MORPHO-CYTOLOGICAL CHARACTERIZATION OF *SECHIUM EDULE* (JACQ.) SW. (CUCURBITACEAE)

By

ASIKHO KISO

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THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN BOTANY OF

DEPARTMENT OF BOTANY

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Nagaland University

(A central university Estd. By the Act of Parliament No. 35 of 1989)

Lumami 798627, Nagaland, India

CERTIFICATE

The thesis entitled **"Morpho-Cytological Characterization of *Sechium edule* (Jacq.) Sw. (Cucurbitaceae)"** submitted by Miss Asikho Kiso, bearing Registration No.781/2017 with effect from 28/7/2016 embodies the results of investigations carried out by her under my supervision and guidance.

Further, certified that this work has not been submitted for any degree elsewhere and that the candidate has fulfilled all conditions laid down by the University.

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19 May, 2022

DECLARATION

I, Asikho Kiso, bearing Ph.D. Registration No. 781/2017 with effect from 28/7/2016, hereby declare that the subject matter of my Ph.D. thesis entitled **"Morpho-Cytological Characterization of *Sechium edule* (Jacq.) Sw. (Cucurbitaceae)"** is the record of original work done by me, and that the contents of this thesis did not form the basis for award of any degree to me or to anybody else to the best of my knowledge. This thesis has not been submitted by me for any Research Degree in any University/ Institute.

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Subject(s)/Paper(s)	Max. Marks	Minimun Qualifying Marks	Marks Secured
Paper No. B.Ph.D -1 Research Methodology	100	35	78
Paper No. B.Ph.D -02 Elective Paper	100	35	68
Paper No. B.Ph.D -03 Review of literature, Report Writing & Seminar	100	35	84
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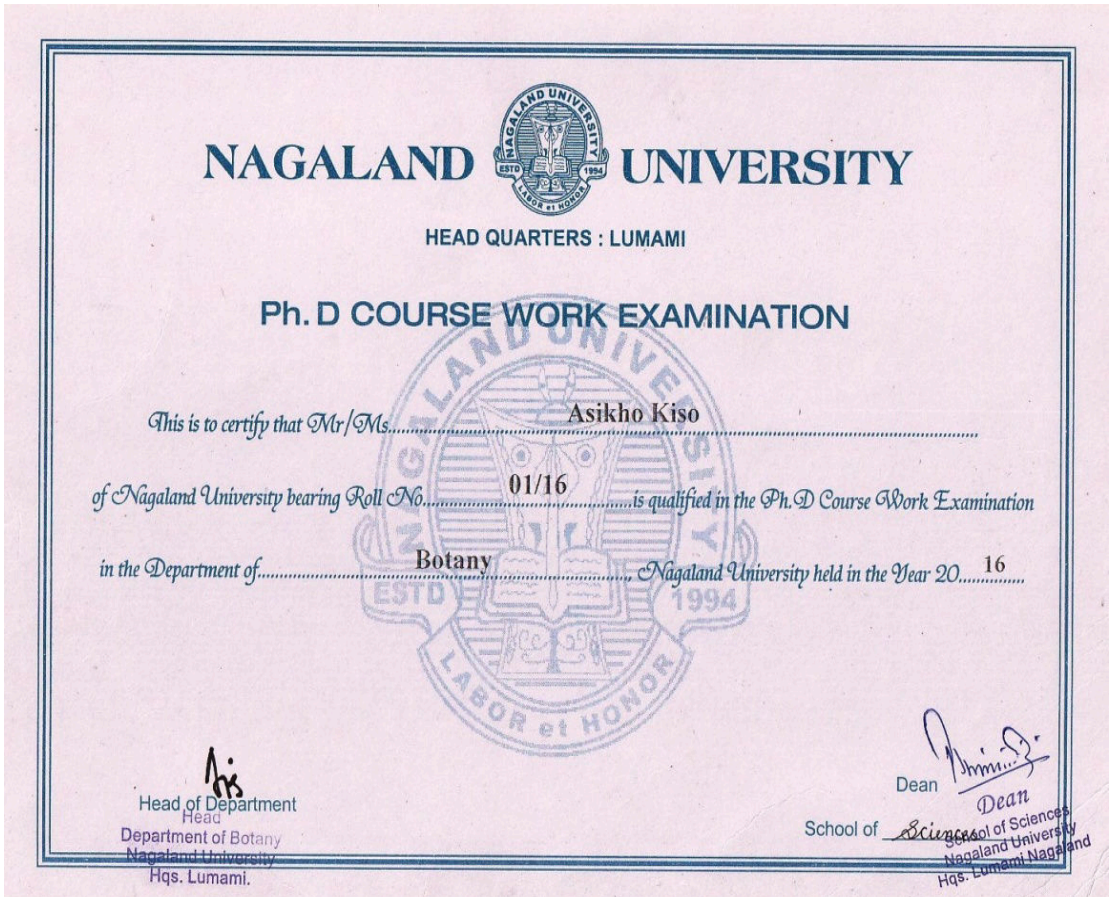

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(Asikho Kiso)

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ABBREVIATIONS

Abbreviation	-	**Full Form**
%	-	Percentage
$\sum CV_{CL}$	-	Summation Coefficient of variation of Chromosome length
∑TLC	-	Summation of total chromosome length
µm	-	Micrometer
A_2	-	Interchromosomal asymmetry index
ANOVA	-	Analysis of Variance
BCN	-	Basic Chromosome Number
CBf	-	Calyx Breadth in female
CBm	-	Calyx Breadth in male
CL	-	Carpel length
CLf	-	Calyx Length in female
CLm	-	Calyx Length in male
CLR	-	Chromosome length range
CS	-	Chromosome size
CV	-	Chromosome Volume
D. I.	-	Dispersion Index
E	-	Environmentability
FA	-	Fruit Area
FC	-	Fruit Circumference
FL	-	Fruit Length
FR	-	Fruit Ridges
FV	-	Fruit Volume
FWd	-	Fruit Width
FWt	-	Fruit Weight
GCV	-	Genotypic coefficient of Variance
GENO	-	Genotypes

GI	-	Gradient Index
H^2	-	Heritability (Broad sense)
LB	-	Leaf Breadth
LL	-	Leaf Length
M	-	Mean
m	-	Minute size chromosome
masl	-	Meter above sea level
ml	-	Millilitre
MLC	-	Mean length of each cromosomes
n	-	Number of analysed chromosomes
NA	-	Number of Anthers
NCf	-	Number of Calyx in female
NCm	-	Number of Calyx in male
No.	-	Number
NPf	-	Number of Petals in female
NPm	-	Number of Petals in male
PBf	-	Petal Breadth in female
PBm	-	Petal Breadth in male
PCA	-	Principal Component Analysis
PCLm	-	Pedicel Length in male
PCV	-	Phenotypic Coefficient of Variance
PDLm	-	Peduncle Length in male
PL	-	Petiole Length
PLf	-	Petal Length in female
PLm	-	Petal Length in male
R	-	Repeatability
S	-	Small size chromosome
S.D.	-	Standard deviation
S.E.	-	Standard Error

SCN	-	Stem Circumference at Node
SL	-	Stamen Length
SND	-	Stem Node Distance
Tno	-	Total tendrils number
Tsp	-	Tendrils in spiral
Tst	-	Tendrils in straight
PGED	-	Paired Group Euclidean Distance
V	-	Variance
V_e	-	Environmental Variance
V_g	-	Genotypic Variance
$V_{g\times e}$	-	Genotype and Environment Interaction Variance
V_p	-	Phenotypic variance
VRC	-	Value of Relative chromatin
Sq.		Square
Km		Kilometer

CONTENTS

ABSTRACT

Sechium edule, fruits are unique as compared to others from Cucurbitaceae and the uniqueness may be compared with their variations in fruit size, fruit colour, presence or absence of spines on outer surface of the fruit and most importantly, viviparous in nature i.e. seed in the fruit sprout out while it is still attached with the parent plant, thus have a suppressive dormancy.

Genus considered as monospecific but other species were also reported by various authors from different regions during 1900s such as *S. edule* sub ssp. *edule*, *S. edule* sub ssp. *sylvestre*, *S. chinatlense*, *S. compositum* and *S. hintonii* and a new species *Sicyos angulatus* L. for Indian flora and *Sechium mexicana* for Mexico respectively. The genus *Sechium* remains very poorly known cytologically and for understanding the inter-relationship amongst different *Sechium* species proper chromosome count is important.

Exploration in flower characters both male and female may reveal the certain cluster of closely related plants; generate the occurrence of variability and specific knowledge about the flowers and plants. It may help in recognizing the practicability of concerned flowering plant to understand plant signal and animal senses and more accessibility towards research.

Plant morphology is an important character for study of habit, habitat and life span of a given plant in space and time and deduces its important features based on its morphological characters and studies had been conducted in the past for various reasons which includes the diversity of leaf shape and colour, adaptive and functional significance within the plant, chlorophyll and photosynthesis rate, physiological, ecological and evolutionary history of the plant.

Fruit characters both qualitative and quantitative, chromosome count, pollen morphology, flower characters both male and female and plant morphological characters were statistically analysed to understand growth, development, association and mass and yield production for genotypes using descriptive statistics (mean ± S.E.), Pearson correlation (2 tailed), analysis of variance (ANOVA), linear regression, paired group euclidean distance (PGED), principle component analysis (PCA). Also, genetic analysis was performed for the purpose using phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve), interaction of genotype and environment variance (Vg×e), phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability (H^2), environment ability (E) and repeatability (R).

Mitosis was studied from the secondary root tips of germinating fruits. Root tips of 2-3 cm in length were pre-treated with α-bromonaphthalene at 6 ± 2 ^{0}C for 3-4h followed by overnight fixation (3:1 ethanol-acetic acid) and preservation (70% ethanol). The root tips were hydrolysed with 1 N HCl for 10-15 min at about 50-60 ^{0}C. The root tips were squashed in 2% acetocarmine. Somatic countable chromosome slides under 100x (emersion oil) were photographed using digital Motic BA210 microscope and recorded. Total chromosome length (µm) were measured for the genotypes with the scale bar of 10µm using ImageJ software and further computation was attempted through windows MS-Excel and with the help of standard formulas for inter and intra chromosomal differences among the chromosome complement of genotypes.

Flower buds were collected and fixed in 6:3:1 ethanol: acetic acid: chloroform fixative for overnight. Anthers were excised and processed in 2% acetocarmine for pollen morphological characters. Spores diameter (µm) was measured with digital

scale attached with Motic BA 210 microscope and with the help of spore diameter computed radius (d/2), circumference ($2\pi r$) and area (πr^2).

FRUIT CHARACTERS

***Descriptive statistics*:** The descriptive statistics reveals high degree of diversity among genotypes at present which can be further explored for crop improvement through molecular techniques, provides an opportunity to enhance its growth and productivity.

Pearson's correlation (2 tailed): Fruit characters FL and FWd showed positive and significant relationship with genotypes at probability level of significance $P \leq 0.05$. Genotypes are highly correlated with each other at both the probability level of significance $P \leq 0.05$ and 0.01 respectively. Spearman's rho correlation matrix for qualitative and quantitative characters of fruit divides in two different independent groups of the characters.

Analysis of variance (ANOVA): ANOVA for the fruit characters suggest that FL was recorded high variation and FA was recorded low variation for all the genotypes.

Regression: The maximum variation was recorded for the character FL with R^2 value (0.347) and regression line y=0.586x+6.908. Similarly, the minimum variation was recorded for the character FWd with R^2 value (0.103) and regression line y=0.059x+4.232.

Paired Group Euclidean Distance (PGED): A single large group suggests approximately similar, especially at morphologically but sub groups indicate towards some differentiation either at the level of genetic constitution or environmental level among the genotypes.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 72.6% of total variations observed in fruit characters.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters of fruit than GCV.

Genetic variances (Vp, Vg, Ve and Vg×e): Phenotypic variance (Vp) was recorded little high than the genotypic variance (Vg) and high environmental variance and genetic constitution may be the reason for the high variation for phenotypes of the characters.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and repeatability was recorded for the characters than the environmentability.

SOMATIC CHROMOSOME COUNT AND POLLEN MORPHOLOGY

Genotype 2 was recorded with 2n=2x=32 and 2n=4x=52, new chromosome count for the *Sechium* and provides hope for the presence of more species in the genus. Spore sizes are in the range of earlier reports.

FLOWER CHARACTERS

Descriptive statistics

Male flower

Genotype 6 was recorded with maximum mean for PLm, PBm, SLm and NAm.

Genotype 5 was recorded with maximum mean for NCm, CLm, and NPm.

Genotype 7 was recorded with maximum mean for CBm, and PDLm.

Genotype 2 was recorded with maximum mean for PCLm.

C.V. was recorded high for the flower characters, PDLm, PCLm and NAm for the genotypes.

Female flower

Genotype 13 was recorded with maximum mean for PLf and PBf.

Genotype 12 was recorded with maximum mean for CBf.

Genotype 6 and 3 was recorded with maximum mean for CL.

Genotype 4 was recorded with maximum mean for CLf.

Pearson's correlation (2 tailed): Flower character, PLm presented an important character which is associated with most of the characters positively or negatively.

Analysis of variance (ANOVA): ANOVA for the flower characters suggest that PDLm was recorded with high variation.

Regression: The maximum and minimum variation was recorded for the character CBm with R^2 value (0.201) for the male flower and NCf with R^2 value (0.001) for female flower.

Paired Group Euclidean Distance (PGED): PGED suggested the genotypes co-phenetically correlated 91.51% and genotype 1 remains ungrouped.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 75.185% of total variations observed in flower characters.

Genetic variances (Vp, Vg, Ve and Vg×e): Genetic constitution and environment are responsible for the variation of flower characters in the genotypes.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters than GCV.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and effect of genetic constitution and environment was recorded for the characters.

MORPHOLOGICAL CHARACTERS

Descriptive statistics**:** Genotype 10, 7, 6 and 2 showed favorable growth for most of the characters. Genotype 3 and 4 has shown less mean value for maximum characters than other genotypes.

Pearson's correlation (2 tailed): Vegetative growth character, LL presented an important character which is associated with most of the vegetative growth characters positively and significantly.

Analysis of variance (ANOVA): ANOVA for the growth characters suggest that LB was recorded high variation and NT was recorded low variation for all the genotypes and NT, SCN and SCI for all the genotypes approximately similar as amount of variation in the character recorded low.

Regression: The maximum variation was recorded for the character PL with R^2 value (0.140) and regression line y=0.191x+7.09 followed by SND with R^2 value (0.136) and regression line y=0.411x+11.98.

Paired Group Euclidean Distance (PGED): PGED suggested the genotypes 7 and 10 are having little variation than others.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 75% of total variations observed in vegetative growth characters.

Genetic variances (Vp, Vg, Ve and Vg×e): Genetic constitution and environment are responsible for the variation of phenotype and growth character in the genotypes.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and repeatability was recorded for the characters.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters than GCV.

CHAPTER-1

INTRODUCTION AND REVIEW OF LITERATURES

The genus *Sechium* belongs to a family Cucurbitaceae which consist of 130 genera and 800 species distributed mainly in the tropical and subtropical regions of the world and the plants of the family is called cucurbits. It was originated in the cool mountains of Central America and domesticated by the Aztecs (Newstrom, 1991). The first description of chayote was given by Francisco Hernández, who was in Mexico from 1550 to 1560, but the crop was not introduced into the southern part of the continent until the arrival of the Spanish (Cook, 1901). The word 'chayote' is a Spanish derivative of the Nahuatl word '*chayohtli'* meaning '*prickly squash*'. The plant was first recorded by modern botanists P. Browne's in 1756. The crop was introduced in Europe, then into Africa, Asia and Australia (Whitaker and Davis, 1962). Though it is a native of Mexico, considerable diversity is also found in the Indian subcontinent where it is grown widely in the North-East and Southern regions of India (Rai *et al.,* 2002).

Chayote is unique among the cultivated cucurbits by bearing a single seeded fruits, exhibiting vivipary and considerable variations in respect of fruit size, shapes, color, present of spines and nutritional composition of the fruits (Aung *et al.,* 1990). Chayote is used for human consumption as fruits, stems and tender leaves (usually known as 'quelites'). The tuberous parts of the adventitious roots are boiled and eaten as vegetable. It has been reported that many nutritional characteristics make chayote, particularly suitable for hospital diets (Liebrecht and Seraphine, 1964).

IMPORTANCE AT INTERNATIONAL LEVEL

Pharmacological properties

The diuretic properties of the leaves and seeds relieve urine retention and burning during urination or to dissolve kidney stones. Through the pharmacological studies, it had been confirmed that leaves and fruits have cardiovascular and anti-inflammatory properties and is used for the treatment of arteriosclerosis and hypertension (Salama *et al.*, 1987; Gordon, 2000). There is a report that leaf juice has transient depressor effect (Khulakpam *et al.*, 2015).

