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## VARIABILITY IN SOUR ORANGE (*CITRUS AURANTIUM* L.) GENOTYPES: A STEP TOWARDS GERMPLASM UTILIZATION

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### ABSTRACT

*Citrus aurantium* Tanaka, a wild endangered and an endemic citrus species native to northeastern India. This endemic species was first collected from the Citrus Gene Sanctuary, located in the buffer zone of the Nokrek Biosphere Reserve in the Garo Hills of Meghalaya. An intensive survey was undertaken during the recent year about the distribution and morpho-phenological and biochemical variability of citrus in the state of Nagaland which is one of the sister states among the eight states of north east India viz. NAMMAST-E (Nagaland, Arunachal Pradesh, Meghalaya, Mizoram, Assam, Sikkim, Tripura of East). The species is unevenly distributed across the surveyed areas and found highly threatened due to lack of knowledge about the importance of this species among the local folks, rapid deforestation in the state and non-commercial areas but observed significant variability among the accessions. There is urgent need the safeguard and initiative for effective conservation strategies of these genetic resources to ensure its future availability to the further citrus crop improvement.

**Keywords :** Sour orange, *Citrus aurantium*, Nagaland, Variability

### Introduction

Sour orange *Citrus aurantium* L., is a significant wild citrus species native to North Eastern India, found in selected pocket in the region. It is believed to be the most primitive citrus species and possibly the ancestor of cultivated Citrus varieties (Singh, 1981). First identified by (Tanaka, 1928), it was later described as a wild citrus native to areas such as Nowgong district, Khasi Hills, and Manipur in Assam (Tanaka, 1937; Bhattacharya & Dutta, 1956). This species is endemic to the North Eastern Himalayas and has been observed in the Naga Hills of Nagaland, the Kaziranga Reserve Forest in Assam, and the Garo Hills of Meghalaya (Singh, 1981). However, surveys suggest its presence is currently limited to the Tura ranges of the Garo Hills in Meghalaya (Upadhyay & Sundriyal, 1998; Singh & Singh, 2003). This region, part of the Nokrek Biosphere Reserve's buffer zone, has been designated as the Citrus Gene Sanctuary (Singh, 1981).

Thirty *Citrus aurantium* genotypes comprising, strains were characterized by using morphological

characters in this study. National Bureau of Plant Genetic Resources (NBPGR) has been developed minimal descriptors for citrus which is used for the study of sixteen morphological characters of leaves, flowers, fruits and seeds as well as eight biochemical attributes of fruits. Sour orange *Citrus aurantium* belongs to the subfamily Aurantioideae of the Rutaceae family and is widespread in the tropical and subtropical areas. It is thought to have originated in Southeast Asia, particularly in areas that stretch from eastern part of Northeast India through the Malay Archipelago and north in China and Japan and southern Australia (Moore, 2001). Presently, it is reported to be endemic to the Tura range of Garo Hills of Meghalaya (Singh and Singh, 2003) and the Garo tribes used the fruits of *C. indica* for medicinal and religious purposes (Malik *et al.*, 2006). According to Rodriguez *et al.* (2009) and Elameen *et al.* (2010), morphological analysis continues to be considered as significant recently and is used as an initial stage in cultivar identification and diversity assessment. Although the development of numerous molecular techniques for analyzing genetic

diversity, Susandarini *et al.* (2013) stressed the practical significance of morphological features in plant systematics and horticultural plant species for the identification of cultivars. The recent study was conducted to evaluate the extent of diversity and relationships across citrus genotypes, which may offer helpful insights for enhancing citrus breeding initiatives going forward.

### Materials and Methods

An extensive exploration drive was carried out by the Department of Horticulture, Nagaland University, SAS, Medziphema campus Nagaland, funded by Department of Biotechnology, Government of India. The climate of Nagaland is hot and humid with hot summer and cool winters. The randomly selected plants and fruit samples collected from different districts (Kohima, Mokokchung, Niuland, Noklak, Tuensang and Wokha). Thirty genotypes of Sour

orange subjected to the present study were presented in Table 1.

Selected twenty-four qualitative and sixteen quantitative traits of leaves, flowers, fruit and seed and eight biochemical characters were studied based on descriptor developed by NBPGR on Citrus guidelines. Biochemical characteristics of fruit such as juice content (%) was measured as volume (ml) per weight (g) basis and converted to percentage, total soluble solid (°B) was measured by hand refractometer (Erma INC.), titratable acidity (%) by Ranganna (1979), ascorbic acid (mg/100g) described by Freed (1966), TSS: Acid ratio, total sugar was determined calorimetrically by the anthrone method, reducing sugar determined by Dinitrosalicylic acid method (Miller, 1972) and non-reducing sugar (%) by Ranganna (1986).

**Table 1:** Different *Citrus aurantium* genotypes identified after survey.

Collector's No.	Vernacular name	Date of collection	District	Latitude (°N)	Longitude (°E)	Altitude (m)	Annual mean temp o(C)	Annual mean rainfall (mm)
CL/I/21/01	Shillong Naring	24-11-2021	Tuensang	26.28510	94.61677	1270.01	14.80	1186.3
CL/I/21/02	Shillong Naring	24-11-2021	Tuensang	26.28507	94.61678	1265.09	14.80	1186.3
CL/I/22/03	Thera konken	05-01-2022	Wokha	26.04240	94.03940	920	17.59	1894.9
CL/I/22/04	Naring	07-01-2022	Mokokchung	26.31666	94.53333	1120	17.68	1997.7
CL/I/22/05	Naring	09-01-2022	Tuensang	26.28507	94.83415	1371	14.80	1186.3
CL/I/22/06	Shillong Naring	09-01-2022	Tuensang	26.24208	94.61298	1337	14.80	1186.3
CL/I/22/07	Chemphen	09-01-2022	Tuensang	26.28507	94.61678	1372	14.80	1186.3
CL/I/22/08	Makanyiu	10-01-2022	Noklak	26.21569	95.02556	1372	14.80	1186.3
CL/I/22/09	Jemben (Haijebou)	11-01-2022	Tuensang	26.35776	94.88168	1372	14.80	1186.3
CL/I/22/10	Jemben (Haijebou)	11-01-2022	Tuensang	26.35776	94.88168	1372	14.80	1186.3
CL/I/22/11	Jemben (Haijebou)	11-01-2022	Tuensang	26.35761	94.88180	1372	14.80	1186.3
CL/I/22/12	Jemben (Haijebou)	11-01-2022	Tuensang	26.35777	94.88168	1372	14.80	1186.3
CL/I/22/13	Jemben (Haijebou)	11-01-2022	Tuensang	94.88177	26.35776	1372	14.80	1186.3
CL/I/22/14	Jemben (Haijebou)	11-01-2022	Tuensang	26.35772	94.88180	1372	14.80	1186.3
CL/I/22/15	Chufu	13-01-2022	Kohima	25.67312	94.03620	1671	13.06	1695
CL/I/22/16	Naring (Tasola)	15-01-2022	Mokokchung	26.37345	94.42923	938	17.68	1997.7
CL/I/22/17	Naring (Tasola)	15-01-2022	Mokokchung	26.37345	94.42923	938	17.68	1997.7
CL/I/22/18	Naring (Tasola)	16-01-2022	Mokokchung	26.29849	94.50132	1120	17.68	1997.7
CL/I/22/19	Shillong Naring	17-01-2022	Tuensang	26.19933	94.77469	1371	14.80	1186.3
CL/I/22/20	Naring	19-01-2022	Mokokchung	26.32204	94.51347	1325	17.68	1997.7
CL/I/22/21	Naring	19-01-2022	Mokokchung	26.33333	94.53458	1325	17.68	1997.7
CL/I/22/22	Naring	20-01-2022	Niuland	25.88578	93.92182	714	25.38	931.2
CL/I/22/23	Shillong Naring	22-01-2022	Tuensang	26.23574	94.81319	1645	14.80	1186.3
CL/I/22/24	Shillong Naring	22-01-2022	Tuensang	26.23572	94.8131	1645	14.80	1186.3
CL/I/22/25	Shillong Naring	22-01-2022	Tuensang	26.28507	94.61678	1264	14.80	1186.3
CL/I/22/26	Fumuo	25-01-2022	Kohima	25.72302	94.03663	1297	13.06	1695
CL/I/22/27	Naring	01-02-2022	Mokokchung	26.33221	94.53458	1325	17.68	1997.7
CL/I/22/28	Naring (Tasola)	03-02-2022	Mokokchung	26.38107	94.43814	1150	17.68	1997.7
CL/I/22/29	Naring (Tasola)	03-02-2022	Mokokchung	26.38108	94.43815	1157	17.68	1997.7
CL/I/22/30	Naring	04-02-2022	Mokokchung	26.24571	94.28157	925.3	17.68	1997.7

