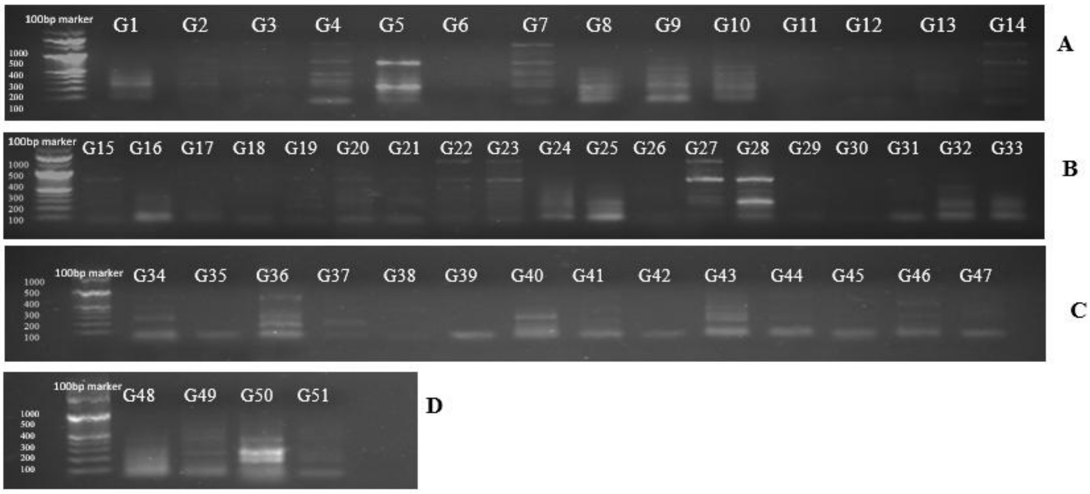


**Plate 6: SSR profiling for genetic diversity of 51 genotypes of *C. pedata*. A, B, C, D: LSR040**

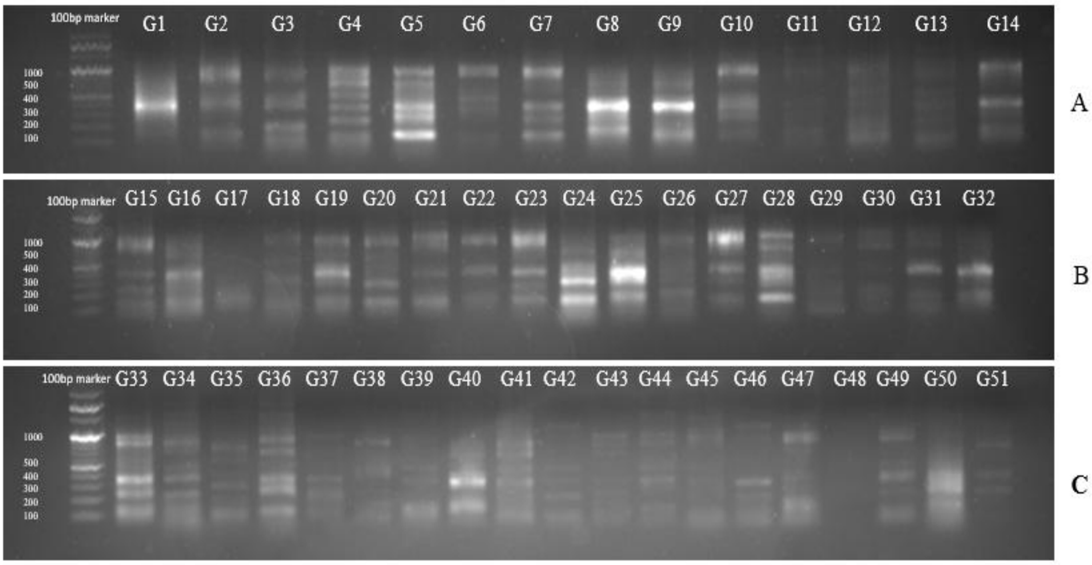


**Plate 7: SSR profiling for genetic diversity of 51 genotypes of *C. pedata*. A, B, C: LSR047**

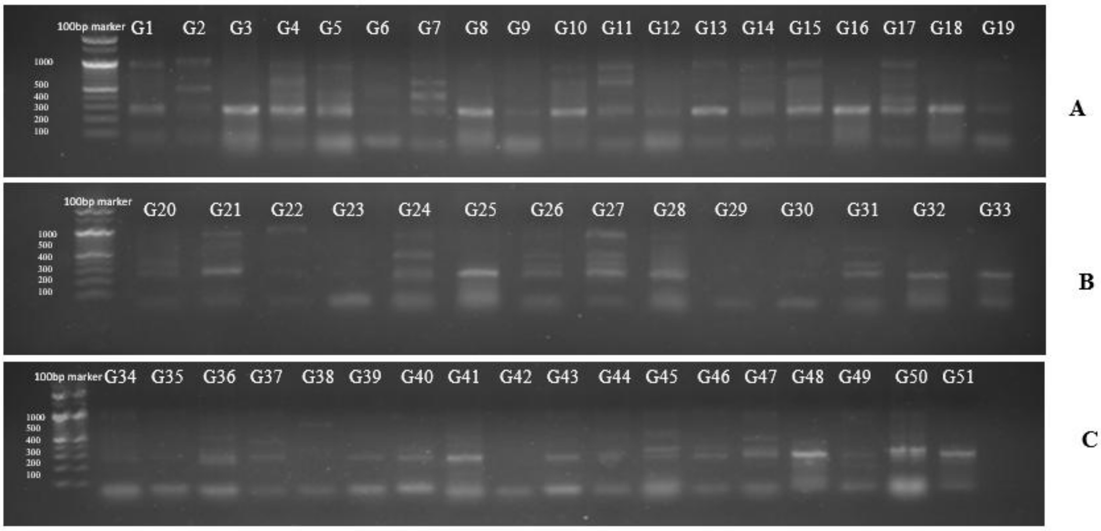


**Plate 8: SSR profiling for genetic diversity of 51 genotypes of *C. pedata*. A, B, C: LSR109**

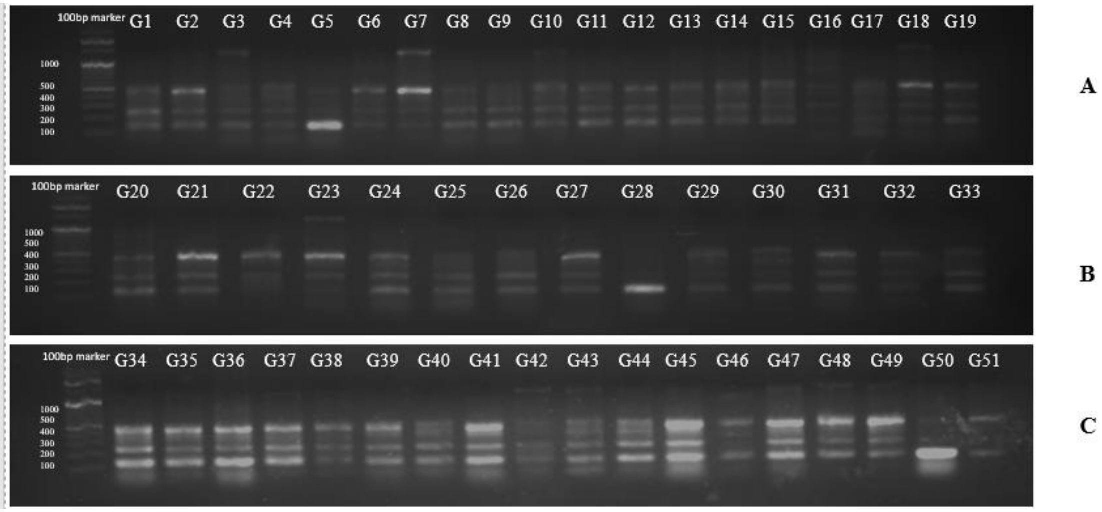


**Plate 9: SSR profiling for genetic diversity of 51 genotypes of *C. pedata*. A, B, C: MC08_106502**

## 4.4 Biochemical analysis of *C. pedata* fruits

### 4.4.1 Estimates of fresh weight, dry weight, and moisture percent

#### *4.4.1.1 Fresh weight (g)*

Fresh weight of *C. pedata* fruits revealed significant variation between the genotypes as shown in Table 4.13. Fresh weight represents significant variation and was in the range of $7.343^{w}\pm1.664$ g to $61.937^{a}\pm5.887$ g. The maximum fresh weight was found in SCP27 ($61.937^{a}\pm5.887$ g), and the minimum value was observed in SCP01 ($7.343^{w}\pm1.664$ g). The general mean for this trait is 20.61. The maximum fresh weight was recorded in genotype SCP27 (61.937a±5.887 g), which significantly outperformed other genotypes. Fresh weight is a crucial trait influencing the marketability and consumer preference of *C. pedata* fruits, as it directly correlates with fruit quality and yield potential.

#### *4.4.1.2 Dry weight*

Similarly, dry weight of *C. pedata* also showed significant variation (Table 4.13). The dry weight of *C. pedata* fruits was recorded between $0.217^{j}\pm0.049$ g to $1.254^{a}\pm0.101$ g. SCP27 ($1.254^{a}\pm0.101$ g) recorded the highest dry weight which was at par with genotypes SCP75 ($1.253^{a}\pm0.087$ g), SCP76 ($1.167^{ab}\pm0.058$ g), SCP77 ($1.127^{abc}\pm0.035$ g), SCP74 ($1.017^{abcd}\pm0.041$ g), SCP78 ($1.027^{abcd}\pm0.194$ g), SCP52 ($0.917^{abcde}\pm0.072$ g), SCP14 ($0.911^{abcde}\pm0.041$ g), SCP59 ($0.88^{abcdef}\pm0.10$ g) and SCP02 ($0.877^{abcdef}\pm0.041$ g), and the lowest dry weight was observed in SCP01 ($0.217^{j}\pm0.049$ g). The mean for this trait is 0.64 g. Dry weight is a critical indicator of the fruit's biomass. The genotypes that contain high dry weight could have maximum efficient photosynthetic mechanisms and better resources for fruit development, leading to the accumulation of more dry matter. It is a desirable trait for applications like drying, processing, or increasing nutritional density in *C. pedata* fruits. The wide range of dry weight percent in *C. pedata* fruits across the genotypes reflects substantial genetic diversity. According to Marcelis et al. (1992) the proportion of total dry matter allocated to fruit production ranged from 40% to 90%, which shows the variation in the moisture content of cucumber fruits. Maeda and Ahn (2021) and Valverde-Miranda et al. (2021) also reported the dry matter content in cucumber fruits, which aligns with the results observed in this study.

#### *4.4.1.3. Moisture percent*

As shown in Table 4.13, moisture percent was found in a range of $93.79^{o}\pm0.21$ to $97.98^{a}\pm0.08$ among different genotypes which was statistically significant. The maximum moisture percent was recorded in SCP12 ($97.98^{a}\pm0.08$) which was at par with SCP27 ($97.97^{a}\pm0.09$) whereas SCP62 ($93.79^{o}\pm0.21$) contained the minimum moisture percentage. The moisture percentage determination among the 51 genotypes of *C. pedata* revealed a statistically significant range, underscoring the intrinsic diversity and physiological plasticity of this species. Moisture content is a heritable trait profoundly influenced by the genotype. The elevated moisture percentage observed in SCP12 and SCP27 may be attributed to inherent genetic mechanisms that could enhance the water retention capacity. Aydin et al. (2011), Abbey et al. (2017), Agatemor et al. (2018) and Saikia & Bhuyan et al. (2022) reported variability in moisture content among the cultivated cucumber varieties studied.