Alcoholic, ethanolic or aqueous extraction properties of Sechium

The extracts of chayote was used as antibacterial, antifungal, antioxidant activity, antihyperglycemic activity, anti-ulcer activity, hepatoprotective effects (Fidrianny *et al.*, 2015), antihypertensive effect (Earl *et al.*, 2014), α-glucosidase activities (Sulaiman *et al.*, 2013), antiproliferative potential (Aquiniga-Sanchez *et al.*, 2015), inhibits lipogenesis and stimulates lipolysis (Wu *et al.*, 2014), β- carotene linoleate model and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging model (Ordonez *et al.*, 2006).

Physiological properties

The physiological properties of chayotewas reported on biosynthesis of cytokinin in the endosperm (Piaggesi *et al.*, 1997), major and trace element composition (Hidalgo *et al.*, 2016), effect of osmotic dehydration (OD) occurrence (Ruiz-Lopez *et al.*, 2010), dynamics of free and bound IAA (Gregorio *et al.*, 1995), yield, physico-chemical, rheological and molecular characteristics (Hernandez-Uribe *et al.*, 2011), trypsin inhibitor activity (Laure *et al.*, 2006), purification and partial

characterization of β-glucosidase (Mateos *et al.*, 2015), palynological character and isolation of starch in the chayote (Alvarado *et al.*, 1992), high composition of polysaccharide (Shiga *et al.*, 2015), nucellar cell degeneration by increase in activity of different classes of proteinases (Lombardi *et al.*, 2007) and ethylene production (Gregorio *et al.*, 1997).

IMPORTANCE AT NATIONAL LEVEL

The importance and other properties of chayote was described by different authors about the introduction and importance of Chayote (Singh *et al.*, 2014), standardization of DNA isolation and RAPD-PCR protocol (Jain *et al.*, 2015), the peels were good source of nutrient content (Nagarajaiah *et al.*, 2015), morphological diversity (Jain *et al.*, 2014), supplements for food and have the potential to improve the health status (Premkumar, 2016), medicinal properties of cultivated and wild Cucurbitaceae crops (Khulakpam *et al.*, 2015), anti- hypertensive and anti oxidant activities (Dhiman *et al.*, 2012), emerging alternative drug of diabetes for its antioxidant and hyperglycemic effect (Pandeya *et al.*, 2013).

IMPORTANCE IN NORTH-EASTERN REGION OF INDIA

It was reported that chayote is a boon crop for Mizoram and Meghalaya, where it is cultivated for its fruits, tender shoots, young leaves and the tuberised roots. They are eaten as vegetable, mixed with meats or as an ingredient of soup and other preparations and also, sustain the socio-economic status of the tribal community (Singh *et al.*, 2015). Diversity of Cucurbits in North Eastern region of India was studied basing on the variation, plant types, morphology, physiology, adaptability,

physic-chemical parameter's reaction to diseases and pests and distribution of horticultural crops (Mishra *et al.*, 2015).

Review of Literatures

HISTORICAL AND TAXONOMICAL ASPECTS OF SECHIUM EDULE (JACQ.) SWARTZ

The first monograph on Cucurbitaceae was published in 1881 (Cogniaux, 1881). The monograph pointed out that *Sechium* was a monospecific genus and contain a single species i.e. *S. edule*. The species *S. edule* was originally discovered in Jamaica during 1756 (Browne, 1756). The species was classified as *Sicyos edulis* and *Chocho edulis* simultaneously and respectively during 1763 (Adanson, 1763; Jacquin, 1788). *Chocho edulis* was changed into the genus *Chayota* and redesignated as *Chayota edulis* (Jacquin, 1788). Later on, it was Swartz (1800) who became the first to include this species into *Sechium* and became *Sechium edule*. The combinations of both proposed by Jacquin (1788) and Swartz (1800) still known today as *Sechium edule* (Jacq.) Swartz. The most accepted term for *Sechium* is Chayote, worldwide.

The most recent classification of *Sechium* was given by Jeffrey (1990). According to this classification, *Sechium* was placed in the sub-tribe Sicyinae (which belongs to Chayote) with other genera. The members of the sub-tribe: Sicyinae (to which Chayote belongs) were characterized by spiny pollen, a single pendulous ovule and single-seeded fruits.

Jeffrey (1978) extended the taxonomic limit of *Sechium* and included many Mexican and Central American taxa which was later differentiated and transferred

into different genera (Cogniaux, 1881; Donnell-Smith, 1903; Pittier, 1910; Standley and Steyermark, 1944; Wilson, 1958; Wunderlin, 1976). All these genera or taxa characterized by the presence of nectaries at the base of the flower receptacle of the both sexes, a complex and variable androecium structure and produce medium to large fleshy-fibrous fruit.

Jeffrey (1978) proposed taxonomic expansion of *Sechium* was not much-admired broadly as no new interest was expected in the genus. Jeffrey's proposal on taxonomic expansion explained the relatives of the *Sechium edule* formally for the first time in the literature, although many authors had been already suggested the closeness of many other species to the *Sechium* species (Standley and Steyermark, 1944). Standley and Steyermark (1944) suggested the closeness of the species *Ahzolia composita* to the *Sechium compositum*. The taxonomic limit expansion of the *Sechium* including the cultivated species [e.g. *S. tacaco* (Pittier) C. Jeffrey] assisted and highlighted the significance and importance for study of *Sechium*. The study of *Sechium* was not only restricted to taxonomic point of view, but also lead to the general perspective of plant genetic resource conservation and exploitation.

As a result of that botanical exploration was augmented and added further representative range as well as variations in the cultivated species in the genus. During 1980s, three wild populations of *Sechium* were reported in Veracruz State (Cruz-Leon, 1985-86). This was the major break through in search for the acquaintance of relationship between wild and cultivated species in the genus. The wild populations of *Sechium* were studied and researched for several years and grouped or defined them as 'wild' type *Sechium edule* (Jacq.) Swartz, without any

specific place in taxonomic group (Cruz-Leon, 1985-86; Cruz-Leon and Querol, 1985).

On the other hand, Newstrom (1985 and 1986) reported his similar findings to those of Veracruz in the Oaxaca and studied them for longer period to find the origin and evolution of *Sechium edule*. In his studies, he included the species or populations of *Sechium* and wild species collected from the Veracruz State, which were transferred to *Sechium* genus by Jeffrey (1978). Later on, the wild species of the Veracruz State were documented as *Frantzia* species (Nee, 1993).

Newstrom (1985 and 1986) restricted the various species of *Sechium* into new taxonomic unit with only three species. The restricted *Sechium* species were *S. edule* (Jacq.) Swartz (represented by wild and cultivated types), *S. compositum* (J.D. Smith) C. Jeffrey and *S. hintonii* (P. G. Wilson) C. Jeffrey. Similarly, he proposed *Frantzia* and *Polakowskia* as independent genera. This proposal leads to the discarding of the categorization given by Wunderlin (1976), where both the genera were kept together. Later, Newstrom (1990) suggested that *Sechium*, *Frantzia* and *Polakowskia* could be considered as a section of *Sechium*, but such classification was never proposed.

During 1990s, most of the botanists were not in the conformity for the taxonomic limit of *Sechium* and caused a range of studies to find out the problems (Lira and Soto, 1991; Alvarado *et al*., 1992; Lira and Chiang, 1992; Mercado *et al*., 1993; Lira *et al*., 1994; Mercado and Lira, 1994; Lira, 1995a; Lira, 1995b). Finally, results of all these studies concluded that *Sechium* is a well defined genus and comprised of 11 species. Out of 11 species, 9 wild species are scattered throughout central and southern Mexico, upto Panama and remaining 2 species are cultivated.

The *Sechium tacaco* is the only cultivated species in Costa Rica while *Sechium edule* is widely cultivated all over the Americas and other regions of the World, with wild populations in southern Mexico. The correct scientific name of chayote is *Sechium edule* (Jacq.) Swartz based on the *Sicyos edule* Jacq. was formally published during 1800. The classifications proposed, debates, correct scientific name and accepted synonyms for the species by various authors in the past tabulated in Table 1.

Jeffrey (1978) classification of *Sechium* based on floral nectaries and arrangement of stamens included seven species and arranged into two sections of *Sechium* and *Frantzia*. These two sections differ in the morphology of the floral nectaries and the arrangement of the stamens.

The section *Sechium* included the species with naked floral nectaries visible from the above, partially or totally joined filaments and free anthers. The following species were included in this section, *Sechium edule* (Jacq) Swartz, *Sechium hintonii* (P.G. Wilson) C. Jeffrey, *Sechium compositum* (J.D. Smith) C. Jeffrey, *Sechium tacaco* (Pittier) C. Jeffrey and *Sechium talamencense* (Wunderlin) C. Jeffrey.

The section *Frantzia* was originally proposed by Wunderlin (1976) for the genus of the same name i.e. *Sechium*, which later on it was placed in *Sechium* synonymy by Jeffrey (1978). This section includes two species *Sechium pittieri* (Cogn.) C. Jeffrey and *Sechium villosum* (Wunderlin) C. Jeffrey. The section *Frantzia* included the species with floral nectaries covered by a cushion or umbrella shaped spongy structure, filaments merged to form a column and anthers merge together to form a globose structure. Wunderlin (1977) described a new species of *Frantzia*

panamensis (genus name was different than the *Sechium*) from Panama and placed this additional species into the section *Frantzia*.

But very soon, generic classification of the species *Frantzia panamensis* in section *Frantzia* by Jeffrey (1978) was discarded by the L.D. Gomez (Gomez and Gomez, 1983). Gomez and Gomez (1983) described a new species from the Costa Rica under the genus *Frantzia* and classified it using binomial classification as *Frantzia venosa* L.D. Gomez and placed it in section Polakowskiasensu (Wunderlin, 1976). Gomez apparently failed to notice that the floral nectaries of this new species (*Frantzia venosa* L.D. Gomez) were covered with the pillow, cushion, or umbrella shaped spongy structure which was the characteristic features of the species in Wunderlin (1976) *Frantzia* section and wrongly placed the species into the section Polakowskiasensu (Wunderlin, 1976). Although none of the two species (*Frantzia panamensis* and *Frantzia venosa*) was reclassified in the *Sechium*, either by C. Jeffrey or by any other author during 1980s, so the generic limit of the genus become indistinct once again (Table 2).

The chayote, a useful plant, was well known for wide variety of names and vernaculars in different languages or ethnic groups for the other parts of the world. The vernacular names of the *Sechium* in different parts of the American continent pointed out by Newstrom (1991). There are effects of the linguistic colonization and almost similar vernacular names were recorded which seems derived from the chayote or chuma of Latin American countries. The vernacular names ‘achocha’ or ‘achojcha’ also given to the *Cucurbita maxima* Duch. Ex Lam., and *Cyclanthera pedata* (L.) Schrad. in Ecuador and other South American countries belong to Cucurbitaceae family and native to the area of the continent.

Newstrom (1986, 1991) made an excellent compilation of the vernacular names from different regions of the world while analyzing the geographical distribution of the species. The pattern of geographical distribution might help to trace out the centre of origin for the species. Lira (1995a) collected additional names and added with Newstrom's names. The list is more elaborated by adding some vernacular names from the India and its different states as well as other parts of the world at this present thesis work. The comprehensive list of vernaculars of this cultivated crop has been tabulated in Table 3.

TRACING THE ORIGIN OF SECHIUM EDULE (JACQ.) SWARTZ

There are no any archeological indications that how long *Sechium edule* cultivated as many other crops. The large fleshy fruit with single soft testa seed does not allow itself to conserve. The pollen grains or other structures are also not reported at the archeological sites. The most commonly and possible source of origin of this species are ethno historic, artistic, linguistic, ecogeographic distribution and genetic diversity of both cultivated and wild species.

Ethno historic records suggested that chayote has been cultivated since the Pre-Colombian times in Mexico. Francisco Hernandez reported its first description of the Chayote, who was in Mexico during 1550 to 1560 (Cook, 1991). The crop was not introduced into the Southern part of the continent till the arrival of the Spanish (Newstrom, 1986 and 1991).

Linguistic records demonstrate that the common names or the vernacular names have been suggested for the purpose in different parts of the Latin America. Linguistic records clearly indicate that species originally concentrated in Mexico and

Central America. The similar common names of the species were used in other parts of the world with slight modifications where species has been introduced (especially Nahuatl origin, 'chayote'). Pre-Colombian decorated pottery with the species clearly indicates the presence of Chayote during that period in Mexico and Central America (Pérez, 1947; Newstrom, 1991).

Ecogeographic distribution of cultivated as well as wild *Sechium edule* provides a maximum indication for establishing the centre of origin for the species. The intense research and exploration for the crop during different times, regions, people and institutes concluded that maximum variety of cultivated *Sechium* was found in the Southern Mexico, Guatemala and Costa Rica at an altitude of 500-1500 masl (León, 1968; Bukasove, 1981; Engels, 1983; Maffioli, 1983; Cruz-León and Querol, 1985; Newstrom, 1985 and 1986; Lira, 1995a).

DISTRIBUTION AND PROPERTIES OF ENDEMIC OR WILD TYPES SECHIUM EDULE OF MEXICO AND AMERICA

There was a little doubt on the distribution and origin of wild and its relative species in this area. Most of the species and morphologically similar species to Chayote were known to grow within the geographic and altitude perimeter. The wild type taxa of *Sechium* which is morphologically close to the Chayote called 'wild types' of *Sechium edule* grow in Southern part of Mexico. Two endemic species [*S. edule* (spiny), *S. chinantlense* (unarmed)] were illustrated from the north of the State of Oaxaca (Lira and Chiang, 1992). The endemic species from the State of Oaxaca were basically recognized as wild types of Chayote previously (Newstrom, 1985, 1986, 1990, 1991). Either the endemic or wild type species from the State of Oaxaca

have the staminate flowers (naked nectaries at the base of the receptacle, partially joined filaments and side branches with other tissue at the apex), which are very similar to the cultivated Chayote. Fruits are large and fleshy with very bitter pulp. They are the only members of the genus (similar to *S. edule*) which have cleft at the apex from which seedlings sprouts once the seed germinated. Newstrom (1986, 1990) suggested the bitter taste of the fruit pulp might be because of the high concentration of secondary chemical 'Cucurbitacine' or the most bitter substances frequently found in the cucurbitaceae family. The high concentration of 'cucurbitacine' might help in the defense against the herbivores (Metcalf and Rhodes, 1990).

There were two more endemic or wild species similar to Chayote i.e. *Sechium compositum* and *Sechium hintonii*. *S. compositum* reported as endemic to the States of Mexico and Guerrero (Wilson, 1958; Lira and Soto, 1991; Lira, 1995a, 1995b), while *S. hintonii* known only from the Mexican state of Chiapas and neighbouring areas of Guatemala (Donnell-Smith, 1903; Standley and Steyermark, 1944; Dieterle, 1976; Lira, 1995a, 1995b). Either endemic or wild species (*S. compositum* and *S. hintonii*) similar to cultivated Chayote in floral nectaries and androecium structure, but the fruits are fibrous and bitter and also do not have cleft at the apex of the fruit.

S. tacaco, a wild species and morphologically similar to cultivated *S. edule* from Veracruz has not been described yet (Nee, 1993). The other wild species of *Sechium edule* are morphologically very similar to other cultivated species and all grow in Southern Central America from Nicaragua to Panama (Pittier, 1910; Wunderlin, 1976, 1977, 1978; Gómez and Gómez, 1983, Newstrom, 1986, 1990, 1991; Lira and Chiang, 1992; Lira, 1995a, 1995b). Ecogeographic distribution of endemic or wild types of *Sechium edule* is tabulated in Table 4.

ECOGEOGRAPHIC DISTRIBUTION, INTRASPECIFIC VARIATION, PHENOLOGY AND POTENTIAL OF ENDEMIC OR WILD TYPE SECHIUM EDULE

(i) ***Sechium edule*** wild types Ecogeographical distribution

Wild types of *S. edule* registered and classified by Cruz-Leon (1985 - 86) for the State of Veracruz on the Gulf of Mexico and later reported in the State of Oaxaca by Newstrom (1985, 1986, 1989 and 1990). Now, it is established that huge and massive populations in thick clusters found in States of Veracruz, Puebla, Hidalgo and Oaxaca in Southern Mexico at an altitude of 500-1700 masl (Lira, 1995a, 1995b). The populations mostly found in the damp habitats such as ravines, waterfalls, rivers or streams and prevails the mountane rainforest. It may occur in the lower parts of ecotone zones with evergreen or semi-evergreen seasonal forest.

Although, it is assumed that some wild species of *S. edule* collected from the Island of Java, Reunion (Backer and Bakhuizen, 1963; Cordenoy, 1895) and some parts of Venezuela (Brűcher, 1989), but these populations have not been backed up through collections and also yet to be confirmed. It could be assumed that these populations might be escaped during the cultivation at least in the Venezuela (Jeffrey, 1990).