### Results and Discussion

#### Qualitative attributes

The data in Table 1 revealed that mostly collected accessions are landraces and occasionally or

abundantly distributed in between 714 m-1671 m altitude except CL/I/22/09 which is rarely found in Tuensang district surrounding at 1372 m altitude. Chetry *et al.* (2021) observed that *Citrus aurantium*

was found to thrive well in some farmers' orchard besides being found abundantly in wild state. It has been claimed that the *Citrus aurantium*, which is native to the northeastern Himalayas, can be found in the wild in the Naga Hills in Nagaland. There had been some information regarding the availability of this species in abundant in some parts of Nagaland earlier, however due to human activities like jhum cultivation, urbanization and less preferences of this species had led to extinction of this species in some particular area. Chetry *et al.* (2021) observed that this species was found to thrive well in some farmers' orchard besides being found abundantly in wild state which may be due to favourable microclimatic condition of that region and was found at elevation ranging between 750 m to 1600 m. Devi *et al.* (2022) also reported that Dailong forest region had comparatively high plant population than two other location. The data pertaining to Table 1, the general range of age variation of the selected accessions was from 7-70 years. Through detailed discussion and inquiry, the oldest tree was noted to be 70 years of age, *i.e.* genotypes CL/I/21/01 and CL/I/21/02 from Tuensang (Tsarü village) and the youngest tree among the genotypes was found to be 7 years, *i.e.* genotype CL/I/22/08 from Noklak (Thang Nokyan village).

#### **Qualitative Characteristics of *Citrus aurantium* genotypes (Tree and Leaf)**

The tree habit showed upright growth habit except the accession CL/I/22/06 from Tuensang district (Kiding village) which showed spreading tree habit. Moreover, the accessions CL/I/22/08 and CL/I/22/21 showed compact growth habit which were from the location Noklak (Thang Nokyan village) and Mokokchung (Dilong ward) respectively as presented in Table 2. Chetry *et al.* (2021) also reported that *Citrus aurantium* varied from upright to compact tree habit among the collected accessions. Devi *et al.* (2022) mentioned about the dissimilarities within same species in regard to nature of the tree which was noted during their findings. Enormous variance among the trees within a district had been documented due to changes in environmental conditions (Dorji, 2011). Plant growth character diversity in distinct genotypes can be linked to genetic traits of particular genotypes and their adaptability to agro climatic conditions (Kumar *et al.*, 2014). The accessions observed were mostly ellipsoid tree shape; however, accessions CL/I/22/06 and CL/I/22/08 showed spheroid tree shape as presented in Table 2. Tree shape is greatly influenced by the environment in which it is growing. The interception of light is directly proportional to the canopy shape of individual tree. Comparable findings

were reported by Chetry *et al.* (2021) where the tree was found to be ellipsoid in shape. These differences had become prominent due to genetic constituent and abiotic factors which varied accordingly to its location. There were variations among the accessions as shown in the data from Table 2. The accession CL/I/22/06 showed compact branch density and CL/I/22/17 was observed to have dense branch density while CL/I/22/21 showed medium branch density. The rest accessions showed sparse branch density. The observations were in congruence with the results reported by Chetry *et al.* (2021) where the density of the branch varied significantly. He registered the branch density to be sparse, medium and dense. In addition, Bhattacharya and Dutta (1956) also stated that *Citrus aurantium* possessed moderately dense foliage. Plant stage, soil and growing conditions all have an impact on morphological characteristics (Devi *et al.*, 2022). These morphological variations may be due to agro-climatic conditions. There was no distinguishable character among the selected genotypes with respect to the leaf type. All the genotypes were observed to have simple leaf type as delineated in Table 2. Devi *et al.* (2021) reported that *Citrus aurantium* collected from Dailong forest, Manipur possessed simple leaf division, which was similar to present findings. Chetry *et al.* (2021) and Malik *et al.* (2006) also observed the same leaf type, *i.e.* simple leaf type in their findings. These findings are in strong agreement with the present results where there were considerable variations within the same species. From these results it may be contented that these phenotypic variations may be due to genetic constituents of the genotypes. No identifiable variation was seen among the selected genotypes with respect to the leaf persistency. All the accessions were observed to have evergreen leaf persistency (Table 2). This observation was found in consonance with the findings of Devi *et al.* (2021), Chetry *et al.* (2021) and Malik *et al.* (2006) wherein they reported evergreen leaf persistency in all the selected accessions. The Table 2 revealed that all the collected 30 accessions did not possess any considerable variation with respect to the leaf form. All the accessions have brevipetiolate (petiole shorter than leaf lamina) leaf form. Similar finding were reported by Devi *et al.* (2021) during characterization of *Citrus aurantium* in Dailong forest area, Manipur wherein they registered the leaf to have brevipetiolate character. The leaflet shape varied from elliptic to lanceolate. Accessions CL/I/22/09, CL/I/22/10, CL/I/22/12, CL/I/22/14, CL/I/22/15, CL/I/22/18, CL/I/22/19, CL/I/22/25, CL/I/22/26, CL/I/22/27, CL/I/22/28 showed lanceolate leaf shape and the rest of the accessions showed elliptic leaf shape (Table 2). Devi *et*