**Table 4.13 Estimation of Fresh weight, Dry weight, and Moisture percent in *C. pedata* genotypes**

| Genotypes | Parameters | | |
|---|---|---|---|
| | **Fresh weight (g)** | **Dry weight (g)** | **Moisture content (%)** |
| **SCP01** | $7.343^{w}\pm1.664$ | $0.217^{j}\pm0.049$ | $96.37^{cdefghijkl}\pm0.51$ |
| **SCP02** | $28.975^{i}\pm0.582$ | $0.88^{abcdef}\pm0.10$ | $96.95^{abcdefghij}\pm0.41$ |
| **SCP03** | $9.257^{uvw}\pm1.248$ | $0.369^{ghij}\pm0.005$ | $95.87^{jklm}\pm0.54$ |
| **SCP04** | $10.527^{stuv}\pm0.298$ | $0.337^{ij}\pm0.036$ | $96.56^{bcdefghijk}\pm0.09$ |
| **SCP05** | $37.987^{ef}\pm1.879$ | $0.842^{abcdefgh}\pm0.051$ | $97.76^{ab}\pm0.22$ |
| **SCP06** | $8.27^{vw}\pm0.502$ | $0.394^{fghij}\pm0.020$ | $95.21^{lmn}\pm0.29$ |
| **SCP07** | $9.04^{uvw}\pm0.658$ | $0.476^{efghij}\pm0.080$ | $94.39^{no}\pm0.67$ |
| **SCP08** | $14.996^{nopq}\pm0.441$ | $0.548^{defghij}\pm0.058$ | $96.32^{defghijkl}\pm0.51$ |
| **SCP09** | $16.166^{no}\pm2.714$ | $0.554^{defghij}\pm0.046$ | $96.36^{cdefghijkl}\pm0.72$ |
| **SCP10** | $21.19^{kl}\pm2.200$ | $0.723^{bcdefghij}\pm0.065$ | $96.55^{bcdefghijk}\pm0.29$ |
| **SCP11** | $34.66^{gh}\pm1.304$ | $0.853^{abcdefg}\pm0.122$ | $97.55^{abcd}\pm0.31$ |
| **SCP12** | $36.783^{fg}\pm1.025$ | $0.746^{bcdefghi}\pm0.042$ | $97.98^{a}\pm0.08$ |
| **SCP13** | $14.327^{nopq}\pm1.175$ | $0.566^{defghij}\pm0.048$ | $96.05^{ghijklm}\pm0.0$ |
| **SCP14** | $39.317^{e}\pm3.724$ | $0.911^{abcde}\pm0.041$ | $97.67^{ab}\pm0.11$ |
| **SCP15** | $9.71^{tuvw}\pm0.661$ | $0.459^{efghij}\pm0.051$ | $95.3^{klmn}\pm0.22$ |
| **SCP16** | $16.953^{mn}\pm3.319$ | $0.507^{efghij}\pm0.142$ | $97.09^{abcdefghij}\pm0.38$ |
| **SCP26** | $12.313^{qrst}\pm0.970$ | $0.473^{efghij}\pm0.058$ | $96.15^{efghijklm}\pm0.44$ |
| **SCP27** | $61.937^{a}\pm5.887$ | $1.254^{a}\pm0.101$ | $97.97^{a}\pm0.09$ |
| **SCP28** | $11.14^{rstuv}\pm1.292$ | $0.453^{efghij}\pm0.020$ | $95.85^{jklm}\pm0.34$ |
| **SCP29** | $20.596^{kl}\pm0.797$ | $0.588^{defghij}\pm0.084$ | $97.16^{abcdefghi}\pm0.36$ |
| **SCP30** | $15.218^{nop}\pm0.179$ | $0.457^{efghij}\pm0.008$ | $96.99^{abcdefghij}\pm0.09$ |
| **SCP31** | $15.078^{nop}\pm0.402$ | $0.422^{efghij}\pm0.040$ | $97.21^{abcdefgh}\pm0.19$ |
| **SCP32** | $8.58^{uvw}\pm1.289$ | $0.416^{efghij}\pm0.093$ | $95.24^{lmn}\pm0.36$ |
| **SCP33** | $27.41^{ij}\pm1.439$ | $0.737^{bcdefghi}\pm0.050$ | $97.31^{abcdefg}\pm0.14$ |
| **SCP34** | $10.327^{stuvw}\pm0.517$ | $0.347^{hij}\pm0.044$ | $96.67^{bcdefghij}\pm0.27$ |
| **SCP35** | $8.653^{uvw}\pm1.045$ | $0.307^{ij}\pm0.038$ | $96.32^{defghijkl}\pm0.75$ |
| **SCP36** | $11.27^{rstu}\pm1.650$ | $0.447^{efghij}\pm0.055$ | $95.94^{hijklm}\pm0.51$ |
| **SCP37** | $14.493^{nopq}\pm0.654$ | $0.567^{defghij}\pm0.022$ | $96.07^{fghijklm}\pm0.27$ |

| | | | |
|---|---|---|---|
| **SCP38** | $10.59^{stuv}$±1.309 | $0.473^{efghij}$±0.015 | $95.38^{klmn}$±0.61 |
| **SCP39** | $19.273^{lm}$±0.537 | $0.75^{bcdefghi}$±0.021 | $96.1^{efghijklm}$±0.17 |
| **SCP40** | $26.953^{ij}$±4.512 | $0.6^{defghij}$±0.059 | $97.6^{abc}$±0.60 |
| **SCP41** | $16.68^{n}$±0.644 | $0.64^{cdefghij}$±0.040 | $96.17^{efghijklm}$±0.13 |
| **SCP42** | $26.173^{j}$±4.140 | $0.907^{abcde}$±0.072 | $96.37^{cdefghijkl}$±0.58 |
| **SCP52** | $25.18^{j}$±2.33 | $1.027^{abcd}$±0.194 | $95.91^{ijklm}$±0.65 |
| **SCP53** | $13.08^{pqrs}$±2.22 | $0.563^{defghij}$±0.007 | $95.36^{klmn}$±0.98 |
| **SCP54** | $13.487^{opqr}$±0.793 | $0.533^{defghij}$±0.028 | $96^{hijklm}$±0.42 |
| **SCP55** | $8.473^{uvw}$±0.318 | $0.413^{efghij}$±0.015 | $95.11^{lmn}$±0.17 |
| **SCP56** | $32.453^{h}$±1.914 | $0.85^{abcdefgh}$±0.120 | $97.34^{abcdef}$±0.47 |
| **SCP57** | $9.06^{uvw}$±0.359 | $0.38^{fghij}$±0.026 | $95.82^{jklm}$±0.13 |
| **SCP58** | $8.813^{uvw}$±1.503 | $0.483^{efghij}$±0.027 | $94.24^{no}$±0.82 |
| **SCP59** | $22.542^{k}$±0.845 | $0.877^{abcdef}$±0.041 | $96.11^{efghijklm}$±0.04 |
| **SCP60** | $9.89^{tuvw}$±0.099 | $0.5^{efghij}$±0.035 | $94.94^{mno}$±0.39 |
| **SCP61** | $8.8^{uvw}$±0.245 | $0.503^{efghij}$±0.012 | $94.27^{no}$±0.19 |
| **SCP62** | $10.663^{stuv}$±0.749 | $0.66^{cdefghij}$±0.029 | $93.79^{o}$±0.21 |
| **SCP72** | $15.43^{nop}$±0.826 | $0.47^{efghij}$±0.032 | $96.96^{abcdefghij}$±0.07 |
| **SCP73** | $10.467^{stuv}$±0.236 | $0.533^{defghij}$±0.015 | $94.9^{mno}$±0.18 |
| **SCP74** | $46.297^{c}$±5.248 | $1.017^{abcd}$±0.041 | $97.76^{ab}$±0.19 |
| **SCP75** | $54.043^{b}$±2.310 | $1.253^{a}$±0.087 | $97.67^{ab}$±0.21 |
| **SCP76** | $47.44^{c}$±0.544 | $1.167^{ab}$±0.058 | $97.54^{abcd}$±0.15 |
| **SCP77** | $43.55^{d}$±5.11 | $1.127^{abc}$±0.035 | $97.36^{abcde}$±0.22 |
| **SCP78** | $39.46^{e}$±4.510 | $0.917^{abcde}$±0.072 | $97.64^{ab}$±21 |
| **Mean** | 20.61 | 0.64 | 96.34 |
| **CD** | 6.046 | 0.176 | 1.13 |
| **SE** | 2.152 | 0.062 | 0.40 |

### 4.4.2 Estimation of Total carbohydrate, TSS, Protein and Vitamin C content in *C. pedata* genotypes

The estimation of total carbohydrate, TSS, protein, and vitamin C content in *C. pedata* genotypes is critical for understanding their nutritional properties and underscores the importance of *C. pedata* as a valuable horticultural crop with multiple applications.

#### *4.4.2.1 Total carbohydrate content (mg/100 g)*

The total carbohydrate content among *C. pedata* genotypes was in significant variation with a range from $85.653^{DE}$ to $309.457^{a}$ mg/100 g (Table 4.14). The highest carbohydrate content was recorded in SCP41 ($309.457^{a}$), which was statistically similar to SCP74 ($306.51^{ab}$), SCP29 ($302.49^{bc}$), and SCP42 ($298.49^{cd}$). Conversely, the lowest carbohydrate content was observed in SCP01 ($85.653^{DE}$). The overall mean carbohydrate content across the genotypes was 201.39 mg/100 g. This significant variability in carbohydrate content could be attributed to genetic differences influencing carbohydrate biosynthesis and accumulation. Genotypes with higher carbohydrate levels, such as SCP41, may be better suited for energy-rich dietary applications or processing into carbohydrate-based food products.

#### *4.4.2.2 Total soluble solids (°B)*

TSS content among the genotypes showed significant variation with observation from $2.433^{k}$±0.088 °B to $5.667^{a}$±0.176 °B (Table 4.14). SCP01 exhibited the highest TSS ($5.667^{a}$±0.176 °B), followed by SCP75 ($4.867^{b}$±0.120 °B) and SCP57 ($4.4b^{cd}$±0.416 °B), while the lowest TSS was recorded in SCP73 ($2.433^{k}$±0.088 °B). The mean TSS value was 3.363 °B. TSS is an essential indicator of sweetness and overall fruit quality, reflecting the soluble sugar content. The high TSS in SCP01 suggests its potential as a preferred genotype for fresh consumption, offering superior sweetness. Variability in TSS may be due to environmental conditions, fruit maturity, and genetic factors.

#### *4.4.2.3 Protein content (µg/ml)*

Protein content ranged from $1.61^{u}\pm0.652$ µg/ml to $16.73^{ab}\pm1.053$ µg/ml among the studied genotypes (Table 4.14). The highest protein content value was recorded in SCP60 ($16.73^{ab}\pm1.053$ µg/ml), which was at par with SCP01 ($14.79^{bc}\pm0.703$ µg/ml). The lowest protein content value was recorded in SCP03 ($1.61^{u}\pm0.652$ µg/ml). The overall mean protein content value was 8.37 µg/ml. Protein content is a critical nutritional parameter, particularly for genotypes like SCP60 and SCP01, which exhibit high levels and may serve as valuable sources of dietary protein. This variability highlights the potential for genetic improvement to enhance the nutritional quality of *C. pedata*.

#### *4.4.2.4 Vitamin C content (mg/100 g)*

Vitamin C content was found to be significant ranging from $0.5^{r}\pm0.0$ mg/100 g to $21.713^{a}\pm0.0787$ mg/100 g (Table 4.14). The highest Vitamin C content was observed in SCP76 ($21.713^{a}\pm0.0787$ mg/100 g), followed by SCP02 ($18.5^{b}\pm1.323$ mg/100 g), SCP54 ($18.167^{bc}\pm1.202$ mg/100 g), and SCP07 ($18.167^{bc}\pm1.202$ mg/100 g). The lowest Vitamin C content was recorded in SCP58 ($0.5^{r}\pm0.0$ mg/100 g). The mean Vitamin C content across the genotypes was 7.16 mg/100 g. Vitamin C is an essential antioxidant and a critical component of the human diet.

**Table 4.14 Estimation of Total Carbohydrate, TSS, Protein and Vitamin C content in *C. pedata***

| Genotypes | Parameters | | | |
|---|---|---|---|---|
| | Total carbohydrate content (mg/100 g) | Total soluble solid (°B) | Protein content (μg/ml) | Vitamin C (mg/100g) |
| **SCP01** | $85.653^{DE}$ | $5.667^{a}\pm0.176$ | $14.79^{bc}\pm0.703$ | $0.833^{qr}\pm0.167$ |
| **SCP02** | $177.442^{st}$ | $3.7^{cdefgh}\pm0.265$ | $10.03^{ghijkl}\pm1.0$ | $18.5^{b}\pm1.323$ |
| **SCP03** | $216.93^{n}$ | $3.533^{defghi}\pm0.285$ | $1.61^{u}\pm0.652$ | $0.667^{r}\pm0.167$ |
| **SCP04** | $196.138^{p}$ | $3.367^{efghijk}\pm0.088$ | $6.78^{opqr}\pm1.520$ | $1.167^{pqr}\pm0.167$ |
| **SCP05** | $167.624^{u}$ | $3.633^{cdefgh}\pm0.088$ | $6.32^{pqr}\pm0.829$ | $6.567^{gh}\pm0.841$ |
| **SCP06** | $188.461^{q}$ | $4.233^{bcdefg}\pm0.120$ | $8.35^{klmnop}\pm1.405$ | $3.3^{klm}\pm0.404$ |
| **SCP07** | $237.361^{k}$ | $3.633^{cdefgh}\pm0.240$ | $9.66^{ghijklm}\pm2.225$ | $18.167^{bc}\pm1.202$ |
| **SCP08** | $200.897^{p}$ | $2.9^{hijk}\pm0.153$ | $5.09^{rst}\pm2.138$ | $7.5^{g}\pm0.173$ |
| **SCP09** | $224.52^{m}$ | $3.133^{hijk}\pm0.033$ | $5^{rst}\pm0.909$ | $15.667^{de}\pm0.167$ |
| **SCP10** | $276.12^{gh}$ | $3.2^{hijk}\pm0.100$ | $9.31^{hijklmn}\pm1.771$ | $15.667^{de}\pm0.167$ |
| **SCP11** | $280.02^{fg}$ | $2.833^{hijk}\pm0.186$ | $12.93^{cdef}\pm3.416$ | $0.5^{r}\pm0.0$ |
| **SCP12** | $251.69^{j}$ | $2.9^{hijk}\pm0.058$ | $5.79^{qr}\pm2.370$ | $3^{klmno}\pm0.0$ |
| **SCP13** | $173.14^{tu}$ | $3.533^{defghi}\pm0.120$ | $10.8^{fghij}\pm2.292$ | $3.5^{jklm}\pm0.50$ |
| **SCP14** | $278.66^{fg}$ | $4.3^{bcde}\pm0.404$ | $6.98^{nopqr}\pm3.527$ | $12.9^{f}\pm0.666$ |
| **SCP15** | $93.337^{C}$ | $2.867^{hijk}\pm0.240$ | $6.58^{opqr}\pm0.098$ | $1.167^{pqr}\pm0.167$ |
| **SCP16** | $100.503^{B}$ | $3.433^{defghij}\pm0.033$ | $9.6^{ghijklm}\pm0.550$ | $3.733^{jkl}\pm0.393.$ |
| **SCP26** | $291.05^{e}$ | $3.067^{hijk}\pm0.120$ | $3.45^{stu}\pm0.088$ | $15.333^{e}\pm0.601$ |
| **SCP27** | $142.507^{w}$ | $2.633^{ijk}\pm0.033$ | $2.15^{u}\pm0.744$ | $16.567^{de}\pm0.869$ |
| **SCP28** | $79.043^{E}$ | $4.533^{bc}\pm0.869$ | $5.34^{rs}\pm1.501$ | $0.667^{r}\pm0.167$ |
| **SCP29** | $302.49^{bc}$ | $3.7^{cdefgh}\pm0.404$ | $12.45^{def}\pm2.593$ | $0.667^{r}\pm0.167$ |
| **SCP30** | $284.687^{f}$ | $2.867^{hijk}\pm0.088$ | $9.82^{ghijkl}\pm1.121$ | $3.967^{jk}\pm0.273$ |
| **SCP31** | $267.353^{i}$ | $2.9^{hijk}\pm0.058$ | $9.88^{ghijkl}\pm0.762$ | $1.833^{nopqr}\pm0.167$ |
| **SCP32** | $179.557^{rs}$ | $3.4^{efghijk}\pm0.115$ | $11.51^{efgh}\pm1.569$ | $3^{klmno}\pm0.0$ |
| **SCP33** | $141.567^{w}$ | $3.033^{hijk}\pm0.186$ | $9.64^{ghijklm}\pm0.930$ | $3.767^{jkl}\pm0.338$ |
| **SCP34** | $108.487^{zA}$ | $2.8^{hijk}\pm0.200$ | $8.62^{jklmnop}\pm0.635$ | $12.533^{f}\pm0.953$ |
| **SCP35** | $217.003^{n}$ | $3.4^{efghijk}\pm0.100$ | $5.74^{qrs}\pm0.183$ | $5.867^{hi}\pm0.133$ |
| **SCP36** | $149.057^{v}$ | $3.6^{cdefghi}\pm0.458$ | $11.66^{defgh}\pm1.698$ | $1.333^{pqr}\pm0.167$ |