Intraspecific variation

S. edule, wild types are morphologically, larger flowers, staminal structures and fruits with cleft at apex similar and identical with cultivated species. The most important visible morphological difference between wild and cultivated types are in

their size that wild plants have more robust leaves, flowers and staminate inflorescence bigger in size than cultivated plants. The fruits found in State of Veracruz have different morphology and strict comparison between the wild and cultivated plants have not been done or very difficult to compare. Yellow or white fruits of wild plants have not been collected. The pulp has bitter taste and more fibrous in wild plants.

The differences could be more emphasized in the wild populations of Oaxaca. The fruits with fibrous pulp and bitter taste are more homogeneous in shape (globulate), colour (dark green) and prickles (very prickly) (Lira, 1995a; Lira, 1995b).

Wild populations differ in their chromosome numbers such as haploid number (n=12) from Veracruz (Palacios, 1987), haploid number (n=13) from Oaxaca (Mercado *et al.*, 1993; Mercado and Lira, 1994). Newstrom (1991) suggested that the morphological variations in populations of wild fruits from Veracruz are very close to the other cultivated areas and expected the hybrid origin of the fruits. Hybrid origin of the some fruits (approx. 6 fruits) was established experimentally.

Phenology

Flowering occurs from April to December and fruits grow from September to January in Wild populations of *Sechium edule*.

Potential importance

Wild plants have potential to improve the crop but much study has not been reported. Crops are morphologically similar, potential for successful hybridization to

cultivated chayotes, the plants could be the first to evaluate for resistance to disease and pests and hybridization should be started with cultivated crops.

(ii) ***Sechium chinantlense*** Lira and Chiang

Ecogeographic distribution

S. chinantlense endemic to a very small region in Mexico, North of the State of Oaxaca at the boundary of State of Veracruz. The species grows at an altitude of 20-800 masl in rainforest of lower areas and higher zone of mountane rainforest. The species may be endangered in near future as it is found in restricted area and threatened.

The representative material of the species was first collected by G. Martinez-Calderón between 1940 and 1941 and R. McVaugh in 1962. The collected representative material was identified as *Ahzolia composita* species (J.D. Smith) Standley and Steyermark. After a long gap, Newstrom (1986, 1989, 1990 and 1991) placed the *Ahzolia composita* species in the '*Sechium edule* Wild types III' group. The structure of stamens, morphological and chemical properties of fruits with apex germination cleft and their reproductive structures of the representative material was not compatible with wild and cultivated types of *S. edule* (Castrejón and Lira, 1992). So the different types of properties (not related or similar to cultivated or wild types) allowed them to identify as a new species i.e. *S. chinantlense* (Lira and Chiang, 1992).

Newstrom (1986, 1989, 1990 and 1991) reported that staminate flower structure, presence of smooth fruit, and chemical properties of the species are very close to the *S. compositum* and might be originated by the hybridization of the species

with cultivated crops. But, in fact, *S. compositum* was not found in Oaxaca, so it may not be a hybrid origin between *S. chinantlense* and other species of genus.

Phenology

Flower blooms from August to November and start fruiting during September to February. Phonological effects have been recorded little early or late depending on the weather and climate of the growing and distributed area.

Potential importance

Plants grow in high relative humidity with small variations in phenology need to be determined its importance in the improvement of the plant. Although there was no report on the hybridization between the *S. chinantlense* and *S. edule*, but hybrids could not be produced from this two species (Casterjón and Lira, 1992).

But on the other hand, both may be crossable as they have different chromosome numbers as *S. edule* (2n=22) and others (2n=24; 2n=26) and *S. chinantlense* (2n=30) have been reported.

(iii) ***Sechium compositum*** (J.D. Smith) C. Jeffrey

Ecogeographic distribution

Sechium compositum could be located in the small patch in the southern most part of the State of Chiapas of Mexico as a Biosphere reserve 'El Triunfo' or conserves in natural condition and the neighbouring areas of the Guatemala such as Quetzaltenango, Escuintla and Suchitepequez in humid areas at an altitude of 50-2100 masl. The species could be easily located in mountane rainforest, rainforest, evergreen

seasonal forest or even in coffee plantations as primary or secondary vegetation (Lira, 1995a; Lira, 1995b).

Intraspecific variation

Morphologically, the fruits of *S. compositum* possess longitudinal ridges with prickles on the ridges (Dieterle, 1976; Donnell-Smith, 1903). Also some fruits were observed without any ridges and prickles, but completely smooth and unarmed in the areas of Chiapas and Guatemala (Lira, 1995a; Lira, 1995b).

Phenology

Flowering occurs in September to January and Fruiting starts during October-November to February.

Application

A mixture of chopped roots and water used as soap substitute and used to kill the horse parasites. Some common names (‘chayote de cabalo’ ‘chayote de burro’ and ‘huisquil de cohi’) suggest that fruit is not too bitter but have high ‘cucurbitacine’ content and used as a cattle feed.

Potential importance

The fruit of the species could be stored for longer duration (several months) without any effect of humidity and turgidity, which seems a most useful feature of the fruit. The transfer of this feature into cultivated plants could be able to solve the problems of storage and conservation of the fruits. The hybridization between *S. compositum* and other cultivated genus has not been explored. However, a hybrid fruit has been produced by crossing between *S. compositum* and a cultivated plant of *S.*

edule in the CATIE gene bank of Costa Rica (Newstrom, 1986). The haploid chromosome number of the species is n=14 (Mercado *et al.*, 1993; Mercado and Lira, 1994).

(iv) *Sechium hintonii* (P. G. Wilson) C. Jeffrey

The species was endemic to a small area of Mexico till it was reported from the Temascaltepec district of the State of Mexico. It was rediscovered in the nearby areas of the localities (Lira and Soto, 1991). A small population of the species recorded from the southern part of the State of the Guerrero (Lira, 1995a). It was recorded at an altitude of 1300-1510 masl in hot to semi hot climatic-vegetation and ecotone transition zone between deciduous seasonal and Quercus forest. The species is threatened by the seasonal agricultural practices in the area.

The specimens collected were differed in many respects from the most common species available e.g. size of fruit and pedicels of the staminate flowers, outline of leaves, shape of the lobules, fruits smaller with prickles only at base, and do not have back-ward-turning prickles.

The specimens were not identified as *S. hintonii*, if identified as *S. hintonii* its distribution could be extended to the north west of Mexico. Since the material is very far in the State of Jalisco, it better suited for conservation in nature than brought to the States of Mexico and Guerrero (Lira, 1995a; Lira, 1995b).

Phenology

Flowers from August to November and produce fruits from October to December. Field observations suggest that aerial part of the species dried up after December and again sprouts just before the commencement of rainy season.

Potential importance

The potential of the species not known much but could be a source of resistance to diseases and pests from the literature. It is also not known that the species could be crossed with other chayotes. It is an endangered species and ex-situ conservation does not exist. The haploid chromosome number of the species is known which is n=14 (Mercado *et al.,* 1993; Mercado and Lira, 1994).

The ecogeographic distribution, intraspecific variation, phenological characteristics and potential importance of endemic or wild types *Sechium edule* from Mexican and American continent tabulated in Table 5.

SCOPE OF THE STUDY

Plant morphology is the morphological characters of plants which can be compared, measured, counted and described to assess the differences or similarities in plant taxa and can be used these characters for plant identification, classification and descriptions. The characters which are used in descriptions or for identification, they are called diagnostic or key characters which can be either qualitative (colour or shape) and quantitative (counted or measured).

A diverse morphological characters and inadequate information on the chromosome count and species in *Sechium*, draws attention of scientist / researchers to explore the plant for better sustainable conservation and identification.

The number, structure and behaviour of chromosomes are of great value in taxonomy, where chromosome count is the most widely used character for the

relationships and classification of organisms using comparative chromosome complements and cell behaviour during mitosis and meiosis.

A very few works related to variability, genetic diversity, and standardization of DNA isolation of *Sechium* has been reported in the literature survey (Sanwal *et al.*, 2008; Kapoor *et al*., 2014; Jain *et al*., 2015). *Sicyos angulatus* L. a new adventives species was recorded for the flora of India (Thakur, 2016) and most probably there is no chromosome count report of *Sechium edule* has been reported in the literature survey of Indian squash.

Internationally, the two important literatures on evidence for the origin of chayote by Newstrom (1991) and origin and evolution of cultivated cucurbits by Bisognin (2002) were informative and related to *Sechium* (Newstrom, 1991; Bisognin, 2002). A new species *S. mexicanum* of *Sechium* section *Frantzia* from Mexico was reported (Lira and Nee, 1999). Moreover, cytogenetic study of six cultivars of *Sechium* had given no evidence of more than one species i.e. *S. edule* (De Donato and Cequea, 1994).

On the basis of information available in the literature regarding its morphology and chromosome count, at present it was tried to verify the earlier results in Indian squashes (*Sechium edule*). The study has been taken to locate the available differences in the morphology, chromosome count, growing season and distribution pattern in Indian climate and phytogeography. Therefore, the plant has been taken to study its morphological and cytological properties and report it, so that, the information could be utilized for identification, description, relationships and classifications purpose.

OBJECTIVES

- To study the morphological characters of *Sechium* for phenotypic and genotypic characterization.
- To study the number, structure, and behaviour of chromosomes for their genotypic characters.

CHAPTER-2

STUDY OF FRUIT QUALITATIVE AND QUANTITATIVE CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

INTRODUCTION

Fruit is a fleshy product of plant that formed from the ovaries after fertilization, in most of the cases, of an angiospermic plant. The main purpose of fruit is the protection and dissemination of the seeds but can be used as a food also. Fruits are widely recommended for dietary and nutritional guidance due to the enough concentration of vitamins, minerals, antioxidants and phytochemicals (Aggarwal, 2011; Kapoor, 2014).

Fruits generally draw attention of the scientists and researchers for certain points as how to promote its production for commercial purpose, hybridization, protection from the pathogens, extraction for its valuable properties, exploration and conservation strategies (Cadena-Iniguez *et al.*, 2007; Jain *et al*., 2017).

Sechium edule, fruits are unique as compared to other species from Cucurbitaceae. The uniqueness of fruits may be compared with their variations in fruit size, fruit colour, presence or absence of spines on covering or outer surface of the fruit and most importantly, viviparous in nature (Martinez-Bauer *et al*., 2021). The seed in the fruit sprout out while it is still attached with the parent plant, thus have a suppressive dormancy (Aung *et al.*, 1990).

The pistillate flower bears a single seeded fruit or sometime two in very rare cases. The fruits may be hand-picked at an immature stage and used as vegetable at home or carry to market for sale (Singh, 2015). This is an observation that early harvesting gives more watery fruit than late harvesting which gives fibrous fruit. This may be the reason that young fruits (fleshy and watery) are given more preferences than matured fruits (fibrous) (Rai *et al*., 2006).

The maturity of the fruit can be checked by pressing the fruit skin with finger nails, and it does not dent inside the pericarp, once reached at maturity. Sometimes, fruits are used as an alternative of potatoes and also known as 'Air-potato' (Jain *et al*., 2017).

The whole plant used by humans for consumption such as fruits, stems, tender leaves (usually known as 'quelites') and tuberous parts of the adventitious roots are boiled and eaten as vegetable (Singh, 2015). Fruits extract have antioxidant, anti-hypertensive, anti- obesity, hepatoprotective, anti- diabetics, anti-microbial, anti-ulcer, anti- epileptic and anti- hyperlipedaemic properties (Vigas *et al.*, 2020). Also, it has been reported that many nutritional characteristics make chayote, particularly suitable for hospital diets (Liebrecht and Seraphine, 1964).

In this chapter, fruit characters both qualitative and quantitative were statistically analysed to understand yield, mass production, association growth and development among genotypes using descriptive statistics, Pearson correlation (2 tailed), analysis of variance (ANOVA), regression, paired group euclidean distance (PGED) and principle component analysis (PCA). Also, genetic analysis was performed for the purpose using phenotypic variance (Vp), genotypic variance (Vg),

environmental variance (Ve), interaction of genotype and environment variance (Vg×e), phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability (H^2), environmentability (E) and repeatability (R).

A) FRUIT QUALITATIVE CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

MATERIALS AND METHODS

Collection site

Sechium edule genotypes were collected from the different parts of Kigwema village of Kohima district, Nagaland and presented the GIS map courtesy google map (Figure 1).

Data collection

Qualitative data on fruit characters were collected at maturity by observation. This included colour, shape, presence and absence of spines, presence and absence of furrows, lenticels, texture of pulp, taste of pulp, and presence or absence of fibres in fruit pulp (Table 6).

Each qualitative character was distributed evenly and assigned a number value. For example, the qualitative character, colour, has been distributed into 5 shades of the colour and assigned a number value from 1 to 5, that is, deep dark green assigned as 1, dark green assigned as 2, green assigned as 3, light green assigned as 4 and yellowish white assigned as 5. Similarly, other characters were also distributed according to their shades and assigned a number value (Table 7).

Statistical analysis

The qualitative characters were analyzed for their descriptive statistics such as mean, standard deviation, variance, ANOVA and correlation.

RESULTS AND DISCUSSION

The descriptive statistics for fruit qualitative characters suggested maximum mean ± S.E. value for the character shape (4.142 ± 0.375) followed by spines (3.785 ± 0.536) and colour (3.285 ± 0.321) (Table 8).

The high mean value of the character shape suggested towards the more stability for the character and provides a definite shape for the fruit either spheroid, flattened, obovoid, pyriform or pear shape, although, there was a little variation in the shape of the fruit. Approximately, six variations in fruit shape was observed for all the genotypes under study and observed that it varies from spheroid to ovoid to pear shape to globous-shaped which was supported by high mean value for the character.

A high variation was recorded for the character spine and supported by the high mean value for the character. Approximately seven different types of spines was recorded on the basis of completely, intermediate, many or few- distributed over the fruit, flexible, strong or absence of distribution.

Similarly, a high variation was recorded for the character colour and supported by the high mean value for the character. Approximately five different variations of colour were recorded for the character. It includes deep dark green, dark green, green, light green and yellowish white.

A little variation in the qualitative characters such as deep, intermediate and shallow furrows, absence and presence of lenticels and fibres of pulp, smooth, solid and soft texture as well as watery and sweet taste of the pulp were also recorded.

Analysis of variance (ANOVA) and regression was performed for the qualitative characters of fruit and found satisfactory at the level of significance and probability of $P \leq 0.05$ (Table 9A and 9B). Variation was recorded high for all the characters except fibres of pulp and lenticels. Large variations in the spine character suggest that presence or absence of spines over the fruit surface could be an inherent property of the genotypes and as well as possibly affected by the environment. According to the Cadena-Iniguez, 2005, variation could be supported by the low or high concentration of gibberellic acid in the fruit. It has been reported that there was a growth of spines over the surface of fruit in the presence of gibberellic acid and vice versa.

Kendall's tau_b correlation coefficient statistics was performed for the association of fruit qualitative characters. The associations among the characters were recorded and suggested that character colour is positively and significantly associated with the shape of the fruit (0.662**) at $P \leq 0.01$ (Table 10).

The association of the characters colour and shape may indicate that at the time of formation of fruit shape (pyriform, spheroid, obovoid), development of colour (deep dark green, dark green, green, light green) starts simultaneously. It was reported that variation in fruit colour may be the result of change in pigment content of chlorophyll or xanthophylls at the time of development of fruit (Hendry, 1993). Also, the presence of calcium and magnesium may cause physiological stress leading to the

low level of chlorophyll and making the fruit colourless (Cadena Iniguez *et al*., 2007). Similarly, a considerable morphological variation is possible in the genotype with respect to fruit shape, colour and size (Dey *et al*., 2006).

Character spine is positively and negatively both associated with texture of the pulp (0.546*) and taste of the pulp (-0.596**) at the level of significance $P \leq 0.05$ and $P \leq 0.01$ respectively. The presence or absence of the spines provides the texture of the pulp such as smooth, solid or soft in texture. Similarly, presence or absence of the spines does not affect the taste of the pulp as it is negatively associated and the taste of the pulp could be watery, watery sweet; light sweet, crunchy or less crunchy. Pulp is associated with the texture such as smoothness, solidness, softness of the fruit. So, it may be suggested that presence or absence of the spines over the surface of the fruit does not affect the taste of the pulp.

The presence of spines over the surface of fruit could be related to the lower amount of gibberellic acid because the application of gibberellic acid induce the spine development over the surface of fruit and suggest its dependency at certain level of presence of hormone (Cadena –Iniguez, 2005).

The presence of furrows character on the fruit surface is negatively correlated to the number of days of harvest (-0.613*) at the level of significance and probability of $P \leq 0.05$. It may suggest the presence of furrows over the surface of the fruit does not form or not related to the number of days to maturity of the fruit. The possible reason may be the genetic makeup of the genotype and environmental effect. The genotypes may differ among the landraces and possible wide geographic distributions (Dey *et al*., 2006).

It could be reported that deep dark green leaves were associated with the development of deep dark green colour of the fruits while plants with light green leaves develop light green fruits by observation (Figure 2).

The other important character of fruit was recorded that fruits start germination when still attached with their parent plant and most probably viviparous (Figure 3).