*al.* (2021) during their research work, they found that the leaflet was elliptic in shape. However, Chetry *et al.* (2021) during their characterization, the leaflet shape was observed to be lanceolate in shape. The extent of variation in morphological characters may be due to genetic constitution of the genotypes and growing environment. Considerable genetic variation was observed in the leaf margin which varied from crenate to dentate. Eleven accessions showed dentate leaf margin, *i.e.* CL/I/22/09, CL/I/22/10, CL/I/22/12, CL/I/22/14, CL/I/22/15, CL/I/22/18, CL/I/22/19, CL/I/22/25, CL/I/22/26, CL/I/22/27 and CL/I/22/28 while the rest of the accessions showed crenate leaf margin as depicted in Table 2. Malik *et al.* (2012) reported that there were variations in leaf margin. The accessions leaf margin varied between dentate to crenate whereas Bhattacharya and Dutta (1956) claimed that the leaf margin of *C. indica* was observed to have crenate margin. There was no significant variation among the collected genotypes as the petiole wings was absent in all the 30 accessions (Table 2). Similar findings were also reported by Chetry *et al.* (2021) where no variation was seen in the accessions during their survey and characterization of Sour orange. All the 30 accessions did not have petiole wings therefore petiole wings shape cannot be characterized (Table 2). Variations were observed in regard to absence and presence of spines along with the size of spines. Almost all the accessions had spines, however the spines were absent in accession CL/I/22/09, CL/I/22/10, CL/I/22/11, CL/I/22/12, CL/I/22/13 and CL/I/22/20 (Table 2). Similarly, *Citrus aurantium* was found to be very thorny with brownish tips (Malik *et al.*, 2012; Bhattacharya & Dutta, 1956). On the contrary, some accessions were observed to have no spines which may be due to genetic makeup. Das *et al.* (2005) investigated fifty four mandarin genotypes from 5 states where they confirmed considerable variations among the genotypes in regard to physical and chemical trait. They opined that these variations observed in the accessions were influenced by environment or abiotic factors.

#### **Qualitative Characteristics of *Citrus aurantium* flowers**

There was no discernible difference in all the 30 accessions with respect to the petals colour. All the accessions had white petals colour as displayed in Table 3. Bhattacharya and Dutta (1956) during classification of *Citrus aurantium* flowers, they stated that the flowers' petals were pure white both inside and outside. These findings were in strong agreement with the current results obtained. All the 30 accession did not show any distinguishable genetic variation with

respect to the flower type. All the accessions were hermaphrodite in nature (Table 3). Bhattacharya and Dutta (1956) also confirmed in one of their findings that the flowers of *Citrus aurantium* were bisexual in nature. No seasonal variation was observed in terms of flowering in all the accessions. Flowering occurred to be in the spring season during January- March. Kumari *et al.* (2021) studied genetic divergence based on floral characteristics on 70 seedlings of acid lime genotypes and they reported that the flowers did not show any considerable variations in respect to qualitative parameters. Dorji *et al.* (2011) also reported that the Bhutanese mandarin accessions which were collected from various location of Bhutan did not show any significant in floral characteristics variations among the genotypes.

#### **Qualitative Characteristics of *Citrus aurantium* fruits**

In regard to fruit shape, it was recorded that all the accessions possessed oblate fruit shape except accession CL/I/22/03 which had spheroid fruit shape as presented in Table 4. Malik *et al.* (2012) also reported that the fruit shape of *C. indica* might vary from spherical to oblate shape. More or less similar findings were also reported by Chetry *et al.* (2021) where the collected accession had oblate fruit shape. Similarly, major differences were also observed in the fruit shape bore on the same tree of Bhutanese mandarine by Dorji *et al.* (2011) during their studies. The fruit base shape varied from concave to truncate shape. All the accessions were observed to have truncate fruit base shape except accessions CL/I/22/01, CL/I/22/02, CL/I/22/16, CL/I/22/17 and CL/I/22/25 which showed concave fruit base shape (Table 4). The findings were in conformity with the observation reported by Chetry *et al.* (2021); Malik *et al.* (2012); Bhattacharya & Dutta (1956), wherein the wild orange fruit base shape varied between concave to truncate shape. The variations observed within the genotypes may be due to climatic conditions and variability in genotypic traits. The results in Table 4 and declared that the fruit apex shape varied from depressed to truncate shape. All the accessions were noticed with truncate fruit apex shape except accessions CL/I/22/04 and CL/I/22/20 which showed depressed fruit apex shape. Devi *et al.* (2022) reported from their recent findings revealed that *C. indica* fruits were truncate apex shape. Similar findings were also reported by Chetry *et al.* (2021) which supported the present findings. Malik *et al.* (2012); Bhattacharya & Dutta (1956) through their findings stated that the fruit of *Citrus aurantium* in regard to its apex shape might significantly vary from depressed to truncate shape. These variations among

the accessions may be due to environmental conditions and genetic make-up. There was no distinguishable difference in all the 30 accession with respect to ripe fruit skin colour. The fruits were observed to have orange skin colour (Table 4). This result is in conformity with the research done by Chetry *et al.* (2021) in characterization of *Citrus aurantium*, where they observed that the fruit skin was orange in colour. Malik *et al.* (2006) also reported that the fruit skin colour was light to dark orange. Significant genetic variations were observed among the accessions, *i.e.* accessions CL/I/22/03, CL/I/22/08, CL/I/22/16, CL/I/22/17, CL/I/22/23, CL/I/22/26 and CL/I/22/27 showed papillate skin surface while the rest accessions had smooth skin surface as depicted in Table 4. Chetry *et al.* (2021) during their survey work noticed some variations in *Citrus aurantium* fruit skin surface. They observed that out of 10 accessions almost all the accessions possessed smooth skin surface but there was two accession with longitudinal grooved and ridges. They opined that these variations within the species may lead to further evolution or increase of advance variety. The adherence of epicarp to mesocarp was slight in almost all the accessions; however it was observed that accessions CL/I/22/19 and CL/I/22/24 had moderate adherence of epicarp to mesocarp (Table 4). The findings of Chetry *et al.* (2021) and Malik *et al.* (2006) were in agreement with the present results, wherein adherence of epicarp to mesocarp were observed to vary from slight to moderate. All the collected 30 accessions did not show any distinguishable variation with respect to mesocarp colour which was pale yellow in colour (Table 4). The observations obtained were in line with the findings of Malik *et al.* (2006) where they recorded that the

mesocarp colour was yellow in colour. Similar findings were observed in *Citrus aurantium* accessions by Chetry *et al.* (2021). There was no distinguishable variation among the genotypes in regard to nature of oil glands. All the accessions/genotypes have conspicuous nature of oil glands (Table 4). Similar characteristics were observed in the nature of oil glands where they found that the *Citrus aurantium* possessed conspicuous oil glands despite being collected at different region, *i.e.* Manipur and Meghalaya by different research workers (Devi *et al.*, 2021; Malik *et al.*, 2006).

### Qualitative Characteristics of *Citrus aurantium* seeds

Variations were observed in the seed shape, *i.e.* accessions CL/I/22/10, CL/I/22/11, CL/I/22/12, CL/I/22/13, CL/I/22/16, CL/I/22/17, CL/I/22/24, CL/I/22/25 and CL/I/22/28 have clavate seed shape while the rest of the accessions showed ovoid seed shape (Table 5). These findings were in close proximity with the results obtained by Devi *et al.* (2021) and Malik *et al.* (2006) wherein they found that the seed shape varied between ovoid and spheroid. It might be possible that the high levels of diversity across different locales and the high levels of variance within the same species were caused by spontaneous mutation and natural hybridization (Zerihun *et al.*, 2009). The data from the Table 5 presented that seed colour of all the collected genotypes did not show any discernible variation and is mostly creamish white in colour. Similar findings were drawn by Devi *et al.* (2021); Chetry *et al.* (2021); Malik *et al.* (2006) where they found that the colour of the seed was cream in colour.