| | | | | |
|---|---|---|---|---|
| **SCP37** | $121.86^{y}$ | $3.067^{hijk}$±0.120 | $9.32^{hijklmn}$±1.367 | $2.167^{mnopq}$±0.441 |
| **SCP38** | $208.203^{o}$ | $2.833^{hijk}$±0.067 | $11.9^{defg}$±1.238 | $0.667^{r}$±0.167 |
| **SCP39** | $230.53^{l}$ | $2.8^{hijk}$±0.100 | $7.21^{nopqr}$±0.846 | $0.5^{r}$±0.0 |
| **SCP40** | $272.017^{hi}$ | $2.8^{hijk}$±0.115 | $13.6^{cde}$±1.338 | $2.5^{lmnop}$±0.50 |
| **SCP41** | $309.457^{a}$ | $4.267^{bcdef}$±0.067 | $9.96^{ghijkl}$±2.478 | $4.1^{jk}$±0.557 |
| **SCP42** | $298.493^{cd}$ | $3.267^{ghijk}$±0.176 | $8.13^{lmnopq}$±1.980 | $4.767^{ij}$±0.384 |
| **SCP52** | $267.267^{i}$ | $2.9^{hijk}$±0.058 | $7.13^{nopqr}$±0.620 | $12.233^{f}$±1.220 |
| **SCP53** | $112.17^{z}$ | $3.333^{efghijk}$±0.088 | $7.38^{mnopqr}$±1.962 | $2.167^{mnopq}$±0.441 |
| **SCP54** | $184.51^{qr}$ | $3.233^{hijk}$±0.437 | $13.87^{cd}$±1.655 | $18.167^{bc}$±1.202 |
| **SCP55** | $223.857^{m}$ | $3.067^{hijk}$±0.133 | $6.53^{opqr}$±0.500 | $7.5^{g}$±0.173 |
| **SCP56** | $129.13^{x}$ | $3.3^{fghijk}$±0.115 | $16.9^{a}$±0.803 | $15.667^{de}$±0.167 |
| **SCP57** | $168.79^{u}$ | $4.4^{bcd}$±0.416 | $7.33^{mnopqr}$±1.381 | $15.667^{de}$±0.167 |
| **SCP58** | $179.89^{rs}$ | $3.5^{defghi}$±0.058 | $7.07^{nopqr}$±1.182 | $0.5^{r}$±0.0 |
| **SCP59** | $279.546^{fg}$ | $3.667^{cdefgh}$±0.067 | $8.51^{jklmnop}$±1.362 | $3^{klmno}$±0.0 |
| **SCP60** | $117.803^{y}$ | $3.3^{fghijk}$±0.351 | $16.73^{ab}$±1.053 | $3.5^{jklm}$±0.50 |
| **SCP61** | $88.97^{CD}$ | $2.833^{hijk}$±0.338 | $8.92^{ijklmno}$±1.406 | $1.667^{opqr}$±0.33 |
| **SCP62** | $105.05^{AB}$ | $2.5^{jk}$±0.200 | $10.77^{fghijk}$±1.801 | $1.5^{pqr}$±0.0 |
| **SCP72** | $81.863^{E}$ | $2.833^{hijk}$±0.167 | $6.63^{opqr}$±3.670 | $3.167^{klmn}$±0.167 |
| **SCP73** | $145.6^{vw}$ | $2.433^{k}$±0.088 | $11.16^{fghi}$±1.814 | $1.7^{opqr}$±0.115 |
| **SCP74** | $306.513^{ab}$ | $3.567^{defghi}$±0.033 | $4.96^{rst}$±1.60 | $17^{cd}$±0.462 |
| **SCP75** | $278.87^{fg}$ | $4.867^{b}$±0.120 | $2^{u}$±0.706 | $13.377^{f}$±0.927 |
| **SCP76** | $280.703^{fg}$ | $3.4^{efghijk}$±0.379 | $2.3^{u}$±1.541 | $21.713^{a}$±0.0787 |
| **SCP77** | $272.74^{hi}$ | $3.567^{defghi}$±0.318 | $3.05^{tu}$±0.842 | $15.533^{e}$±0.273 |
| **SCP78** | $296.17^{de}$ | $3^{hijk}$±0.058 | $5.39^{rs}$±0.370 | $17.867^{bc}$±1.534 |
| **Mean** | 201.39 | 3.363 | 8.37 | 7.16 |
| **CD** | 11.779 | 0.674 | 4.29 | 1.591 |
| **SE** | 33.098 | 0.240 | 1.527 | 0.566 |

The high Vitamin C levels in SCP76 and SCP02 highlight their potential as nutrient-dense genotypes for promoting health benefits and combating oxidative stress.

### 4.4.3 Estimation of essential elements present in C. pedata fruits

#### *4.4.3.1 Elemental analysis of K, Ca, Mg, Na, Fe, Mn, Cu and Zn*

The elemental composition analysis of *C. pedata* genotypes revealed significant variation across the eight elements studied, as detailed in Table 4.15. These results highlight the diverse nutrient profiles of different genotypes and show their potential for dietary value.

Potassium showed more variability, from 104.26 mg/kg (SCP06) to 940.09 mg/kg (SCP27). Potassium is critical for plant physiological functions. High potassium content genotypes like SCP27 could be advantageous for promoting plant stress resilience. As reported by Rivas et al. (2013), 7400 mg/100g and Oliveira et al. (2014), 2183 mg/100g, significantly exceed those observed in the present study, which could be due to potential methodological or environmental factors influencing potassium content.

Magnesium levels were observed to be highest in SCP39 (79.26 mg/kg), followed by SCP27 (75.23 mg/kg). The lowest magnesium concentration was recorded in SCP06 (8.66 mg/kg). These results could be due to differential efficiency in magnesium absorption from the soil and its subsequent transport to the plant. Magnesium is vital for chlorophyll synthesis and enzymatic processes, and its variability among genotypes may be linked to differences in root uptake efficiency. The observed range is consistent with Valadares et al. (2020), who reported 2.1 mg/100g. Genotypes with high magnesium levels, such as SCP39, are valuable for enhancing dietary intake.

Calcium content peaked in SCP27 (18.79 mg/kg), followed by SCP74 (13.62 mg/kg). Whereas it was minimum in SCP06 (1.02 mg/kg). Calcium distribution could be influenced by genotypic differences in calcium-binding proteins or translocation efficiency. Compared to previous studies by Oliveira et al. (2014) and Rivas et al. (2013), the calcium content observed in this study is lower, reflecting differences in calcium availability or plant genetics.

Sodium content was significantly highest in genotype SCP62 (5.01 mg/kg), followed by SCP11 (3.72 mg/kg) and SCP59 (3.61 mg/kg). The lowest sodium content was obtained from SCP72 (0.88 mg/kg). The variation in sodium content may be caused due to genetic factors influencing sodium uptake and accumulation in plant tissues. The comparison with Rivas et al. (2013), who reported significantly higher sodium (77 mg/100g), indicates possible environmental or methodological differences.

The highest Iron content was recorded in SCP60 (2.83 mg/kg), followed by SCP57 (2.58 mg/kg) and SCP56 (2.24 mg/kg). The lowest was found in SCP62 (0.01 mg/kg). These differences might be linked to genotypic capacities for iron storage and assimilation. These findings are in accord with Oliveira et al. (2014), which suggest a stable genetic influence on iron content.

Manganese levels were highest in SCP42 (2.15 mg/kg), with SCP16 showing the lowest concentration (0.12 mg/kg). Manganese is essential for photosynthesis and enzyme activation. The result aligns with the findings of Valadares et al. (2020) (2.9 mg/100g), underscoring the role of genotype in influencing manganese accumulation.

Copper concentration was significantly higher in SCP42 (1.05 mg/kg), with the lowest levels found in SCP52 (0.04 mg/kg). The differences could reflect variations in the genotypes' ability to transport or sequester copper ions. Similar findings by Oliveira et al. (2014) further support the influence of genetic factors on copper content.

Zinc levels were highest in SCP03 (2.63 mg/kg), followed by SCP55 (2.48 mg/kg) and SCP11 (2.11 mg/kg). The lowest zinc content was found in SCP53 (0.12 mg/kg). Zinc is an important cofactor for several enzymes, and its variability may depend on genetic factors or soil zinc bioavailability. The observed results emphasizing the importance of genotype selection for enhancing zinc biofortification.