Single seeded large size of the endosperm (Figure 4 A-D) and root growth (Figure 4 E-H) from the seed of the fruit reported (Figure 4).

It was observed that genotypes develop flowering in the months of April, May and June, beginning and starting of fruiting in May, June, and July while ends of fruiting occurs in the month of December and January by observation (Table 11).

The morphological variations among the genotypes based on the qualitative characters were reported in other species and findings are in agreement with those of others (Shankar and Synrem, 2012; Abdoulaye *et al*., 2016).

B) FRUIT QUANTITATIVE CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

MATERIALS AND METHODS

Collection of fruit samples of Sechium edule genotypes

Sechium edule genotypes were collected from the different parts of Kigwema village of Kohima district, Nagaland. The mean value of the latitude (25.61 $^{\circ}$ N), longitude (94.35 $^{\circ}$ E) and altitude (1538 masl) of collection site was recorded. Fruit

samples were collected randomly from the habitat (shrub) of the *Sechium edule* (Figure 5 A and B).

Quantitative data collection

Quantitative data on fruit characters were recorded and includes fruit length, FL (cm), fruit width, FWd (cm), fruit circumference, FC (cm), fruit area, FA (cm^2), fruit weight, FWt (g), fruit volume, FV (cm^3) and fruit ridges, FR (count) (Figure 6).

Statistical analysis

The data were subject to statistical analyses such as descriptive statistics, correlation, analysis of variance (ANOVA), Pearson's correlation and regression.

Both qualitative and quantitative data were analyzed for their relationship using Spearman's rho correlation, Paired Group Euclidean Distance and principle component analysis (PCA).

Also, phenotypic variance (Vp), genotypic variance (Vg), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2), repeatability (R) and environmentability (E) were determined.

Mathematical calculation

FC= $2\pi r$

FA= $4\pi r^2$

FV= $\frac{4}{3}\pi r^3$

Genetic analysis,

Genotypic variance

Vg = Mean square (between) - Mean square (group) ÷ Number of replications (r)

Where,

Vg = genotypic variance

r = number of replications

Phenotypic variance

$$Vp = Vg + Ve/r$$

Where, Vp = phenotypic variance

Vg = genotypic variance

Ve = environmental variation

r = number of replications

Genotypic coefficient of variation (GCV)

Burton (1952)

$$GCV = \sqrt{Vg}/ X * 100$$

Where,

Vg = genotypic variance

X = mean

Phenotypic coefficient of variation

(Burton 1952)

$$PCV = \sqrt{Vp}/ X * 100$$

Where,

Vp = phenotypic variance

X = mean

Broad Sense Heritability (H^2)

(Falconer and Mackay, 1996)

$H^2 = Vg/Vp * 100$

Where,

Vp = phenotypic variance

Vg = genotypic variance

Vg*e = variation due to genotype and environment interaction

Repeatability = $\frac{Vg+Vg \times e}{Vp}$ (Falconer,1981)

Environmentability = 1- Heritability

RESULTS AND DISCUSSION

Descriptive statistics

The maximum mean value of each character was compared and found that fruit length, FL has the highest mean ± S.E. (17.5 ± 0.223) for the genotype 13. Mean value were recorded for Fruit width, FWd (10.2 ± 0.2), Fruit circumference, FC (25.4 ± 10.244), Fruit area, FA (2.044 ± 3.987), Fruit weight, FWt (6.042 ± 33.435), Fruit volume, FV (2.751 ± 8.075), and Fruit ridges, FR (6.2 ± 0.2) respectively.

The high mean value of the characters indicated that the characters are successfully reached towards maturity without any hindrance towards their growth

and development or in the climatic conditions available for their morphological or phenotype expression.

Soil and climatic factors such as soil pH, light intensity, rainfall and soil nutrients provided favorable condition towards the particular characters and helped in growth and development of character as well as the genotypes.

Genotype 13 is well adapted for its fruit length, FL in available soil and environmental factors, Genotype 8 and 12 well supported for its fruit width, FWd. Also, Genotype 8 has favourable condition for its FC, FA and FV. Genotype 5 was supported for its fruit weight, FWt and Genotype 9 for its fruit ridges, FR.

Overall, Genotype 8 was supported very well for the fruit characters in terms of FWd, FC, FA and FV growth and development.

The low mean value was recorded for characters as FL (1.246 ± 0.509), FC (18.8 ± 0.374), FA (1.123 ± 4.425), and FV (1.121 ± 6.807) for genotype 4, FWt (1.568 ± 10.116) for genotype 1 and FR (3.6 ± 0.244) for Genotype 1 and FWd (7.2 ± 0.2) for both genotype 1 and 4 respectively. Lower mean value could be related with the less growth and unfavorable conditions prevailing for the particular character and creates some hindrance towards their growth and development towards their expression (Table 12).

High variations recorded for all the characters for the genotypes, possibly because of some soil, a range of soil pH 7.3 - 7.9, environmental and climatic factors. There was a report on the high variation in the FL and FWt reached upto 45 cm and 1 kg respectively from Costa Rica and Mexico (Engels, 1983). The variation was not

observed in the present study. Overall, other variations recorded for the characters are in agreement with the earlier reports (Pakhrou *et al*., 2017; Yatrib *et al*., 2017; Rix *et al*., 2015; Singh *et al*., 2014). Although morphological characters are generally employed to estimate genetic diversity but morphological characters have its own limitations and these characters are heavily influenced by the environmental conditions and climatic factors influencing the growth and development of the species (Cadena Iniguez and Arevalo Galarza, 2011).

The characters were computed for their variability using standard deviation (S.D.) and the characters such as FL, FWd, FC and FR measured deviations from mean ≤ 1 suggest the distribution of data linearly over or very near to the measured linear line, while characters FA, FWt and FV measured deviations from mean > 1 and represented that data are not distributed linearly and stretched very far from the measured linear line.

The possibility of stretched distribution of the data from the linear line is due to the possible human error in the measurement as well as various shapes of the fruits at different growth stage of the particular character. This may lead to the probability of error and stretched data collection.

The coefficient of variation (C.V.) showed similar trend as in S.D. measurement that is low in FL, FWd, FC and FR than FA, FWt and FV (Table 12).

The lower C.V. for the characters suggest that their relative variability is very low than FA, FWt and FV, while higher C.V. for the characters FA, FWt and FV suggest the measurement of fruit characters, FA , FWt and FV are highly variable. The possible reason may be as measurement has taken on different fruit sizes, which

may be mature or immature which could be possibly correlated with high relative variability in the characters (Abdoulaye *et al*., 2016; Bahloul *et al*., 2014; Metougui *et al*., 2017).

Pearson correlation

The quantitative fruit characters were analyzed for Pearson's correlation to establish a relationship among the characters and their role for growth and development. All the characters were positively and significantly correlated with each other at the level of probability of significance $P \leq 0.05$ and $P \leq 0.01$ (2 tailed) (Table 13).

Fruit length, FL was positively and significantly associated with FWd (0.642*), FC (0.587*), FA (0.575*), FV (0.566*) and genotypes (0.598*) at probability level of significance $P \leq 0.05$ and FWt (0.788**) at $P \leq 0.01$ respectively.

Fruit character, FL presented an important character which is associated with other characters of fruit and genotypes positively and significantly except FR.

Fruit width, FWd was positively and significantly associated with FC (0.973**), FA (0.972**), FWt (0.78**) and FV (0.973**) at the probability level of significance $P \leq 0.01$ and genotype (0.549*) at probability level of significance $P \leq 0.05$ respectively.

Fruit character, FWd association with other characters indicates its involvement in increase of FC, FA, FWt, and FV.

FWd presented an important character which is associated with other characters of fruit and genotypes positively and significantly except FR.

Fruit character, FC was positively and significantly associated with FA (0.99**), FWt (0.799**), FV (0.997**) at probability level of significance $P \leq 0.01$ and FR (0.573*) at probability level of significance $P \leq 0.05$ respectively.

FC may be directly involved as positive association was observed for FA, FWt, FV and FR and suggest that increase in FC would directly affect FA, FWt, FV and FR of the fruits.

FA associated with FWt (0.796**), FV (0.999**) at $P \leq 0.01$ and FR (0.564*) at $P \leq 0.05$ respectively.

FWt correlated with FV (0.796**) and FR (0.714**) at $P \leq 0.01$.

FV is directly associated with FR (0.557*) at $P \leq 0.05$.

Fruit characters FL and FWd (only two characters) showed positive and significant relationship with genotypes at probability level of significance $P \leq 0.05$.

The positive and significant correlation indicates that increase or decrease in a character or genotype would affect the other character or genotype simultaneously. Both characters will move simultaneously depending on the increasing or decreasing value, if value decreasing both the characters will decrease, and if value increases both the characters increase together.

Overall, the positive and significant associations recorded for the characters are in agreement with the earlier reports (Alim *et al*., 2016; Higaki *et al*., 2016; Brophy *et al*., 2018; Glover, 2000; Jacques and Vissenberg , 2014; Mitchison, 2016; Julien and Boudaoud, 2018; Sapala *et al*., 2019; Zhang *et al*., 2011).

Pearson's correlation for the genotypes based on the fruit characters suggested genotypes are positively and significantly associated with each other at both the probability level of significance $P \leq 0.05$ and $P \leq 0.01$ respectively (Table 14).

Analysis of variance (ANOVA)

Fruit characters showed good amount of variations (mean square value) at the probability level of significance $P \leq 0.05$. FL was recorded maximum variation with mean square value of 17.354 and minimum value was recorded for FA with mean square value 0.111 and could be correlated with less amount of variation in the FA (Table 15).

ANOVA for the fruit characters suggest that FA for all the genotypes approximately similar as amount of variation in the character recorded low.

ANOVA for the fruit characters are in agreement with the earlier reports (Khan *et al*., 2018).

Regression

Fruit characters were regressed against the genotypes to record the variations in the characters and for the genotypes. The maximum variation was recorded for the character FL with R^2 value (0.347) and regression line y=0.586x+6.908. Similarly, the minimum variation was recorded for the character FR with R^2 value (0.103) and regression line y=0.059x+4.232 (Table 16).

The maximum variation was recorded for the character FL with R^2 value (0.347) and regression line y=0.586x+6.908. Similarly, the minimum variation was

recorded for the character FWd with R^2 value (0.103) and regression line y=0.059x+4.232 (Figure 7).

Spearman's rho correlation

Spearman's rho correlation was analyzed for the both qualitative and quantitative characters for its significant association either positive or negative with each other (Table 17).

Character colour is significantly and positively associated with the other character shape at the probability level of significance $P \leq 0.01$.

The association suggests that increase in a definite shape character, there will increase in colourcharacter at maturity. The character colour may increase from deep dark green, dark green, green, light green or yellowish white, simultaneously shows its dependency on spheroid shape, small spheroid shape, intermediate spheroid shape, spheroid shaped, pyriform shape and small obovoid shape. Overall, all type of shapes have some colour at maturity.

Spines, positively and significantly associated with texture (0.587*) at $P \leq 0.05$, but negatively correlated with taste (-0.751**) at $P \leq 0.01$ respectively.

The positive correlation of the spines with texture could be correlated that fruit may have any texture smooth, solid, or soft and there is no much effect of the distribution of the spines over the surface of fruit. But, on the other hand, negative correlation between taste and spines indicates that taste of the fruit may change if there is strong distribution of the spines over the surface of the fruit. The taste of the fruit may be watery, watery sweet or watery light sweet by the absence or presence of the spines over the surface of the fruit.

Furrows are negatively correlated with harvest (-0.683**) at $P \leq 0.01$. The presence of furrows over the surface of fruit doesn't mean it becomes mature. There is no correlation between the harvest of fruit at maturity and the number of furrows over the surface of fruit.

Taste is negatively correlation with the harvest (-0.550*) at $P \leq 0.05$ suggest that taste may not be good at the time of harvest or at maturity of the fruit.

Quantitative characters such as FL, FWd, FC, FA, FWt, FV, FR and genotypes have not shown any dependency or association over the qualitative characters of the fruit.

The quantitative characters are positively and significantly correlated with each other at both level of significance $P \leq 0.05$ and $P \leq 0.01$ respectively. Characters showed a similar pattern of association as in Pearson's correlation (2 tailed) reported.

Spearman's rho correlation matrix for qualitative and quantitative charactersof fruit divides in two different independent groups of the characters. Both the groups are involved in the growth and development of fruit as well as genotypes independently.

Paired Group Euclidean Distance

Qualitative and quantitative characters of fruitwere analyzed for Paired Group Euclidean Distance. The analysis was based on both the qualitative and quantitative data (Figure 8).

The co-phenetic correlation was recorded 72.6 %, suggest that genotypes are approximately similar with each other and only 22.84 % variation was recorded for characters and genotypes.

Similarity or dis-similarity among the characters or genotypes may be based on many factors including soil factor, climate factorand any physical or chemical factor.

Paired Group Euclidean Distance classified genotypes into a single large group except genotype 4.

Large group sub-classified into two sub-groups I and II with 5 and 8 genotypes each.

A single large group suggests approximately similar especially at morphologically but sub groups indicate towards some differentiation either at the level of genetic constitution or environmental level among the genotypes.

Principle component analysis (PCA)

PCA is a mathematical procedure that transformed a number of correlated variables into a number of uncorrelated variables. The transformation of variables occurs from large number of correlated variables to a small number of uncorrelated variables which is called Principal Component (Esposito *et al.,* 2007). The first principal component accounts for as much of the variability in the data as possible and each succeeding components accounts for as much of the remaining variability as possible. Moreover, the objective of PCA to discover or to reduced the dimensionality of the data sets and to identify new meaningful variables (Jollife, 2002).

The qualitative and quantitative characterswere involved in PCA for component 1 and 2, which indicates the maximum variability in component 1 and the

remaining possible variation in component 2 and their loadings are represented in figure 9 (A and B).

Eigen values are recorded for all the possible principal components which indicates the percent of variations available in the fruit character and it has being observed that component 1 has shown 58.5 % of total variation of the fruit traits and component 2 has shown 14 % of total variation of the fruit trait. All together Principle Components 1 and 2 had shared 72.6 % of total variations observed in fruit characters (Table-18).

To identify, the involvement of the components for the total variations of the fruit character either qualitative or quantitative a scree plot was drawn which showed 2 components has taken maximum variations in the fruit character (Figure 10).

Principle Component graph analysis indicates G4 as the only genotype which suggested maximum variations for the fruit characters in component 1 and component 2 of PCA. Along with G4, G3, G6, G7, G10, G9, G11, G1 and G2 showed a little higher variations which are involved in the variations of the fruit trait in component 1 and 2 in Principal Component Analysis. The other genotypes, G12, G13, G8, G14 and G5, these genotypes are also involved in the variation of the trait of fruit. But their involvement is less as compared to other genotypes (Figure 11). The Principal Component Analysis and multivariate statistical methods having successfully used to classify qualitative and quantitative variations in many crop species (Chandran and Padya, 2000; Cravero *et al*., 2002; Rosso and Pagano, 2005; Bhargava *et al*., 2007; Harris, 2001).

Principal Component Analysis measures the contribution of each component or independent impact of a particular character to the total variants observed in a given population in relation to the character of interest to the breeder. PCA has been used to determine the optimum numbers of clusters, to compliment cluster analysis and to investigate patterns of genetic diversity (Thompson *et al*., 1998; Lombard *et al*., 2000; Mohammadi and Prasanna, 2003). PCA was used to reduce the complexity of the data set and to partition the observed variation within characters based on their degree of importance (Ringner, 2008).

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

Phenotypic coefficient of variance was recorded higher for all the characters of fruit than GCV.The estimation of PCV was slightly greater than the corresponding GCV for all characters of fruit indicating the role of environment in their expression of these characters. The present finding for GCV and PCV are in agreement with those of others (Chhetri *et al*., 2019; Singh *et al*., 2018; Rahman *et al*., 2016) (Table 19).

Phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve) ***and interaction of genotype and environmental variance (Vgxe)***.

ANOVA was computed for the quantitative characters and reported environmental variance (Ve) was high for the characters suggested high effect of environment in the variations of the characters therefore it is represented as Ve = high. Since environmental variation is high, the interaction of genotype with environment variations is found to be too high. Therefore, the effect of environment

from the genotype will be high and environmental effect may cause the genotype variations through the variations in fruit characters (Table 20).

Similarly, phenotypic variance (Vp) was recorded little high than the genotypic variance (Vg) and high environmental variance and genetic constitution may be the reason for the high variation for phenotypes of the characters.

Heritability (H^2), Environmentability (E) and Repeatibility (R)

High heritability and repeatability was recorded for the characters than the environmentability (Table 20).

High heritability indicates selection of characters is easy for the transmission of character from parent to offspring (Singh, 2011; Sabesan *et al*., 2009).

The high heritability of the genotypes suggests that all the traits of fruits are highly influenced by the genetic make-up of the genotype and because of the genetic make-up of each and every genotype differs from each other.

The character which is constantly within individuals as well as differing between individuals corresponds to selection under repeatability.