**Table 2:** Qualitative Characteristics of *Citrus aurantium* genotypes (Tree and Leaf)

Collector's No.	Tree Habit	Tree Shape	Branch Density	Leaf Type	Leaf Persistency	Leaf Form	Leaf/Leaflet shape	Leaf Margin	Petiole Wings	Petiole Wings Shape	Spine
CL/I/21/01	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/21/02	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/03	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/04	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/05	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/06	Spreading	Spheroid	Compact	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/07	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/08	Compact	Spheroid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/09	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Absent
CL/I/22/10	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Absent
CL/I/22/11	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Absent
CL/I/22/12	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Absent
CL/I/22/13	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Absent
CL/I/22/14	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/15	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/16	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/17	Compact	Spheroid	Dense	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/18	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present

CL/I/22/19	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/20	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Absent
CL/I/22/21	Compact	Ellipsoid	Medium	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/22	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/23	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/24	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/25	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/26	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/27	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/28	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Lanceolate	Dentate	Absent	-	Present
CL/I/22/29	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present
CL/I/22/30	Upright	Ellipsoid	Sparse	Simple	Evergreen	Brevipetiolate	Elliptic	Crenate	Absent	-	Present

**Table 3 :** Qualitative Characteristics of *Citrus aurantium* flowers

Collector's No.	Colour of Petals	Type of Flower	Season of Flowering
CL/I/21/01	White	Hermaphrodite	Spring
CL/I/21/02	White	Hermaphrodite	Spring
CL/I/22/03	White	Hermaphrodite	Spring
CL/I/22/04	White	Hermaphrodite	Spring
CL/I/22/05	White	Hermaphrodite	Spring
CL/I/22/06	White	Hermaphrodite	Spring
CL/I/22/07	White	Hermaphrodite	Spring
CL/I/22/08	White	Hermaphrodite	Spring
CL/I/22/09	White	Hermaphrodite	Spring
CL/I/22/10	White	Hermaphrodite	Spring
CL/I/22/11	White	Hermaphrodite	Spring
CL/I/22/12	White	Hermaphrodite	Spring
CL/I/22/13	White	Hermaphrodite	Spring
CL/I/22/14	White	Hermaphrodite	Spring
CL/I/22/15	White	Hermaphrodite	Spring
CL/I/22/16	White	Hermaphrodite	Spring
CL/I/22/17	White	Hermaphrodite	Spring
CL/I/22/18	White	Hermaphrodite	Spring
CL/I/22/19	White	Hermaphrodite	Spring
CL/I/22/20	White	Hermaphrodite	Spring
CL/I/22/21	White	Hermaphrodite	Spring
CL/I/22/22	White	Hermaphrodite	Spring
CL/I/22/23	White	Hermaphrodite	Spring
CL/I/22/24	White	Hermaphrodite	Spring
CL/I/22/25	White	Hermaphrodite	Spring
CL/I/22/26	White	Hermaphrodite	Spring
CL/I/22/27	White	Hermaphrodite	Spring
CL/I/22/28	White	Hermaphrodite	Spring
CL/I/22/29	White	Hermaphrodite	Spring
CL/I/22/30	White	Hermaphrodite	Spring

**Table 4 :** Qualitative Characteristics of *Citrus aurantium* fruits

Collector's No.	Fruit Shape	Fruit Base Shape	Fruit Apex Shape	Fruit Skin Colour	Fruit Skin Surface	Adherence of Epicarp to Mesocarp	Mesocarp Colour	Nature of Oil Glands
CL/I/21/01	Oblate	Concave	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/21/02	Oblate	Concave	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/03	Spheroid	Truncate	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/04	Oblate	Truncate	Depressed	Orange	Smooth	Slight	White	Conspicuous
CL/I/22/05	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/06	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/07	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/08	Oblate	Truncate	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/09	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/10	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/11	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous

CL/I/22/12	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/13	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/14	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/15	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/16	Oblate	Concave	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/17	Oblate	Concave	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/18	Oblate	Concave	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/19	Oblate	Truncate	Truncate	Orange	Smooth	Moderate	Yellow	Conspicuous
CL/I/22/20	Oblate	Truncate	Depressed	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/21	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/22	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/23	Oblate	Truncate	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/24	Oblate	Truncate	Truncate	Orange	Smooth	Moderate	Yellow	Conspicuous
CL/I/22/25	Oblate	Concave	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/26	Oblate	Truncate	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/27	Oblate	Truncate	Truncate	Orange	Papillate	Slight	Yellow	Conspicuous
CL/I/22/28	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/29	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous
CL/I/22/30	Oblate	Truncate	Truncate	Orange	Smooth	Slight	Yellow	Conspicuous

**Table 5 :** Qualitative Characteristics of *Citrus aurantium* seeds

Collector's No.	Seed Shape	Seed Colour
CL/I/21/01	Ovoid	Cream
CL/I/21/02	Ovoid	Cream
CL/I/22/03	Ovoid	Cream
CL/I/22/04	Ovoid	Cream
CL/I/22/05	Ovoid	Cream
CL/I/22/06	Ovoid	Cream
CL/I/22/07	Ovoid	Cream
CL/I/22/08	Ovoid	Cream
CL/I/22/09	Ovoid	Cream
CL/I/22/10	Clavate	Cream
CL/I/22/11	Clavate	Cream
CL/I/22/12	Ovoid	Cream
CL/I/22/13	Clavate	Cream
CL/I/22/14	Ovoid	Cream
CL/I/22/15	Ovoid	Cream
CL/I/22/16	Clavate	Cream
CL/I/22/17	Clavate	Cream
CL/I/22/18	Ovoid	Cream
CL/I/22/19	Ovoid	Cream
CL/I/22/20	Ovoid	Cream
CL/I/22/21	Ovoid	Cream
CL/I/22/22	Ovoid	Cream
CL/I/22/23	Ovoid	Cream
CL/I/22/24	Ovoid	Cream
CL/I/22/25	Ovoid	Cream
CL/I/22/26	Ovoid	Cream
CL/I/22/27	Ovoid	Cream
CL/I/22/28	Clavate	Cream
CL/I/22/29	Ovoid	Cream
CL/I/22/30	Ovoid	Cream

**Quantitative attributes**

Genotype CL/I/22/17 (9.54 cm) possessed the highest leaf length, which was found statistically at par with genotypes CL/I/22/25 (10.52 cm) and CL/I/22/21 (10.50 cm). The lowest leaf length was recorded in CL/I/22/04 (7.01 cm). Similar findings were noted by Chetry *et al.* (2021) where the leaf length of the collected accessions ranged from 7.17-9.99 cm. The

variation in leaf length may be due to influence of abiotic factors and genetic variabilities. Genotype CL/I/22/06 (4.57 cm) was significantly having the highest leaf width, which was statistically at par with CL/I/22/26 (4.14 cm) and CL/I/22/27 (3.71cm). Significantly, the lowest leaf width value was recorded in CL/I/22/07 (2.83cm). Chetry *et al.* (2021) also reported the range of leaf width in between 3.68 cm to