**Table 4.15 Elemental analysis of fifty-one genotypes of *C. pedata***

| Genotypes | K (mg/kg) | Mg (mg/kg) | Ca (mg/kg) | Na (mg/kg) | Fe (mg/kg) | Mn (mg/kg) | Cu (mg/kg) | Zn (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| **SCP01** | 165.21[t] | 9.61[uv] | 4.65[ghijklm] | 1.9[bcd] | 0.84[cde] | 0.11[e] | 0.11[bc] | 0.67[cde] |
| **SCP02** | 299.4[op] | 26.79[klmnopqr] | 4.65[ghijklm] | 1.43[cd] | 0.69[cde] | 0.16[de] | 0.05[c] | 0.29[de] |
| **SCP03** | 213.15[s] | 78.56[b] | 7.89[defghij] | 2.11[abcd] | 0.57[de] | 1.12[abcde] | 1.01[ab] | 2.63[ab] |
| **SCP04** | 409.36[l] | 21.76[nopqrstu] | 4.91[ghijklm] | 1.12[d] | 0.56[de] | 0.16[de] | 0.05[c] | 0.27[de] |
| **SCP05** | 629.21[fg] | 42.35[hij] | 7.21[efghijkl] | 2.64[abcd] | 0.06[e] | 0.67[bcde] | 0.44[abc] | 1.39[bcde] |
| **SCP06** | 104.26[u] | 8.66[v] | 2.55[klm] | 1.14[d] | 0.21[e] | 0.1[e] | 0.03[c] | 0.24[de] |
| **SCP07** | 349.25[m] | 21.7[a] | 7.14[efghijkl] | 1.48[cd] | 0.08[e] | 0.57[bcde] | 0.3[abc] | 1.71[abcde] |
| **SCP08** | 311.37[mno] | 20.55[opqrstuv] | 4.35[hijklm] | 1.39[cd] | 0.92[bcde] | 0.16[de] | 0.07[c] | 0.36[de] |
| **SCP09** | 351.04[m] | 48.56[efgh] | 13.05[bcd] | 1.35[cd] | 1.02[bcde] | 0.25[de] | 0.07[c] | 0.36[de] |
| **SCP10** | 423.96[kl] | 23.26[mnopqrs] | 5.88[fghijkl] | 1.85[bcd] | 0.84[cde] | 0.16[de] | 0.05[c] | 0.38[de] |
| **SCP11** | 109.55[u] | 36.27[hijkl] | 4.38[hijklm] | 3.72[abcd] | 0.13[e] | 1.2[abcde] | 0.63[abc] | 2.11[abcd] |
| **SCP12** | 590.16[g] | 22.49[nopqrst] | 5.4[ghijkl] | 2.73[abcd] | 0.54[de] | 0.18[de] | 0.05[c] | 0.34[de] |
| **SCP13** | 239.82[qrs] | 14.5[rstuv] | 3.23[jklm] | 1.22[cd] | 0.51[de] | 0.1[e] | 0.05[c] | 0.2[e] |
| **SCP14** | 321.69[mno] | 35.71[ijklm] | 6.41[efghijkl] | 1.02[d] | 0.76[cde] | 0.2[de] | 0.05[c] | 0.28[de] |
| **SCP15** | 255.03[qr] | 19.95[opqrstuv] | 4.22[hijklm] | 1.37[cd] | 0.44[de] | 0.15[de] | 0.04[c] | 0.24[de] |
| **SCP16** | 396.45[l] | 17.22[qrstuv] | 3.17[jklm] | 1.38[cd] | 0.48[de] | 0.12[de] | 0.05[c] | 0.29[de] |
| **SCP26** | 328.87[mno] | 18[qrstuv] | 5.97[fghijkl] | 1.34[cd] | 0.59[cde] | 0.19[de] | 0.05[c] | 0.24[de] |
| **SCP27** | 940.09[a] | 75.23[bc] | 18.79[a] | 2.99[abcd] | 2.03[abcd] | 0.48[bcde] | 0.13[abc] | 0.96[bcde] |
| **SCP28** | 525[h] | 48.4[efghi] | 9.68[bcdefg] | 1.74[bcd] | 0.99[bcde] | 0.23[de] | 0.05[c] | 0.34[de] |
| **SCP29** | 262.71[pq] | 16.01[qrstuv] | 3.46[hijklm] | 1.44[cd] | 0.54[de] | 0.13[de] | 0.06[c] | 0.21[de] |
| **SCP30** | 630.7[fg] | 64.75[cd] | 14.5[ab] | 1.53[cd] | 1.27[abcde] | 0.31[cde] | 0.08[c] | 0.38[de] |
| **SCP31** | 621.32[fg] | 27.41[klmnopq] | 7.24[efghijkl] | 1.37[cd] | 0.1[e] | 0.95[abcde] | 0.45[abc] | 1.47[abcde] |
| **SCP32** | 347.79[mn] | 15.61[qrstuv] | 3.84[hijklm] | 1.94[bcd] | 0.46[de] | 0.14[de] | 0.04[c] | 0.24[de] |
| **SCP33** | 854.78[c] | 33.72[jklmn] | 7.2[efghijkl] | 2.96[abcd] | 0.08[e] | 0.39[bcde] | 0.3[abc] | 1.52[abcde] |
| **SCP34** | 899.45[b] | 14.7[qrstuv] | 2.32[klm] | 2.32[abcd] | 0.1[e] | 1.76[ab] | 0.58[abc] | 1.71[abcde] |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **SCP35** | 615.58 fg | 40.94 hij | 8.42 defghi | 3.27 abcd | 0.97 bcde | 0.26 de | 0.06 c | 0.28 de |
| **SCP36** | 612.49 qrs | 20.33 opqrstuv | 3.2 jklm | 2.44 abcd | 0.05 e | 0.53 bcde | 0.26 abc | 1.48 abcde |
| **SCP37** | 243.26 qrs | 20.76 opqrstuv | 6.62 efghijkl | 1.25 cd | 0.46 de | 0.14 de | 0.04 c | 0.21 de |
| **SCP38** | 455.78 jk | 62.21 d | 4.16 hijklm | 1.92 bcd | 0.07 e | 0.51 bcde | 0.27 abc | 1.55 abcde |
| **SCP39** | 594.11 g | 79.26 a | 3.14 jklm | 1.61 cd | 0.05 e | 0.5 bcde | 0.27 abc | 1.97 abcde |
| **SCP40** | 113.7 u | 63.77 cd | 2.49 klm | 4.11 abc | 0.14 e | 1.51 abcd | 0.82 abc | 1.39 bcde |
| **SCP41** | 253.13 qrs | 31.28 jklmnop | 6.69 efghijkl | 0.94 d | 1.25 abcde | 0.24 de | 0.12 bc | 0.47 de |
| **SCP42** | 130.6 tu | 55.84 defg | 3.44 hijklm | 4.64 ab | 0.16 e | 2.15 a | 1.05 a | 3.32 a |
| **SCP52** | 238.17 qrs | 16.52 qrstuv | 3.28 ijklm | 0.97 d | 0.36 e | 0.17 de | 0.04 c | 0.27 de |
| **SCP53** | 252.98 qrs | 13.28 stuv | 4.06 hijklm | 1.18 d | 0.47 de | 0.09 e | 0.05 c | 0.12 e |
| **SCP54** | 220.65 rs | 13.31 stuv | 3.85 hijklm | 0.93 d | 0.29 e | 0.12 de | 0.04 c | 0.16 e |
| **SCP55** | 649.51 f | 61.44 d | 5.28 ghijkl | 1.92 bcd | 0.07 e | 0.98 abcde | 0.44 abc | 2.48 abc |
| **SCP56** | 508.26 hi | 43.33 ghij | 10.61 bcdef | 3.13 abcd | 2.24 abc | 0.28 de | 0.1 bc | 0.54 de |
| **SCP57** | 538.77 h | 46.51 fghi | 11.27 bcde | 3.02 abcd | 2.58 ab | 0.25 de | 0.12 bc | 0.61 cde |
| **SCP58** | 306.97 no | 19.53 pqrstuv | 3.91 hijklm | 0.91 d | 0.57 de | 0.14 de | 0.05 c | 0.23 de |
| **SCP59** | 701.1 e | 32.59 jklmno | 6.03 fghijkl | 3.61 abcd | 0.01 e | 0.11 e | 0.09 bc | 0.47 de |
| **SCP60** | 478.81 ij | 60.27 de | 8.54 cdefgh | 3.05 abcd | 2.83 a | 0.41 bcde | 0.16 abc | 0.89 bcde |
| **SCP61** | 312.45 mno | 10.31 tuv | 6.12 efghijkl | 1.12 d | 0.04 e | 0.46 bcde | 0.12 bc | 1.26 bcde |
| **SCP62** | 141.56 tu | 58.93 def | 1.02 m | 5.01 a | 0.01 e | 0.13 de | 0.08 c | 0.51 de |
| **SCP72** | 263.47 pq | 16.48 qrstuv | 3.89 hijklm | 0.88 d | 1.12 bcde | 0.56 bcde | 0.11 bc | 0.69 cde |
| **SCP73** | 754.54 d | 18.75 pqrstuv | 8.22 defghij | 1.17 d | 0.05 e | 1.69 abc | 0.35 abc | 1.5 abcde |
| **SCP74** | 230.6 qrs | 47.36 fghi | 13.62 bc | 1.08 d | 1.37 abcde | 0.39 bcde | 0.09 bc | 0.58 cde |
| **SCP75** | 613.41 fg | 36.57 hijk | 2.18 lm | 1.92 bcd | 0.04 e | 0.77 abcde | 0.34 abc | 1.63 abcde |
| **SCP76** | 304.78 o | 41.97 hij | 8.31 defghij | 1.25 cd | 1.25 abcde | 0.22 de | 0.07 c | 0.5 de |
| **SCP77** | 617.74 fg | 26.55 klmnopqr | 7.36 efghijk | 1.58 cd | 0.8 cde | 0.22 de | 0.06 c | 0.33 de |
| **SCP78** | 426.22 kl | 23.63 lmnopqrs | 7.25 efghijkl | 1.07 d | 0.69 cde | 0.2 de | 0.05 c | 0.35 de |
| **Mean** | **402.98** | **37.5** | **6.15** | **1.95** | **0.64** | **0.45** | **0.19** | **0.84** |
| **SE** | **14.79** | **5.61** | **1.88** | **1.01** | **0.81** | **0.69** | **0.49** | **0.87** |

# Chapter 05

# Summary and Conclusion

The present investigation, titled "Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening", was undertaken to characterize *C. pedata* genotypes collected from Sikkim. The study aimed to explore the genetic diversity and nutritional potential of this underutilized crop to enhance its horticultural value.

The objectives of the study included conducting a survey and interviews with local farmers to understand their perceptions of desirable horticultural traits in *C. pedata*. This understanding was crucial for identifying genotypes of interest and gathering baseline information on farmers' preferences. The genotypes were subsequently collected and evaluated through morphological and molecular characterization to assess their genetic diversity.

Along with morphological studies, biochemical and elemental analyses were performed to ascertain the nutritional value of the collected genotypes. These analyses also offered key insights into the potential health benefits and horticultural value of the *C. pedata*.

The experimental findings of this investigation are summarized below:

## 5.1 Farmers' perception of desirable horticulture traits of *C. pedata*

Women dominated *C. pedata* cultivation (57%), emphasizing their critical role in agriculture. Most respondents were married (71%) and in the 30–39 age group. A majority (55%) were unemployed, relying on agriculture for livelihood, with some involvement from service professionals (11%) and students (2%). Family size averaged five members (52%). Education levels varied, with 32% having primary education, 22% having high school education, and 19% being graduates or higher. Farming experience ranged, with 46% having over ten years of experience. On average, 1.5 family members participated in *C. pedata* cultivation per household.

Farmers emphasize traits related to productivity, ease of cultivation, and market suitability of *C.pedata*. These insights highlight the need for breeding programs to prioritize traits such as long vines, spineless and soft-textured fruits, short fruit length, and mild taste. Additionally, sustainable practices, local climatic adaptability, and socio-economic factors should guide the development of improved varieties.

## 5.2 Morphological characterization of *C. pedata* genotypes

The morphological evaluation of fifty-one *C. pedata* genotypes revealed significant variability across all studied traits, highlighting the genetic diversity within the collected population. Vine length ranged from 4.01 m (SCP01) to 11.91 m (SCP27), with a mean of 7.80 m, suggesting its potential use in breeding for trellis-based or low-input systems. The number of primary branches varied from 9.0 (SCP09) to 19.60 (SCP36), averaging 14.75, indicating its relevance for productivity enhancement. Internodal length showed a wide range, with SCP01 having the longest

(12.73 cm) and SCP06 the shortest (7.64 cm), emphasizing its role in plant architecture and yield potential. Days to 50% flowering, ranging from 85.30 days (SCP01) to 214.70 days (SCP42), with a mean of 108.06 days, demonstrated variability in flowering time, impacting early yield and adaptability to cropping systems.

Node number for the first staminate flower ranged from 4.50 (SCP02) to 10.10 (SCP27), with a mean of 6.45, while the node number for the first pistillate flower ranged from 3.90 (SCP53) to 10.20 (SCP27), averaging 6.15. These traits are vital for determining reproductive timing and yield potential. Peduncle length varied significantly, with SCP41 exhibiting the longest (2.65 cm) and SCP32 the shortest (0.89 cm), averaging 1.46 cm, which is important for flower positioning and harvesting. Fruit length ranged from 5.75 cm (SCP03) to 18.56 cm (SCP33), with a mean of 8.75 cm, while fruit girth varied between 2.14 cm (SCP01) and 5.12 cm (SCP75), averaging 3.35 cm. These traits provide opportunities for market-specific breeding programs.

The number of seeds per fruit ranged from 7.3 (SCP72) to 13.2 (SCP78), with a mean of 10.4, while 100-seed weight varied from 4.6 g (SCP04) to 10.8 g (SCP74), with a mean of 7.1 g. These attributes are essential for seed production and marketing strategies. Average fruit weight is in the range of 8.7 g (SCP61) to 51.2 g (SCP74), with a mean of 22.2 g, reflecting its association with fruit size and yield. The number of fruits per plant varied widely, with SCP36 producing the maximum (414.7) and SCP78 the minimum (47.1), averaging 177.7, indicating its impact on overall productivity. Petiole length ranged from 0.50 cm (SCP01) to 1.22 cm (SCP40), with a mean of 0.78 cm, which influences leaf positioning and photosynthesis.

Finally, yield per plant was recorded from 0.99 kg (SCP72) to 8.41 kg (SCP39), with an average of 3.31 kg. High-yielding genotypes like SCP39 and SCP40 demonstrated superior traits, including optimal fruit size, fruit count, and reproductive efficiency, making them suitable for commercial cultivation and breeding programs. The significant variability observed across these traits underscores the genetic diversity in *C. pedata* and its potential for targeted breeding to develop improved varieties for different ecological and market needs.

## 5.3 Phenotypical and genotypic correlation matrix of 15 quantitative traits

The phenotypic and genotypic correlation analyses of various traits in *C. pedata* revealed significant relationships among yield-related characteristics. Traits such as the number of fruits per plant, primary branches, peduncle length, and petiole length showed strong positive correlations with fruit yield, suggesting their importance in selection for higher yields. In contrast, traits like days to 50% flowering and node number to first flower exhibited negative correlations with yield, indicating that earlier flowering and lower flower node positions may slightly favor higher yields. Other traits like vine length and internodal length were found positively correlated with reproductive and vegetative traits but had minimal direct impact on yield. Overall, the findings highlight key traits for breeding programs aimed at improving yield, emphasizing the balance between vegetative growth and reproductive efficiency.