The high heritability and estimated repeatability may be correlated with the overestimated or very high values for genotype and environment interaction, environmental effect on genotype and phenotype.

CONCLUSION

FRUIT QUALITATIVE CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

The morphological diversity evolution such as variation in colour, spines, shape, ridges, etc. provides valuable information to be added in the future to molecular diversity studies for the conservation of genetic resources (Kumar and Gupta, 2019).

FRUIT QUANTITATIVE CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

***Descriptive statistics*:** The descriptive statistics for quantitative fruit characters reveals high degree of diversity among genotypes at present which can be further explored for crop improvement through molecular techniques, provides an opportunity to enhance its growth and productivity.

Pearson's correlation (2 tailed): Fruit characters FL and FWd showed positive and significant relationship with genotypes at probability level of significance $P \leq 0.05$. Genotypes are highly correlated with each other at both the probability level of significance $P \leq 0.05$ and 0.01 respectively. Spearman's rho correlation matrix for qualitative and quantitative characters of fruit divides in two different independent groups of the characters.

Analysis of variance (ANOVA): ANOVA for the fruit characters suggest that FL was recorded high variation and FA was recorded low variation for all the genotypes and

FA for all the genotypes approximately, similar as amount of variation in the character recorded low.

Regression: The maximum variation was recorded for the character FL with R^2 value (0.347) and regression line y=0.586x+6.908. Similarly, the minimum variation was recorded for the character FWd with R^2 value (0.103) and regression line y=0.059x+4.232.

Paired Group Euclidean Distance (PGED): A single large group suggests approximately similar especially at morphologically but sub groups indicate towards some differentiation either at the level of genetic constitution or environmental level among the genotypes.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 72.6 % of total variations observed in fruit characters.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters of fruit than GCV.

Genetic variances (Vp, Vg, Ve and Vg×e): Phenotypic variance (Vp) was recorded little high than the genotypic variance (Vg) and high environmental variance and genetic constitution may be the reason for the high variation for phenotypes of the characters.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and repeatability was recorded for the characters than the environmentability.

CHAPTER-3

SOMATIC CHROMOSOME COUNT, KARYOMORPHOLOGICAL BEHAVIOUR AND POLLEN MORPHOLOGICAL STUDIES OF *SECHIUM EDULE* GENOTYPES

INTRODUCTION

Sechium genus first published in cucurbitaceae monograph in 1881 suggested that, the genus is monospecific (with a single species) and represented as *Sechium edule* (Cogniaux, 1881). In later years, literature survey supported that it is monopecific and there is no reports of more than one species in the genus (De Donato and Cequea, 1994). The most accepted term for *Sechium* is 'Chayote' worldwide. Historically, the monospecific genus, *Sechium* was originally recorded from Jamaica (Browne, 1756). Since then this monospecific genus has been classified variously by authors. Initial classification of the genus has been recorded as *Sicyos edulis* and *Chocho edulis* simultaneously (Adanson, 1763; Jacquin, 1788). The genus *Chocho* has been changed to *Chayota* and redesignated as *Chayota edulis* (Jacquin, 1788). Later on, *Chayota edulis* redesignated as *Sechium edule* (Swartz, 1800). At present, the species is known as a combination of both Jacquin and Swartz i.e. *Sechium edule* (Jacq.) Sw.

The monospecificity of the genus had shattered, when other species were reported for the genus by various authors from different regions during 1900s. A few of them are *S. edule* sub spp. *edule*, *S. edule* sub spp. *sylvestre*, *S. chinatlense*, *S. compositum* and *S. hintonii* (Goldblatt, 1990; Singh, 1990; Mercado *et al.*, 1993;

Mercado and Lira, 1994). Recently, a new species *Sicyos angulatus* L. has been reported for Indian flora and *Sechium mexicana* for Mexico respectively (Thakur, 2016; Lira and Nee, 1999). The reported species were morphologically very similar and never verified for the presence of a new species in the genus. This may be considered as a big lacuna for the genus which could not be carried out to identify the presence of new species at the molecular level.

Cytologically, genus *Sechium* was tried to differentiate in to more than one species. Many authors reported the different chromosome count for the genus *Sechium* with base chromosome number x=12, 13, 14, and 15. The base chromosome number x=11 has also been reported for the genus by Singh (1990). On the basis of reported base chromosome number and chromosome count, it has been suggested that genus *Sechium* may be categorized into 5 species (*S. edule* sub ssp. *edule*, *S. edule* sub ssp. *sylvestre*, *S. chinatlense*, *S. compositum* and *S. hintonii*) and two new species *S. mexicana* and *Sicyos angulatus*. The chromosome count and base chromosomes number suggest that it remains unresolved and needs thorough examination cytologically. Therefore, at present, this is an attempt to extend chromosome information on the genus *Sechium*.

The number, structure, and behaviour of chromosomes is of great value in taxonomy with chromosome number being the most widely used character for the relationships and classification of organisms using comparative studies of chromosomes, cell behaviour and also their behaviour during mitosis and meiosis.

Similarly, pollen mother cells, PMCs (2n) undergo meiosis to produce microspores (n) or male spores or male portions are powdery yellowish in colour,

found in the anther and responsible for reproduction in the plant. They are the microscopic structure varying in size and shape. It consists of outer exine and inner intine coat. Exine is rough and spiky in nature and contains sporopollenin, whereas the intine is smooth, made up of cellulose. Presence or absence of pollen grains may change in amount of production at harvest and also allows the plant to survive in a specific environment. They can be used to determine the mechanism of pollination which is a source zone of the pollinators.

Therefore, a diverse morphological characters and incomplete information on the chromosome count as well as pollen characters were statistically analysed for its morphological features which may be useful for better sustainability, conservation and identification.

MATERIALS AND METHODS

In the study, *Sechium* fruit samples, a shrub climber, were collected randomly from Kigwema village, Kohima, Nagaland (India) at an average altitude (1538 masl), latitude (25.61 $^{\circ}$N) and longitude (94.35 $^{\circ}$E). Mitosis was studied from the secondary root tips of germinating fruits. Root tips of 2 - 3 cm in length were pre-treated with α-bromonaphthalene at 6 ± 2 $^{\circ}$C for 3 – 4 hours followed by overnight fixation (3:1 ethanol-acetic acid) and preservation (70 % ethanol). The root tips were hydrolysed with 1 N HCl for 10-15 min at about 50-60 $^{\circ}$C. The root tips were squashed in 2 % acetocarmine. Three somatic countable chromosome slides under 100x (emersion oil) were photographed using digital Motic BA210 microscope and recorded. Somatic chromosome count of each genotype reported from the chromosomes (Figure 12 - 27)

were analysed for their total length (TCL), Inter and Intra chromosomal indices and chromosome classification for each genotype and tabulated in Table 21-24.

Further, flower buds were collected and fixed in 6:3:1 ethanol: acetic acid: chloroform fixative for overnight. Anthers were excised and processed in 2 % acetocarmine for pollen morphological characters. Photographs of pollen morphological characters were clicked at the magnification of 100X (immersion oil) using Motic BA 210 microscope (Figure 28-29).

Spores diameter (µm) was measured with digital scale attached with Motic BA 210 microscope and with the help of spore diameter computed radius (d/2), circumference ($2\pi r$) and area (πr^2) (Table 25).

CHROMOSOMAL STATISTICAL ANALYSIS

Total chromosome length (µm) were measured for the genotypes with the scale bar of 10 µm using ImageJ software and further computation was attempted through windows MS-Excel and with the help of standard formulas for inter and intra chromosomal differences among the chromosome complement of genotypes.

Rec $= \frac{Total\ sum\ length\ of\ each\ chromosome \div Longest\ chromosome}{Total\ number\ of\ chromosomes}$ x 100 (Greilhuber and Speta, 1976)

$A_2 = \frac{Standard\ deviation\ of\ chromosome\ length}{Mean\ chromosome\ length}$ (Romero – Zarco, 1986)

$CV_{CL} = \frac{Standard\ deviation\ of\ chromosome\ length}{Mean\ chromosome\ length}$ x 100 (Lavania and Srivastava, 1999; Paszko, 2006)

Disparity Index (Dis.I) $=\frac{Longest\ chromosome-Shortest\ chromosome}{Longest\ chromosome+Shortest\ chromosome}$ x 100 (Mohanty *et al*., 1991)

VRC = ∑ Total Length of chromosome / n (Dutta and Bandyopadhyay, 2014)

Where, n=somatic chromosome count

Gradient Index = $\frac{Shortest\ chromosome}{Longest\ chromosome}$ x 100 (Lavania and Srivastava, 1992)

Chromosome volume = $\pi r^2 h$ (where, h = total length of chromosome) (Toijam *et al*., 2013)

RESULTS

The chromosome numbers for each genotype, smallest and longest chromosome length, the value of Stebbin's classification, Disparity index, Gradient Index, VRC and chromosome volume were recorded (Table 21-24). Somatic chromosome count reported in Figure 12-27.

The chromosome number of G1 is 2n = 26. The smallest chromosome length is 0.501 µm, the longest chromosome length is 0.764 µm with the mean chromosome length 16.733 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 20.79, 65.575, 0.643 and 16.708, respectively.

The chromosome number of G2 is 2n = 26. The smallest chromosome length is 0.583 µm, the longest chromosome length is 1.043 µm with the mean chromosome length 20.919 µm. The value of Stebbin's classification, Disparity Index, Gradient

Index, VRC and chromosome volume are 1A, 28.29, 55.896, 0.804 and 20.877, respectively.

The chromosome number of G2 is 2n = 32. The smallest chromosome length is 0.295 µm, the longest chromosome length is 0.855 µm with the mean chromosome length 18.099 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1B, 70.289,31.929, 0.565 and 18.062.

The chromosome number of G2 is 2n=4x = 52. The smallest chromosome length is 0.342 µm, the longest chromosome length is 0.803 µm with the mean chromosome length 28.884 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 40.174, 42.643, 0.555 and 28.826.

The chromosome number of G3 is 2n = 30. The smallest chromosome length is 0.696 µm, the longest chromosome length is 0.343 µm with the mean chromosome length 29.618 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 31.731, 51.824, 0.987 and 29.121, respectively.

The chromosome number of G4 is 2n = 26. The smallest chromosome length is 0.642 µm, the longest chromosome length is 1.113 µm with the mean chromosome length 22.89 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 26.837, 57.681, 0.88 and 22.84, respectively.

The chromosome number of G5 is 2n = 28. The smallest chromosome length is 0.628 µm, the longest chromosome length is 1.086 µm with the mean chromosome

length 23.47 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 26.721, 57.826, 0.838 and 23.423, respectively.

The chromosome number of G6 is 2n = 28. The smallest chromosome length is 0.651 µm, the longest chromosome length is 1.209 µm with the mean chromosome length 26.08 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 30.00, 53.846, 0.931 and 26.037, respectively.

The chromosome number of G7 is 2n = 28.The smallest chromosome length is 0.583 µm, the longest chromosome length is 0.876 µm with the mean chromosome length 16.733 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 20.082, 66.552, 0.725 and 20.279, respectively.

The chromosome number of G8 is 2n = 24. The smallest chromosome length is 0.521 µm, the longest chromosome length is 0.904 µm with the mean chromosome length 16.274 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 26.877, 57.632, 0.678 and 16.241, respectively.

The chromosome number of G9 is 2n = 24. The smallest chromosome length is 0.541 µm, the longest chromosome length is 0.863 µm with the mean chromosome length 16.899 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 22.934, 62.688, 0.704 and 16.865, respectively.

The chromosome number of G10 is 2n = 28. The smallest chromosome length is 0.591 µm, the longest chromosome length is 0.805 µm with the mean chromosome length 19.276 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 15.329, 73.416, 0.688 and 19.237, respectively.

The chromosome number of G11 is 2n = 26. The smallest chromosome length is 0.6 µm, the longest chromosome length is 1.202 µm with the mean chromosome length 23.784 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1B, 33.407, 49.916, 0.914 and 23.736.

The chromosome number of G12 is 2n = 30. The smallest chromosome length is 0.62 µm, the longest chromosome length is 1.097 µm with the mean chromosome length 23.784 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 27.781, 56.517, 0.891 and 26.693.

The chromosome number of G13 is 2n = 26. The smallest chromosome length is 0.568 µm, the longest chromosome length is 0.946 µm with the mean chromosome length 20.009 µm. The value of Stebbin's classification, Disparity Index, Gradient Index, VRC and chromosome volume are 1A, 24.966, 87.925, 0.769 and 19.968.

The chromosome number of G14 is 2n = 28. The smallest chromosome length is 0.603 µm, the longest chromosome length is 0.939 µm with the mean chromosome length 21.956 µm. The value of Stebbin's classification, Disparity Index, Gradient Index , VRC and chromosome volume are 1A, 21.789, 64.217, 0.784 and 21.912.

Intrachromosomal asymmetry index could contain more number of acrocentric or telocentric chromosomes than the metacentric and submetacentric chromosome

which could be the result of change in position of centromere. The change in centromere position brings the rearrangement in the chromosomes and may lead to increase in karyotype asymmetric percent (Rane *et al*., 2012).

The intra chromosomal asymmetry depends on exact identification of the centromere and the chromosomal morphology and not only on the chromosome size. The extreme symmetry (Ideal karyotype A) or asymmetry (Ideal karyotype C) of karyotype measure in nature (Stebbin, 1971). However, the present analysis indicates an extreme symmetric karyotype (1A) among the genotypes except genotypes G2 and G11 and may be classified as ideal karyotype B of Stebbin's classification (Table 22).

The presence of asymmetric karyotype may be the result of chromosomal structural changes particularly centric fusion or fission may lead to the symploid or agmatoploid chromosome rearrangement during the course of evolution in plant species. The centric fusion and fission is also suggested as cause of frequent disploidy or pseudoaneuploidy among plant genera or species (Peruzzi and Altinordu, 2014).

The intrachromosomal index values suggested that karyotypes of two genotypes deviated from symmetric to asymmetric and are in agreement with the hypothesis of Stebbin's classifications (1971). According to the hypothesis, asymmetric karyotypes most probably, being originated from the symmetrical karyotypes over a period of time and due course of evolution. Similar work has been reported earlier and in agreement that primitive member with symmetrical karyotype gives rise to advance members with the asymmetrical karyotype (Levitzky, 1931; Kumar and Kumar, 2014).

Both Disparity index (DI) and Gradient index (GI) parameter were used for the evaluation of karyotype symmetry and could be an indication of nature of

evolutionary process occurring or occurred among the plant genus or species. Also, could be used to show the trend of evolution has taken place in cyto-types (Sinha, 2018).

Comparatively, lower and higher value of DI and GI suggested symmetrical nature of the karyotype among the genotypes except G2 with 2n=32 and 52 and supports Stebbins hypothesis. Therefore, both DI and GI showed high degree of symmetry which may lead to the lesser degree of chromosomal variation and evolution (Stebbin's, 1971).

Value of relative chromatin (VRC) was recorded maximum (0.987) for the G3 with 2n=30 and minimum (0.565) for G2 with 2n = 32. Similarly the chromosome volume was recorded maximum (29.121) for G3 with 2n = 30 and minimum (16.241) in G8 with 2n = 24. More chromosome volume could be correlated with high chromatin material in the genotype G3.

Chromosome size was classified into small and minute types of chromosomes for the genotypes according to Kutarekar and Wanjari (1983). Chromosome size classification was used as minute (less than 1 µm), small (1-3 µm), medium (3-5 µm) and large (more than 5 µm) (Table 23).

The chromosome size of chayote varies from 0.7 to 0.9 µm, reported by Sanjappa, 1979 (Cadena- iniguez *et al*., 2007). The Chromosome of chayote (*S. edule* ssp. *edule*) n=12, 2n=24 (Sugiura, 1938, 1940; Sobti and Singh, 1961; Goldblatt, 1981, 1984, 1990), n=13, 2n=26 (Goldblatt, 1990), 2n=28 (Giusti *et al*., 1978), 2n=22 (Singh, 1990). In wild population, n=12 (Palacios, 1987) and in *Sechium edule* ssp. *sylvestre* 2n=24 (Palacios, 1987) and n=13 (Mercado *et al*., 1993; Mercado and Lira,

1994). In *Sechium chinantlense*, 2n=30 was reported (Mercado *et al.*, 1993). Similarly, *Sechium compositum* and *Sechium hintonii*, n=14 (Mercado *et al.*, 1993; Mercado and Lira 1994) and *Sechium edule*, 2n=28 (De Donato and Cequea, 1994) were reported. Hybridization between cultivated *Sechium edule* and *Sechium chinantlense* had failed due to the differences in their chromosome number (Saade, 1996).

Interchromosomal Index (A_2 index) was computed for all the chromosomes of genotypes and produces a value close to zero. The index near to zero indicates the conservation of chromosome size in the karyotype complement. Low variation in the chromosomes size of a karyotype suggests interchromosomal asymmetry remains constant. Similarly, interchromosomal Index (Rec index) measures the resemblance between the chromosomes and the average degree of symmetry over the whole karyotype (Huziwara, 1962). The summation of Rec index value recorded high for the chromosomes. The high value of Rec index indicates heterogeneity in the chromosomes of a karyotype complement (Table 24).