4.44 cm which was in consonance with the findings in the present result. The genotype CL/I/22/14 (2.08 cm) was having the longest spine length followed by CL/I/22/27 (1.80 cm), CL/I/22/08 (1.23 cm) and CL/I/22/18 (1.17 cm). The shortest spine length was observed on genotype CL/I/22/22 (0.53 cm). However, the spine was absent in some accessions, *i.e.* CL/I/22/09, CL/I/22/10, CL/I/22/11, CL/I/22/12, CL/I/22/13 and CL/I/22/20. Similar findings were registered by Marboh *et al.* (2015) during their investigation in fifty citrus genotypes by morphological characterization. The results did not show any ample variation in the number of petals. The number of petals of the selected genotype was 5.00 in number. The present findings were in strong agreement with the investigations done by Malik *et al.* (2012); Bhattacharya & Dutta (1956) who revealed that *Citrus aurantium* flowers bore 5 numbers of petals. Similar observations were also reported by Dorji & Yapwattanaphun (2011) where they found that the number of petals per flowers were 5 in all the mandarin genotypes. These similar findings may be due to their genetic make-up. The petal length which ranged from 0.78 cm to 1.15 cm wherein overall mean was 0.94 cm and the fifteen genotypes surpassed the general mean. CL/I/22/06 (1.15 cm) was significantly having the longest pedal length followed by CL/I/22/11 (1.05 cm) and CL/I/22/18 (1.05 cm). Significantly the shortest pedal length was recorded in CL/I/22/24 (0.78 cm). Malik *et al.* (2012) recorded the petal length to be measuring about 1.2 cm which was in consonance with the present findings. Fluctuations in day and night temperatures during growth period, genetic make-up of the genotype as well as biotic and abiotic factors may have possibly contributed to the diversity in these traits. The mean was 0.27 cm and fourteen genotypes were found to exceed the general mean of the petal width. CL/I/22/06, CL/I/22/15 and CL/I/22/19 (0.32 cm) were significantly having the highest petal width followed by CL/I/21/02 and CL/I/22/16 (0.31 cm) whereas the lowest value of petal width was recorded in CL/I/22/24 (0.21cm). Variations in these characteristics may be attributable to fluctuations in day and night temperatures during the period of growth, the genetic make-up of the genotype as well as biotic and abiotic factors.

Genotype CL/I/22/29 (3.78 cm) was significantly having the longest fruit length, followed by CL/I/22/17 (3.77cm) and CL/I/22/13 (3.74 cm). CL/I/22/06 (2.07 cm) was having the shortest fruit length. Chetry *et al.* (2021) also reported on the variation found in fruit length which ranged from 2.11 cm to 3.88 cm with 2.8 cm as the general mean of the accessions. Similarly, (Sarkar *et al.*, 2023) recorded the average fruit length

of Sour orange as 3.3 cm during his research. The genotype CL/I/22/29 exhibited the highest fruit width with the value of 5.89 cm followed by CL/I/22/20 (5.88 cm), CL/I/22/11 (5.71 cm) and CL/I/22/27 (5.70 cm). The lowest fruit width value was recorded from the genotype CL/I/22/19 (2.98cm). Results were in partial agreement with the findings of Chetry *et al.* (2021) where they recorded that the range of fruit width was 2.66 cm to 4.30 cm. Malik *et al.* (2006). The mean of the fruit weight was 33.43 g. CL/I/22/11 (39.51 g) was significantly having the maximum fruit weight which was statistically at par with CL/I/22/29 (39.11 g) and CL/I/22/07 (38.91 g). CL/I/22/19 (13.67 g) was recorded to have the minimum fruit weight. These results were approximate to the findings obtained by Chetry *et al.* (2021) where they recorded accession from L-09 weight maximum, *i.e.* 36.50 g while minimum weight was recorded from L-04, *i.e.* 11.66 g. Maiti *et al.* (2001) during their study on pummelo genetic variability, recorded significant variation among the pummelo genotypes in respect to fruit weight. They opined that the phenotypic variability was governed by genetic makeup and environmental or climatic conditions (particularly temperature and rainfall). The genotype CL/I/22/11 (11.80) contained more number of fruit segments, which was at par with CL/I/22/17 (11.66), CL/I/22/26 (11.60), CL/I/22/27(11.60) and CL/I/22/28 (11.60). The lesser number of fruit segments was observed in genotype CL/I/22/13 (9.40). Similar findings could be supported by Malik *et al.* (2006) who recorded the mean number of segments of *Citrus aurantium* to be 10.60 and the segments of the collected accessions were in the range from 9-12. The amount of juice contained in the selected genotype ranged from 2.76 ml to 17.88 ml wherein the average juice content was 11.11 ml. The highest juice content was observed in CL/I/22/11 (17.88 ml) which was followed by CL/I/22/09 (15.08ml), CL/I/22/13 (15.67ml), CL/I/22/10 (15.53 ml) and CL/I/22/12 (15.22 ml). The lowest juice content was observed in CL/I/22/19 (2.76 ml). The highest rind thickness was recorded in CL/I/22/26 (3.18 mm) followed by CL/I/22/29 (3.10 mm), CL/I/22/25 (3.08 mm), CL/I/22/18 (3.07 mm) and CL/I/22/21 (3.06 mm) and the lowest rind thickness was recorded in CL/I/22/04 (1.96mm). Present findings could be a match to the results confirmed by several research workers who did their investigation on *Citrus indica* characteristics. Devi *et al.* (2021) reported that the rind thickness ranged from 0.17 to 0.25 mm. Climatic factor coupled with temperature and rainfall were reported to be the major cause of variations in the thickness of the rind (Nauer *et al.*, 1975).

The genotype CL/I/22/11 (28.06) was having the maximum number of seeds per fruit which was significantly different with other accessions followed by CL/I/22/09 (25.00). Significantly, the minimum number of seeds per fruit was recorded in CL/I/22/19 (7.00). Chetry *et al.* (2021) reported the highest number of seed as 12.53 on accession L-05 which was in contrary with the present findings. The gap or variation between the research findings may be due to the genetic makeup between the accessions in addition to abiotic factors. CL/I/22/18 (1.50 g) was significantly having the highest seed weight which was found at par with CL/I/22/27 (1.36 g), CL/I/22/13 (1.26 g) and CL/I/22/29 (1.22 g). The lowest value of seed weight was recorded in CL/I/22/24 (0.60 g). The present findings are in partial agreement with the results recorded by Malik *et al.* (2006) where they observed that the seed weight ranged from 1.45 to 2.16 g. These variations among the genotypes may be due to genotypic differences inherited within the species.

Genotype CL/I/22/05 (12.05 mm) was significantly having the longest seed length followed by CL/I/22/18 (11.35 mm) and CL/I/22/13 (11.15 mm). Significantly, the shortest seed length was recorded in the accession CL/I/22/02(9.48mm). The overall average number of the seed length was 10.43 mm. Similar results were found by Devi *et al.* (2021) where the seed length varied from 10.00 mm to 12.4 mm. Malik *et al.* (2006) also confirmed that the seed length of *Citrus aurantium* varied from 9-13 mm with mean value of 10.80 mm. Genotype CL/I/22/05 (7.30 mm) was significantly having the highest seed breadth followed by CL/I/22/09 (6.05 mm) and CL/I/21/01 (6.03 mm). The lowest value of seed breadth was recorded in CL/I/22/22(4.80 mm). The mean seed breadth was 5.45 mm. Present observation was in partial agreement with the findings by Malik *et al.* (2006) where the significantly highest seed breadth recorded was 8.00 mm.