## 5.4 Direct and indirect effect of traits on yield per plant at phenotypic and genotypic level

Path coefficient analysis at both phenotypic and genotypic levels revealed key insights into the direct and indirect effects of various traits on yield per plant in *C. pedata*. The number of fruits per plant and average fruit weight were identified as the primary direct contributors to yield, with strong positive effects, while traits like fruit girth, internodal length, and node number to the first flower showed negative direct effects. Some traits with negative direct effects such as fruit girth and internodal length, exhibited positive indirect effects, suggesting their potential for enhancing yield through indirect pathways. Notably, traits like peduncle length, seed number per fruit, and primary branches had substantial indirect positive effects on yield. The analysis accounted for a significant variation of the phenotypic (81.61%) and genotypic (87.61%) in yield, indicating that the selected traits are powerful predictors of yield. This highlights the importance of optimizing both the direct and indirect effects of these traits in breeding process to improve yield potential.

## 5.5 Estimation of genetic divergence using Tocher method of $D^2$

Genetic divergence among 51 genotypes of *C. pedata* was assessed using the Tocher method of $D^2$ statistics, revealing significant genetic diversity. The genotypes were grouped into 8 clusters, with Cluster I and II containing the most genotypes (30 and 15, respectively), indicating genetic similarity among genotypes within these clusters. However, Clusters III to VIII, each containing only one genotype, represented highly distinct genotypes with unique trait combinations. The highest intra-cluster diversity was observed in Cluster II (1.83), followed by Cluster I (1.34), while Clusters with a single genotype had zero intra-cluster diversity. Inter-cluster distances showed the greatest divergence between Clusters II and V (7.02), suggesting that crossing genotypes from these clusters could yield significant genetic variation and hybrid vigor. These findings highlight the potential for selecting diverse genotypes for breeding, particularly those with unique trait combinations from the smaller clusters.

## 5.6 Qualitative traits analysis of fifty-one genotypes of *C. pedata*

Evaluation of various qualitative traits in 51 *C. pedata* genotypes reveals both variability and uniformity. Fruit shape showed significant diversity with 90% of genotypes exhibiting elongated fruits with pointed tips, while 44% were fruit spines and 2 % spineless. Medium green was the most common fruit colour (49%), followed by deep green (42%), and light green (9%). Leaf colour showed similar trends, with medium green found dominant (57%), over deep green (29%) and light green (14%) observed less frequently. Traits like the number of leaf lobes, stem shape, stem pubescence, leaf shape, leaf blade, flower color, and seed color were all uniform across genotypes, with 100% occurrence in specific forms such as five leaf lobes, angular stem shape, absence of stem pubescence, palmately compound leaf shape, serrated leaf blade, yellowish-white flowers, and black seeds. These consistent traits highlight the genetic stability and conservation within the species, with strong genetic control and minimal environmental influence. Such traits can be reliable markers for species identification and breeding efforts.

## 5.7 Marker attributes and genetic diversity analysis of *C. pedata* genotypes

24 SSR markers were employed to analyze the genetic diversity of 51 *C. pedata* genotypes, revealing a high level of genetic variability. Out of the 24 markers, 21 were polymorphic, with PIC values above 0.5, indicating significant polymorphism. The markers EC49 and LSR015 exhibited the highest and lowest PIC values, 0.969 and 0.576, respectively. Other markers like LSR108, LSR112, and EC11 showed 100% polymorphism. The average PIC value was 0.84, and the average number of different alleles was 1.85. The study shows that SSR markers are highly effective for assessing genetic diversity in *C. pedata*, with markers like EC49, LSR108, and LSR112 emerging as particularly important for future genetic studies and marker-assisted selection.

## 5.8 Biochemical Analysis of *C. pedata* fruits

The biochemical analysis of 51 *C. pedata* genotypes revealed significant variation in several key nutritional traits. Fresh weight ranged from 7.34 g to 61.94 g, with SCP27 exhibiting the highest value, which is crucial for the quality of fruit and consumer preference. Dry weight was in the range from 0.22 g to 1.25 g, with SCP27 showing the highest dry weight, indicating better resource accumulation for fruit development. Moisture content ranged from 93.79% to 97.98%, with SCP12 and SCP27 having the highest moisture retention. Carbohydrate content varied between 85.65 mg/100 g and 309.46 mg/100 g, with SCP41 having the highest carbohydrate content.

Total soluble solids (TSS) ranged from 2.43 °B to 5.67 °B, with SCP01 exhibiting the highest TSS, indicating superior sweetness. Protein content ranged from 1.61 µg/ml to 16.73 µg/ml, with SCP60 showing the highest protein level, while Vitamin C was found in the range of 0.5 mg/100 g to 21.71 mg/100 g, with SCP76 having the highest Vitamin C content, highlighting its potential as a nutrient-dense genotype. These findings demonstrate substantial genetic diversity in the biochemical constituents of *C. pedata*, offering opportunities for selection and improvement of desirable traits for nutritional and market value.

The elemental composition analysis of *C. pedata* genotypes revealed significant variation in the content of eight key elements, offering insights into their nutritional potential. Genotype SCP27 exhibits the highest value potassium and calcium ranging from 104.26 mg/kg to 940.09 mg/kg, and 1.02 mg/kg to 18.79 mg/kg, respectively. Magnesium from 8.66 mg/kg to 79.26 mg/kg, with SCP39 showing the highest concentration. Sodium content ranged from 0.88 mg/kg to 5.01 mg/kg, with SCP62 having the highest. Iron content ranged from 0.01 mg/kg to 2.83 mg/kg, with SCP60 having the highest level. SCP42 genotypes showed the highest manganese and copper levels, ranging from 0.12 mg/kg to 2.15 mg/kg, and 0.04 mg/kg to 1.05 mg/kg, respectively, while Zinc content varied from 0.12 mg/kg to 2.63 mg/kg, with SCP03 exhibiting the highest level. These variations reflect genetic differences influencing the uptake, transport, and accumulation of these essential elements, highlighting the potential genotypes with desirable nutrient profiles for dietary and agricultural applications.

# Conclusion

The study "Exploitation of genetic diversity of stuffing cucumber [*Cyclanthera pedata* (L.) Schrader] for horticultural traits through morpho-molecular screening" has furnished significant insights into the genetic diversity, nutritional potential, and crop adaptability of *C. pedata*. The findings emphasize the importance of utilizing superior genotypes to enhance productivity, market value, and nutritional benefits while addressing the needs of socio-economic and ecology of the farming community.

Farmers prioritize higher yield, ease of cultivation, and market suitability in *C. pedata* cultivation. Likewise, traits such as long vines for trellising systems, spineless, soft-textured fruits, mild taste, adaptation to local climatic conditions, and sustainable cultivation practices, are considered crucial. These preferences align with the socio-economic reality of farming households, where women predominantly manage to cultivate *C. pedata*, which highlights its significance in the farming community. Farmers' preferences offer foundational baseline information for breeding programs aimed at developing varieties improved with traits that cater to local needs and market demands.

The research marks a significant milestone as the first to employ SSR markers for diversity analysis in *C. pedata*. The findings reveal a significant level of genetic variability, with 21 out of 24 SSR markers being polymorphic and showing high PIC values (average 0.84). Markers like EC49 and LSR108 as particularly highly informative markers due to their high PIC values. The pioneering use of SSR markers in this study lays the basis for future molecular research on this underutilized crop.

Beyond being a culinary component, *C. pedata* offers medicinal benefits, supported by its biochemical profile. High vitamin C content, proteins, and essential minerals like magnesium and potassium underscore its nutritional and therapeutic potential. Its cultivation thus aligns with both food security and health improvement goals.

Furthermore, genotypes with traits, such as SCP39 and SCP40, demonstrated optimal fruit size, fruit yield, and reproductive potency combinations, which play a crucial role for improving yield and productivity. For instance, the number of fruits per plant ranged from 47.1 (SCP78) to 414.7 (SCP36), with an average of 177.7, further underscoring the diversity within the population.

By examining genetic, horticultural, and nutritional attributes this study has contributed to a deeper understanding of the genetic architecture and potential of *C. pedata*. *C. pedata* stands out as a climate-resilient species within the cucurbit family. Its ability to remain productive despite changing environmental conditions highlights its potential as a sustainable crop. Observations such as green vines and continued yield under stress conditions reinforce its role as a critical genetic material for developing climate-adaptive cultivars. *C. pedata* is an exclusive crop for the September-October period, making it a unique addition to cropping calendars. It can be seamlessly integrated into relay cropping systems, optimizing land use and enhancing farm incomes. Its suitability for such practices further demonstrates its versatility in diverse farming systems.

The findings presented herein lead to the following recommendations. Firstly, targeted breeding programs focusing on traits like yield, nutrient content, and spineless fruits should be prioritized to enhance the crop’s market and nutritional value. Secondly, awareness campaigns and support for farmers cultivating *C. pedata* can be implemented to promote its cultivation and conservation. By adopting these recommendations, farmers, researchers, and policymakers can benefit from the increased utilization and value of this underexploited crop.

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# Annexure

**Annexure I: Questionnaire “Farmers' perception of desirable traits of *Clyanthera pedata*”**

**Geographical information of Village:**

1. Name of village: ________________________
2. GPS location: Latitude: ________ Longitude: ________ Altitude: ___________.

**Basic Information of Farmers:**

1. Name of the farmer:
2. Age:
3. Sex: M/F
4. Duration of Crop growing:
5. Time since they are growing the crop:

**Perception of farmers on the crop's desirable traits:**

| Sl. No. | Characteristics | Category | Response |
|---|---|---|---|
| 1. | Main vine length | Short (less than 3 m)/Medium (3-7 m)/Long (more than 7m) | |
| 2. | Number of primary branches | Least (less than 10)/Moderate (10-20)/Highest (more than 20) | |
| 3. | Internodal length | Short (less than 5cm)/Medium (5-8 cm)/Long (more than 8 cm) | |
| 4. | Fruit length | Short (5-10 cm)/Medium (10-15 cm)/Long (15-20 cm) | |
| 5. | No. of fruit per plant | Least (70-90)/Moderate (90-100)/ Highest (150) | |
| 6. | Fruit diameter | Thin/medium/thick | |
| 7. | Fruit spines | Least/Many/Least | |
| 8. | Fruit taste | Bitter/mild/sweet | |
| 9. | No. of seed per fruit | Least (5-10)/Medium (15-25)/ Highest (25-30) | |
| 10. | Whether seediness/seedless | Yes/no | |
| 11. | Harvesting Time | Early (85-90)/mid (100-120)/late (130-160) | |
| 12. | Stem shape | Angular/Rounded | |
| 13. | Flesh texture | Soft/medium/hard | |
| 14. | Skin thickness | Thin/medium/thick | |
| 15. | Seed weight | Brown/Black | |
| 16. | Flower color | Green/ White/ Yellow | |
| 17. | Fruit color | White/creamy white/ light green/green | |
| 18. | Average fruit weight | 8 g/15 g/ 25 g | |

**Annexure II: Respondent (Farmers) Information**

| Sl. No. | Respondent name | Village name | District |
|---|---|---|---|
| 1. | Suraj Subba | Yakten | Pakyong |
| 2. | Laxuman Subba | Yakten | Pakyong |
| 3. | Phul Maya Limboo | Yakten | Pakyong |
| 4. | Sangay Sherpa | Yakten | Pakyong |
| 5. | Gyamtsho Sherpa | Yakten | Pakyong |
| 6. | Laxuman Gurung | Yakten | Pakyong |
| 7. | Suk Raj Subba | Yakten | Pakyong |
| 8. | Arun Subba | Yakten | Pakyong |
| 9. | Bishnu Prasad Chettri | North Kartok | Pakyong |
| 10. | Harka Bdr. Gurung | North Kartok | Pakyong |
| 11. | Ramesh Gurung | North Kartok | Pakyong |
| 12. | Suk Dhoj Subba | Kartok | Pakyong |
| 13. | Garja Man Subba | Kartok | Pakyong |
| 14. | Ganga Ram Rai | Gaucharan | Pakyong |
| 15. | Khush Narayan Rai | Gaucharan | Pakyong |
| 16. | Easter Lepcha | Gaucharan | Pakyong |
| 17. | Arun Chettri | Namcheypong | Pakyong |
| 18. | Chandra kala Sharma | Pacheykhanu | Pakyong |
| 19. | Bidur Rai | Lower Pachak | Pakyong |
| 20. | Bhakta Bdr. Rai | Pachak | Pakyong |
| 21. | Arpan Chettri | Singtam | Pakyong |
| 22. | Mangal Singh Shankar | Bikmat | Namchi |
| 23. | Dal Bahadur Gurung | Bikmat | Namchi |
| 24. | Kalpana Rai | Bikmat | Namchi |
| 25. | Monu Subba | Bikmat | Namchi |
| 26. | Sujan Limboo | Bikmat | Namchi |
| 27. | Bubbha L Chettri | Bikmat | Namchi |
| 28. | Ratna Limboo | Bikmat | Namchi |
| 29. | Santi Limboo | Bikmat | Namchi |
| 30. | Pirthiman Rai | Bikmat | Namchi |
| 31. | Kali Maya Sharki | Khayerbotay | Namchi |
| 32. | Som Maya Subba | Khayerbotay | Namchi |
| 33. | Prem Kumari Pradhan | Namthang | Namchi |
| 34. | Shanti Rai | Namthang | Namchi |
| 35. | Prakash Chettri | Nagi | Namchi |
| 36. | Divya Chettri | Rateypani | Namchi |
| 37. | Man Maya Tamang | Kolbung | Namchi |
| 38. | Garjaman Lal Chettri | Upper Syari | Gangtok |
| 39. | Puran Chettri | Upper Syari | Gangtok |
| 40. | Prabha Sarma | Namli | Gangtok |
| 41. | Kamal Subba | Namli | Gangtok |
| 42. | Bikash Chettri | Luing | Gangtok |
| 43. | Basant Chettri | Luing | Gangtok |
| 44. | Dhan kumari Sharma | Luing | Gangtok |
| 45. | Birman Gurung | Luing | Gangtok |
| 46. | Bek Raj Thapa | Nandok | Gangtok |