In the present study, *Sechium edule* reports somatic chromosome count 2n=2x=32 and 2n=4x=52 (genotype 2) and perhaps first report for *Sechium edule*. Chromosome numbers are significantly different for the haploid chromosome count in the range of x=12-16 for the species except n=x=11 and 16 reported earlier and may need rigorous and precise confirmation for the chromosome count. Somatic chromosome counts are in agreement and comparable according to the earlier reports in other genotypes (Bisognin, 2002; Lira and Nee, 1999; Newstrom, 1991).

The presence of differences, if any, in cultivated or wild forms of the *Sechium*, possibly could have been originated through the chromosomal evolutionary factors in due course of time with the help of primary, secondary, agmatoploidy, symploidy, dysploidy or pseudoaneuploidy evolutionary factors and needs to be verified through cytological and molecular techniques.

Similarly, *Sechium edule* suggested ploidy nature 2n=4x=52 (genotype 2) of the species. Ploids are not reported earlier in the *Sechium edule* and most probably this is the first report for the species. The findings of ploidy suggest towards the whole genome content change, diversification, evolution and speciation in the genus. Also, the fact that to establish a new species from the pre-existing ones through evolution and diversification required reproductive or genetic isolation from the progenitors through various evolutionary events of primary, secondary and dysploid alterations of chromosome numbers such as chromosome fusion, fission, deletion, duplication, inversion, translocation, rearrangements and ascending or descending dysploidy. In the presented work, origin, diversification, genetic isolation or possibility of interbreeding of tetraploid genotype 2 needed to be explored.

Further, mean diameter was found highest in G12 with 76.485 µm, followed by G13 (75.44 µm) and G11 (74.60 µm) and the lowest mean diameter was recorded in G7 with 55.705 µm. Genotype G12 has the highest value of the radius, area and circumference with 38.24 µm, 4591.614 μm^2, 240.147 µm and the lowest radius, area and circumference in G7 with 27.85 µm, 2438.454 μm^2 and 174.898 µm (Table 25).

Spores morphology indicates that spines are the characteristic feature of the all type of spores, circular in shape, both exine and intine spore layers and germ pores (dispersal unit) present in genotypes (Figure 28-29).

It has been reported that spore size in *Sechium edule* ranged from 55-76 µm which supports the present result of spore size. Spores outer layer, exine contains sporopollenin a water resistant substance and inner layer, intine contains cellulose and pectin. It is also reported that pollens are colpate with longitudinal furrow in the outer layer exine of spores (Lira and Nee, 1999). Pollen size may vary and depends on certain factors such as hydration, chromosome number, genetic variation in plants and environmental factors. The present results of spore morphology are in agreement with the earlier reports (Lira and Nee, 1999). A few reports were available on the origin and evolution of cultivated cucurbits and the evidence suggested Mexico, Central America and Guatemala as the origin of centre for this crop. Earlier, *Sechium edule* was considered as monotypic and native to New World, but now it includes as many as eight species and cultivated throughout tropical and subtropical regions of the world and not explored extensively (Newstrom, 1990). The report of new species gives a hope for the presence of more species in the genus, *Sechium*. A very few or negligible reports are available on genus *Sechium* from India (Sanwal *et al*., 2008; Kapoor *et al*., 2014; Jain *et al*., 2015)

The genus *Sechium* remains very poorly known cytologically and for understanding the inter-relationship amongst different *Sechium* species proper chromosome count is important.

CONCLUSION

Genotype 2 was recorded with 2n=2x=32 and 2n=4x=52, new chromosome count for the *Sechium* and provides hope for the presence of more species in the genus. Spore sizes are in the range of earlier reports.

CHAPTER-4

STUDY OF MALE AND FEMALE FLOWER CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

INTRODUCTION

Flower is a seed bearing part surrounded by colourful corolla with green calyx extended on pedicel (stalk that holds individual flower) attached with stem or peduncle (main stalk of inflorescence). Among the various other parts of the plant, Flower of the respective plant is considered to be the most beautiful God's creation. It serves as the food to pollinators by acquiring nectaries and pollen, provides relief of emotions and beautifies the environment, bears fruits and vegetables for the survival of the fruit or vegetable dependent creatures including human beings. Flower facilitates reproduction in the plants (Guitian *et al*., 1997; Thomas *et al*., 2020).

Sechium edule flowers are specialized with the presence of nectaries at the base of the flower in both male and female which leads to the attraction of pollinators The anther of the male flower is fused when young but become unfused when attain maturity. The stigma of the *Sechium* flower is as big as the fused anther but it is found only in the female flower separately. Flowers maintain five petals which are green to greenish white in colour as well as widely triangular in shape. Similarly, it contains 4-5 sepals which are green in colour and narrow triangular in shape (Martinez - Bauer *et al.,* 2021) (Figure 30).

Female flowers are borne solitarity or in pairs but remains on the same axil from where male flower arises. Male flowers are borne in clusters of 20-30 flowers on peduncle (Figure 30).

Also, flower characters such as occurrences of both pistilate and staminate flowers in the same plant, presence of nectaries and conspicuousness may draw attention towards the more exploration in those characters. Further exploration in flower characters may reveal the certain cluster of closely related plants. It may also generate the occurrence of variability and specific knowledge about the flowers and plants. It may help in recognizing the practicability of concerned flowering plant to understand plant signal and animal senses and more accessibility towards research (Barrera-Guzman *et al*., 2007).

In this chapter, flower characters were statistically analysed to understand their role in yield and mass production, growth, development and association among genotypes using descriptive statistics, Pearson correlation (2 tailed), analysis of variance (ANOVA), regression, paired group euclidean distance (PGED), principle component analysis (PCA). Also, genetic analysis was performed for the purpose using phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve), interaction of genotype and environment variance (Vg×e), phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability (H^2), environmentability (E) and repeatability (R).

MATERIALS AND METHODS

Quantitative data collection

Quantitative data on flower characters were recorded and includes number of calyx, NC (count), calyx length, CL (cm), calyx breadth, CB (cm), number of petals, NP (count), petal length, PL (cm), petal breadth, PB (cm), stamen length, SL (cm),

peduncle length, PDL (cm), pedicel length, PCL (cm), number of anther, NA (count) and carpel length, CL (cm).

Statistical analysis

The data were subject to statistical analyses such as descriptive statistics, Pearson's correlation, and analysis of variance (ANOVA), regression, Paired Group Euclidean Distance and principle component analysis (PCA). Also, phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (H^2), repeatability (R) and environmentability (E) were determined.

Genetic analysis,

Genotypic variance

Vg = Mean square (between) - Mean square (group) ÷ Number of replications (r)

Where,

Vg = genotypic variance

r = Number of replications

Phenotypic variance

Vp = Vg+ Ve/r

Where, Vp = phenotypic variance

Vg = genotypic variance

Ve = Environmental variation

r = Number of replications

Genotypic coefficient of variation (GCV)

(Burton, 1952)

$$GCV = \sqrt{Vg} / X * 100$$

Where,

Vg = genotypic variance

X = mean

Phenotypic coefficient of variation

(Burton, 1952)

$$PCV = \sqrt{Vp} / X * 100$$

Where,

Vp = phenotypic variance

X = mean

Broad Sense Heritability (H^2)

(Falconer and Mackay, 1996)

$$H^2 = Vg/Vp * 100$$

Where,

Vp = phenotypic variance

Vg = genotypic variance

Vgxe = variation due to genotype and environment interaction

Repeatability = $\frac{Vg+Vg \times e}{Vp}$ (Falconer, 1981)

Environmentability =1- Heritability

RESULTS AND DISCUSSION

Descriptive statistics

Mean with standard error (S.E.), standard deviation (S.D.) and co-variance (C.V.) were recorded for both the male and female flower of *Sechium edule* (Table 26-27).

Male flower

The mean value of flower character, NCm ranges from 4.8 ± 0.200 to 5.4 ± 0.244.

S.D. from the mean ranges from 0.447 to 0.836 and C.V. ranges in between 8.277 to 9.312 except genotype 2 and 14.

The mean value of flower character, CLm was recorded high (5.96 ± 0.222) for genotype 5. S.D. from the mean recorded the value less than 1 in genotypes and C.V. range in between 2.454 to 17.745.

The mean value of flower character, CBm was recorded high (2.68 ± 0.135) for genotype 5. S.D. from the mean recorded the value less than 1 in genotypes and C.V. recorded high for the genotype 8 and 10.

The mean value of flower character, NPm was recorded high (5.40 ± 0.244) for genotype 5. S.D. from the mean recorded the value less than 1 and C.V. range in between 8.596 to 17.416.

Both the flower characters, NCm and NPm showed similar trend for their descriptive statistics possibly because of similar count for both the characters.

The mean value of flower character, PLm was recorded high (7.880 ± 0.149). S.D. from the mean recorded the value less than 1 and C.V. recorded high for the genotype 4.

The mean value of flower character, PBm was recorded high (4.78 ± 0.156). S.D. from the mean recorded the value less than 1 except 1.059 (genotype 2) and C.V. recorded high 31.517 (genotype 2).

The mean value of flower character, SLm was recorded high (5.80 ± 0.200). S.D. from the mean recorded the value less than 1 and C.V. range in between 9.312 to 15.194.

The mean value of flower character, PDLm was recorded high (17.80 ± 2.059). S.D. from the mean recorded the value range from 2.509 to 5.674 and C.V. was recorded high for the character.

C.V. was recorded high for the flower character, PDLm in all genotypes.

The mean value of flower character, PCLm was recorded high (3.400 ± 0.200) and in the range of 2.1 to 3.4. S.D. from the mean recorded the value less than 1 and C.V. was recorded high for the character.

C.V. was recorded high for the flower character, PCLm in all genotypes. Similar result was reported earlier and in agreement with Ashfag *et al*., 2014.

The mean value of flower character, NAm was recorded high (5.200 ± 0.200) and in the range of 3.6 to 5.2. S.D. from the mean recorded the value less than 1 except genotypes 5 and C.V. was recorded high for the character.

C.V. was recorded high for the flower character, NAm in all genotypes.

The high mean value of the characters indicated that the characters are successfully reached towards maturity without any hindrance towards their growth and development or in the climatic conditions available for their morphological or phenotype expression.

Soil and climatic factors such as soil pH, light intensity, rainfall and soil nutrients provided favorable condition towards the particular characters and helped in growth and development of character as well as the genotypes.

Lower mean value could be related with the less growth and unfavorable conditions prevailing for the particular character and creates some hindrance towards their growth and development towards their expression.

The characters were computed for their variability using standard deviation (S.D.) and measured deviations from mean ≤ 1 suggest the distribution of data linearly over or near to the measured linear line, while high S.D. estimation from mean represented that data are not closely distributed towards the measured linear line. Some other characters showed very high deviations from the mean.

The possibility of stretched distribution of the data from the linear line is due to the possible human error in the measurement as well as various shapes of the fruits

at different growth stage of the particular character. This may lead to the probability of error and stretched data collection.

Higher C.V. for the characters suggests the measurement of characters is highly variable. The possible reason may be as measurement has taken on different genotypes, could be possibly correlated with high relative variability in the characters.

The descriptive statistics for flower characters reveals high degree of diversity among genotypes which can be further explored for crop improvement through molecular techniques, provides an opportunity to enhance its growth and productivity.

Female flower

The mean value of flower character, NCf was recorded high (5.4 ± 0.244) and ranges from 4.6 ± 0.244 to 5.4 ± 0.244. S.D. from the mean recorded the value less than 1 and C.V. range in between 8.596 to 11.891.

The mean value of flower character, CLf was recorded high (6.88 ± 0.326) for genotype 4. S.D. from the mean recorded the value less than 1 except genotype 2 and C.V. recorded high for most of the genotypes.

The mean value of flower character, CBf was recorded high (2.200 ± 0.200). S.D. from the mean recorded the value less than 1 and C.V. recorded high for most of the genotypes.

The mean value of flower character, NPf was recorded high (5.40 ± 0.244) and in the range of 4.6 to 5.4. S.D. from the mean recorded the value less than 1 and C.V. range in between 8.596 to 11.891.

Both the flower characters, NCf and NPf showed similar trend for their descriptive statistics possibly because of similar count for both the characters.

The mean value of flower character, PLf was recorded high (7.180 ± 0.149). S.D. from the mean recorded the value less than 1 and C.V. range in between 4.357 to 16.637.

The mean value of flower character, PBf was recorded high (4.30 ± 0.126). S.D. from the mean recorded the value less than 1 and C.V. range in between 4.659 to 13.255.

The mean value of flower character, CL was recorded high (5.60 ± 0.244). S.D. from the mean recorded the value less than 1 and C.V. range in between 4.551 to 19.00.

***Pearson correlation* (both male and female flower)**

Both male and female flower characters were analyzed for Pearson's correlation to establish a relationship among the characters and their role for growth and development. Characters were significantly correlated either positively or negatively with each other at the level of probability of significance $P \leq 0.05$ and $P \leq 0.01$ (2 tailed) (Table 28).

Number of calyx, NCm was positively and significantly associated with CBm (0.597*), and NPm (0.977**) at probability level of significance $P \leq 0.05$ and $P \leq 0.01$ respectively. Calyx Length, CLm significantly and positively associated with Calyx Breadth, CBm (0.724**) at $P \leq 0.01$ and also associated with carpel length, CLm (0.533*) of female flower at $P \leq 0.05$. Calyx Breadth, CBm positively associated with NPm (0.556*) at $P \leq 0.05$.

Petal Length, PLm significantly and positively associated with PBm (0.896**), SL (0.653*), NA (0.584*), CLf (0.619*), PLf (0.589*), CL (0.640*), at P ≤ 0.01 and P ≤ 0.05 and negatively with CBf (-0.774**) at P ≤ 0.01. Petal Breadth, PBm positively and significantly associated with NA (0.621*) and CL (0.588*) at P ≤ 0.05 and negatively associated with CBf (-0.892**) at P ≤ 0.01. Stamen Length, SL positively and significantly associated with PLf (0.541*) at P ≤ 0.05 Number of calyx, NCf positively and significantly associated with NPf (0.913**) at P ≤ 0.01. Calyx Length, CLf significantly associated with PLf (0.648*) at P ≤ 0.05. Calyx breadth, CBf negatively but significantly associated with CL (-0.573*) at P ≤ 0.05.

The genotypes were analyzed for Pearson's correlation based on the both male and female flower characters and established that genotypes were positively and significantly correlated with each other at the level of significance P ≤ 0.01 (2 tailed) (Table 29).

The high association for all the genotypes indicates that the climatic factors such as soil factor, water holding capacity, pH of the soil, temperature, light of the given area are suitable for the growth of the genotypes.

The positive and significant correlation indicates that increase or decrease in a character or genotype would affect the other character or genotype simultaneously. Both characters will move simultaneously depending on the increasing or decreasing of value, if value decreasing both the characters will decrease, and if value increases both the characters increase together.

Analysis of variance (ANOVA)

Both male and female flower characters were analyzed for ANOVA and showed a good amount of variations (mean square value) at the probability level of significance $P \leq 0.05$ (Table 30).

PDLm was recorded maximum variation with mean square value of 8.008 and minimum value was recorded for NCm with mean square value 0.048.

ANOVA for the flower characters suggest that NCm has approximately similar or very less variation as amount of variation in the character recorded low than others.

Regression

Both male and female characters were regressed against the genotypes to record the variations in the characters for the genotypes (Table and Figure 31-32).

The maximum variation was recorded for the character CBm with R^2 value (0.201) and regression line y=-0.046x+2.025. Similarly, the minimum variation was recorded for the character NCf with R^2 value (0.001) and regression line y=0.002x+5.001.

Paired Group Euclidean Distance

The genotypes were analyzed for Paired Group Euclidean Distance based on the male and female flower characters data (Figure 33).

Paired Group Euclidean Distance classified genotypes into a 5 sub groups descended from main 3 groups.

Four sub groups are interrelated with each other indicates the almost similar morphological variation based on the flower characters. Although, some differentiation either at the level of genetic constitution or environmental level among the genotypes.

The 5 groups showed co-phenetic correlation of 91.51 % based on morphological characteristics of flower. Genotype 1 remains ungrouped

Principle component analysis (PCA)

Both male and female flower characters were involved in PCA for component 1 and 2, which indicates the maximum variability in component 1 and the remaining possible variation in component 2 and their loadings are represented (Figure 34-36).

To identify, the involvement of the components for the total variations of the flower character a scree plot was drawn which showed 2 components has taken maximum variations in the flower character.

Graphic representation of components 1 and 2 recorded and showed that genotypes 6, 7 and 3 are the farthest from the centre of the axis.

Eigen values are recorded for all the possible principal components which indicates the percent of variations available in the flower character and it has being observed that component 1 and 2 has shown 54.352 % and 20.833 % of total variation of the flower characters. Both Principle Components 1 and 2 had shared 75.185 % of total variations observed in flower characters (Table 33).

