COLLECTION, CHARACTERIZATION AND EVALUATION OF SELECTED *BEGONIA* SPECIES OF SIKKIM HIMALAYAS FOR ITS ORNAMENTAL VALUE

A Thesis Submitted
To
Sikkim University

In Partial Fulfilment of the Requirement for the
Degree of Doctor of Philosophy

By
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DECLARATION

I, Mr. Bikash Bhattarai, hereby declare that the research work embodied in the thesis titled **"Collection, Characterization and Evaluation of selected *Begonia* species of Sikkim Himalayas for its ornamental value"** submitted to Sikkim University for the award of the degree of Doctor of Philosophy under the supervision of Dr. Manju Rana, Assistant Professor, Department of Horticulture, School of Life Sciences is my original research work and solely carried out by me. The thesis has not been submitted for any other degree or diploma in any other University/Institution.

Date: 20.08.2020

Place: Gangtok

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

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

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"Collection, Characterization and Evaluation of selected *Begonia* species of Sikkim Himalayas for its ornamental value"

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

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Bikash Bhattarai

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ABBREVIATIONS

@	at the rate of
h	hour
t	tonne
ha	hectare
g	gram
mg	milligram
kg	kilogram
cm	centimeter
%	Percentage
°C	Degree Centigrade
µg	Micro gram
µl	Micro litre
µM	Micro mole
cm	Centimeter
mm	Milimetre
mM	Milimole
nm	Nanometer
ml	Milliliter
L	Liter
v/v	Volume/volume
v/w	Volume /weight
A.O.A.C	Association of Official Analytical Chemists
A.A.E	Ascorbic acid equivalent
CD	Critical difference
CV	Coefficient of variance
SEm	Standard error mean
SD	Standard deviation
e.g	example

et al.	Co-workers
NR	No response
i.e	That is
MS	Murashige and Skoog media
I.U	International unit
G.A.E	Gallic acid equivalent
QE	Quercetin equivalent
ITS	Internal transcribed spacer
IUCN	International Union for conservation of nature
Viz.	videlicet (namely)
cv.	cultivar
IC50	The half maximum inhibitory concentration
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
FRAP	Fluorescence recovery after photobleaching
BHT	Butylated hydroxytoluene
DPPH	2,2-diphenyl-1-picrylhydrazyl
p^H	Potential of hydrogen
TLC	Thin layer Chromatography
CNMR	Carbon 13 nuclear magnetic resonance
Chl	Chlrophyll
$M^{-2}s$	Meter square per second
sp	species
TPC	total phenolic content
TFC	Total flavonoid content
NAA	1-Naphthalene acetic acid
IAA	Indole 3-acetic acid
BAP	6-Benzylamino purine
TDZ	Thidiazuron
2,4-D	2, 4-Dicholrophenoxy acetic acid

PGR	Plant growth regulator
IBA	Indole 3-butyric acid
GA_3	Gibberellic acid
ANOVA	Analysis of variance
RBD	Randomized block design
CRD	Completely Randomized design
UV/Vis	Ultraviolet-visible
FCR	Folin Ciocalteu reagent
BSA	Bovine serum albumin
HCl	Hydrochloric acid
MSL	Mean Sea Level
DM	Dry Matter
FM	Fresh matter
S	Satisfactory
NS	Not satisfactory

CHAPTER 1

INTRODUCTION

India is bestowed with rich flora and fauna due to its varied agro-climatic and regional topography (Jain, 1983) and Sikkim is one of the best examples of that. Sikkim Himalayas lies between $27^0$10`- $28^0$5` N and $88^0$30`- 89^0 E. This region is famous for its varietal ornamental plants, unique natural and geographical conditions (Rawat and Tambe, 2011). The State is recognized among the 200 globally important regions (Olson and Dinerstein, 1998). Nearly 82% of the area of Sikkim is under forest cover land. About 4450 species of ornamental plants have been recorded from Sikkim which falls under 197 families, 1371 genera (Singh and Sanjappa, 2011). It is also estimated that 3% of the plants known from Sikkim are endemic to this region (Chatterjee, 1939). Nearly 165 species have been named after the state, as they were first collected or known to occur in Sikkim.

Sikkim Himalayas harbors great importance for ornamental horticulture. Wild plant species include plants of sub-tropical, temperate, and alpine having vast diversity in morphological characters. Many of them have been introduced into European countries, which includes the species like *Hedychium gardenerianum, Luculia gratissima, Allium wallichii, Acer oblongum, Anemone vitifolia, Arisaema graffithii, Berberies aristata, B. asiatica, B. sikkimensis, Bergina ciliata, Betula utilis, Boenninghausenia albifolia, Cauteleya gracilis, C. spicata, Cymbidium cyperifolium, Daphne bholua, Juniperus recurva, Mahonia acanthifolia, Meconopsis bella, Mellia thyrsiflora, Paris polyphylla, Pleione paraecox, Polygonatum oppositifolium, Prunus cerasoides, Rhododendron*

anthopogon, R. arboretum, R. barbatum, R. hodgsonni, R. nivale, R. macrophylla, R. sericea, Vandopsis undulata, Viburnum cordifolium, Primula spp. and many more. But still, several species of *Corydalis, Gentiana,* and *Primula* being of interest and beauty, await introduction (Srivastava, 1998). Apart from these still some potent wild species found in Sikkim Himalayas like wild *Orchids,* Lilies, *Cucurma, Arisaema, Allium, Jasminum, Begonia,* etc. are of great importance for introduction in ornamental horticulture and yet to explore.

The richness in plant diversity of Sikkim has encouraged and fascinated the researchers and plant collectors from different parts of the world. Literature reveals that besides botanists, several horticulturists and amateur researchers have widely explored this area since Griffith's visit in 1843. During that period plant exploration team explored the plant species like *Rosa, Primula, Sencio, Rhododendron,* Orchids, etc. and also many beautiful herbs, shrubs, and trees for introduction in their garden (Srivastava, 1998).

In this experiment, wild *Begonia* species of Sikkim Himalayas has been targeted to evaluate for the morphological characterization and potential use in the ornamental horticulture industry. *Begonia* L. genus under Begoniaceae is widely distributed and had reported morphologically diversity. This genus is recognized for its horticultural importance and used as ornamental plants throughout the world. *Begonia* genus is known for its showy foliage having different patterns and its flowers in diverse variations of white, yellow, pink to scarlet. *Begonia* L. is the sixth largest angiosperm genera of flowering plants (Frodin, 2004; Hughes, 2008; Moonlight *et al.,* 2018), with more than 1800 recorded species around the world (Averyanov *et al.,* 2019). *Begonia* by nature is shade loving herbs and shrubs which are also used as edible and medicinal plants in many parts of the world.

Begonia is native to moist subtropical to tropical climates; some species are commonly grown indoors as ornamental house plants in cold climates. More than 10,000 registered artificial hybrids of *Begonia* were used for commercial purposes, even though wild species are cultivated and can be used as parents for new hybrids.

Introduction and acclimatization of wild ornamental plants are protecting resources, providing ecological balance, and also benefits to the florist and horticulturist for various purposes. Investigation and application of wild ornamental plants plays important role in landscape and ornamental purposes. Product novelty is an important attribute that makes the ornamental industry unique among agricultural industries (Halevy, 1999). The challenging task of a new crop researcher is beginning with a blank slate and developing the evaluations necessary to accumulate pertinent production information. Species that are presently under cultivation for economic, medicinal, or ornamental purposes have come from wild germplasm resources during the medieval period. The introduction of new crops requires identification, exploration, and collection of new plants, with interesting characteristics that may be economically important. Cultivation of wild ornamental not only provides a potential source of income but also offers potential conservation management; these plants can be used directly for their ornamental value or as genetic resources for plant breeding programs (Bridgen, 2001).

Hybridization and selection from a breeding program or introduction of new cultural technology (Roh and Lawson 1987) are important steps in the introduction of a new crop. Nowadays novel crop research is becoming a key interest among scientists, growers, and the general public. Attempts have been made to describe the identification of wild *Begonia* species with potential value for the successful introduction of the wild plants in the

floriculture industry. These trials also provide the opportunity to collect data pertinent labeling information for a garden plant such as maximum expected plant height, flowering time, flowering duration and most importantly *in vitro* regeneration protocol of wild *Begonia* species.

The continuous demand for new and special products in the floricultural market encourages this discovery of new genetic sources in areas that are less exploited. Sikkim presents a unique botanical condition with an enormous potential due to its high number of endemic species not yet exploited genetically. Keeping above in mind, an attempt has been made to evaluate some selected wild *Begonia* of the Sikkim Himalayas for their ornamental value.

There is no adequate information regarding the morphological study and *in-vitro* micropropagation of selected wild *Begonia* species of Sikkim Himalayas for horticultural purposes. The main focus of the research was to document the above lacking information about the selected wild *Begonia* species for horticultural purposes. By keeping above points in view, the present study "**Collection, Characterization and Evaluation of selected *Begonia* species of Sikkim Himalayas for its ornamental value**" was undertaken with the following objectives:

- To select and collect *Begonia* species for ornamental purposes
- To study the morphological characters of the selected species
- To conduct biochemical analysis of the selected species
- To develop the micro-propagation protocol of selected species for the production of elite planting material

CHAPTER 2
REVIEW OF LITERATURE

This chapter highlights the major findings of literature review for the present work on **"Collection, Characterization and Evaluation of Selected *Begonia* species of Sikkim Himalayas for its ornamental value"**. Introduction of noble floriculture traits in horticulture sector begins with the procurement of both foreign and domestic species having distinct and attractive characteristics. If some plant species may possess potential as floriculture crops than it can be fully evaluated through a research. Researcher may have potential to recognize the success of a species while at the same time other may be discarded with little chance of acceptance in near future (Armitage, 1988).

2.1 Potential as wild ornamental crops

Floriculture is a money-spinning industry comprising thousands of species and varieties of ornamental plants in both cultivated and wild one (Chin and Tay, 2007). Most of the developed countries comprising varieties of ornamental plant with great diversity, in return they can fetch added value in near future, either through direct development of commercial floriculture or through breeding of newer crops (Baudoin *et al.,* 2007). Floriculture industry is expanding rapidly around the world and new cut flowers and potted plant introductions are in great demand. Floriculture sector mostly deals with the noble traits in crops, whether noble traits or new crop having floriculture importance. Even todays people are not aware of true value of ornamental plants which possess great potential in

floriculture industry, due to their beautiful flowers and can also be of great value as medicinal plants, herbs, vegetables or for other industrial uses (Chin and Tay, 2007). The word “wild” in contrast to the plant species refers to those that grow impulsively in self maintaining populations in natural ecosystems (Heywood, 1999).