| | | | |
|---|---|---|---|
| 47. | Binod Sharma | Nandok | Gangtok |
| 48. | Umesh Chettri | Nandok | Gangtok |
| 49. | Tilak Bahadur Chettri | Nandok | Gangtok |
| 50. | Sanchaman Limboo | Nandok | Gangtok |
| 51. | Karma Bhutia | Tashiding | Gyalshing |
| 52. | Pintso Lepcha | Tashiding | Gyalshing |
| 53. | Pintso Bhutia | Tashiding | Gyalshing |
| 54. | Amit Gurung | Tashiding | Gyalshing |
| 55. | Arun Subba | Tashiding | Gyalshing |
| 56. | Anita Limboo | Tashiding | Gyalshing |
| 57. | Ash Kumari Gurung | Tashiding | Gyalshing |
| 58. | Susmita Pradhan | Upper Langang | Gyalshing |
| 59. | Sashi kala Rai | Upper Langang | Gyalshing |
| 60. | Sumitra Devi Chettri | Upper Langang | Gyalshing |
| 61. | Jan maya Rai | Upper Langang | Gyalshing |
| 62. | Tshering Bhutia | Upper Langang | Gyalshing |
| 63. | Namgyal Dorjee Bhutia | Upper Langang | Gyalshing |
| 64. | Zongmu Bhutia | Upper Langang | Gyalshing |
| 65. | Sancha Rani Subba | Ogeng | Soreng |
| 66. | Anuman Subba | Ogeng | Soreng |
| 67. | Dhan Maya Subba | Ogeng | Soreng |
| 68. | Kumari Limboo | Ogeng | Soreng |
| 69. | Man Maya Sherpa | Ogeng | Soreng |
| 70. | Ashish Subba | Upper Ogeng | Soreng |
| 71. | Phip Raj Limboo | Choktay Gaon | Soreng |
| 72. | Mangal Singh Subba | Upper Tambong | Soreng |
| 73. | Chandra Singh Limboo | Lower Tambong | Soreng |
| 74. | Birman Subba | Lower Tambong | Soreng |
| 75. | Chandra Subba | Upper Fambong | Soreng |
| 76. | Sumila Nembang | Lower Fambong | Soreng |
| 77. | Buddha Hangma Limboo | Lower Fambong | Soreng |
| 78. | Jit Bdr Pradhan | Daramdin | Soreng |
| 79. | Laykheka Subba | Daramdin | Soreng |
| 80. | Gun Maya Subba | Ambotay | Soreng |
| 81. | Kul Bdr. Limboo | Ambotay | Soreng |
| 82. | Som Kumar Subba | Ambotay | Soreng |
| 83. | Ruk Bdr. Gurung | Upper Singling | Soreng |
| 84. | Til Maya Biswakarma | Upper Singling | Soreng |
| 85. | Dike Lepcha | Lower lingdong | Mangan |
| 86. | Tshering Gyatso Lepcha | Upper lingdong | Mangan |
| 87. | Lhamu Lepcha | Upper burfok | Mangan |
| 88. | Gyatso Tshering Lepcha | Lower burfok | Mangan |
| 89. | Laki Pem Lepcha | Nom | Mangan |
| 90. | Sonam Lepcha | Ponong | Mangan |
| 91. | Phur Diki Lepcha | Khulas | Mangan |
| 92. | Pema lhamu Lepcha | Manzing | Mangan |
| 93. | Passang Tshering Lepcha | Gyathang | Mangan |
| 94. | Ongmu Lepcha | Gyathang | Mangan |

| | | | |
|---|---|---|---|
| 95. | Wangdi Lepcha | Lower Dzongu | Mangan |
| 96. | Karma Gyatso Lepcha | Upper Dzongu | Mangan |
| 97. | Phur Diki Lepcha | Upper Dzongu | Mangan |
| 98. | Pintso Lepcha | Sangdong | Mangan |
| 99. | Zampo Bhutua | Bichu | Mangan |
| 100. | Diki Lepcha | Bichu | Mangan |

**Annexure III: Coordinated details of site of collection of *C. pedata* genotypes**

| Sl. no | Place name | N | E | MSL |
|---|---|---|---|---|
| 1 | Ambotay | 27°08’22” | 88°09’32” | 1211 |
| 2 | Assam linzey | 27°17’07” | 88°37’53” | 1289 |
| 3 | Beechu | 27°39’40” | 88°43’48” | 2466 |
| 4 | Bikmat | 27°07’48” | 88°26’50” | 712 |
| 5 | Sumbuk | 27°06’16” | 88°22’48” | 675 |
| 6 | Daramdin | 27°08’17” | 88°09’58” | 1017 |
| 7 | Dikling | 27°13’14” | 88°35’25” | 1417 |
| 8 | Gaucharan | 27°17’04” | 88°38’40” | 1402 |
| 9 | Gyathang | 27°28’21” | 88°30’33” | 1172 |
| 10 | Kartok | 27°14’08” | 88°36’19” | 1276 |
| 11 | Kayerbotay | 27°10’41” | 88°23’30” | 1843 |
| 12 | Kolbung | 27°12’34” | 88°18’28” | 1132 |
| 13 | Lingthem | 27°31’34” | 88°30’14” | 1336 |
| 14 | Lower Barfok | 27°29’45” | 88°30’17” | 1496 |
| 15 | Lower fambong | 27°07’37” | 88°09’33” | 1062 |
| 16 | Lower lingdong | 27°30’29” | 88°31’22” | 891 |
| 17 | Namcheybong | 27°15’19” | 88°35’13” | 1194 |
| 18 | Namthang | 27°09’46” | 88°28’53” | 1221 |
| 19 | North kartok | 27°14’05” | 88°36’20” | 1242 |
| 20 | Ogeng | 27°10’39” | 88°11’46 | 1791 |
| 21 | Pachak | 27°13’11” | 88°34’01” | 1290 |
| 22 | Palitam | 27°10’00” | 88°28’41” | 1418 |
| 23 | Passingdang | 27°31’38” | 88°30’53” | 917 |
| 24 | Singtam | 27°08’09” | 88°23’42” | 1830 |
| 25 | Sombaria | 27°08’27” | 88°08’47” | 1579 |
| 26 | Timburbong | 27°09’39” | 88°10’55” | 1517 |
| 27 | Upper dzongu | 27°30’39” | 88°26’17” | 2738 |
| 28 | Upper singling | 27°10’29” | 88°12’12” | 1664 |
| 29 | Upper langang | 27°17’38” | 88°14’07” | 1646 |
| 30 | Tashiding | 27°18’36” | 88°17’25” | 1159 |

## Annexure IV: Metrological Data

### Overall Metrological data of Sikkim for Year 2020

| Year-2020 | | | | | |
|---|---|---|---|---|---|
| | | Temperature ($^0$C) | | Relative Humidity | |
| Months | Rainfall(mm) | Maximum | Minimum | Maximum | Minimum |
| January | 44.80 | 25.00 | 8.50 | 100.00 | 47.00 |
| February | 3.70 | 28.10 | 9.20 | 99.00 | 43.00 |
| March | 3.60 | 33.10 | 14.30 | 92.00 | 23.00 |
| April | 172.50 | 34.10 | 13.50 | 95.00 | 21.00 |
| May | 278.40 | 33.10 | 14.50 | 99.00 | 50.00 |
| June | 310.20 | 31.90 | 17.50 | 100.00 | 67.00 |
| July | 391.20 | 31.10 | 17.50 | 100.00 | 69.00 |
| August | 360.50 | 31.20 | 21.00 | 100.00 | 73.00 |
| September | 239.70 | 33.10 | 15.50 | 99.00 | 63.00 |
| October | 424.90 | 31.60 | 17.00 | 100.00 | 73.00 |
| November | 66.60 | 28.70 | 11.10 | 100.00 | 38.00 |
| December | 0.00 | 26.80 | 10.00 | 100.00 | 51.00 |

### Overall Metrological data of Sikkim for Year 2021

| Year-2021 | | | | | |
|---|---|---|---|---|---|
| | | Temperature ($^0$C) | | Relative Humidity | |
| Months | Rainfall(mm) | Maximum | Minimum | Maximum | Minimum |
| January | 5.40 | 25.80 | 25.80 | 97.00 | 42.00 |
| February | 0.00 | 28.20 | 28.20 | 93.00 | 32.00 |
| March | 74.30 | 33.40 | 33.40 | 90.00 | 21.00 |
| April | 61.40 | 34.50 | 34.50 | 97.00 | 17.00 |
| May | 176.60 | 32.90 | 32.90 | 95.00 | 51.00 |
| June | 367.60 | 30.80 | 30.80 | 97.00 | 67.00 |
| July | 491.50 | 32.60 | 32.60 | 100.00 | 75.00 |
| August | 450.40 | 30.70 | 30.70 | 99.00 | 72.00 |
| September | 332.30 | 32.40 | 32.40 | 99.00 | 59.00 |
| October | 107.90 | 32.60 | 32.60 | 99.00 | 63.00 |
| November | 4.70 | 28.90 | 28.90 | 99.00 | 39.00 |
| December | 0.00 | 24.10 | 7.40 | 92.00 | 34.00 |

**Annexure V: ANOVA Table for pooled data for Morphological characterization**

| | | df | | | Sum of squares | | |
|---|---|---|---|---|---|---|---|
| **Sl. No.** | **Characters** | **Replication + Environment + Interaction** | **Genotypes** | **Error** | **Treatment** | **Replication** | **Error** |
| 1 | Vine length | 9 | 50 | 450 | 1195.28*** | 196.62 | 3085.41 |
| 2 | No. of primary branches | 9 | 50 | 450 | 3145.89*** | 51.69 | 2477.91 |
| 3 | Internodal length | 9 | 50 | 450 | 646.92*** | 3263.19 | 1979.10 |
| 4 | Days taken to 50% flowering | 9 | 50 | 450 | 180870.04 | 17894.94 | 1338187.26 |
| 5 | Node to first staminate flower | 9 | 50 | 450 | 863.87*** | 1206.99 | 1971.50 |
| 6. | Node to first pistillate flower | 9 | 50 | 450 | 1046.27*** | 1448.12 | 1842.98 |
| 7 | Peduncle length | 9 | 50 | 450 | 10054.57*** | 38.77 | 5474.16 |
| 8. | Fruit length | 9 | 50 | 450 | 344885.39*** | 13485.87 | 784618.42 |
| 9. | Fruit girth | 9 | 50 | 450 | 32901.71*** | 2398.75 | 8151.16 |
| 10 | No. of seeds per fruit | 9 | 50 | 450 | 862.29*** | 90.041 | 519.67 |
| 11 | 100 seed weight | 9 | 50 | 450 | 1029.36*** | 15.62 | 272.69 |
| 12 | Average fruit weight | 9 | 50 | 450 | 73206.25*** | 1809.71 | 15457.51 |
| 13 | No. of fruit per plant | 9 | 50 | 450 | 5590893.80*** | 36290.47 | 3247416.63 |
| 14 | Petiole length | 9 | 50 | 450 | 870.73*** | 238.26 | 1729.22 |
| 15 | Yield/plant | 9 | 50 | 450 | 1669727707.30*** | 73798624.69 | 1662871182.04 |