The Principal Component Analysis and multivariate statistical methods successfully used to classify flower characters variations in many crop species (Chandran and Padya, 2000; Cravero *et al*., 2002; Rosso and Pagano, 2005; Bhargava *et al*., 2007; Harris, 2001).

Principal Component Analysis measures the contribution of each component or independent impact of a particular character to the total variants observed in a given population in relation to the character of interest to the breeder. PCA has been used to determine the optimum numbers of clusters, to compliment cluster analysis and to investigate patterns of genetic diversity (Thompson *et al*., 1998; Lombard *et al*., 2000; Mohammadi and Prasanna, 2003). PCA was used to reduce the complexity of the data set and to partition the observed variation within characters based on their degree of importance (Ringner, 2008).

Phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve) ***and interaction of genotype and environmental variance (Vgxe)***

ANOVA was computed for the flower characters and reported phenotypic variance (Vp) values are more than the genotypic variance (Vg) which may be considered the effect of environment on the genotypes. Both genetic constitution and environment are responsible for the variation of phenotype and flower character in the genotypes.

The high value for the interaction of genotypes and environment variance (Vg×e) suggest, genotypes may be influenced by both their genetic constitutions as well as environmental factors (Table 34).

Heritability (H^2), Environmentability (E) and Repeatibility (R)

High heritability and repeatability was recorded for the characters. High heritability indicates selection of characters is easy for the transmission of character from parent to offspring (Singh, 2001; Sabesan *et al*., 2009).

The high heritability of the genotypes suggests that all characters are highly influenced by the genetic make-up of the genotype and because of the genetic make-up of each and every genotype differs from each other. The character which is constantly within individuals as well as differing between individuals corresponds to selection under repeatability.

The high heritability and estimated repeatability may be correlated with the over estimated or very high values for genotype and environment interaction, environmental effect on genotype and phenotype.

The value of repeatability indicates that the variation observed in the genotypes most probably due to the differences in the genotypes which are affected by their genetic constitution and environmental conditions (Table 34).

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

Phenotypic coefficient of variance (PCV) was recorded higher for all the flower characters than GCV. The estimation of PCV was slightly greater than the corresponding GCV for all characters indicating the role of environment in their expression of these characters (Table 35). The present finding for GCV and PCV are in agreement with those of others (Chhetri *et al*., 2019; Singh *et al*., 2018; Rahman *et al*., 2016).

CONCLUSION

Descriptive statistics

Male flower

Genotype 6 was recorded with maximum mean for PLm, PBm, SLm and NAm.

Genotype 5 was recorded with maximum mean for NCm, CLm, and NPm.

Genotype 7 was recorded with maximum mean for CBm, and PDLm.

Genotype 2 was recorded with maximum mean for PCLm.

C.V. was recorded high for the flower characters, PDLm, PCLm and NAm for the genotypes.

Female flower

Genotype 13 was recorded with maximum mean for PLf and PBf.

Genotype 12 was recorded with maximum mean for CBf.

Genotype 6 and 3 was recorded with maximum mean for CL.

Genotype 4 was recorded with maximum mean for CLf.

The descriptive statistics for flower characters reveals high degree of diversity among genotypes which can be further explored for crop improvement through molecular techniques, provides an opportunity to enhance its growth and productivity.

Pearson's correlation (2 tailed): Flower character, PLm presented an important character which is associated with most of the characters positively or negatively.

Analysis of variance (ANOVA): ANOVA for the flower characters suggest that PDLm was recorded with high variation.

Regression: The maximum and minimum variation was recorded for the character CBm with R^2 value (0.201) for the male flower and NCf with R^2 value (0.001) for female flower.

Paired Group Euclidean Distance (PGED): PGED suggested the genotypes co-phenetically correlated 91.51 % and genotype 1 remains ungrouped.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 75.185 % of total variations observed in flower characters.

Genetic variances (Vp, Vg, Ve and Vg×e): Genetic constitution and environment are responsible for the variation of flower characters in the genotypes.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters than GCV.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and effect of genetic constitution and environment was recorded for the characters.

CHAPTER-5

STUDY OF VEGETATIVE MORPHOLOGICAL CHARACTERS FOR PHENOTYPIC AND GENOTYPIC CHARACTERIZATION

INTRODUCTION

There are various external characters or phenotype which can be seen by our naked eye and its study could be correlated with physical form or morphology and external structure of the organism. Plant morphology (phyto-morphology) could be differentiated into vegetative (root system and shoot system) and reproductive (flower or inflorescence, fruit and seed) morphology.

Shoot system includes stems are the part of the plant which supports buds and leaves. It helps in conducting water, minerals and food (photosynthates). Generally, stem grow above the ground, but in some cases it grows below ground in the form of tubers or rhizomes with both the nodes and internodes. A node is an area or part of the stem where flower, buds and leaves are located and helps the plant by destroying while pruning the plant. Internode is an area or part of stem between the two nodes and its length depends on the division of the stem, intensity of light, seasonal changes and soil fertility. Leaves are generally attached with the petiole which arises from the node and plays an important role of photosynthesis process for the plant. Leaf shape may be considered to be the evidence for the origin of Angiospermic flowers, for instance, flattened leaf (size dependent) determines the amount of heat absorbance and its effect on environment (Adrienne *et al*., 2011).

External morphology of plant also known as plant morphology includes the study of shape, size, structure and its parts such as root, shoot, leaf, flower, fruit and seed as indicated above. Morphological characters represent the study of plant growth, development, form and structure and using those characters it may be possible to interpret similarity or dissimilarity and origin of the plant (Barclay, 2015).

Plant morphology is an important character for study of habit, habitat and life span of a given plant in space and time and deduces its important features based on its morphological characters. Morphological character and its study have great importance in taxonomy and determination of crop productivity. Morphological characters may be correlated with the habitats of living as well as fossil plant which could be helpful for the association and distribution of fossil in space and time. It could be used to determine significant association and clustering through phylogeny.

Plant morphology is the morphological characters of plants which can be compared, measured, counted and described to assess the differences or similarities in plant taxa and can be used these characters for plant identification, classification and descriptions. The characters which are used in descriptions or for identification, they are called diagnostic or key characters which can be either qualitative (colour or shape) and quantitative (counted or measured).

Phytomorphological studies had been conducted in the past for various reasons which includes such as the diversity of leaf shape and colour, adaptive and functional significance within the plant, chlorophyll and photosynthesis rate, physiological, ecological and evolutionary history of the plant (Fidrianny *et al.*, 2015; Jain and Manohar, 2014).

Sechium edule, plant is a perennial climber with a tuberous root system. Stems are angular grooved, thick at the base and become woody when matured, but at the tip, it produce tender green colour soft stem crawling over the trellis with the support of tendrils. Presence of tendrils supports the plant to grow vigorously over the trellis leading to good fruit harvest (Newstrom, 1989). Leaves are 3-5 angled or lobed with broadly cordate base having four sharp corners (Veigas *et al*., 2020). The tender leaves and shoots of *Sechium edule* were used as a favourable vegetable (Booth *et al.,* 1992; Chadha, 2009) (Figure 37).

In this chapter, plant morphological characters were statistically analysed to understand the growth and development among genotypes based on vegetative growth characters using descriptive statistics, Pearson correlation (2 tailed), analysis of variance (ANOVA), regression, paired group Euclidean distance (PGED), principle component analysis (PCA). Also, genetic analysis was performed for the purpose using phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve), interaction of genotype and environment variance (Vg×e), phenotypic and genotypic coefficient of variation (PCV and GCV), broad sense heritability (H^2), environmentability (E) and repeatability (R).

MATERIALS AND METHODS

Quantitative data collection

Quantitative data on vegetative growth characters were recorded which includes leaf length, LL (cm), leaf breadth, LB (cm), petiole length, PL (cm), length of tendril straight LTst, (cm), length of tendril spiral, LTsp (cm), number of tendrils,

NT (count), stem node distance, SND (cm), circumference node, SCN (cm) and circumference internode, SCI (cm).

Statistical analysis

The data were subject to statistical analyses such as descriptive statistics, Pearson's correlation, and analysis of variance (ANOVA), regression, Paired Group Euclidean Distance and principle component analysis (PCA). Also, phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), broad sense heritability (H^2), repeatability (R) and environmentability (E) were determined.

Genetic analysis,

Genotypic variance

Vg = Mean square (between) -Mean square (group) ÷ Number of replications (r)

Where,

Vg = genotypic variance

r = Number of replications

Phenotypic variance

Vp = Vg+ Ve / r

Where, Vp = phenotypic variance

Vg = genotypic variance

Ve = Environmental variation

r = Number of replications

Genotypic coefficient of variation (GCV)

(Burton, 1952)

$$GCV = \sqrt{Vg}/ X * 100$$

Where,

Vg = genotypic variance

X = mean

Phenotypic coefficient of variation

(Burton, 1952)

$$PCV = \sqrt{Vp} / X * 100$$

Where,

Vp = phenotypic variance

X = mean

Broad Sense Heritability (H^2)

(Falconer and Mackay, 1996)

$$H^2 = Vg/Vp * 100$$

Where,

Vp = phenotypic variance

Vg = genotypic variance

$Vg_{*}e$ = variation due to genotype and environment interaction

Repeatability = $\frac{Vg+Vg \times e}{Vp}$ (Falconer, 1981)

Environmentability = 1- Heritability

RESULTS AND DISCUSSION

Descriptive statistics

The maximum mean value of each character was compared and found that length of tendril straight, LTst has the highest mean ± S.E. (32.80 ± 1.210) for the genotype 12. Mean value were recorded for leaf length, LL (19.30 ± 0.815), leaf breadth, LB (28.20 ± 0.860), petiole length, PL (14.00 ± 1.151), length of tendril spiral, LTsp (21.20 ± 2.159), number of tendril, NT (4.40 ± 0.244), stem node distance, SND (20.10 ± 1.600), circumference node, SCN (3.88 ± 0.182) and circumference internode, SCI (3.56 ± 0.136) respectively (Table 36 and Figure 37).

The high mean value of the characters indicated that the characters are successfully reached towards maturity without any hindrance towards their growth and development or in the climatic conditions available for their morphological or phenotype expression.

Soil and climatic factors such as soil pH, light intensity, rainfall and soil nutrients provided favorable condition towards the particular characters and helped in growth and development of character as well as the genotypes.

Genotype 10, 7, 6 and 2 showed favorable growth for most of the characters.

The low mean value was recorded for growth characters as LL (13.00 ± 0.474), LB (13.50 ± 0.447), PL (5.60 ± 0.678), LTst (26.90 ± 1.873), LTsp (14.40 ±

7.31), NT (3.3 ± 0.200), SND (11.50 ± 0.866), SCN (2.62 ± 0.374) and SCI (2.26 ± 0.040) respectively (Table 36).

Genotype 3 and 4 has shown less mean value for maximum characters than other genotypes. Lower mean value could be related with the less growth and unfavourable conditions prevailing for the particular character and creates some hindrance towards their growth and development towards their expression (Devcota and Jha, 2009).

The characters were computed for their variability using standard deviation (S.D.) and the characters such as NT, SCN and SCI measured deviations from mean ≤ 1 suggest the distribution of data linearly over or near to the measured linear line, while characters LL, LB and PL measured deviations from mean between 1 ≤ S.D. ≤ 2 and represented that data are not closely distributed towards the measured linear line. Other characters (LTst, LTsp and SND) showed very high deviations from the mean.

The possibility of stretched distribution of the data from the linear line is due to the possible human error in the measurement as well as various shapes of the fruits at different growth stage of the particular character. This may lead to the probability of error and stretched data collection.

The coefficient of variation (C.V.) was recorded high in all the characters. Higher C.V. for the characters suggests the measurement of characters is highly variable. The possible reason may be as measurement has taken on different genotypes, could be possibly correlated with high relative variability in the characters.

Pearson correlation

The growth characters were analyzed for Pearson's correlation to establish a relationship among the characters and their role for growth and development. All the characters were positively and significantly correlated with each other at the level of probability of significance $P \leq 0.05$ and $P \leq 0.01$ (2 tailed) (Table 37).

Leaf length, LL was positively and significantly associated with LB (0.917**), and SND (0.736**) at probability level of significance $P \leq 0.01$ and LTst (0.567*), SCN (0.609*), and SCI (0.571*) at $P \leq 0.05$ respectively.

Vegetative growth character, LL presented an important character which is associated with other vegetative growth characters positively and significantly.

Leaf breadth, LB was positively and significantly associated with SND (0.643*) at the probability level of significance $P \leq 0.05$.

Stem node distance, SND association with SCN (0.751**) and SCI (0.729**) at probability level of significance $P \leq 0.01$ indicates its involvement for the growth of circumference of node and internode.

Circumference node, SCN positively and significantly associated with circumference internode SCI (0.987**) at probability level of significance $P \leq 0.01$.

The genotypes were analyzed for Pearson's correlation based on the vegetative growth characters and established that genotypes are highly significant and positively correlated with each other at the level of significance $P \leq 0.01$ (2 tailed) (Table 38).

The positive and significant correlation indicates that increase or decrease in a character or genotype would affect the other character or genotype simultaneously.

Both characters will move simultaneously depending on the increasing or decreasing of value, if value decreasing both the characters will decrease, and if value increases both the characters increase together. Overall, the positive and significant associations recorded for the characters are in agreement with the earlier reports (Higaki *et al*., 2016; Zhang *et al*., 2011; Brophy *et al*., 2017; Glover, 2000; Jacques and Vissenberg, 2014; Mitchison, 2016; Alim *et al*., 2016; Julien and Boudaoud, 2018; Sapala *et al*., 2019).

Analysis of variance (ANOVA)

Vegetative growth characters showed good amount of variations (mean square value) at the probability level of significance $P \leq 0.05$. LB was recorded maximum variation with mean square value of 13.594 and minimum value was recorded for NT with mean square value 0.122 and could be correlated with less amount of variation in the NT (Table 39).

ANOVA for the growth characters suggest that NT, SCN and SCI for all the genotypes approximately similar or very less variation as amount of variation in the character recorded low as compared to other characters.

Regression

Vegetative growth characters were regressed against the genotypes to record the variations in the characters and for the genotypes. The maximum variation was recorded for the character PL with R^2 value (0.140) and regression line y=0.191x+7.09 followed by SND with R^2 value (0.136) and regression line y=0.411x+11.98. Similarly, the minimum variation was recorded for the character LL with R^2 value (1E- 05) and regression line y=0.003x+14.85 (Figure 38 and Table 40).

Paired Group Euclidean Distance

The genotypes were analyzed for Paired Group Euclidean Distance based on the vegetative growth characters data (Figure 39).

Paired Group Euclidean Distance classified genotypes into a 5 sub groups descended from main two groups.

Four sub groups are interrelated with each other indicates the almost similar morphological variation except genotype 7 and 10 based on the vegetative growth characters. Although, some differentiation either at the level of genetic constitution or environmental level among the genotypes.

Principle component analysis (PCA)

The vegetative growth characters were involved in PCA for component 1 and 2, which indicates the maximum variability in component 1 and the remaining possible variation in component 2 and their loadings are represented (Figure 40).

Eigen values are recorded for all the possible principal components which indicates the percent of variations available in the growth character and it has being observed that component 1 and 2 has shown 62.022 % and 12.989 % of total variation of the vegetative growth characters.

Both Principle Components 1 and 2 had shared 75 % of total variations observed in growth characters (Table 41).

To identify, the involvement of the components for the total variations of the growth character a scree plot was drawn which showed 2 components has taken maximum variations in the vegetative growth character (Figure 41).

PCA graphic representation of components 1 and 2 were recorded (Figure 42). The Principal Component Analysis and multivariate statistical methods successfully used to classify vegetative growth characters variations in many crop species (Chandran and Padya, 2000; Cravero *et al*., 2002; Rosso and Pagano, 2005; Bhargava *et al*., 2007; Harris, 2001).

Principal Component Analysis measures the contribution of each component or independent impact of a particular character to the total variants observed in a given population in relation to the character of interest to the breeder. PCA has been used to determine the optimum numbers of clusters, to compliment cluster analysis and to investigate patterns of genetic diversity (Thompson *et al*., 1998: Lombard *et al*., 2000: Mohammadi and Prasanna, 2003). PCA was used to reduce the complexity of the data set and to partition the observed variation within characters based on their degree of importance (Ringner, 2008).

Phenotypic variance (Vp), genotypic variance (Vg), environmental variance (Ve) ***and interaction of genotype and environmental variance (Vgxe)***

ANOVA was computed for the vegetative growth characters and reported environmental variance (Ve) in the range of value 2-10. Since, phenotypic variance (Vp) was recorded little high than the genotypic variance (Vg), it may be considered the effect of environment on the genotypes. Both genetic constitution and environment are responsible for the variation of phenotype and growth character in the genotypes (Table 42).