**Table 6:** Leaf and flower characters of different *Citrus aurantium* genotypes.

Collector's No.	Leaf Length (cm)	Leaf Width (cm)	Spine Length (cm)	No. of Petals	Petal Length (cm)	Petal Width (cm)
CL/I/21/01	10.17	3.50	0.65	5.00	0.95	0.27
CL/I/21/02	9.23	3.27	0.73	5.00	1.02	0.31
CL/I/22/03	9.28	3.34	0.74	5.00	0.93	0.23
CL/I/22/04	9.37	3.61	0.79	5.00	0.95	0.25
CL/I/22/05	9.08	3.49	0.91	5.00	0.92	0.27
CL/I/22/06	10.83	4.57	0.78	5.00	1.15	0.32
CL/I/22/07	8.89	2.83	0.81	5.00	1.04	0.24
CL/I/22/08	9.90	3.38	1.23	5.00	0.99	0.30
CL/I/22/09	10.26	3.47	0.00	5.00	0.93	0.31
CL/I/22/10	8.44	3.12	0.00	5.00	0.82	0.29
CL/I/22/11	8.74	3.56	0.00	5.00	1.05	0.29
CL/I/22/12	9.65	3.37	0.00	5.00	0.93	0.27
CL/I/22/13	8.15	3.28	0.00	5.00	1.03	0.23
CL/I/22/14	10.43	3.60	2.08	5.00	0.92	0.27
CL/I/22/15	9.44	3.45	0.93	5.00	0.91	0.32
CL/I/22/16	9.47	3.57	0.57	5.00	0.95	0.31
CL/I/22/17	8.81	3.48	0.79	5.00	0.87	0.25
CL/I/22/18	10.04	3.45	1.17	5.00	1.05	0.29
CL/I/22/19	7.01	3.07	0.87	5.00	0.95	0.32
CL/I/22/20	10.00	3.60	0.00	5.00	0.94	0.26
CL/I/22/21	10.50	3.26	1.07	5.00	0.84	0.29
CL/I/22/22	7.97	3.70	0.53	5.00	0.87	0.21
CL/I/22/23	9.88	3.56	0.74	5.00	0.84	0.22
CL/I/22/24	8.99	3.28	0.93	5.00	0.78	0.25
CL/I/22/25	10.52	3.47	0.56	5.00	0.93	0.27
CL/I/22/26	9.88	4.14	0.87	5.00	0.97	0.29
CL/I/22/27	9.79	3.71	1.80	5.00	0.80	0.29
CL/I/22/28	8.43	3.11	0.91	5.00	0.95	0.25
CL/I/22/29	8.19	3.55	0.62	5.00	0.99	0.30
CL/I/22/30	9.43	3.67	0.75	5.00	0.98	0.23
SEm±	0.21	0.09	0.02	0.00	0.03	0.01
CD at 5%	0.58	0.27	0.05	0.00(NS)	0.07	0.02
CV	3.80	4.71	4.31	0.00(NS)	4.78	4.83

**Table 7 :** Fruit and seed characters of different *Citrus aurantium* genotypes

Collector's No.	Fruit Length (cm)	Fruit Width (cm)	Fruit Weight (g)	Number of Segments	Juice Content (ml)	Rind Thickness (mm)	Number of Seeds	10 Seed Weight (g)	Seed Length (mm)	Seed Breadth (mm)
CL/I/21/01	2.83	3.70	26.17	10.80	6.17	2.16	8.40	1.04	10.80	6.03
CL/I/21/02	2.80	3.17	34.40	10.20	7.37	2.28	8.02	0.91	9.12	5.67
CL/I/22/03	2.22	3.04	34.30	11.00	7.33	2.22	15.00	0.72	9.48	5.30
CL/I/22/04	2.44	3.24	17.60	10.09	4.60	1.96	7.80	0.90	9.95	5.35
CL/I/22/05	2.36	4.36	34.57	10.60	13.83	2.29	16.60	0.84	12.05	7.30
CL/I/22/06	2.07	3.66	38.47	11.60	12.90	2.14	16.40	0.84	10.15	5.34
CL/I/22/07	2.44	4.13	38.91	10.00	12.57	2.15	14.20	0.84	10.15	5.28
CL/I/22/08	3.68	5.06	32.38	11.00	13.10	2.21	17.80	0.81	10.07	5.00
CL/I/22/09	3.42	4.96	38.05	10.40	15.80	2.20	25.00	0.95	10.95	6.05
CL/I/22/10	3.58	4.74	35.32	9.80	15.53	2.08	9.80	1.14	11.05	5.55
CL/I/22/11	3.62	5.70	39.51	11.80	17.88	2.10	28.06	1.10	11.04	5.05
CL/I/22/12	3.38	5.04	37.61	10.80	15.22	2.38	18.80	1.14	10.70	5.25
CL/I/22/13	3.74	5.36	36.89	9.40	15.67	2.37	17.40	1.26	11.15	5.30
CL/I/22/14	3.54	5.28	33.84	11.20	14.80	2.42	15.80	1.12	11.03	5.48
CL/I/22/15	3.50	5.20	37.57	11.18	13.00	2.13	18.29	0.93	10.93	5.36
CL/I/22/16	2.88	4.00	24.00	10.20	7.60	2.30	14.60	0.94	10.50	5.54
CL/I/22/17	3.77	5.18	38.76	11.66	9.70	2.19	17.20	0.75	9.70	5.05
CL/I/22/18	3.64	5.68	38.36	10.40	9.11	3.07	12.20	1.50	11.35	6.00
CL/I/22/19	2.30	2.98	13.67	10.10	2.76	2.33	7.00	0.71	9.25	5.25
CL/I/22/20	3.64	5.88	35.93	11.00	12.67	3.04	16.40	1.05	10.45	5.30
CL/I/22/21	3.26	4.46	30.46	10.40	10.12	3.06	12.00	0.95	9.80	5.40
CL/I/22/22	3.32	4.10	31.20	10.60	11.00	2.49	14.60	0.82	9.60	4.80
CL/I/22/23	3.50	5.34	37.67	10.20	11.53	2.67	13.80	0.87	10.85	5.50
CL/I/22/24	2.84	3.62	20.40	9.40	5.85	2.44	11.60	0.60	9.55	4.85
CL/I/22/25	3.14	4.66	30.80	11.00	6.97	3.08	17.40	0.75	9.90	5.10
CL/I/22/26	3.30	5.10	36.70	11.60	12.05	3.18	16.60	0.94	10.85	6.00
CL/I/22/27	3.50	5.70	37.46	11.60	9.47	2.33	18.80	1.36	10.65	5.85
CL/I/22/28	3.24	5.16	36.25	11.60	12.27	1.98	14.80	0.96	10.75	4.95
CL/I/22/29	3.78	5.89	39.11	10.20	14.27	3.10	18.40	1.22	10.35	5.25
CL/I/22/30	2.96	4.58	36.60	10.00	12.10	2.06	15.20	1.15	10.60	5.20
SEm±	0.07	0.12	0.77	0.17	0.27	0.03	0.39	0.01	0.22	0.13
CD at 5%	0.19	0.35	2.19	0.48	0.77	0.09	1.10	0.03	0.61	0.37
CV	3.65	4.62	4.01	2.78	4.22	2.22	4.41	2.19	3.60	4.21