**Annexure VI: Morphological characterization data**

**First year mean table for morphological characterization of *C.pedata***

| Genotypes | VL (m) | NPB | IL (cm) | D50%F | NFSF | NFPF | PL (cm) | FL (cm) | FG (cm) | NSPF | 100SW (g) | AFW (g) | NFPP | PtL (cm) | Y/P (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP01** | 4.1 | 12.4 | 13.3 | 74.6 | 6.0 | 5.2 | 0.81 | 5.13 | 1.99 | 7.6 | 6.7 | 7.6 | 157.0 | 4.0 | 1.16 |
| **SCP02** | 7.0 | 12.6 | 12.6 | 81.2 | 6.4 | 8.0 | 1.48 | 9.64 | 3.56 | 11.0 | 7.8 | 19.9 | 81.8 | 6.9 | 1.68 |
| **SCP03** | 5.1 | 9.8 | 10.5 | 99.6 | 6.8 | 6.8 | 1.00 | 5.62 | 2.53 | 7.2 | 6.5 | 7.9 | 178.6 | 5.5 | 1.43 |
| **SCP04** | 6.9 | 13.2 | 11.3 | 110.6 | 7.2 | 7.4 | 0.88 | 7.03 | 2.71 | 11.2 | 4.6 | 13.0 | 211.0 | 7.5 | 4.80 |
| **SCP05** | 8.3 | 14.0 | 11.6 | 106.8 | 9.0 | 9.6 | 2.19 | 11.73 | 3.94 | 12.6 | 8.0 | 37.3 | 69.0 | 9.4 | 2.58 |
| **SCP06** | 6.4 | 13.2 | 8.8 | 116.4 | 6.0 | 6.0 | 1.01 | 6.36 | 2.23 | 8.3 | 5.6 | 8.5 | 167.0 | 5.1 | 1.40 |
| **SCP07** | 6.2 | 13.0 | 13.0 | 91.0 | 5.6 | 5.2 | 0.94 | 6.41 | 2.54 | 8.9 | 6.0 | 10.4 | 172.6 | 10.2 | 1.79 |
| **SCP08** | 7.3 | 8.8 | 10.4 | 121.6 | 11.0 | 11.0 | 1.65 | 7.76 | 2.69 | 10.9 | 7.8 | 12.8 | 128.8 | 5.8 | 1.65 |
| **SCP09** | 8.9 | 10.0 | 11.1 | 113.6 | 7.2 | 7.4 | 1.07 | 7.64 | 2.38 | 9.7 | 7.0 | 14.2 | 133.6 | 7.5 | 1.96 |
| **SCP10** | 7.6 | 17.2 | 12.9 | 110.2 | 5.2 | 4.6 | 1.77 | 9.59 | 3.12 | 10.3 | 7.1 | 18.0 | 98.8 | 6.6 | 1.76 |
| **SCP11** | 8.1 | 17.4 | 11.9 | 109.6 | 9.0 | 9.0 | 1.60 | 10.41 | 3.56 | 11.2 | 9.4 | 33.9 | 76.2 | 9.6 | 2.16 |
| **SCP12** | 7.8 | 14.0 | 15.2 | 107.2 | 7.6 | 6.8 | 1.77 | 14.52 | 3.50 | 9.8 | 8.1 | 31.4 | 97.2 | 10.3 | 3.03 |
| **SCP13** | 7.9 | 18.0 | 14.9 | 90.2 | 6.4 | 6.4 | 1.38 | 7.39 | 2.92 | 10.2 | 6.1 | 13.0 | 293.8 | 11.1 | 3.90 |
| **SCP14** | 8.5 | 11.2 | 10.8 | 126.6 | 5.8 | 5.8 | 1.87 | 12.14 | 4.59 | 11.3 | 6.8 | 34.8 | 71.2 | 8.4 | 2.47 |
| **SCP15** | 7.4 | 11.2 | 14.7 | 97.6 | 6.2 | 6.4 | 0.89 | 5.56 | 2.54 | 8.7 | 6.4 | 9.4 | 147.0 | 6.5 | 1.36 |
| **SCP16** | 7.2 | 12.4 | 12.3 | 95.0 | 5.6 | 5.4 | 1.22 | 7.21 | 2.67 | 9.2 | 7.4 | 14.4 | 167.2 | 8.1 | 2.57 |
| **SCP26** | 8.6 | 15.4 | 12.5 | 87.4 | 7.2 | 6.2 | 1.20 | 7.14 | 2.82 | 8.8 | 6.4 | 15.6 | 322.0 | 8.3 | 5.05 |
| **SCP27** | 10.9 | 16.6 | 14.1 | 100.2 | 15.8 | 16.0 | 1.12 | 9.67 | 3.84 | 12.7 | 9.9 | 46.2 | 54.4 | 8.7 | 2.44 |
| **SCP28** | 7.5 | 14.0 | 13.3 | 71.6 | 6.8 | 6.2 | 1.22 | 7.06 | 2.71 | 9.9 | 6.1 | 12.4 | 191.4 | 8.4 | 2.36 |
| **SCP29** | 9.0 | 15.4 | 15.9 | 87.6 | 7.4 | 7.8 | 1.44 | 7.27 | 3.02 | 10.1 | 6.6 | 18.9 | 382.8 | 9.8 | 7.18 |
| **SCP30** | 10.0 | 13.4 | 15.7 | 96.6 | 7.2 | 8.4 | 2.24 | 9.59 | 3.20 | 10.7 | 5.5 | 15.8 | 467.8 | 11.1 | 7.37 |
| **SCP31** | 7.6 | 13.2 | 14.0 | 78.4 | 7.0 | 6.0 | 1.54 | 7.57 | 2.92 | 9.7 | 5.6 | 12.9 | 522.6 | 8.3 | 6.75 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP32** | 5.2 | 10.6 | 13.7 | 91.4 | 5.8 | 5.0 | 0.85 | 6.38 | 2.47 | 8.8 | 5.5 | 10.3 | 133.8 | 6.5 | 1.37 |
| **SCP33** | 9.2 | 16.4 | 16.5 | 87.6 | 6.0 | 6.0 | 3.48 | 10.08 | 1.87 | 10.3 | 7.5 | 26.4 | 166.0 | 13.0 | 4.38 |
| **SCP34** | 6.4 | 15.8 | 14.8 | 109.4 | 6.6 | 6.4 | 0.94 | 6.67 | 2.64 | 9.5 | 5.7 | 13.9 | 181.2 | 8.4 | 2.59 |
| **SCP35** | 5.2 | 15.4 | 10.4 | 83.2 | 7.0 | 6.6 | 0.94 | 5.83 | 2.54 | 8.0 | 5.0 | 10.2 | 263.8 | 6.0 | 2.72 |
| **SCP36** | 7.1 | 20.2 | 14.3 | 108.8 | 7.0 | 7.0 | 1.16 | 7.29 | 2.87 | 9.0 | 5.1 | 16.8 | 523.6 | 9.7 | 9.16 |
| **SCP37** | 5.7 | 19.4 | 13.2 | 91.0 | 6.2 | 5.8 | 1.06 | 7.71 | 3.31 | 9.8 | 7.1 | 14.8 | 549.0 | 9.4 | 8.14 |
| **SCP38** | 6.4 | 17.8 | 13.0 | 74.2 | 7.4 | 7.2 | 1.29 | 6.73 | 2.51 | 7.6 | 6.3 | 11.3 | 390.0 | 8.6 | 4.46 |
| **SCP39** | 7.4 | 19.0 | 15.9 | 98.0 | 7.8 | 7.8 | 1.45 | 8.40 | 3.27 | 11.1 | 7.4 | 20.4 | 496.8 | 11.6 | 10.11 |
| **SCP40** | 6.2 | 16.6 | 14.3 | 115.0 | 6.0 | 6.0 | 1.60 | 8.18 | 3.33 | 9.0 | 7.8 | 28.2 | 166.0 | 12.7 | 4.79 |
| **SCP41** | 7.2 | 13.2 | 13.9 | 117.8 | 6.2 | 6.0 | 1.93 | 10.02 | 3.22 | 10.2 | 8.2 | 16.1 | 86.6 | 12.4 | 1.41 |
| **SCP42** | 6.9 | 15.0 | 12.1 | 114.2 | 10.4 | 11.0 | 1.80 | 10.49 | 3.61 | 10.8 | 8.4 | 26.4 | 119.6 | 8.3 | 3.13 |
| **SCP52** | 6.0 | 12.2 | 12.2 | 113.8 | 7.4 | 7.8 | 1.29 | 10.06 | 3.94 | 11.4 | 7.3 | 26.8 | 62.6 | 6.5 | 1.67 |
| **SCP53** | 7.2 | 16.8 | 13.5 | 97.8 | 5.8 | 5.6 | 1.25 | 7.05 | 3.11 | 11.4 | 5.3 | 14.4 | 80.6 | 8.6 | 1.16 |
| **SCP54** | 9.1 | 15.0 | 13.2 | 95.4 | 8.8 | 8.8 | 1.23 | 7.09 | 3.00 | 8.8 | 6.6 | 13.6 | 167.4 | 7.2 | 2.28 |
| **SCP55** | 6.0 | 14.2 | 11.8 | 93.0 | 7.0 | 6.4 | 1.23 | 6.35 | 2.36 | 9.4 | 5.6 | 8.4 | 109.4 | 7.7 | 0.92 |
| **SCP56** | 7.1 | 12.8 | 13.3 | 129.6 | 9.0 | 8.0 | 2.24 | 10.72 | 4.45 | 11.5 | 8.8 | 34.6 | 65.2 | 12.2 | 2.26 |
| **SCP57** | 7.1 | 13.8 | 12.5 | 105.0 | 8.2 | 8.2 | 1.28 | 7.02 | 2.75 | 9.4 | 5.1 | 8.8 | 91.4 | 6.7 | 0.80 |
| **SCP58** | 7.7 | 14.4 | 12.7 | 104.8 | 8.4 | 8.0 | 1.21 | 6.24 | 2.66 | 9.3 | 5.4 | 10.4 | 119.6 | 7.5 | 1.25 |
| **SCP59** | 7.4 | 17.8 | 9.5 | 124.0 | 13.0 | 12.8 | 1.65 | 8.93 | 3.74 | 12.4 | 9.9 | 24.6 | 60.2 | 7.3 | 1.49 |
| **SCP60** | 7.6 | 18.2 | 12.3 | 97.0 | 8.4 | 7.6 | 1.47 | 7.70 | 2.63 | 9.7 | 6.0 | 10.0 | 123.0 | 9.2 | 1.23 |
| **SCP61** | 6.0 | 16.4 | 13.6 | 108.8 | 8.2 | 7.4 | 1.10 | 5.68 | 2.33 | 7.9 | 5.8 | 8.3 | 443.0 | 8.5 | 3.71 |
| **SCP62** | 6.4 | 11.6 | 12.3 | 98.2 | 7.4 | 7.0 | 1.08 | 6.64 | 2.70 | 9.9 | 6.6 | 12.7 | 115.4 | 7.7 | 1.49 |
| **SCP72** | 5.5 | 11.0 | 11.4 | 92.6 | 6.2 | 6.0 | 1.53 | 8.88 | 2.30 | 7.6 | 6.0 | 18.9 | 45.2 | 6.2 | 0.85 |
| **SCP73** | 7.4 | 14.6 | 12.6 | 70.0 | 7.0 | 6.6 | 0.92 | 6.13 | 2.70 | 8.3 | 6.5 | 10.8 | 120.0 | 7.5 | 1.29 |
| **SCP74** | 8.2 | 13.2 | 15.2 | 113.6 | 11.4 | 11.4 | 2.15 | 10.89 | 4.70 | 11.2 | 10.3 | 49.5 | 49.8 | 10.7 | 2.45 |
| **SCP75** | 7.0 | 10.2 | 13.1 | 106.6 | 15.6 | 15.2 | 2.07 | 11.33 | 5.08 | 12.1 | 9.4 | 53.8 | 48.8 | 7.5 | 2.62 |
| **SCP76** | 6.8 | 14.2 | 12.7 | 119.0 | 12.8 | 12.0 | 1.88 | 10.29 | 4.49 | 11.6 | 9.9 | 45.9 | 47.2 | 7.7 | 2.19 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP77** | 8.7 | 12.4 | 15.1 | 113.0 | 11.0 | 11.4 | 1.69 | 10.10 | 4.27 | 11.7 | 10.2 | 44.5 | 72.8 | 11.1 | 3.16 |
| **SCP78** | 9.9 | 11.8 | 14.0 | 107.6 | 12.8 | 12.8 | 2.07 | 11.39 | 4.84 | 12.8 | 8.6 | 40.8 | 47.8 | 10.2 | 1.97 |
| **Mean** | 7.31 | 14.24 | 13.02 | 101.06 | 7.98 | 7.83 | 1.45 | 8.29 | 3.13 | 10.01 | 7.03 | 20.39 | 179.17 | 2.98 | 0.85 |
| **C.D. 5%** | 2.1 | 3.7 | 2.3 | 7.9 | 1.8 | 1.8 | 0.30 | 0.89 | 0.36 | 1.2 | 0.9 | 4.5 | 72.8 | 2.2 | 1.65 |
| **SE(m)** | 0.8 | 1.3 | 0.8 | 2.8 | 0.6 | 0.7 | 0.11 | 0.32 | 0.13 | 0.4 | 0.3 | 1.6 | 26.1 | 0.8 | 0.59 |
| **Range Lowest** | 4.1 | 8.8 | 8.8 | 71.6 | 5.2 | 5.0 | 0.81 | 5.13 | 1.87 | 7.2 | 4.6 | 7.6 | 45.2 | 4.0 | 0.85 |
| **Range Highest** | 10.9 | 20.2 | 16.5 | 129.6 | 15.8 | 16.0 | 3.48 | 14.52 | 5.08 | 12.8 | 10.3 | 49.5 | 549.0 | 13.0 | 10.11 |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.