New plant species from the wild, botanical gardens, nurseries or plant collector can provides the new application for existing plant species and those species neglected by the horticulture industry (Middleton, 2011). Botanical gardens and special plant collectors constitute a rich source of plant material and can be used for cultivating new ornamental plant (Halevy, 1999). Species which were often overlooked in consideration of the development potential of wild plants. Due to rapid expansion of horticulture and flower trade around the world, novelty is important criteria in cut flower, potted plants/bedded plants introduction, which are in constant demand. While considering the facts, often overlooked in wild cultivars may have considerable role in world trade and have a great potential in introducing new varieties/type in farming systems in many parts of the world. Most of the species are commercially harvested from the wild and cultivated (Heywood, 1999).

The large and rich biodiversity of the indigenous plants of Sikkim Himalayas offers a valuable source for investigation into new crops. But the deficit of research funding for indigenous crops for evaluations and trials into commercialized new crops, but worthwhile may achieved with proper taxonomic documentation. Likewise, the rich diversity of indigenous plants of South Africa offers great potential for investigating the new crops (Reinten and Coetzee, 2002). Research has been conducted by the Agricultural Research Council of South Africa in the field of floriculture, they evaluated many indigenous species

including, *Protea, Leucadendron, Leucospermum, Serruria, Aulax, Mimetes, Paranomus, Ornithogalum, Lachenalia, Amaryllis belladonna, Nerine, Crytanthus, Gladioulus, Lapeirousia* and *Crinum.*

New varieties and species are another major source of new ornamental plants (Brickell, 2001). *Pelargonium* and *Gerbera* are such example of perennials which had undergone intensive cultivation programme and selection process over many years and resulted novelties of such general available in the market worldwide.

Kannan *et al.* (2019) evaluated *Heliconia* genotypes for genetics, yield and quality attributes. Due to its attractiveness and much attention for its floricultural potential as a source of cut flowers and flowering pot plants. Authors evaluated twelve genotypes of *Heliconia* viz., *H. angusta, H. psittacorum, H. densiflora, H. stricta, H. orthotricha, H. latispatha, H. rostrata, H. bihai, H. caribaea, H. latispatha, H. wagneriana, H. marginata,* for fifteen different parameters to observe the genetic variability and association among the characters and concluded that the genotypes *H. wagneriana* 'Wagneriana Red', *H. rostrata* 'Lobster Claw' and *H. stricta* 'Iris' can be recommended for commercial cultivation.

Tian *et al.* (2018) reported the vulnerability of *Begonia* species in near future posed by continuous over exploitation and illegitimate assemblage for ornamental or medicinal purposes. Authors mentioned that the rapid development of internet commerce is making role to trade the endemic flora. Authors also suggested that these species should be identified and require immediate protection strategies either through *ex situ* or *in situ* conservation.

According to Singh and Abhilash (2018a) wild species are the treasure home of genetic resources for crop improvement and they are tolerant or resistant to various diseases and abiotic factors such as drought, flood, heat-sensitive, salinity etc. Singh (2017) also suggested that the wild relatives may be cultivated with less inputs and agronomic practices than most of the modern cultivars/varieties. Such wild genotypes may be used for breeding and modifying the cultivated crops to improve their traits (Dubey *et al.,* 2016; Singh and Abhilash, 2018b; Whitney *et al.*, 2018).

Park *et al.* (2014) carried out an experiment to identify genetic resources of wild species of chrysanthemum and commercial cultivars for white rust resistant (*Puccinia horiana*). After evaluation for resistance to white rust, a total 41 spray cultivars and three wild species of chrysanthemum were identified as resistant. Author concluded that the use of resistant cultivars is the most efficient strategies to overcome white rust diseases and these genetic resources can be used in crossbreeding programs for developing white rust resistant chrysanthemum cultivars.

The quest for new genetic resources from the existed and conserved germplasm is highly valued for the use in breeding programmes. The genotypes developed out of the desired germplasm with significant promising traits play a major role in varietal development. The introduction of genetic tools to diversify the production, colour and size of ornamental plants is therefore a primary concern (Pedapati *et al.,* 2018).

It has been learned that the inferior wild species are highly superior than commercial varieties in crop improvement in conserving many of desirable genes (Maloupa *et al.,* 1999; Shillo, 1999). The genetic improvement of the wild plants leads to

the production of diseases resistant, vigorous and useful traits which are then introduced into commercial floriculture (Littlejohn *et al.,* 1999). Thus, characterization and evaluation of wild species and utilization of their novel traits are of one of the leading strategies in plant breeding to meet the demand. Therefore, exploration of wild species is key to conservation and management strategies for crop improvement (Hajar and Hodgkin, 2007; Singh, 2017; Teso *et al.,* 2018).

Cervelli *et al.* (2012) conducted an experiment to evaluate the development of new ornamental crops from native Mediterranean flora for pot plant, cut flower or cut foliage production. Five genera showed interesting results in terms of availability of new germplasm (species or genotypes), efficiency of propagation, cultivation requirements, morphology of end products. Some clones of *Myrtus communis* were selected with different fruit colours, plant compactness and leaf morphology for pot plant production or cut foliage production. In the *Arbutus* genus, one hybrid species (*A. etrachnoides*) was selected for cut foliage, one variety (*A. unedo* 'Compacta') for potted plants with flowers and fruits. Five species of *Helichrysum* (H. *stoechas*, *H. hyblaeum*, *H. scandens*, *H. italicum subsp. microphyllum*, *H. errerae*) were considered ideal for the development of potted plants with flowers; three of them have uniform grey foliage and *Limonium serotinum* exhibited interesting features for cut flower production.

In the post-globalization era floriculture has become an important commercial venture in the agricultural sector (Harisha, 2017). The practice of floriculture has been marked as a viable and lucrative business with the potential to enable self-employment among low- and middle-income farmers and earn the important foreign exchange in developing countries like India. The world production of floriculture is rising at a time.

World production of floriculture is increasing at a rate of 10 per cent per annum. Almost 45 to 50 countries are active on a large scale in the production of floriculture.

India is on the 18th rank with contributing 0.6 percent share in global floriculture trade. During the last decade, export increased at a Compound Annual Growth Rate (CAGR) of 4.33 percent (Vahoniya *et al.,* 2018). According to APEDA (2019) the Indian total export of floriculture was estimated worth Rs. 571.38 Crores/ 81.94 USD Millions in the year 2018-2019. The major importing countries were United States, Netherlands, United Kingdom, Germany, and United Arab Emirates.

India's native ornamental plants are *Orchids*, Musk rose, Lotus, Water lily, *Crossandra, Clerodendron, Tabernamontana, Begonia, Clitoria* and *Clematis*. India is ideally suited for growing many varieties of flowers in different season, with its varied climatic zones. New flowers such as *Iris, Curcuma, Liatris* etc. have recently been added as ornamental germplasm. The traditional system of germplasm introduction is recommended in specific regions used mostly in ornamental plant breeding. (Flavia *et al.*, 2015; Pedapati *et al.,* 2018).

Prasad and Thomas (2015) highlighted the diversity of ornamental potential plants based on their attractive flower colour, good looking habit and various plant parts with their beautiful appearance from Meenachil taluk of Kottayam district, Kerala, India. During the investigation, 98 taxa belonging to 80 genera in 37 families were documented from the region.

Reddy *et al.* (2015) researched potential wild ornamental species of Convolvulaceae which were identified and reported from Andhra Pradesh, India's Eastern

Ghats. A total of 61 plants belonging to 11 genera have been systematically identified and described. From the research area they identified the potentials of wild ornamental plants with beautiful flowers. The study clearly indicated that this research could assist researchers and individuals who are interested in wild ornamental plants and that there is ample scope for indoor as well as outdoor gardening and landscape practice.

Palanisamy and Arumugan (2014) carried out floristic exploration of wild ornamental plants and identified 137-wild ornamental species belonging to 99 genera and 42 families with potential artistic ornamental value. Ornamental potential was evaluated on the basis of distinctive features includes flowers, fruits and foliage. Authors suggests that with the help of this work, it will assist researcher or any individuals interested in agriculture and horticulture.

The demand for ornamental crops will certainly increase in coming years and it is clear that creative efforts in production and marketing are required. Works needs to be focused mainly on the growth new commercial varieties in a new production area with a diverse environment. It is also important to look for a new source of genes from the already collected and conserved germplasm, with respect to the use of germplasm in breeding programmes. However, research on the evaluation of wild *Begonia* species for the potential use in commercial floriculture are in very early stages and detail study of their cultivation practices are yet to explore.

2.2. *Begonia*

Begonia L. is one of the largest angiosperm genera with more than 1900 pantropically distributed species currently identified (Thomas, 2010). Although, the genus

showed greater diversity, which can be found from dry desert bush to wet rainforest at an altitude over 3000 mean sea level (Tebbitt, 2005). The species of the genus also displayed high rates of speciation and shows wide range of variation among the species level, it may be due to limited seed dispersal mechanisms and low level of gene flow in fragmented populations (Hughes *et al.,* 2003; Matolweni *et al.,* 2000; Hughes and Hollingsworth, 2008). The pantropically distributed *Begonia* species showed a great horticulture importance, which make an exceptional system for the study of plant evolution in tropical conditions (Neale *et al.*, 2006). Some new reported *Begonia* species were cited below;

Begonia is one of the ten largest plant genera found throughout the tropics- wet tropics and is represented by over 1600 species. They can be easily distinguished from each other on the basis of morphological parameters, which share similar floral morphology with swollen or caudex stem base. At the generic level, Begonias can easily be differentiated by asymmetry of the type of the leaf, succulent petioles, unisexual flowers borne on the same inflorescence and winged capsules (Aswathy and Murugan, 2015).

As per the report of Phutthai and Hughes (2016) and Tian *et al.* (2017) the genus *Begonia* is considered as the fifth or sixth largest angiosperm genus and one of the diverse plants with more than 1800 accepted species in around the globe. According to Tian *et al.* (2018) the count of species in the genus *Begonia*, has showed increasing trend over the past two decades and predicted that the number of *Begonia* species may between 2000 to 2500.

According to Hilinske (2016) *Begonia* was awarded as “Year of the” crops in the year 2016 and recognized as most attractive ornamental plants due to its beautiful foliage during the program hosted by National Garden Bureau of USA.

Nautiyal *et al.* (2009) studied two rare species known from Eastern Himalayas *viz.* *Begonia satrapis* C.B. Clarke and *B. scutata* Wall in which former being an endemic to Sikkim and Darjeeling Himalayas and latter belonged to Sikkim and the adjacent Nepal Himalayas. The authors studied the various factors leading to the extinction and disappearance of many species and also investigated the collection history and real time conservation strategies etc.

Ten new species of *Begonia* has been documented by Averyanov and Nguyen (2012) from Laos and they also assessed the *Begonia* species from eastern Indo-China region to be around 180-200 species.

Weihua and Kaiyun (2013) conducted an experiment to understand the status of variegated *Begonia* species of China. Out of 203 taxa, 84 species were found variegated, having different colours of variegation ranging from light green, silvery green, silvery white to white. From horticulture point of view, variegated species of Chinese *Begonia* were divided into different types on the basis of variegation features. Authors also discussed about the mechanism and the hereditary property lies in variegation of Chinese *Begonia* and found to be rich diversity of the variegated *Begonia* germplasm in China. They suggested that the genetic characteristics of the species will be understood through research to achieve the selective breeding of new varieties with specific ornamental traits.