Heritability (H^2), Environmentability (E) and Repeatibility (R)

High heritability and repeatability was recorded for the characters. High heritability indicates selection of characters is easy for the transmission of character from parent to offspring (Singh, 2001; Sabesan *et al*., 2009).

The high heritability of the genotypes suggests that all characters are highly influenced by the genetic make-up of the genotype and because of the genetic make-up of each and every genotype differs from each other.

The character which is constantly within individuals as well as differing between individuals corresponds to selection under repeatability (Table 42).

The high heritability and estimated repeatability may be correlated with the over estimated or very high values for genotype and environment interaction, environmental effect on genotype and phenotype.

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

Phenotypic coefficient of variance was recorded higher for all the growth characters than GCV (Table 43). The estimation of PCV was slightly greater than the corresponding GCV for all characters indicating the role of environment in their expression of these characters. The present finding for GCV and PCV are in agreement with those of others (Chhetri *et al*., 2019; Singh *et al*., 2018; Rahman *et al*., 2016; Jangde *et al*., 2018).

CONCLUSION

***Descriptive statistics*:** Genotype 10, 7, 6 and 2 showed favorable growth for most of the characters. Genotype 3 and 4 has shown less mean value for maximum characters than other genotypes. The descriptive statistics for growth characters reveals high degree of diversity among genotypes at present which can be further explored for crop improvement through molecular techniques, provides an opportunity to enhance its growth and productivity.

Pearson's correlation (2 tailed): Vegetative growth character, LL presented an important character which is associated with most of the vegetative growth characters positively and significantly.

Analysis of variance (ANOVA): ANOVA for the growth characters suggest that LB was recorded high variation and NT was recorded low variation for all the genotypes and NT, SCN and SCI for all the genotypes approximately similar as amount of variation in the character recorded low.

Regression: The maximum variation was recorded for the character PL with R^2 value (0.140) and regression line y=0.191x+7.09 followed by SND with R^2 value (0.136) and regression line y=0.411x+11.98.

Paired Group Euclidean Distance (PGED): PGED suggested the genotypes 7 and 10 are having little variation than others.

Principle component analysis (PCA): Principle Components 1 and 2 had shared 75 % of total variations observed in vegetative growth characters.

Genetic variances (Vp, Vg, Ve and Vg×e): Genetic constitution and environment are responsible for the variation of phenotype and growth character in the genotypes.

Broad sense heritability, Environmentability and Repeatability (H^2, E and R): High heritability and repeatability was recorded for the characters.

Phenotypic and genotypic coefficient of variance (PCV and GCV): Phenotypic coefficient of variance was recorded higher for all the characters than GCV.

CHAPTER-6

SUMMARY

Nagaland state involves the former Naga Hills district of Assam situated at the extreme North-Eastern part of India carrying an area of 16,527 sq. km. It is mostly covered by high altitude mountains with average height of the peaks between 900 and 1200 masl. It consists of 11 districts with different altitudes, lowest being Dimapur with 260 masl and Kiphire with 3840 masl as the highest. The luxuriant growth of all the 14 genotypes of *Sechium edule* recorded from the Kohima district with an average altitude of 1538 masl with latitude (25.60690 ° N) and longitude (94.34250 ° E).

The reason for the luxuriant growth could be long duration of the rainy season or monsoon season from May to September in the State. Nagaland gets medium to heavy rainfall depending on the location of the region and its relation to the surrounding mountains with average rainfall between 18 - 22 centimeters and heaviest rainfall recorded in August and September. The other reason could be the type of soil distributed and probably two major landscapes i.e. Alluvium-Colluvium and Sandstone are recognized with 46 numbers of soil series and about 89 % area under forest followed by 6.3 % under Jhum cultivation. Soils have been classified under 6 physiographic classes and mostly falls under undifferentiated hill side slopes which taxonomically classified into four orders i.e. alfisols [highly fertile soil with aluminium (Al) and Iron (Fe) mix, pH 5-6], entisols (immature soils, pH variable), inceptisols (altered from parental material, pH variable) and ultisols (red soil with mixed vegetation, pH variable). The high fertile soil types available in the forest and slopes may be played at best role for the growth

of squash (*Sechium edule*) luxuriantly in the Eastern Himalayan Region (Kant, 2004; Kharwal and Rawat, 2013; Pradhan, 1986).

Morphologically, male and female flower emerges on the different branches from the same nodal area or separately or single on different branches. Male flowers are in groups or always grow in cluster form and female flowers generally single or 1-2 arises at the node. Female flower has thalamus at base with prominent stigma and grows separately. Male flowers have fused or unfused anthers with fused stamens. The nectar glands were found in both male as well as female flowers. The tendrils growth (3-4 tendrils from the node) was straight at young, but becomes coiled towards the maturity of the crop. The characteristics of flowers are similar to the reports of the Mexican squashes. The presence of nectaries in both the male and female flower provides major rewards for the pollinators in the *Sechium* plant. Bees (*Apis mellifera*) are most popular insurance pollinators (Martinez-Bauer *et al*., 2021). *Sechium edule* flower have similar morphological characteristics like that of *Sechium compositum* and *Sechium chinantlense* (Lira *et al*., 1999).

Mean range of days to harvest was recorded from 90-120 days but it may differ depending on the rainfall, soil types and cultivation season of the local area. Two genotypes (G2 and G10) recorded with watery pulp taste, most probably altered glucose synthesis in the genotypes and could be processed further for fruit improvement to the diabetic patients as vegetable without sweet. The variation in furrows on the fruits might be the presence of gene and need to be explored. Two genotypes (G8 and G12) recorded with smooth surface and rest were with spines encouraged us to identify the wild genome for breeding program and improvement. Shapes could be made better through hybridization between pyriform and spheroid. The variation in colour of the fruit might be genotypic and expected deep dark green wild genotype.

At present no genotype with bitter taste of pulp recorded on any fruit color which is generally accepted that dark fruit colour with bitter taste might be the wild type. It suggests that all the 14 genotypes collected from Kigwema village, Kohima district, Nagaland of North-East India are cultivated type. The qualitative characters of the squashes most probably seem to be similar as found in the Mexican squashes as revealed from the literature.

The variation in mean quantitative traits of fruits may the result of types of soils, rainfall and cultivation season of the local region. The present genotypes were collected at an average altitude of 1538 masl, but earlier reports indicates little variation in its altitudes of collection as low as 20 masl to 2100 masl high. The mean range value of various quantitative characters of all the genotypes is found to be almost similar to the earlier reports.

The Spearman's rho correlation was conducted for the fruit qualitative vs. quantitative characters and relation between and within the characters reported. The positive correlation between colour and shape indicate that spheroid shape will be darker than the pyriform shape. On the other hand, earlier reports suggested that more spheroid shape is little bitter in taste than the pyriform. Therefore, sweeter taste may be explored through hybridization and improvement in the fruit. Fruits with spines may be good in taste as shown good correlation between them than smooth surface fruits. At maturity furrows are much clear or it may the indication of maturity of the fruit and could be harvested, but it does not mean that fruit will be sweet at harvest as there is negative correlation between taste and harvest.

Fruit morphology varies considerably from small spheroid to small ovoid to pyriform with overall starting months of flowering from April to June, starting months of fruiting from May to July and ending months of fruiting are December and January. The phenology of

collected genotypes differs a little from Chayote recorded in the Mexico suggested the effect of climate, weather and adaptation properties of the two different continent. Moreover, there is a need to work out on the role of relative humidity and phenological variations for the crop improvement.

Earlier reports suggest that the close accession of *Sechium edule* wild type is with *Sechium hintonii* but the varieties of domesticated *Sechium edule* are phylogenetically related to *Sechium chinantlense* and *Sechium compositum*. Also, if more spines on the fruit, then there is more giberellic acid content in it and if less spines on the fruit, GA content is less. Yellow fruit has less chlorophyll a and b content compare to the light green and dark green fruit (Barrera-Guzman *et al*., 2021).

The sweetness in the *Sechium* fruit is due to the presence of fructose and glucose in the mesocarp and bitterness is due to cucurbitacin and raffinose and sucrose are present in cotyledons (Cadena- Iniguez *et al*., 2011). When there is more cucurbitacin content, then the fruit will not be viviparous in nature (only found in wild) and wild chayotes are the ancestors of all the domesticated chayote (Cadena- Iniguez *et al*., 2011). All the domesticated variations may be due to the environmental changes leading to the differences in colour in both leaves and fruit, appearances (thorns) and flavor in the fruit (Cadena- Iniguez *et al*., 2011).

The worldwide presence and high morphological variation with different *Sechium edule* varieties may be due to the interaction of its population with the environment, soil, geographical region as well as there are some risk that they are the products of hybridization of *Sechium edule* with *Sechium chinantlense* and *Sechium compositum* leading to its varietal population (Barrera-Guzman *et al*., 2021). *Sechium edule* and *Sechium tacaco* (Brownie) both are the cultivated

species among the genus *Sechium*. Weak environmental influence indicates high values of heritable characters and vice versa. All the genotypes have viviparous fruit (Cadena- Iniguez *et al*., 2008).

An extensive variation of chromosome number has been reported for *Sechium edule* in the literature. A few studies suggest that the haploid and diploid number of the species are n=12 and 2n=24 (Sugiura, 1938, 1940; Sobti and Singh, 1961; Goldblatt, 1981, 1984), while others work describes n=13 or 2n=26 (Goldblatt, 1990), 2n=28 (Giusti *et al*., 1978) and 2n=22 (Singh, 1990) respectively. At present, somatic chromosome count varies from n=12 to n=15 for all the 14 genotypes. Out of 14 genotypes, 5 counted n=13, 5 counted n=14, 2 counted n=12 and 2 counted n=15. The chromosome counts are similar to earlier reports except n=11 reported by Singh 1990, but rigorous chromosome count by expanding the area of collection may give positive results with n=11. As it has been reported in the literature that chromosome count with n=12 and n=13 belongs to the *Sechium edule* wild type (G8, G9, G1, G2, G4, G11 and G13), n=15 belong to *S. chinantlense* Lira and Chiang (G3 and G12) and n=14 belongs to *S. compositum* (J.D. Smith) C. Jeffrey or *S. hintonii* (P.G. Wilson) C. Jeffrey (G5, G6, G7, G10 and G14).

At present, chromosome count established that 3 species are recorded from the 14 genotypes of the collected *Sechium*. Morphologically, they are so similar that difficult to recognize as separate species. There is a need to identify *S. compositum* and *S. hintonii* with same chromosome number, n=14 or merge to a single species. There is a possibility of more number of species within the genus *Sechium* and may not be a single species genus.

Although chromosome size is very small but efforts have been put to count the chromosomes and found that a ploidy level 4x (2n=4x=52) and n=16 was observed in the genotype 2, since sampling was random. Therefore, rigorous study is required to establish the phylogenetic relationship among the morphologically similar and genetically different genus *Sechium*. The entire chromosome for the 14 genotypes were observed, counted and analysed. The chromosome count is reported as 2n=24, 2n=26, 2n= 28, 2n=30, 2n=32 and 2n=4x=52 and the most occurring chromosome numbers are 2n =26 (G1, G2, G4, G11, G13) and 2n= 28 (G5, G6, G7, G10 and G14). The least occurring chromosome number in 2n = 24 (G8 and G9). The critical problem is that the chromosomes are extremely small in size and most probably due to the secondary metabolites, clustering occurs within the *Sechium* chromosomes increasing the difficulty level for chromosome count (Thrup, 2000; Olvera-Vazquez *et al*., 2019).

Pollen morphology of *Sechium* was described as spiny and verified that all genotypes spores are spiny and approximately similar to the earlier reports. However, some variations may prevail and required to reveal the variations using sophisticated and molecular techniques.

Although chayotes are morphologically similar but there is high chances of some more species in the genus *Sechium* than earlier and this present verification of species *S. edule*, *S. chinantlense*, *S. compositum* and *S. hintonii*. Molecular cytogenetic and other studies must be undertaken to answer this question with accuracy and to determine the true relationships among *Sechium* species. There is a need to identify wild and cultivated crops with precision of high similarity. There is no doubt wild species have high potential to improve the cultivated crops through hybridization which has not been tried. Inter or intra specific hybridization among the chayote may be able to resolve the existing problems of phenological variations and fruiting, fruit storage, resistance to diseases and pests and meager genetic variation.

At last but not the least, there is no report on the hybridization of wild×cultivated crops. There was a report that fruits of *S. compositum* could be stored for longer duration, crossing with other chayotes may solve the problem of storage and conservation of fruits. Similarly, fruits of other *Sechium* species could be used as resistance to diseases and pests. Moreover, the importance of the wild types or wild species and related genetic resources must be established for their genetic correlation with the cultivated species of Chayote. The cross breeding research among the wild and cultivated species must be established to find the potential for improvement among the species.

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Appendix-I

GPS Co-ordinates of the Sechium genotypes collection sites* (*Kigwema Village, Kohima, Nagaland):

Genotype	LATITUDE (N°)	LONGITUDE (E°)	ALTITUDE (masl)
G1	25.60836°	94.12756°	1565
G2	25.60708°	94.12798°	1544
G3	25.60661°	97.12952°	1526
G4	25.60667°	94.12926°	1540
G5	25.60724°	94.12785°	1528
G6	25.60656°	94.12944°	1530
G7	25.60727°	94.12771°	1531
G8	25.60690°	94.12749°	1524
G9	25.60720°	94.12790°	1530
G10	25.60759°	94.12893°	1504
G11	25.60692°	94.12761°	1520
G12	25.60575°	94.12788°	1585
G13	25.60661°	94.12898°	1547
G14	25.60590°	94.12694°	1557
	25.606904 °	**94.342503°**	**1537.929**

Appendix II

Instruments and equipments used in study:

1. Autoclav - BioGene India
2. Camera - Nikon
3. Digital microscope - Motic BA210
4. Light microscope - HoverLabs MD500
5. Weighting balance - Sartorius weighing balance, made in USA
6. Classmate ruler
7. Stage calibrator
8. Carmine GPS

Softwares:

1. SPSS 16.0
2. Window Excel Microsoft
3. ImageJ
4. URKUND

LIST OF PUBLICATIONS

Kumar, S. and Kiso, A. 2018. Quantitative evaluation of mangoes (*Mangifera indica* L.) in temperate areas of North-East India. *Research in Environment and Life Sciences* **11**(5): 144-151.

Kumar, S., Kiso, A. and Kithan, N.A. 2021. Chromosome banding and mechanism of chromosome aberrations, *Cytogenetics - Classical and molecular strategies for analysing heredity material* (Eds: Marcelo L. Larramendy and Sonia Soloneski). IntechOpen, DOI: 10.5772/intechopen.96242. https://www.intechopen.com/chapters/75292

Kumar, S., Kiso, A. and Asenla, L. 2022. Genetic variation, heritability, principal component analysis, correlation and path coefficient analysis in the fruit samples of *Sechium edule* (Jacq.) Sw. genotypes. Yuzuncu Yil University Journal of Agricultural Sciences **32**(1): 164-174.

Kumar, S. and Kiso, A. 2022. New reports of somatic chromosome number and symmetric or asymmetric karyotype estimation of *Sechium edule* (Jacq.) Sw. (Cucurbitaceae). Caryologia (Accepted)

List of Paper/ Poster presented in Seminars/ Conferences

1. Poster Presentation on the topic "Morphological and cytogenetical analysis of *Sechium edule* (Jacq.) Swartz**"** at Sixth International conference on Plants and Environmental Pollution held on November 27 to November 30, 2018, organized by International Society of Environmental Botanists (ISEB) and CSIR- National Botanical Research Institute (CSIR-NBRI) Lucknow-226001, India.
2. Presented Paper (Oral) on the topic "Studies on diversity of *Sechium edule* varieties in Nagaland, their uses and medicinal properties" in the National Seminar on "Recent Trends of Research in Medicinal Botany" held on October 4 to October 5, 2019 at Department of Botany, Ramjas College, University of Delhi, India.

List of Seminar /online Webinar/ workshops attended

1. Participated in National Seminar on Advances in Biological Research held during February 28 to March 01, 2017 by the Department of Botany, Nagaland University, Lumami– 798627, Nagaland, India.
2. Participated in Hands on training on "Functional Genomics" held on November 14 to November 21, 2017 organized by the Institutional Biotech Hub, Nagaland University, Lumami- 798627, Nagaland, India.
3. Participated in Workshop on 'Skill and Entrepreneurial Development of the Tribal Youth" with the theme 'Value additions to Rich Bio- Resources with special References to Medicinal and Aromatic Plant' held on July 25 to July 28, 2018 at Nagaland University, Lumami- 798627, Nagaland, India.

4. Participated in the National conference of stakeholders on conservation, cultivation, resource development and sustainable utilization of medicinal plants of North- Eastern India held on March 6 to March 7, 2019 at Nagaland University, Lumami- 798627, Nagaland, India.
5. Attented in the Webinar on the topic “Introduction to the realms of research” held on November 7, 2020, by the Department of Chemistry, Immanuel College, Dimapur, Nagaland, India.