### Biochemical attributes

The average percentage of juice content was 29.84%, with the highest juice content (%) observed on accession CL/I/22/11 (41.82%), which was comparable to CL/I/22/28 (41.73 %) and CL/I/22/09 (39.66%), and the lowest was on CL/I/22/19 (15.25 %). Out of 30 accessions, fourteen genotypes exceeded the overall average juice content of the genotypes. The findings appeared to be in partial agreement with the results registered by Chetry *et al.* (2021) temperature, rainfall along with mineral nutrition, growing period and the progress of fruit development might be the reason for variability in the genotypes. CL/I/22/15 (62.65 mg/100g) had the highest ascorbic acid content, followed by CL/I/22/26 (61.67 mg/100g), CL/I/22/11 (50.48 mg/100g), and CL/I/22/25 (49.83 mg/100g), while CL/I/22/22 (17.76 mg/100g) had the lowest ascorbic acid content. Twelve genotypes confirmed higher ascorbic acid content than the general average of the genotypes. The variation found in ascorbic acid content may be due to factors such as seasonal

conditions which were favourable and the locations where they were grown (Kamatyanatti *et al.*, 2015; Sinclair 1984). The ascorbic acid concentration of the fruit had also been shown to be affected by light intensity as (Sites, 1949) found the most ascorbic content in fruit that had received the most sunlight. The highest TSS was detected in CL/I/22/26 (10.00°B) followed by CL/I/22/21 (9.55°B), CL/I/21/02 (9.50°B) and CL/I/22/29 (9.44°B) and the lowest TSS was recorded in CL/I/22/03 (4.54°B). Similar findings were recorded by (Sarkar *et al.*, 2023) where the TSS of the *C. indica* fruit was 10.36 °Brix. The variations may be due to genetic constituent among the fruits besides difference in temperature at different locales, as more or less levels of differences in this phenomenon could be seen even within same location. The highest titratable acidity was recorded on accessions from CL/I/22/03 (7.68 %) followed by CL/I/21/02 (5.84 %), CL/I/22/04 (4.72 %) and CL/I/22/06 (4.64 %) and lowest on accessions from CL/I/22/17 (1.54%). Eighteen accessions exceeded the overall average of

titratable acidity of the genotypes, *i.e.* 3.66 %. Sarkar *et al.*, 2023 found out that the average titratable acidity of fruit was 3.16 % during his biochemical analysis of *C. indica* genotype. Variations were observed in the data, as even within a small district or location the climatic conditions could vary which might have great impact on the quality of the fruit. Besides, edaphic factor and light intensity might play a critical role in variations. The highest ratio of TSS to acid was recorded in CL/I/22/21 (5.51) followed by CL/I/22/17 (5.38) and CL/I/22/29 (4.56) while the lowest ratio was recorded in CL/I/22/03 (0.59). The fine balance between TSS and acid also plays a crucial role in determining quality of the fruit in respect to palatability. Some accessions were found to have higher TSS to acid ratio as it may be due to high TSS content in the fruit. Environmental conditions might be the fundamental cause of variations in TSS to acid ratio. The total sugar analysis showed fifteen accessions were recorded to have higher percent of total sugar than the general mean of all the accessions, *i.e.* 3.31 %. The highest total Sugar was registered in CL/I/22/29 (5.28 %) which was at par with CL/I/22/26 (5.15 %), CL/I/22/27 (5.06 %), CL/I/22/15 (4.65 %) and CL/I/22/17 (4.32 %). The lowest Total Sugar was significantly recorded in CL/I/22/24 (1.71%). Sarkar *et al.*, 2023 determined total sugar of *C. indica* fruit and recorded 5.71% as the average value. The variation in total sugar content

across districts may be due to changes in primary and secondary metabolites whose synthesis is influenced by temperature and humidity (Ram *et al.*, 2004). Highest per cent of reducing sugar was recorded on accessions from CL/I/22/27 (4.93 %) which was followed by CL/I/22/26 (4.88 %), CL/I/22/15 (4.39 %) and CL/I/22/06 (4.04 %) and lowest on accessions from CL/I/22/24 (1.45 %). Present findings matched with the result reported by (Sarkar *et al.*, 2023) who reported that the average percent of reducing sugar was 2.96 %. Similar variations among the genotypes within the same species were also observed by Chetry *et al.* (2021). These variations may be the outcome of changes in meteorological information, such as temperature, precipitation, location, soil depth and age of the tree in addition to genetic makeup. Non-Reducing sugar was found highest on accession from CL/I/22/29 (1.39 %) followed by CL/I/22/17 (1.33 %), CL/I/22/28 (1.06 %) and CL/I/22/30 (0.98 %) while the lowest percent was recorded on accession from CL/I/22/22 (0.01 %). Eleven accessions were registered to have higher percent of non-reducing sugar than the mean value of the genotypes. Chetry *et al.* (2021) also recorded wide variation in non-reducing sugar during their studies. These wide variations among the genotypes may be due to differences either in the genetic constituent and environment in or around the growing location.

**Table 8:** Biochemical characteristics of *Citrus aurantium* fruits.

Collector's No.	Juice Content (%)	Ascorbic Acid (mg/100 ml)	TSS (°B)	Titratable Acidity	TSS: Acid Ratio	Total Sugar (%)	Reducing Sugar (%)	Non-Reducing Sugar (%)
CL/I/21/01	24.83	44.41	9.10	4.09	2.22	2.45	1.87	0.67
CL/I/21/02	22.38	28.61	9.50	5.84	1.63	2.22	1.62	0.68
CL/I/22/03	23.58	39.47	4.54	7.68	0.59	2.83	2.29	0.65
CL/I/22/04	26.17	39.93	8.81	4.72	1.87	3.50	3.14	0.52
CL/I/22/05	32.16	43.40	9.19	3.82	2.40	3.35	2.69	0.80
CL/I/22/06	31.66	46.37	9.23	4.64	1.99	4.31	4.04	0.47
CL/I/22/07	31.59	37.49	8.74	3.98	2.20	3.65	3.04	0.76
CL/I/22/08	29.68	44.90	8.54	3.79	2.25	3.61	3.38	0.40
CL/I/22/09	39.66	38.48	8.95	3.72	2.41	3.60	3.35	0.42
CL/I/22/10	37.51	47.36	8.45	3.83	2.21	3.39	2.97	0.57
CL/I/22/11	41.82	50.81	8.51	3.95	2.16	3.25	3.07	0.33
CL/I/22/12	37.13	36.01	8.44	4.16	2.03	3.79	3.58	0.38
CL/I/22/13	36.26	40.45	8.74	4.07	2.15	3.26	3.13	0.29
CL/I/22/14	29.13	35.03	8.35	3.48	2.40	3.05	2.88	0.31
CL/I/22/15	28.33	62.65	9.00	2.88	3.13	4.65	4.39	0.48
CL/I/22/16	31.65	30.33	8.70	3.97	2.19	3.35	2.79	0.70
CL/I/22/17	24.15	31.08	8.27	1.54	5.38	4.32	3.15	1.33
CL/I/22/18	20.88	40.95	9.02	2.97	3.04	2.76	2.53	0.35
CL/I/22/19	15.25	31.57	8.75	4.33	2.02	1.87	1.75	0.21
CL/I/22/20	27.60	23.68	9.35	4.05	2.31	2.59	2.33	0.38
CL/I/22/21	32.30	38.48	9.55	1.73	5.51	1.96	1.78	0.27
CL/I/22/22	35.22	17.76	6.55	3.03	2.16	1.74	1.68	0.14
CL/I/22/23	29.67	30.59	7.15	3.90	1.83	3.67	3.40	0.44
CL/I/22/24	28.91	22.20	6.75	2.28	2.96	1.71	1.45	0.35
CL/I/22/25	22.08	49.83	8.65	3.09	2.80	2.89	1.98	1.01