Tebbitt (2015) reported a morphologically distinct group of 10 species within the *Begonia* L. sect. *Eupetalum* (Lindl.) A. DC. is identified and informally named as the *B. octopetala* L'Hér. species group. Two new species of *Begonia* (*B. pseudopleiopetala* Tebbit and *B. marinae* Tebbit) were also described.

Utley and Utley (2011) described four new species for the Begoniaceae, *Begonia campanensis* Burt-Utley & Utley and *B. fortunensis* Burt-Utley & Utley from Panama; *B. matudae* Burt-Utley & Utley from Chiapas, Mexico; and *B. makrinii* Burt-Utley & Utley from Oaxaca, Mexico.

Utley and Utley (2012) again reported 10 new species of *Begonia* are described, discussed, and illustrated: *Begonia wilburi*, *B. gentryi*, *B. liesneri*, *B. mcphersonii*, *B. pseudopeltata*, *B. guabuenensis*, *B. sukutensis*, *B. panamensis*, *B. gracilioides* and *B. tenuis. B. militaris* were evaluated and *B. sciadophora* is synonymized with it, while *B. pustulata* Liebm. and *B. ludicra* A. DC. are recognized as species endemic to México.

Low *et al.* (2015) reported a new species of *Begonia*, *B. jamilahana* Y.W. Low, Joffre & Ariffin based on a collection from Ladan Hills Forest Reserve, Tutong, Brunei Darussalam. This new species is closely related to *B. conniegeriae* S. Julia & Kiew and *B. papyraptera* Sands, but differs in morphological characters.

Girmansyah (2012) described two new species *of Begonia* from Sumatra, Indonesia which belonged to *Begonia* section *Petermannia* namely, *Begonia trigintico Uium Girm.* belongs to *Begonia* section *Bractei Begonia* and *Begonia do Uchocarpa Girm.*

Nakamura (2013) described *Begonia tandangii*, a new species of *Begonia* sect. *Baryandra* from the Mountain range of the Philippines. *B. tandangii* shows close resemblance to *B. fenicis* in ross morphology, differ in leaf margin sparsely fringed with minute hairs (vs. glabrous or with minute hairs only on teeth) and capsules with broadly-ovate outline and an acuminate apex (vs. capsules with broadly-obovate outline and a rounded to truncate apex). Phylogenetic analysis of Philippines species of sect. Baryandra based n ITS sequences revealed that *B. tandgii* was clearly separated from *B. fenicis*.

Phutthai *et al.* (2014) discovered *Begonia kanburiensis* Phutthai, a new species which belongs to *Begonia* section *Diplocinium,* from Kanchanaburi Province during surveys for a revision of the genus for the Flora of Thailand and noted that the species was recognized as limestone endemic and its IUCN status was considered as 'Vulnerable'.

The genus *Begonia* (Begoniaceae) has around 750 species in Asia, with new species being frequently published, especially from limestone areas (Peng *et al*., 2014; Phutthai and Sridith, 2010; Sang *et al*., 2013).

Peng *et al.* (2015) discovered the six new species of *Begonia* from Northern Vietnam which were being studied for diagnostic feature to differentiate the various relative species morphologically and genetically as somatic chromosomes. The species were *B. caobangensis*, B. *circularis, B. melanobullata, B. langsonensis, B. locii* and *B. montaniformis.*

Rajbhandary *et al.* (2010a) described and discovered three new species of *Begonia* from Nepal namely; *B. panchtharensis* S. Rajbhandary, *B. nuwakotensis* S. Rajbhandary and *B. taligera* S. Rajbhandary belongs to *Begonia* section *Platycentrum*.

Sosef and Miyono (2010) described a new, yellow-flowering species of *Begonia i.e. B. aequatoguineensis* from the Monte Alen region in Equatorial Guinea which belongs to the section *Loasi Begonia.*

Thomas *et al.* (2011) studied and classified the nine new species of *Begonia* as Vulnerable, Least Concern and Data Deficient. Of which, the species like *Begonia comestibilis*, *B. nobmanniae*, *B. prionota*, *B. sanguineopilosa* and *B. vermeulenii* considered as Vulnerable, *B. lasioura, B. rantemarioensis* and *B. torajana* as Least Concern and *B. insueta* as Data Deficient. These species were derived from South and West Sulawesi, Indonesia, belongs to *Begonia* section *Petermannia*.

Ardi and Hughes (2010) discovered a new species of *Begonia* from the Indonesian island of Sumatra which is considered as vulnerable by IUCN. The species is *B. droopiae* Ardi which belongs to *Begonia* sect. *Reichenheimia.*

A new species, *Begonia mysteriosa* L. Kollmann & A.P. Fontana from the Atlantic Forest of Espírito Santo, known only from the municipality of Sao Roque do Canaa in the Atlantic Forest of the state of Espírito Santo, Brazil, was described and illustrated by Kollmann and Fontana (2008). It was noted that a newspecies was probably related to *Begonia barckleyana* L.B.Sm., section *Knesebeckia*, from which it differs byits leaf shape, stipule size, stigma more than two branches and pistillate flowers with six tepals.

During the floristic survey on Kurung Kumey District of Arunachal Pradesh by Dash and Mao (2011), six species were collected which was only known from the type locality, out of the six species, *Begonia silhetensis* shows extended distribution. It was found that the species was first collected by I.H. Burkill from the Abor Hills of Arunachal

Pradesh on 1911 and was known only from its type locality till it was recollected from Kameng District of Arunachal Pradesh on 1957 by G. Panigrahi. While exploring the Kurung Kumey District, the species was again collected and a good population was found scattered along the primary forest floors.

Lin *et al.* (2014) reported a new species called *Begonia hosensis* from Sarawak, Malaysia. Its morphological resemblance was found to be similar with the *B. andersonii*, however differed in its floral architecture. These two species are ecologically separable due to its inhabitation characters. The former thrives on granitic cliffs and the latter was found only in limestone.

Hughes *et al.* (2015a) reported a new species, *Begonia yapenensis* M. Hughes, in *Begonia* section *SymBegonia* (Begoniaceae) is described and diagnosed against *Begonia sympapuana*. The new species is endemic to Yapen Island, Papua, Indonesia, and is currently known from a single collection.

Fifteen new species of *Begonia* L. has been described by Hughes *et al.* (2015b) from Sumatra in *Begonia* sect. *Bractei Begonia* using the IUCN category, out of fifteen, six were considered as Least Concern, five were under Vulnerable and four were considered as Data Deficient.

Wilde *et al.* (2011) described a new *Begonia* species from Laos and Thailand, belongs to *Begonia* sect. Tetraphila, along with 30 other species which are all endemic to Africa. This was the first record of any of the 65 currently accepted sections in *Begonia* transgressing continental borders. The discovery of *Begonia afromigrata* emphasized the importance of chance in the assembly of tropical floras.

Hainan is the largest island of the Indo-Burma Biodiversity Hotspot and has the best preserved and most extensive tropical forests in China. A study on distribution of endangered species in China identifies southern Hainan as one of eight hotspots for plant conservation in the country. In continuation of studies of Asian *Begonia*, Peng *et al.* (2014) reported the discovery of an attractive undescribed species, *B. wuzhishanensis* from Hainan.

Kollmann and Peixoto (2014) registered four new *Begonia* species from Espirito Santo, Minas Gerais and Bahia states. *Begonia dietrichiana, B. glabra* and *B. platanifolia* to Espírito Santo state and *Begonia admirabilis* to Bahia and Minas Gerais states. Authors described their descriptions, illustrations, geographic distribution data, maps, information on the habit, phenology, sections and vegetative differences.

Liu *et al.* (2019) reported first natural hybrid of *Begonia*, *B.* × *kapangan* from *B. balangcodiae* with *B. crispipila* in sect. Petermannian from Philippines. It was confirmed from the molecular analyses that the former contributed the maternal genome while the latter provided the paternal genome.

Tan *et al.* (2018) reported new species of *Begonia* (B. *yenyeniae*) of horticulturally value known from Endau Rompin National park, Peninsular Malaysia. It was confirmed by comparing its morphological characters with three similar species viz; *B. rajah, B. foxworthyi* and *B. reginula* and molecular analysis using the *ndhF-rpl132* chloroplast markers confirms the four species as distinct.

Lin *et al.* (2017) reported eleven new species of *Begonia* from Borneo. Due to its diverse terrain and varied microhabitats in Borneo, which occupies 200 known *Begonia*

species. During the field exploration of Sarawak, Borneo, eleven new speces were discovered and described namely; *B. aiensis, B. dinosauria, B. hirsuticarpa, B. iridifolia, B. lawii, B. lichenora, B. magnicarpa, B. metallicolor, B. nix, B. superciliaris* and *B. wallacei*, all of which belonging to section *Petermannia*.

Phutthai and Hughes (2017) reported four new species of *Begonia* from Northern, peninsular and Southern Thailand and are described. Out of four, three species belong to *Begonia* section *Diploclinium* (*B. exposita* Phutthai & M. Hughes, *B. pengchingii* Phutthai & M Hughes, *B. pseudosubperfoliata* Phutthai & M. Hughes) and one to *Begonia* section *ApteroBegonia* (*B. phutthai* M. Hughes).

Ambrish and Amadudin (2006) during the floristic exploration under flora on upper Subansiri District of Arunachal Pradesh, an interesting *Begonia* species was collected. Later it was identified as *Begonia tessaricarpa* C. B. Clarke. The plant was first discovered by C.B Clarke in 1879 and again in 1890 on the basis of single specimen collected by Griffith. Since then it was rediscovered after a century and categorized as endangered plant.

2.3. Biochemical studies in *Begonia*

In plant physiology and metabolism, biochemical compounds and secondary metabolites such as proteins, amines, polyamines, complex carbohydrates, organic acids, lipids, phenols, flavonoids, terpenoids, aromatic compounds, mineral products, hormones and vitamins play an important role.

Most of the ornamental plant species possess enormous sources of antioxidants, which able to remove the negative effect of free radicals. In flowers, polyphenols,

carotenoids, and vitamin C represent essential compounds with antioxidant activity and anti-inflammatory properties (Mlcek and Rop, 2011). Polyphenols are among the most common naturally occurring antioxidants (Haslam, 1998) Flowers with their pigments are rich in phenolics which include phenolic acids, flavonoids and anthocyanins (Cavaiuolo *et al.,* 2013).

Many ornamental flowers are rich in antioxidant compounds, often much higher than common horticultural crops. Estimation of total phenols contents of the plants is one of the parameters used to determine the antioxidant contents. Flowers with higher total phenolics content are *Antigonon leptopus, Bougainvillea glabra, Tagetes erecta, Cosmos sulphureus, Prunus mume* and *Sophora viciifolia* with values >100 mg g^{-1} DW (Cavaiuolo *et al.,* 2013).