**Second year mean table for morphological characterization of *C.pedata***

| Genotypes | VL (m) | NPB | IL (cm) | D50%F | NFSF | NFPF | PL (cm) | FL (cm) | FG (cm) | NSPF | 100SW (g) | AFW (g) | NFPP | PtL (cm) | Y/P (kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP01** | 3.9 | 12.2 | 12.2 | 96.0 | 6.8 | 6.8 | 1.06 | 6.88 | 2.28 | 8.1 | 5.9 | 10.2 | 200.2 | 5.9 | 2.02 |
| **SCP02** | 6.7 | 13.4 | 10.2 | 96.8 | 2.6 | 5.4 | 2.19 | 14.80 | 4.79 | 14.0 | 8.1 | 49.9 | 87.4 | 7.3 | 4.40 |
| **SCP03** | 4.7 | 11.2 | 11.8 | 99.6 | 4.4 | 5.4 | 1.13 | 5.89 | 2.76 | 8.1 | 7.3 | 12.6 | 189.4 | 9.0 | 2.41 |
| **SCP04** | 6.0 | 14.8 | 9.4 | 120.8 | 7.2 | 5.0 | 1.19 | 7.35 | 3.30 | 10.3 | 4.7 | 14.7 | 249.8 | 7.9 | 3.63 |
| **SCP05** | 8.6 | 11.8 | 7.5 | 111.6 | 6.8 | 6.2 | 2.22 | 11.79 | 4.32 | 11.9 | 8.3 | 54.6 | 128.4 | 8.1 | 6.97 |
| **SCP06** | 7.1 | 14.6 | 6.5 | 114.0 | 5.2 | 4.8 | 1.12 | 7.38 | 2.93 | 8.1 | 5.1 | 13.2 | 288.0 | 5.9 | 3.84 |
| **SCP07** | 16.2 | 13.2 | 11.7 | 99.4 | 4.2 | 4.2 | 1.21 | 6.66 | 2.81 | 11.3 | 6.8 | 12.5 | 236.8 | 6.8 | 3.01 |
| **SCP08** | 7.4 | 9.2 | 10.5 | 101.8 | 2.8 | 3.0 | 1.50 | 8.41 | 3.09 | 12.0 | 7.7 | 14.4 | 170.4 | 8.5 | 2.41 |
| **SCP09** | 9.9 | 10.8 | 8.6 | 101.0 | 5.0 | 4.0 | 1.55 | 11.72 | 3.30 | 10.2 | 7.8 | 18.5 | 176.4 | 9.0 | 3.30 |
| **SCP10** | 8.1 | 15.4 | 7.1 | 106.4 | 5.0 | 5.0 | 3.26 | 14.19 | 4.97 | 10.1 | 7.2 | 22.6 | 96.6 | 6.7 | 2.19 |
| **SCP11** | 8.5 | 16.6 | 7.6 | 105.2 | 4.0 | 3.2 | 2.08 | 14.28 | 5.58 | 10.1 | 10.4 | 41.6 | 129.0 | 6.7 | 5.21 |
| **SCP12** | 6.2 | 15.4 | 7.1 | 115.8 | 4.6 | 4.0 | 2.85 | 14.74 | 5.06 | 10.6 | 9.9 | 34.8 | 157.6 | 7.9 | 5.52 |
| **SCP13** | 8.4 | 15.4 | 8.6 | 110.2 | 5.0 | 4.6 | 1.15 | 8.15 | 3.30 | 12.1 | 6.3 | 18.6 | 348.6 | 6.7 | 6.43 |
| **SCP14** | 9.3 | 11.4 | 6.4 | 112.8 | 5.0 | 4.2 | 1.72 | 11.68 | 4.81 | 11.6 | 7.9 | 54.4 | 50.2 | 6.9 | 2.75 |
| **SCP15** | 8.7 | 13.4 | 9.5 | 101.2 | 5.2 | 5.6 | 1.48 | 6.78 | 3.38 | 10.0 | 5.6 | 19.4 | 121.2 | 6.5 | 2.33 |
| **SCP16** | 7.3 | 12.6 | 8.5 | 108.2 | 6.4 | 7.0 | 1.22 | 7.69 | 2.58 | 9.9 | 7.3 | 14.9 | 239.4 | 7.0 | 3.51 |
| **SCP26** | 7.1 | 19.0 | 7.0 | 116.0 | 5.8 | 4.2 | 0.96 | 7.29 | 2.95 | 9.6 | 5.8 | 16.9 | 35.6 | 5.1 | 0.58 |
| **SCP27** | 12.9 | 16.2 | 7.3 | 105.4 | 4.4 | 4.4 | 1.14 | 9.62 | 4.48 | 12.6 | 8.9 | 49.1 | 44.6 | 5.9 | 2.21 |
| **SCP28** | 9.1 | 15.6 | 5.8 | 106.8 | 4.0 | 2.8 | 0.83 | 5.80 | 2.38 | 9.9 | 5.0 | 11.0 | 117.0 | 5.6 | 1.34 |
| **SCP29** | 11.2 | 14.6 | 8.2 | 119.2 | 5.2 | 4.4 | 1.08 | 6.86 | 3.11 | 10.7 | 7.0 | 26.3 | 312.8 | 5.2 | 8.26 |
| **SCP30** | 9.1 | 13.6 | 7.8 | 107.2 | 5.8 | 4.6 | 1.29 | 7.50 | 2.68 | 11.4 | 6.7 | 24.3 | 246.6 | 7.0 | 6.54 |
| **SCP31** | 9.7 | 12.6 | 8.5 | 99.0 | 3.8 | 4.0 | 0.85 | 5.26 | 2.40 | 10.8 | 7.0 | 11.4 | 267.2 | 5.0 | 2.96 |
| **SCP32** | 5.8 | 11.4 | 7.9 | 101.8 | 6.0 | 5.0 | 0.92 | 7.56 | 2.53 | 9.7 | 6.6 | 16.0 | 301.6 | 7.1 | 4.80 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP33** | 10.8 | 17.0 | 6.1 | 106.2 | 4.4 | 4.0 | 1.31 | 9.03 | 3.35 | 10.3 | 7.8 | 27.8 | 183.6 | 5.8 | 4.96 |
| **SCP34** | 7.7 | 17.0 | 7.8 | 119.0 | 4.6 | 4.0 | 0.86 | 5.64 | 2.62 | 10.7 | 6.6 | 16.7 | 259.0 | 6.2 | 4.27 |
| **SCP35** | 7.6 | 17.0 | 6.1 | 118.6 | 5.6 | 3.4 | 1.03 | 5.96 | 2.74 | 11.0 | 6.5 | 14.1 | 301.0 | 6.6 | 4.36 |
| **SCP36** | 8.1 | 19.0 | 5.9 | 113.0 | 5.6 | 4.6 | 1.03 | 6.19 | 2.31 | 9.5 | 6.6 | 18.0 | 305.8 | 6.7 | 5.60 |
| **SCP37** | 5.4 | 18.6 | 6.5 | 128.4 | 6.0 | 4.8 | 1.20 | 6.98 | 2.99 | 10.5 | 7.7 | 15.6 | 171.4 | 7.2 | 2.68 |
| **SCP38** | 8.4 | 16.4 | 7.6 | 104.0 | 3.2 | 3.6 | 1.18 | 7.38 | 3.28 | 11.1 | 6.8 | 14.7 | 242.6 | 7.3 | 3.52 |
| **SCP39** | 8.5 | 18.4 | 8.8 | 103.2 | 4.8 | 4.2 | 1.12 | 6.98 | 3.30 | 13.3 | 7.8 | 21.4 | 312.6 | 8.0 | 6.71 |
| **SCP40** | 7.6 | 18.2 | 7.2 | 120.2 | 6.8 | 4.2 | 1.68 | 9.60 | 4.14 | 12.5 | 7.4 | 40.0 | 273.8 | 12.3 | 11.16 |
| **SCP41** | 7.9 | 15.4 | 6.4 | 114.6 | 5.8 | 3.8 | 3.37 | 12.37 | 4.21 | 11.9 | 8.8 | 42.8 | 175.6 | 9.5 | 7.56 |
| **SCP42** | 7.5 | 15.8 | 4.7 | 117.2 | 5.8 | 4.6 | 1.65 | 11.01 | 4.50 | 12.0 | 9.1 | 34.8 | 117.0 | 6.8 | 4.19 |
| **SCP52** | 7.7 | 13.4 | 6.6 | 102.6 | 3.6 | 3.4 | 1.34 | 8.51 | 3.45 | 12.0 | 8.4 | 25.1 | 112.0 | 7.5 | 2.84 |
| **SCP53** | 5.8 | 15.4 | 7.8 | 101.8 | 4.2 | 2.2 | 1.25 | 7.17 | 3.12 | 11.7 | 6.1 | 13.7 | 214.4 | 6.3 | 2.91 |
| **SCP54** | 10.9 | 16.2 | 8.6 | 105.2 | 3.8 | 2.6 | 1.36 | 7.79 | 3.25 | 9.4 | 5.9 | 23.7 | 157.4 | 7.0 | 3.70 |
| **SCP55** | 7.8 | 15.6 | 7.0 | 104.6 | 4.6 | 5.6 | 1.32 | 9.06 | 3.55 | 11.6 | 5.1 | 9.0 | 150.8 | 7.0 | 1.37 |
| **SCP56** | 9.2 | 15.4 | 7.6 | 106.4 | 4.8 | 5.6 | 1.46 | 10.36 | 4.13 | 11.5 | 8.3 | 33.4 | 177.6 | 7.3 | 5.95 |
| **SCP57** | 7.0 | 16.8 | 9.5 | 105.6 | 6.4 | 4.6 | 1.25 | 7.21 | 3.13 | 10.0 | 6.1 | 10.7 | 161.8 | 7.2 | 1.71 |
| **SCP58** | 10.4 | 16.0 | 8.4 | 118.8 | 4.6 | 3.8 | 1.20 | 5.91 | 2.81 | 9.1 | 5.7 | 15.7 | 113.8 | 7.8 | 1.79 |
| **SCP59** | 8.6 | 17.0 | 6.6 | 109.4 | 5.6 | 4.2 | 1.39 | 7.79 | 4.23 | 10.8 | 9.3 | 32.3 | 76.6 | 6.8 | 2.46 |
| **SCP60** | 9.0 | 17.6 | 8.1 | 95.4 | 3.0 | 3.8 | 1.33 | 7.81 | 3.19 | 11.7 | 6.8 | 16.7 | 207.8 | 6.3 | 3.47 |
| **SCP61** | 7.4 | 18.2 | 9.0 | 88.4 | 4.2 | 3.2 | 1.44 | 8.97 | 3.91 | 11.1 | 5.3 | 9.0 | 355.4 | 5.8 | 3.19 |
| **SCP62** | 8.7 | 11.6 | 7.3 | 103.0 | 3.0 | 1.6 | 1.01 | 5.64 | 2.48 | 10.1 | 5.9 | 11.5 | 251.6 | 6.0 | 2.91 |
| **SCP72** | 5.9 | 12.0 | 8.5 | 123.8 | 5.0 | 5.8 | 1.10 | 7.47 | 2.28 | 7.0 | 5.6 | 19.5 | 58.4 | 6.1 | 1.14 |
| **SCP73** | 8.2 | 15.4 | 7.9 | 109.0 | 4.8 | 4.8 | 1.24 | 8.32 | 3.23 | 9.6 | 7.5 | 8.9 | 149.4 | 5.8 | 1.30 |
| **SCP74** | 9.2 | 15.4 | 8.0 | 114.8 | 4.8 | 5.6 | 2.10 | 12.20 | 5.39 | 11.3 | 11.3 | 53.0 | 49.4 | 7.4 | 2.67 |
| **SCP75** | 8.4 | 11.2 | 6.7 | 114.4 | 3.2 | 4.2 | 1.84 | 12.18 | 5.17 | 13.4 | 8.8 | 28.2 | 65.0 | 7.3 | 1.87 |
| **SCP76** | 6.3 | 15.8 | 8.4 | 112.2 | 7.0 | 6.2 | 1.88 | 11.92 | 4.62 | 10.0 | 8.7 | 31.5 | 59.0 | 7.3 | 1.82 |
| **SCP77** | 9.6 | 13.0 | 8.5 | 111.2 | 5.2 | 5.2 | 1.81 | 12.71 | 5.49 | 11.7 | 9.1 | 30.1 | 41.8 | 7.1 | 1.23 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SCP78** | 11.7 | 9.2 | 9.6 | 104.8 | 5.4 | 6.6 | 1.75 | 11.23 | 4.93 | 13.5 | 8.0 | 40.6 | 46.4 | 6.4 | 1.88 |
| **Mean** | 8.30 | 14.75 | 7.98 | 108.47 | 4.92 | 4.47 | 1.46 | 8.86 | 3.56 | 10.81 | 7.26 | 23.93 | 176.19 | 6.99 | 3.64 |
| **C.D. 5%** | 4.1 | 2.0 | 2.2 | 9.9 | 2.1 | 1.9 | 0.20 | 0.91 | 0.36 | 1.1 | 0.9 | 5.3 | 88.1 | 2.0 | 2.26 |
| **SE(m)** | 1.5 | 0.7 | 0.8 | 3.5 | 0.7 | 0.7 | 0.07 | 0.33 | 0.13 | 0.4 | 0.3 | 1.9 | 31.6 | 0.7 | 0.81 |
| **Range Lowest** | 3.9 | 9.2 | 4.7 | 95.4 | 2.6 | 1.6 | 0.85 | 5.26 | 2.28 | 7.0 | 4.7 | 8.9 | 44.6 | 5.0 | 0.58 |
| **Range Highest** | 16.2 | 19.0 | 12.2 | 128.4 | 7.2 | 7.0 | 3.37 | 14.80 | 5.58 | 14.0 | 11.3 | 54.4 | 355.4 | 12.3 | 11.16 |

Note: VL - Vine length; NPB - No. of primary branches, IL- Internodal length, D50%F - Days to 50% flowering, NFSF - Node no. to 1st staminate flower, NFPF - Node no. to 1st pistillate flower, PL - Peduncle length, FL - Fruit length, FG - Fruit girth, NSPF - No. of seed per fruit, 100SW - 100 seed weight, AFW - Average fruit weight, NFPP - No. of fruit per plant, PtL - Petiole length, Y/P - Yield/plant.