CL/I/22/26	29.82	61.67	10.00	2.88	3.48	5.15	4.88	0.51
CL/I/22/27	21.01	30.09	8.87	2.35	3.78	5.06	4.93	0.38
CL/I/22/28	41.73	25.16	9.03	3.50	2.58	3.11	2.16	1.06
CL/I/22/29	30.06	26.15	9.44	2.07	4.56	5.28	4.01	1.19
CL/I/22/30	33.02	34.04	8.97	3.73	2.40	3.02	4.15	0.98
SEm±	0.76	1.06	0.12	0.07	0.04	0.05	0.04	0.01
CD at 5%	2.15	3.00	0.34	0.21	0.11	0.13	0.13	0.03
CV	4.41	4.87	2.46	3.49	2.67	2.44	2.69	2.85

### Genetic parameters

The high estimate of heritability (> 80%) was recorded with different traits in Sour orange, viz., Leaf length (cm), Spine length (cm), Petal width (cm), Fruit length (cm), Fruit weight (g), No. of segments, Juice content (ml), Rind thickness (mm), No. of seeds, 10 seed weight (g), Seed breadth (mm), Juice Content (%), Ascorbic Acid (Mg/100ml), TSS (°B), Titratable Acidity, TSS: Acid Ratio, Total Sugar (%), Reducing Sugar (%) and Non-Reducing Sugar (%) among physico-chemical characters, which indicated the least influence of environment on these traits. On contrary, moderate heritability was observed for leaf width and seed length and low heritability in petal length indicating relatively more influence of environment on these traits. The high value of genetic advance (>20%) as percent of mean was recorded for Spine length (138.79%), Petal width (21.87%) among leaf characters, for fruit length (32.81%), Fruit weight (40.09%), Juice content (68.70%), rind thickness (31.50%) among fruit characters, for No. of seed (61.57%), 10 seed weight (42.93%) for juice content (43.20%), ascorbic acids (57.40%), TSS (25.10%), titratable acidity (66.35%), TSS:Acid ratio (81.69), total sugar (59.82%), reducing sugar (67.25%) and non-reducing sugar (113.06%) among physico chemical characters indicating that the influence of additive gene effects in their genetic control. Moderate estimate of genetic advance (>15%) was recorded for leaf length (17.45%) and leaf width (15.75%) among leaf characters, for seed breadth (16.07%) among leaf

characters. On contrary, low value of genetic advance was recorded for petal length (14.47%) among fruit character. For no. of segments (11.58%) among fruit characters, for seed length (11.11%) among fruit characters. Similar associations between traits studied have also been reported in citrus genotypes by Rabha *et al.*, 2013 in pummelo by Mitra *et al.*, 2011 and in guava by Mrinalini & Tiwari (2008). High heritability coupled with high genetic advance as percent of mean was recorded for petiole length and leaf lamina width among leaf characters, anther length, pedicel length, stamen length and petal length among flower characters, diameter of fruit axis, rind thickness, fruit weight and width of epicarp among fruit characters, average number of seeds per fruit, seed width and seed weight among seed characters and ascorbic acid content, titratable acidity, total sugars, reducing sugars and ratio of soluble solids to titratable acidity may be effective traits due to the genetic control of additive gene effects. The results are in conformity of the findings of Rabha *et al.*, 2013 in citrus genotypes, Mitra *et al.*, 2011 in pummelo and Ranpise and Desai (2003) in acid lime. The remaining characters had moderately low heritability coupled with low genetic advance in the present study indicating the limited scope of selection for these traits as the gene effects were found to be non-additive in nature. The present study showed high genetic diversity in sweet orange accessions for different plant, leaf, flower, fruit, seed and physicochemical traits.

**Table 9:** Variability in physico chemical character of leaf and fruit among different genotypes.

	Range			Variance		Heritability (Percent)	Genetic Advance	Genetic Advance value % means
	Mean	Max	Min	Phenotypic	Genotypic			
Leaf length(cm)	9.36	10.83	7.01	9.927	9.172	85.373	1.634	17.458
Leaf width(cm)	3.48	4.57	2.83	9.893	8.698	77.303	0.549	15.754
Spine length(cm)	0.73	2.08	0.00	67.692	67.554	99.594	1.013	138.879
Petal length (cm)	0.94	1.15	0.78	9.445	8.146	74.377	0.136	14.471
Petal width (cm)	0.27	0.32	0.21	12.489	11.517	85.036	0.06	21.877
Fruit length(cm)	3.15	3.78	2.07	16.723	16.32	95.243	1.036	32.81
Fruit weight (g)	33.43	39.51	13.67	20.259	19.858	96.082	13.405	40.098
No. of segments	10.66	11.08	9.40	6.764	6.167	83.124	1.235	11.583
Juice content (ml)	11.11	17.88	2.76	33.876	33.612	98.449	7.631	68.701
Rind thickness (mm)	2.41	3.18	1.96	15.61	15.45	97.969	0.76	31.503
No. of seeds	15.27	28.06	7.00	30.526	30.206	97.917	9.392	61.574

10 seed weight (g)	0.97	1.50	0.60	21.077	20.958	98.881	0.417	42.932
Seed length (mm)	10.43	12.05	9.12	7.192	6.228	74.984	1.158	11.11
Seed breadth (mm)	5.45	7.30	4.80	9.642	8.673	80.908	0.875	16.07
Juice content (%)	29.84	41.82	15.25	21.861	21.412	95.939	12.893	43.204
Ascorbic acid (mg/100ml)	37.63	62.65	17.76	28.694	28.277	97.114	21.603	57.405
TSS (°b)	8.57	10.00	4.54	12.664	12.422	96.227	2.152	25.103
Titrateable acidity	3.66	7.68	1.54	32.581	32.395	98.857	2.428	66.35
TSS/acid ratio	2.62	5.51	0.59	39.837	39.748	99.555	2.142	81.698
Total sugar (%)	3.31	5.28	1.71	29.246	29.143	99.298	1.982	59.824
Reducing sugar (%)	2.88	4.93	1.45	32.868	32.758	99.327	1.937	67.253
Non-reducing sugar (%)	0.57	1.33	0.01	55.033	54.96	99.736	0.649	113.068

## Conclusion

*Citrus aurantium* is the wild endangered species of citrus found in North-East states particularly and present exploration enumerated some new genotypes of *Citrus aurantium* from various unexplored area of Nagaland. These genotype populations are in a highly threatened in the state of Nagaland due to lack of conservation facilities of these species *in vivo* and clearing of forest cover at an alarming rate for the jhum cultivation and urbanization. There is urgent need to adapt complementary conservation strategies to safeguard the genotypes through *in situ* and *ex situ* gene sanctuary after molecular characterization and evaluation of those genotypes for further advanced citrus improvement programme.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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