A key molecule in plant metabolism is the ascorbic acid, it has been recognized to play a crucial role in several physiological processes such as photosynthesis, photo-protection, cell division, plant development, stress responses, regeneration of other essential molecule (Cavaiuolo *et al.,* 2013; Gallie, 2013). Barth *et al.* (2004) suggested that Ascorbic acid is important in the regulation of development senescence and plant defense against pathogens.

It has also been reported to serve as a co-factor for the biosynthesis of important plant hormones such as ethylene, gibberellic acid and abscisic acid and as a substratum for oxalate and tartrate biosynthesis (Davey *et al.,* 2000; Conklin, 2001; Barth *et al.,* 2006; Gallie, 2013). The ascorbic acid was reported to be involved in the metabolism of cell walls (Smirnoff, 1996).

Flavonoids, along with chlorophylls and carotenoids, are a major pigment in higher plants. Although there have been reports of 8,000 kinds of flavonoids in nature, anthocyanins, chalcones, aurones and some flavonols act as major flower pigments. The flavonoids are present in many flowers as main components. In comparison, colorless or very pale-yellow flavones and flavonols act as copigment substances (Iwashina, 2015).

According to Shairi *et al.* (2011) the fresh and dry mass, water content and soluble carbohydrates of flowers of *Helleborus orientalis* cv. Olympicus showed an increase in the sepal tissues during the process of floral development from bud to fully open bloom after which a decreasing trend was detected during senescence. The reducing and non-reducing sugars of sepal tissues was increased from tight bud sage to partially green stage and declined during senescence. The concentration of non-reducing sugars was found lower as compared to reducing sugars, whereas, soluble proteins concentrations was slightly more during the tight bud to half open stage and increased at full open stage and declined thereafter during senescence. The amount of α-amino acid displayed a small increased during the tight bud to half open stages and then declined afterwards. The concentration of total phenols increased during the tight bud to half open stages, decreased during flower opening and increased afterwards senescence progressed.

As per the Sood (2006) the content of reducing sugar found to be lowers in younger petals and increased rapidly in *Rosa damascena* Mill and *R. bourboniana* Desport. The role of sugars in flower development may have multifunctions, they act as energy source (Moalem-Beno *et al.,* 1997), osmotic regulators (Bieleski, 1993) and as precursors for metabolic processes.

According to Weiss (2001) anthocyanin accumulation is an integral part of lower development in most of the plants and seems to be regulated by same factor that controls petal growth.

As per the Sood (2006) the starch content in both the species of *Rosa* increased from younger to mature stage. Same kind of findings were found during maturation of the perianth leaves of gladiolus flowers (Ferreira *et al.,* 1986), roses (Ho and Nichols, 1997) and carnation (Tirosh and Mayak, 1988) Further, as a function of stages of flower development in carnation, starch content decreased and soluble sugars increased concomitantly and hence, increased sink strength of the developing flower is coordinated with increased mobilization of stored reserves as well as supply of photosynthates. In same findings of Sood (2006) the amount of protein per unit dry weight was highest in the youngest stage and lower in the latter stages of development. A loss of protein during development of daylily petals has been reported by Ley-Yee *et al.* (1992) and *Sandersonia* flower by Eason and Webster (1995).

The size of respirable substrate pool, which is composed, mostly of sugars is affected by the rate of hydrolysis of starch and other polysaccharides (Ho and Nichols, 1977) translocated to the petals on the one hand (Nichols and Ho, 1975) and translocation out of the flower to other plant parts on the other.

Cai and Wang (1998) isolated five compounds were isolated from *Begonia limprichtii* Irmsh. On the basis of chemical and spectral evidences, they identified as stigmast-3-O-beta-D-glucopyanosyl-6-hexadecanoate (1), stigmast-3-O-beta-D-glucopyranoside (2), rutin (3), stigmasterol (4) and dausterol (5).

Wu *et al.* (2004) isolated and characterized three new compounds viz. begonanline, nantoamide. and methyl (S)-glycerate and 44 known compounds has been isolated and characterized from the rhizomes of *Begonia nantoensis.* Authors revealed that some compounds showed cytotoxicity against four human cancer cell lines (cucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, dihydrocucurbitacin E, cucurbitacin I. and (-)-auranamide). They also proved that some compounds showed significant activity against HIV replication in H9 lymphocyte cells (3β,22α-Dihydroxyolean-12-en-29-oic acid, indole-3-carboxylic acid, 5,7-dihydroxychromone, and (-)-catechin).

Hosaka *et al.* (2012) isolated nine anthocyanins, delphinidin 3-O-glucoside, delphinidin 3-O-rutinoside, cyanidin 3-O-glucoside, cyanidin 3-O-rutinoside, petunidin 3-O-glucoside, petunidin 3-O-rhamnosylglucoside, malvidin 3-O-glucoside, malvidin 3-O-rhamnosylglucoside and malvidin 3-O-malonylglucoside, three flavonols, myricetin 3-O-glucoside, kaempferol 3-O-rutinoside, kaempferol 3-O-glucoside, and a flavone, luteolin 4′-O-glucoside, from the black flowers of *Alcea rosea* 'Nigra'

Inomata *et al.* (2013) isolated flavones and anthocyanins from the leaves and flowers of fourteen *Ajuga* taxa (Lamiaceae), which are all native of Japan. 11 out of 13 flavones obtained from the leaves were characterized as apigenin, luteolin, 6-hydroxyluteolin and acacetin glycosides. Ten flavones and anthocyanin has been isolated from the flowers. Out of 10 isolated anthocyanin, six were identified as acylated delphinidin glycosides and four were shown to be acylated cyanidin glycosides.

Aswathy and Murugan (2015) attempted to compare the contents of anthocyanin and morphological traits among three species of *Begonia*. They reported that *B.*

malabarica. *B. rex* 'baby rainbow' and 'black beauty' (69.6 and 70.6 mg g^{-1} FW, respectively) display remarkable anthocyanin content and similar morphological characters in contrast to *Begonia rex* and *Begonia heracleifolia* cultivars.

Ramesh *et al.* (2002) isolated and identified six known compounds *viz.* friedelin, epi-friedelinol, beta-sitosterol, luteolin, quercetin and beta-sitosterol-3-beta-D-glucopyranoside from the leaves of *Begonia malabrica.* According to studies, the plants found to be effective in treating respiratory tract infections, diarrhea and dermal infection by bacteria.

According to Aswathy *et al.* (2016) plant-based antioxidants are fascinated in pharmaceutical industries which may possess nutraceutical potential. The genus *Begonia* due its diverse group form is usually distinguished on the basis of morphological parameters. In this experiment, authors selected two *Begonia* viz. *Begonia rex-cultorum* (Baby rainbow) and *Begonia malabarica*, which exhibits highest antioxidant activities. The IC_{50} value related with DPPH and metal chelating activities of the *B. rex-cultorum* (Baby rainbow) extract are 32.3 μg mL^{-1} and 18.7 μg mL^{-1} respectively. Authors displayed that remarkable scavenging potentialities against metal chelating, β-carotene bleaching, ABTS radical, FRAP assays, comparing with synthetic antioxidant like BHT. Results from this experiment showed that the antioxidant potentialities of the cultivars are positively correlates with anthocyanin concentration.

According to Khoo *et al.* (2017) anthocyanins are colored water-soluble pigments of phenolic group. The pigments are in glycosylated forms. Different colours of the plants is due to the presence of anthocyanin pigments. Cyanidin-3-glucoside is the major

anthocyanin found in most of the plants. The colored anthocyanin pigments have been traditionally used as a natural food colorant. The color and stability of these pigments are influenced by pH, light, temperature, and structure. In acidic condition, anthocyanins appear as red but turn blue when the pH increases. Besides the use of anthocyanidins and anthocyanins as natural dyes, these colored pigments are potential pharmaceutical ingredients that give various beneficial health effects.

Zhang *et al.* (1997) isolated six compounds from *Begoniaevansiana* through TLC, mp, 1H and 13 CNMR and identified as β sitosterol, β amyrin, daucosterol, stigmasterol, stigmasterol 3 O β D glucopyranoside and 4′, 5′,7 trihydroxy flavone 6 O β D glucopyranoside.

Maridass (2009) studied the antibacterial activity through disk- diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* of the leaves extract of *Begonia albo-coccinia B. cordifolia, B. dipetala B. fallax* and *B. floccifera.* Author reported that best results was observed from *B. fallax* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* and also similar results showed in *B. floccifera*. The antibacterial activity was observed might be due to the active constituents of alkaloids and anthraquinones found to be in the all species of *Begonia*.

Jeeva and Marimuthu (2012) investigated the antibacterial activity and phytochemical properties of *Begonia floccifera* methanolic flower extracts. They revealed that the presence of different compounds in the methanolic extracts of *Begonia floccifera* exhibited the anti-bacterial activity. Phenol, tannins, xanthoproteins, steroids, tannins,

steroids, phytosterols, triterpenoids, sapogenins, coumarins and carbohydrates were found during phytochemical analysis.

Singh *et al.* (2013) conducted an experiment were dye sensitized solar cells has been fabricated by sensitizing with dye extract of *Begonia* plant from Eastern Himalayas. It was evident from the current studies that the presence of anthocyanin in *Begonia* dye has been confirmed. Thermal studies of the dye extract reveal that it was stable upto 150 °C. The fabricated cells have efficiency in the range 1.73–1.86%, which was highest known efficiency in anthocyanin based natural dye sensitized solar cell.

Zhang *et al.* (2010) used two genotypes of *Begonia semperflorens* to study the effects of anthocyanins on tolerance of high-light stress. Results revealed that the maximum quantum yield of PSII (Fv/Fm) in red leaves was significantly higher than that in green leaves during and after high-light stress. High light also induced significant increases in anthocyanins in both genotypes, but chlorophyll content and Chl a/b ratio did not differ between red and green leaves. It was observed lower xanthophyll pool size and enzymatic antioxidant activity in red leaves than in green leaves after high light stress. However, non-enzymatic antioxidant activity by DPPH assay was found significantly higher in red leaves than in green leaves. Authors noted that the changes in DPPH activity were closely correlated with changes in anthocyanin content during and after high-light stress. Results suggested that anthocyanins primarily function as light filters rather than as antioxidant molecules during high-light stress in *B. semperflorens*.

Aswathy and Murugan (2017) isolated bioactive anthocyanin from *Begonia malabarica* and *Begonia rex-cultorum* ‘Baby rainbow’ and purified and fractionated by

using amberlite column chromatography and LC-MS/MS analysis. The major anthocyanins identified as anthocyanidin Malvidin 3 –diglucoside as the major compound. They reported that the purified anthocyanin exhibited free-radical scavenging activity against DPPH, hydroxyl radicals and superoxide anions.

Silva *et al.* (2017) conducted their first study of essential oil of *Begonia reniformis.* They identified various compounds in the essential oil extracted from the leaves of *B. reniformis* by hydro-distillation through GC-MS. Of which, Sesquiterpenes silphiperfol-4,7(14)-diene (15.7%) and β-vetispirene (21.0%) were found in major percentage. The oil was studied for anti-bacterial properties for which it has shown weak response against *Bacillus subtilis* and *Pseudomonas aeruginosa* with minimum inhibitory concentrations of 625μg mL^{-1} for each of the two bacteria.

Nisar *et al.* (2017) conducted experiment on physiological and biochemical aspects of flower development and senescence in *Nicotiana plumbaginifolia* Viv. Results showed that the increase concentration of soluble protein from stage I to stage IV (8.48 mg g^{-1}f.m.) to stage IV (8.84mg g^{-1}f.m.) and thereafter significant decrease was observed as the flower development progress towards senescence. The decrease in the soluble protein content towards senescence was observed in some ethylene sensitive (*Alstroemeria, Petunia, Dianthus*) and ethylene in-sensitive (*Hemerocallis, Iris*) flower systems (Ahmad and Tahir 2015; Dar *et al.,* 2014).

To validate the traditional use of *Begonia picta*, Shrestha *et al.* (2018) conducted an experiment and results showed that the methanolic extract possesses anti-hyperglycemic potential and has anti-inflammatory activities. The studies showed that the extracts of *B.*

picta viz. hexane and methanol were found to be mildly toxic in nature based on brine shrimp lethality bioassay.

Zhang *et al.* (2018) laid out an experiment to investigate the response of the morphological and physiological characteristics of *Begonia semperflorens* while exposing to different levels of shading. Results showed that the leaf area, water content, superoxide anion production rate, malondialdehyde (MDA) content and plasma membrane permeability increased as the level of shading increased. In comparison, anthocyanin, soluble sugar, starch, and the superoxide dismutase activity showed increasing patterns. However, the number of flowers per plant, the amount of chlorophyll, nitrate reductase, and peroxidase activities initially increased but subsequently dropped.

Naik *et al.* (2019) carried out research work on different genotypes of Heliconia under shade house conditions. Results found that among multiple genotypes, inflorescence length (26.18cm), number of spikes clump^{-1} 4.50), number of bracts spike^{-1} (9.56), stomatal conductance (0.38 mol m^{-2} s^{-1}), rate of photosynthesis (9.23 µmol m^{-2} s^{-1}), transpiration rate (4.17 mmol m^{-2} s^{-1}) and anthocyanin content in flowers (3.64 mg 100 g^{-1} tissue) recorded highest in genotype G_6.

Gong *et al.* (2020) investigated the potential ecological functions of reddish colour patterns in young leaves to determine the capacity for chemical defense, tannins concentration and anthocyanin of both leaves. Results revealed that young red leaves possess significant higher anthocyanin and tannins content and lower herbivore damages than non-red leaves. Results suggested that the red colouration of young leaves protects

from insects by chemical defense due to high concentration of tannins and anthocyanin in it.

2.4 Uses / Importance

The Genus *Begonia* are much importance and uses apart from the ornamental point of view. Most of the wild species have cooked or taken raw and some having medicinal properties too. Important literature related to uses and importance of *Begonia* species has been provided below.

Rop *et al.* (2012) studied antioxidant and contribution to popularize the some selected 12 edible flower of ornamental plant species including *Begonia bolivensis*. They found positive response and found some flower of selected plant had high level of mineral elements.

Velusamy Kalpansdevi *et al.* (2012) concluded that the methanol extract of *Begonia malabarica* and *B. floccifera* had exhibited *in vitro* anti-oxidant property based on various models of test namely DPPH, hydroxide, super oxide, and ABTS radicle scavenging activity.

According to Meyanungsang (2010) *Begonia picta* Smith, belongs to Begoniaceae Family possess medicinal properties in their leaves. Leaves are used to cleanse hands by crushing between palms and also used in cooking for their sour taste.

Begonia picta recognized as medicinally important species and has been traditionally used, young shoots were crushed and the paste applied on the affected part to treat the wound and juice is rubbed on the forehead to treat headache (Manandhar, 1994;

Shrestha and Dhillion, 2003). Juice of *B. picta* is use in headache, leaf were use in sore nipples and juice of root is use in conjunctivitis (Sapkota, 2013). Leaves are used to cleanse hands by crushing between palms and also leaves are used in cooking for their sour taste (Malewska, 2014).

To validate the traditional use of *Begonia picta,* Shrestha *et al.* (2018) conducted an experiment and results showed that the methanolic extract possesses anti-hyperglycemic potential and has anti-inflammatory activity.

In Nagaland, the luke warm juice is used to treat ulcers and bristles of the mouth or stomatitis, juice is drunk to cure diarrhoea or dysentery, leaves are eaten as a vegetable and mature root stalks are used in the making of red dyes with the leaves of *Impatiens sp* (Rao and Jamir, 1982; Changkija, 1999; Deorani and Sharma, 2007).

Doskotch *et al.* (1969) has traced the presence of cucurbitacin B (1) from the alcoholic extract residue from tubers of *Begonia tuberhybrida* Voss var. alba. Further Cucurbitacin D (2) and dihydrocucurbitacin B were also isolated and identified. Doskotch and Hufford (1970) isolated compound from *Begonia tuberhybrida* which has shown to have the structure of hexanorcucurbitacin D (3).

According to Aswathy and Murugan (2017) anthocyanins are the most common flavonoid molecules of vegetables and fruits, especially berries. Human consumption of anthocyanins represents the highest among the flavonoids. Epidemiological studies have suggested that the consumption of anthocyanins lowers the risk of life style disorders like cardiovascular disease, diabetes, arthritis and cancer. *Begonia malabarica* Lam. of

Begoniaceae, is used traditionally as anti-hypoglycemic, antimicrobial, wound healing and in the treatment of anemia.

Pandikumar *et al.* (2009) studied the effects of hexane, ethyl acetate and methanol extracts of *Begonia malabarica* against hypoglycemic and antihyperglycemic activity. Results showed the decreasing trend in the plasma glucose levels of the animals treated with methanol extracts. However, the serum insulin levels and liver glycogen level had increased significantly compared to the diabetic controls. The authors suggested the use of methanol extracts as aid in treating diabetes.

According to Kingston (2009) *Begonia floccifera* belongs to the family Begoniaceae is endemic to Western Ghats and being used as a wild edible by the Kani tribe of Kanyakumari district (Hediat *et al.,* 2009). According to Ayyanar and Ignacimuthu (2008) tribal peoples of Tirunelveli Hills of Western Ghats used *B. floccifera* plant to cure venereal diseases and to reduce body heat by providing cooling effects.

Ariharan *et al.* (2012) studied the ethano botanical obtained from the Kannikar tribes regarding the medicinal and other uses of *Begonia floccifera* and *B. malabarica*. The ethno botanical studies, phytochemical analysis and the antibacterial evaluation showed that the two plants contain Vitamin C which could be exploited as a potential natural source. The Vitamin C content of *Begonia flocciferra*was 1.62 mg g^{-1} and *Begonia malabarica* was 1.42 mg g^{-1} on fresh weight basis.

According to Ganapthy (2013) *Begonia laciniata* Roxb is found in tropical and sub-tropical regions, especially in America and also found in Sikkim, Arunachal Pradesh, Assam, Meghalaya, Nagaland and Manipur, ascending to an altitude to 2100 m. As per the

Ganapthy (2013) a decoction of the root is given for liver diseases and ever and the extract from succulent stalks is used for venereal diseases in folk medicine. Fresh shoots are chewed for tooth troubles and aqueous extracts of the leaves and flowers of *Begonia* sp are active against Gram-positive and Gram-negative bacteria, Christoper (2003).

According to Bisht and Bhatt (2012), *Begonia venusta* anannual climber with the help of tip tendril, flowers brightly coloured and showy, which leaves and flowers are used as antidote of snake bite.

Shrestha *et al.* (2016) studied to extend the scientific knowledge for utilization of traditionally used medicinal plant *Begonia picta*. The result of the present study revealed that the ethyl acetate extract of *B. picta* has highest TPC (66.994 mg $GAEg^{-1}$) and hexane extract of *B. picta* has highest TFC value (33.3617 mg QEg^{-1}) of extract. Antioxidant activity was determined by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging method. Inhibitory concentrations (IC_{50}) were calculated. The sequence of antioxidant activity of *B. picta* extracts were; ethyl acetate (with IC50 value 40.949μgml^{-1}) > methanol (IC50 value 45.7184μgml^{-1}) > hexane (IC50 value 248.365μgml^{-1}). Antibacterial activity was evaluated by cup diffusion method in which only ethyl acetate extract *B. picta* showed the indicative antibacterial activity against *Salmonella typhi.*

Hong Shan *et al*. (2015) purposed to explore the effect of the extract of *Begonia fimbristipula* on the development of diabetic nephrophathy rats. The extract of *Begonia fimbristipula* can improve kidney filtration function, and has obviously improved the diabetic nephrophathy rats' clinical symptoms.

Rahman (1998) had described *Begonia malabarica* as important medicinal plant whose main secondary metabolites are luteolin, quercetin and β-sitosterol. Gamborg and Phillips (2004) and Ramesh *et al.* (2012) also mentioned that *B. malabrica* leaves are used to treat respiratory infections, dysenteries, blood cancer and skin diseases.

2.5. Micropropagation

The concept of totipotency in plant cells is proposed by Haberlandt (1902), he suggests that any plant cells are capable to produce entire new plants provided with appropriate stimulus and a suitable environment for growth. The technique of growing plant cells, tissues or organs in an artificially prepared nutrient medium under aseptic conditions is known as tissue culture or micropropagation or cell culture.

Tissue culture techniques is used to eliminate virus, for clonal propagation, gene conservation, invitro fertilization, mutation, induction for genetic diversity, genetic transformation, protoplast isolation, and somatic hybridization, secondary metabolite production etc.

Plant tissue culture techniques are the most frequently used tools in the areas of agriculture, horticulture and plant biotechnology and is an ideal method of propagation for rapid multiplication and high rate of propagation within a short duration and limited space, it will provide the plants throughout the year, also protect the plants from pests and pathogen under controlled environmental conditions.

Germplasm conservation is also one of the most important advantages of plant tissue culture. Genetics and plant breeders utilize *in vitro* culture techniques to carryout breeding strategies for the genetic improvement.

With the advance of the plant tissue culture technology, it is now possible to propagate different crops from tiny plantlets. In ornamental crops, orchids, carnation, anthurium, gerbera, gladiolus etc. has been commercially grown through tissue culture techniques. Besides these, globally, the commercial mass production of indoor plants is a growing interest among the horticulturists. There is a high demand for ornamental plants in both domestic and international markets and was significantly increased over the last decades (Jain, 2002). In developed countries, tissue cultures played a great role to propagate the various plats such as *Ficus*, *Begonia*, *Saintpaulia*, *Chrysanthemum*, *Rosa*, *Anthurium* and *Spathiphyllum* were micrpropagated in large scale in the nurseries. Even though, many indoor horticultural species were mass propagated through tissue culture techniques to eliminate the diseases and production of true to type planting materials (Awasthy and Murugan, 2019). This propagation protocols are indeed applicable and beneficial to difficult to propagate and also economically important for those species which are propagated easily (Awal, 2009).

Therefore, plant tissue culture is most promising techniques for the production of large-scale plantlets for use as pot plants (Pederson *et al.*, 1996). Plant tissue culture can also be divided into several methods, which includes meristematic culture, vegetative explant culture, callus induction, suspension culture, direct and indirect somatic embryo induction, synthetic seed production, in-vitro flowering, *in vitro* mutation breeding, protoplast and somatic hybridization process. Awal (2009) suggested that the some of these

techniques can be apply to selected plants to overcome generation incapability of the plants. For tissue culture raised pots plant production, priority will be given to obtain early, synchronize and profuse flowering with homogeneous plant size. It will also provide the to meet the demands of the nursery industries (Pederson *et al*., 1996; Awal, 2009).

Thus, plant tissue culture techniques are always required to meet the demand of potted / indoor plants of floriculture industries and is widely applied in both the research and development of improved crops (Maliro and Lameck, 2004). It has been reported that more than 156 ornamental genera have been propagating through tissue culture techniques in around the world (Route *et al.,* 2004).

For successful production of tissue culture plants, it required many factors to manipulate including media and environmental factors, whereas media factors include, media constituents, macronutrients, micronutrients, vitamins, amino acids, carbon source, gelling agents, activated charcoal, plant growth regulators and pH of the medium whereas, environmental factors include temperature and illumination of the culture room, agitation process and incubation period of the culture. The success or failure of the culture can be determined by both the factors. Apart from these factors, many other processes also need to keep in mind during the tissue culture techniques, which includes source of explant, surface sterilization procedure, medium selection and preparation. Some important literature related to tissue culture of *Begonia* species has been listed below.

The regeneration of adventitious shoots from petiole segments of *Begonia* sp., is also possible on a solid culture medium Murashige and Skoog supplemented with hormonal addition of 0.1 mgL^{-1} NAA and 0.5 mgL^{-1} BA. With a low concentration of 0.1

mgL^{-1} BA, less strains were regenerated from explants, but increasingly more stem in exchange on a culture medium with the addition of 0.1 mg L^{-1} BA were produced 50% more buds, which were regenerated in explants larger than 1 cm from the culture medium with a higher concentration of BA (0.5 mgL^{-1}) (Simmonds, 1984).

Buritt and Leung (1996) studied caulogenesis and rhizogenesis in petiole explant of *Begonia* × *erythrophylla*. They reported that *in vitro* culture of *Begonia* × *erythrophylla* petiole explants showed highly organogenic, shoots and roots were directly regenerate from the epidermal cells.

Jain (1997) used two cytokinin (Kinetin and Zeatin) for regeneration of plantlets of *Begonia* × *elatior* from leaf disc callus. He suggested that plant characteristics was not affected by the use of cytokinin in medium.

According to Nakano *et al.* (1999) leaf and petiole explants of *Begonia* x *tuberhybrida* Voss produced adventitious shoots after culturing in MS medium supplemented with 0.54µM NAA and 0.44 µM BA. Successful plantlets were achieved on half strength MS media containing 0.54µM NAA and solidified with 8 gL^{-1}agar.

Wealander (2006) studied *in vitro* culture of petiole explants from 17 cultivars of *Begonia* × *hiemalis* on a basal agar medium with different combination of NAA and BA. He observed that the explants from short day treated stock plants did not showed any differentiation whereas from long day treated stock plants root, shoot and both root and shoot initiation was recorded after 55 days.

By using leaves and petioles explants of *Begonia* × *hiemalis* Fotsch, Awal *et al.* (2008) successfully induced direct somatic embryogenesis and *in vitro* plant regeneration. They found that higher concentration of cytokinin (0.5 to 10 mgL^{-1}BAP) and low concentration of auxin (0.1 mgL-1 2,4-D) induced direct somatic embryogenesis.

According to Mathan *et al.* (2009) developed a rapid and high yield *in vitro* propagation of *Begonia malabrica* on Murashige and Skoog medium in combination of different concentration of BA and IAA or in alone. They found that the maximum shoots were produced from culture in MS media in combination 4.4 mgL^{-1} BA and 1.4 mgL^{-1} IAA through nodal segments.

Mendi *et al.* (2009) conducted an *in vitro* tissue culture of *Begonia elatoir* cv. Toran orange to enhance regeneration protocol. Authors revealed that the best regeneration and growth was obtained from MS media supplemented with 2.0 mgL^{-1} BA (70%) and 1.0 mgL^{-1} NAA (50%) which was followed by 1.0 mgL^{-1} BA and 0.5 mgL^{-1} NAA. They reported that BAP in combination of NAA gave better cell division and regeneration than the combination of BA and IAA.

Nhut *et al.* (2009) suggested that after eight weeks of culture shoots were elongated and produced 210 ± 9.7 shoots per segments through thin cell layer system. Authors also reported that within eight months of period 10,000 plantlets can be produced from five petiole segments of *Begonia* plants through micropropagation of the axillary buds of one plant.

As per the experiment conducted by Romocea *et al.* (2010) on *in vitro* propagation of *Begonia erythrophylla* L., they found that rooting was absent in mineral basic medium

culture MB -MS medium in combination of cytokinin (1 mgL^{-1} BAP) alone. Further it was observed by the authors that the organogenesis was achieved by culturing in a basic mineral culture media without PGR's and Murashige and Skoog (1962) basic mineral media supplemented with 1 mgL^{-1} IBA.

Nhut *et al.* (2010) established a highly efficient protocol for micropropagation of *Begonia* using thin cell layer system. Authors suggested that by optimizing the size of the tissue and applying an improved selection procedure, shoots can be elongated within 8 weeks of culture with an average of 210 ±9.7 shoots per segments.

High frequency *in vitro* propagation of *B. tuberhybrida* through petioles and leaves explant has been developed by Nada *et al.* (2011). They found that maximum shoots number per leaf explant (132) was reported in MS medium in combination of 1.0 mgL^{-1} NAA and 2.0 mgL^{-1} TDZ. Whereas, 33 shoots per explant through petioles explant has been recorded in MS medium supplemented with 0.5 mgl^{-1} NAA and 2.0 mgL^{-1} TDZ. Successful rooting was observed in MS media supplemented with 0.5 mgL^{-1} NAA.

As per the Ghimire *et al.* (2012) leaf explant of *Solanum aculeatissimum* Jacq. resulted better regeneration rate as compare to petiole explant with highest number of shoot number per explant (11.33±2.21) in MS media supplemented with 0.1 mgL^{-1} and NAA and 2.0 mgL^{-1} BA which contains 3 % sucrose and 0.8 % agar.

Kumaria *et al.* (2012) established *in vitro* plant regeneration protocol from leaf and petiole explants of *B. rubrovenia* var meisneri C.B. Clarke- a rare and endangered ornamental plant of Meghalaya, India. Multiple shoots was found on MS medium supplemented with various concentration of BAP and TDZ. Results found that 65 shoots/

petiole was formed on MS+ 0.1 mgL^{-1} TDZ. Rooting was observed in MS medium + 0.1 mgL^{-1} IAA with 13.8 roots per shoots.

According to Sakhanokho *et al.* (2013) synthetic seeds were produced by culturing shoot tips of two *in vitro* grown *Begonia* cultivars using 3% sodium alginate in Murashige and Skoog medium (MS) salt solution as the gel matrix and 100mM calcium chloride for complexation. Synthetic seed formation was achieved by releasing the sodium alginate per explant combination into 100mM calcium chloride ($CaCl_2 \cdot H_2O$) solution for 30 or 45 min. The best results was obtained by storing synthetic seeds at 4^0C and germinated on MS medium. Regenerated plantlets were successfully established in potting soil.

Awal *et al.* (2013) studied efficient shoot bud formation (94.5 ± 7.59%), *in vitro* regeneration and production of flowers were obtained from sterile plants of *Begonia* × *hiemalis* Fotsch. An *in vitro* regeneration was attempted using immature reproductive organs, which were not commonly used before, such as young inflorescences, peduncles and petals of flowers collected in the field. The present study revealed that floral parts (inflorescence, peduncle and petals) could also be used as a source of explants besides the commonly used tissues such as leaf, stem, shoot and root segments.

According to Rosilah *et al.* (2014) leaf segments of *Begonia pavonina* plants cultured on MS medium in combination of 1.0 and 2.0 mgL^{-1} 2.4-D with 1.0 mgL^{-1} BAP successfully induced somatic embryos. They germinated approximately 90 % for the development of somatic embryos into complete plantlets.

Rowe and Gallone (2016) investigates the effects of NAA and BA on *Begonia rex* Fedor explant. Initially the culture explants was placed in optimum conditions 23.0°C

±1.5°C with photoperiod of 16 hours light and 8 hours dark for eight weeks. Results showed that the highest leaf regeneration (2.7 per plant) was obtained from leaf lamina explants placed in NAA 0.5 µm and BA 0.98 µm. the longest mean root length (2.24cm) was observed in 0.28 µm NAA and 0.49 µm BA. Longest vegetative expansion was noted in leaf lamina explants placed in 0.7 µm NAA and 0.6 µm BAP.

Murugan *et al.* (2016) established *in- vitro* seed germination of *B. malabrica* Lam by breaking dormancy. Results showed that half strength MS medium germinated quicker and expressed good germination rate. Further 3 % sucrose in the culture medium was found optimum for maximum growth of plantlets.

Kumari *et al.* (2017) developed an *in vitro* regeneration protocol of highly valued ornamental and medicinal plant (*B. homonyma*). Authors reported that MS medium in combination with 15 µm BA and 5 µm NAA from leaf explant produced shoot regeneration. They also reported that highest number of shoots (37.2 per explant) was obtained from shoot explants cultured on MS media with 5 µm GA_3 and 0.5 µm BA. Rooting of shoots was also obtained in MS media with 15 gL^{-1} sucrose, 2 µm IBA and 0.5 µm NAA.

Aswathy and Murugan (2017) conducted an experiment of *in vitro* cell suspension culture, isolation, purification of anthocyanin and its antioxidant potential of *Begonia malabarica*, *Begonia rex-cultorum* 'Baby Rainbow'. Explants such as leaves and nodes were cultured on MS medium with various phytohormones for callus induction. Leaf explants of *Begonia* cultured on MS medium fortified with 2, 4-D and BAP showed significant callus induction and also in terms of fresh and dry weights. Significant reddish

coloured callus was achieved in cultures initiated from nodal explants in MS medium supplemented with 2, 4-D. Cell suspension cultures were also established in liquid MS medium. After 14 days of culture, cell suspension was obtained with optimal biomass accumulation.

Nabieva *et al.* (2018) reported morphological studies of *Begonia sutherlandii* brood bud differentiation in tissue culture-controlled conditions. Authors found that cool treatment of bulbils at 2-4° C, followed by application of 0.2 mgL^{-1} 6-BAP was considered to have notable effect on their germination *in vitro*. They found that bulbils induced by three stage treatment formed numerous shoots after three months compared with intact bulbils germinated after five months of dormant period. Using immature buds in different concentration of IBA, BAP, TDZ, adenine sulfate and the macro elements composition of modified MS and N6 media resulted direct shoot organogenesis, flowering in-vitro, and callusogenesis. Results revealed that the best multiple shoot induction (3.73±0.38 shoots/explant) was achieved from cutting flower buds in N6 modified medium supplemented with 50 mgL^{-1} adenine sulfate, 0.5 mgL^{-1} TDZ or 1.0 mgL^{-1} BAP in combination with 0.25 mgL^{-1} IBA. Further it was observed that the transition from vegetative to flowering phase during *B. sutherlandii* micropropagation was induced when 0.2 mgL^{-1} BAP and 40 mgL^{-1} adenine sulfate added to the N6 medium.

Lai *et al.* (2018) conducted an experiment to develop optimum conditions for *in vitro* propagation of *Begonia montaniformis* × *Begonia ningmingensis* var. bella F_1 progeny. Authors reported that maximum explants regeneration of shoots were obtained after eight weeks from half strength MS medium in combination of 2.0 μM BAP and 0.8

μM (NAA). Authors also reported successful rooting on rooting medium after four weeks in half strength MS medium supplemented with different concentrations of NAA.

Karpova *et al.* (2019) designed a protocol of *Begonia fischeri* var *palustris* through *in vitro* seed culture and evaluated the flavonoid content and antimicrobial properties of *in vitro* plantlets. Highest percentage of seed germination (92.5%) was recorded in the half strength MS medium and 83.3% in agar medium in comparison with 50-60 % in greenhouse conditions. Results revealed that flavonoid composition of the leaves of *in vitro* plantlets (13.6 mg g^{-1} of dry weight) insignificantly differed from the leaves of the greenhouse plants and with higher yield of total flavonoids per plant (189.2 g in comparison with 174.0 g).

CHAPTER 3

MATERIAL AND METHODS

The experiment entitled **"Collection, Characterization and Evaluation of selected *Begonia* species of Sikkim Himalayas for its ornamental value**" was carried out in the Department of Horticulture, Sikkim University, Gangtok, Sikkim, India from 2015 onwards. The details of the experimental methodology adopted during the course of the study are discussed herewith.

3.1. Sample collection

Thorough investigation of *Begonia* was carried out with the help of information collected from the various research institutes and available literature review. Mature plants about 1-2 years old of *Begonia* from the different region of Sikkim Himalayas were collected and evaluated for their ornamental value. Collected *Begonia* germplasm from various natural sources was maintained in the departmental field to study the growth and morphological pattern of the plants, and further multiplication of the species for experimental purpose.

3.2. Morphological characters

The systematic utilization of descriptive morphological characters of the *Begonia* species has enabled the characterization and identification of species. For description preparation of selected *Begonia* species, nineteen qualitative traits and nineteen

quantitative traits were studied. The morphological characters used were selected on the basis of the following:

3.2.1. Quantitative traits

Nineteen quantitative traits for morphological descriptions were selected for this experiment. Fully matured plants about two years old plant was selected for the study and characters were recorded during active flowering season of each species. The data of quantitative traits of morphological characters were subjected to analysis of variance (ANOVA) for randomized block design (RBD) at level of 5% CD. Average mean was calculated and data was presented in Mean±SD. Quantitative traits recorded in this experiment were presented in table with its acronym (Table 3.1).

3.2.2. Qualitative traits

Nineteen qualitative traits for morphological descriptions were selected for this experiment. Fully matured plants about two years old plant was selected for the study and characters were recorded during active flowering season of each species. Qualitative traits recorded in this experiment were plant habit, plant form, leaf lamina, leaf margin, leaf base, leaf apex, inflorescence, leaf colour upper surface, leaf colour lower surface, leaf pubescent, stipules colour, petioles colour, petioles hair, flower colour, flower pubescent, capsule colour, capsule hair, peduncle colour and peduncle hair. Descriptor was prepared by compiling the Dorrensbos *et al.*, (1998). Data sheet of the descriptor were attached in annexure 1.

Table 3.1. Quantitative traits with its acronym

Sl. No	Quantitative Traits	Acronym
1.	Plant height (cm)	PH
2.	Plant spread (cm)	PS
3.	Length of midrib (cm)	LM
4.	Leaf length (cm)	LL
5.	Leaf blade (cm)	LB
6.	Petiole length (cm)	PTL
7.	Stipule length (cm)	SL
8.	Peduncle length (cm)	PL
9.	Number of leaves per plant (no.)	NLP
10.	Number of female flowers per plant (no.)	NFF
11.	Number of male flowers per plant (no.)	NMF
12.	Male flower length (cm)	MFL
13.	Male flower width (cm)	MFW
14.	Female flower length (cm)	FFL
15.	Female flower width (cm)	FFW
16.	Flower bud development (days)	FBD
17.	Blooming (days)	BL
18.	Wilting of flowers (days)	WL
19.	Flowering durations (days)	FD

3.3. Survey on the basis of consumer preference of *Begonia* species

On the basis of morphological characters different *Begonia* species were selected for the evaluation of consumer preference by preparing questionnaire and total 100 respondents were randomly selected and interviewed. Data were presented on the basis of percentage of respondents for liking and disliking the *Begonia* species. Questionnaire were attached in annexure 2.

3.4. Biochemical studies

3.4.1. Total protein content

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

- **Reagent A**- 2.0 % Na_2CO_3 mixed in 0.1 N NaOH (2g of sodium carbonate and 4 g of sodium hydroxide in distilled water and volume was made up to 1 liter).
- **Reagent B**- 1 % $CuSO_4.5H_2O$ in 2 % sodium potassium tartarate (1g of copper sulphate was dissolve in 100 ml of distilled water and 2g of sodium potassium tartarate was dissolved in 100 ml distilled water then the working solution of regent B was prepared by mixing the solution of 1 % $CuSO_4.5H_2O$ in 2 % sodium potassium tartarate).
- **Reagent C**- Alkaline Copper solution: prepared by mixing 50 ml of reagent A and 1 ml of reagent B prior to use.
- **Reagent D**- Folin-Ciocalteu Reagent

- **Protein Stock Solution**- BSA mg ml^{-1} (100 mg of Bovine serum albumin was weighed accurately and dissolved 100 ml water).
- **Working standard**- Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. One ml of this solution contains 200 µg protein.

Procedure:

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

b) Preparation of standard curve and estimation of protein:

- From the BSA stock solution 0.1, 0.2, 0.4, 0.6, 0.8 was pipette out and 1 ml of working standard and poured into the series of test tubes.
- Sample extract (0.5 ml) was pipetted out in other set of test tubes.
- Volume was made up to 1 ml in all the test tubes by adding distilled water. A tube with 1 ml of water served as a blank.
- Reagent C (5 ml) was added in each tube and was mixed well and waited for 10 minutes.
- FCR (Reagent D) at the rate of 0.5 ml was added in each tube and mixed well then incubated at room temperature for 30 minutes and reading was taken at 660 nm.

3.4.2. Total Sugar content

One hundred mg plant sample was homogenized in 10 ml of 80 per cent ethanol and centrifuged at 4000 rpm for 20 minutes. The supernatant was collected and the residue

reextracted with 10 ml of 80 per cent ethanol and centrifuged again at 4000 rpm for 20 minutes. Both the supernatant was mixed together. Known amount of ethanol extract (0.1 to 0.2 ml) was evaporated to dryness in a test tube on water bath and cool it to room temperature. 1 ml of distilled water was added to each test tube and mixed thoroughly. To each test tube 4 ml of anthrone reagent was added along the wall of the test tube and mixed gently, heated on a water bath at 100 °C for 10 minutes, cooled rapidly under running cold water and absorbance was measured at 620 nm against reagent blank. The amount of total soluble sugar present in the extract was calculated using standard curve prepared from graded concentration of glucose and expressed as mg g^{-1}.

3.4.3. Starch content

Homogenize 0.1 to 0.5g of the sample in hot 80% ethanol to remove sugars. Centrifuge and residue were retained. Washed out residue with hot 80% ethanol. Dried out the residue well over a water bath. To the residue added 5.0 mL of water and 6.5 mL of 52% perchloric acid. Extract at 0°C for 20 min. After centrifuge supernatant were saved. Extraction were repeated using fresh perchloric acid. Again, centrifuge and pool the supernatant and made up to 100 mL volume. Pipetted out 0.1 or 0.2 mL of the supernatant and made the volume to 1 mL with water. Standard were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 mL in each tube with water. Added 4 mL of anthrone reagent to each tube and heated for eight minutes in a boiling water bath. After cooling, read the intensity of green to dark green color at 630 nm by using UV/VIS Spectrophotometer, Perkin Elmer, Lamda 35 UV/VIS spectrometer. For calculation, multiplied the value by a factor 0.9 and starch content was expressed as mg g^{-1}.

3.4.4. Ascorbic acid

Ascorbic acid content was determined by using method as described in the Handbook of Analysis and Quality control for fruit and vegetable products (2012).

3.4.5. Anthocyanin content

For the estimation of anthocyanin content of *Begonia* plant. 1g of fresh leaf, stem and tuber/ rhizome sample were weigh and then homogenized in 3 ml methanol with 1% HCl and the anthocyanin content was quantified by the standard protocol of Sutharut and Sudarat (2012). The absorbance of each dilution was read at 510 and 700 nm against blank distilled water by using UV/VIS Spectrophotometer, Perkin Elmer, Lamda 35 UV/VIS spectrometer. Total anthocyanin content was expressed as mg L^{-1}. The results were calculated using the following equation:

$$A = (\mathrm{A500} - \mathrm{A700})\mathrm{pH}\ 1.0 - (\mathrm{A_{500}\text{-}A_{700}})\ \mathrm{pH}\ 4.5$$

The total anthocyanin level of the extract was calculated by the formula:

$$Total\ Anthocyanin\ \frac{(mg)}{L} = \frac{A \times BM \times DF \times 1000}{\epsilon \times \iota}$$

Where:

BM =Molecular weight of cyanidin 3-gulucoside

DF =Dilution factor

ϵ = Molar absorptivity of cyanidin 3-gulucoside

ι = Cuvette thickness

3.4.6. Total flavonoid content

Total flavonoid content in the leaf, stem and tuber/rhizome extract of *Begonia* were estimated by using the aluminum chloride colourimetric method (Lamaison and Carnet 1990), with some modifications. Briefly, the test samples were individually dissolved in a different solvent. Then, the sample solution (2 mL) was mixed with 2 mL of 2% Aluminum chloride ($AlCl_3$). The absorbance of the solution was measured at 435 nm by using UV/VIS Spectrophotometer, Perkin Elmer, Lamda 35 UV/VIS spectrometer after 10 min of incubation at ambient temperature. The flavonoid content was expressed as milligram quercetin equivalent (mg QE g^{-1} extract).

3.4.7. Total Phenol content

Total phenolic content of *Begonia* plant extract was determined by using Folin–Ciocalteu assay (Meda *et al.*, 2005). An aliquot of each extract (100 μL; 1 mg mL^{-1}) were taken individually in test tubes. To this solution, 2.5 mL of 10-fold diluted Folin–Ciocalteu reagent was added, and the test tubes were thoroughly shaken. After 3 min, 2.0 mL of 7.5 % Na_2CO_3 solution was added and the mixtures were incubated for 30 min for colour development. The absorbance of the reaction mixtures was measured at 760 nm by using UV/VIS Spectrophotometer, Perkin Elmer, Lamda 35 UV/VIS spectrometer. Gallic acid was used as a standard and TPC of *Begonia* extracts was expressed in milligram gallic acid equivalents (mg $GAEg^{-1}$ extract).

3.4.8. Statistical analysis

The statistical analysis of biochemical analysis was done in Microsoft excel with CRD at level of 5% CD with three replications. Average mean was calculated and data was presented in Mean ± SD.

3.5. Micropropagation of selected *Begonia* species for the production of elite planting material.

3.5.1. Plant material

Three economically and medicinally important *Begonia* species were selected for the micropropagation for the production of elite planting material namely; *B. xanthina* Hook. F., *B. hatacoa* D. Don and *B. palmata* D. Don. All the plants were collected from the various natural habitat of Sikkim Himalayas. During the experimental period, all the plants were planted in pot in the departmental field and maintain their germplasm, which was kept under natural light conditions.

3.5.2. Source of explant

From the healthy two years old mother stock plants, diseases free and healthy intact explants were selected for the tissue culture. Generally, petioles, leaf disc and immature flower buds were utilized to identify the best explant for *in vitro* regeneration of *Begonia*.

3.5.3. Sterilization of medium

Medium was sterilized by heating in an autoclave for 15 minutes at 121.4ºC at 15 psi (pounds per square inch). After sterilization of culture media, media were left for cooling,

after cooling, the medium containers were stored at 4-15ºC in cold room and were used after 3-4 days of preparation.

3.5.4. Tissue culture media preparation

3.5.4.1 Glassware's and plastic wares

Glassware's used for the present study were of the borosilicate quality and obtained from Borosil Glass works Ltd. Bombay and Tarsons Products Private Limited India. Volumetric flasks, plankton, culture tubes, beakers, measuring cylinders, pipettes, funnels, bottles for storage of solutions, petri plates, glass rods, aluminum foil, glass markers, wrapping paper, filter paper were used.

3.5.4.2 Instruments and accessories

The various instrument used for the present investigation were water distillation unit, refrigerators, weighing balances, hot plates, hot air oven and microwave oven, magnetic stirrer, incubators, autoclave, pH meter, chemical storage cabinets, scalpels holder, sterile scalpels blades, blunt forceps, pointed forceps and needles.

3.5.4.3 Culture room

The culture room was maintained at 23 ± 2ºC temperature with the help of air conditioners, photoperiodic controller with 16 hours light and 8 hours dark photoperiod, racks fitted with 1000 lux white fluorescent tubes and laminar air flow.

3.5.5. Maintenance of aseptic conditions

3.5.5.1 Laminar air flow

Laminar air flow was used for aseptic *in vitro* culture by blowing filter sterilized air through laminar flow cabinets and was installed in culture room. The platform of laminar flow cabinet was sterilized with 70 % alcohol by wiping thoroughly before and after use. The working area was irradiated with UV for 30 minutes prior to inoculation and sub-culturing and other culture associated work.

3.5.5.2 Culture room

Culture room was kept under sterilized condition by fumigation once a month with 3% $KMnO_4$ in butanol: formic acid (1:1) and kept closed overnight in a glass petridish. Following day, the room was cleaned with 20% savlon and then with 70% alcohol.

3.5.5.3 Cleaning and Sterilization of Glassware

Glassware were cleaned by dipping in detergent or by dipping in chromic acid overnight and kept under running tap water for 30 minutes to 1 hour in the following day to eradicate all the traces of acid and thoroughly washed with a suitable brush, followed by a rinse with distilled water and dried in hot air oven at 170-180^{o}C for 2 hours and stored in dust proof cabinets. The stainless-steel instruments like scalpels and forceps were subjected to boiled for 30 minutes in single distilled water and cleaned properly with absolute alcohol prior to packing for autoclaving.

3.5.5.4 Autoclaving

Petriplates, forceps, conical flask, beakers, scalpels and distilled water were sterilized by heating in an autoclave to 121.4ºC at 15 lb pressure for 15 minutes. Before autoclaving the petriplates, scalpels and forceps were cleaned with 70% alcohol and then wrapped with brown paper. Culture vessels containing media or without media were also sterilized by heating in an autoclave.

3.5.5.5 Flame sterilization

Instruments like stainless steel forceps, scalpels, were used for inoculation were dipped in alcohol (95%) and then flamed and cool before use. Repeatedly sterilization of these instruments was done at the time of operation to avoid maximum contamination during culture. The instrument may also be sterilized by exposing to UV light.

3.5.6 Explants sterilization

Medium-sized healthy petioles, leaves and flower buds were collected from the stock plants and used as explants to initiate cultures. Sterilization protocol was also established to obtain healthy *in vitro* plantlets to overcome contamination problems during incubation period. Different stages of sterilization procedures were carried out using different solvents such as Tween 20, 15% Sodium hypochlorite and 70% alcohol were applied as sterilizing agents.

The explants were initially surface sterilized for thirty minutes under running tap water to remove the dust particles, followed by stirring in 500 ml distilled water containing 1.0 ml l^{-1} Tween 20 for 15-20 minutes. Explant were immersed with 15% sodium hypochlorite

mixed with two drops of Tween twenty for 20 minutes and then rinsed five times with sterile distilled water. Finally, the explants were rinsed with 70 % ethanol and followed by five times in sterile distilled water. After that explants were rinsed in 0.1% mercuric chloride solution for 30 seconds and again rinsed the explants five times with sterile distilled water. Each rinsed time lasted approximately for one minute.

3.5.7 Preparation of PGR's stock solutions

The stock solutions were prepared by dissolving auxin 100 mg (NAA) in few drops of absolute alcohol and then volumes were made to 100 ml. Likewise, the stock solutions were prepared by cytokinin (BAP) 100 mg by dissolving in few drops of dilute NaOH and final volumes were made to 100 ml with distilled water. Both hormones contained 1.0 mgl^{-1}. Stock solutions of PGR's were stored in a refrigerator at 4º C in liquid phase.

3.5.8 Media composition

The appropriate media used for the present study were Murashige and Skoog medium (1962). The composition of the medium largely determines the success of the culture. Generally, media used for the present experiment consists of MS medium one liter containing 34. 4 g powder.

3.5.9 Direct organogenesis

Different combinations and concentrations of NAA and BAP were used to obtain a good combination range between NAA and BAP for shoot formation. By using five combinations of NAA and BAP, the explants from intact plants were cultured in the selected media. All media contained 3.0 % (w/v) sucrose and 0.8 % (w/v) technical agar. The pH was adjusted to 5.8 before autoclaving.

Leaf discs, petiole and immature flower segments were cultured in the media supplemented with combinations of NAA and BAP. There were 24 treatments in this experiment, with combinations of three levels of NAA (0.1, 0.5 and 1.0 mg l^{-1}) and five levels of BAP (0.1, 0.5, 1.0, 1.5 and 2.0 mg l^{-1}) with one control with five replications per treatment.

All cultures were incubated in the tissue culture room supplied with standard photoperiod and temperature *i.e.* under 16 hours photoperiod at illumination of 1 000 Lux and temperature maintained at 23 ± 1 ºC. The details list of different concentrations and combinations of NAA and BAP that were used in this study were given in table 3.2.

3.5.10 Parameters recorded

Parameters recorded for the present experiment includes survival rate, days required for shoot initiation, shoot length (cm), number of shoots per explant, number of plantlets per explant, days required for root initiation and root length were studied during the in vitro culture of selected *Begonia* species (*B. palmata, B. hatacoa and B. xanthina*)

3.5.11 Statistical analysis

The statistical analysis was done by employing the O.P Stat software packages at level of 5% CD. Average mean was calculated and data was presented in Mean ± SEm.

Table 3.2 Treatments combination of MS medium with different plant growth hormones for micropropagation of *Begonia*

Treatments	Media and plant growth hormone combination
T_1	MS + 0.0 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_2	MS + 0.1 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_3	MS + 0.5 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_4	MS + 1.0 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_5	MS + 1.5 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_6	MS + 2.0 mg l^{-1} BAP + 0.0 mg l^{-1} NAA
T_7	MS + 0.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_8	MS + 0.1 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_9	MS + 0.5 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_{10}	MS + 1.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_{11}	MS + 1.5 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_{12}	MS + 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA
T_{13}	MS + 0.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{14}	MS + 0.1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{15}	MS + 0.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{16}	MS + 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{17}	MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{18}	MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA
T_{19}	MS + 0.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA
T_{20}	MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA
T_{21}	MS + 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA
T_{22}	MS + 1.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA
T_{23}	MS + 1.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA
T_{24}	MS + 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA

CHAPTER 4

RESULTS

The present investigation titled **"Collection, Characterization and Evaluation of Selected *Begonia* species of Sikkim Himalayas for its ornamental value"** was undertaken to characterize the *Begonia* species collected from Sikkim Himalayas and to evaluate their ornamental values, biochemical parameters and conservation through tissue culture. The results of the different parameters observed during the experiment have been presented below:

4.1 Survey and Collection of *Begonia* species from Sikkim Himalayas

Sample collection was carried out from the different regions of Sikkim Himalayas using GPS. A thorough investigation of *Begonia* was carried out with the help of information collected from the various research institutes and available literature from the related field. To carry out this experiment, species of different *Begonia* were collected with the help of GPS coordinates and the altitudinal range was recorded. Collection of *Begonia* sample was based on random sampling methods and collected species were planted in the departmental field for germplasm establishment of *Begonia* species and each species were tagged with their name and location. Details data of collected species of *Begonia* were given in Annexure 3.

4.2. Morphological characters of the *Begonia* species

The morphological characters study of collected *Begonia* species for characterization and identification of species have been carried out. For description preparation of selected *Begonia* species 19 quantitative traits and 19 qualitative traits have been studied and presented below:

4.2.1. Quantitative traits

4.2.1.1. Plant height (cm)

As per the experimental data pertaining to plant height (Table 4.1) revealed that among the analyzed species, plant height varies from 18.66±0.79 cm to 114.40±8.24 cm. Among the analyzed *Begonia* species maximum plant height was found in *B. roxburghii* (114.40 ± 8.24 cm) and minimum was observed in *B. picta* (18.66±0.79 cm).

Results revealed that the diverse range of *Begonia* species was found in Sikkim Himalayas, which includes short, medium and tall heights. After characterizing the plant height of all the analyzed *Begonia* species, *B. picta*, and *B. satrapis* were found short heighted, whereas for medium height were *B. hatacoa, B. josephi, B. megaptera, B. cathcartii, B. xanthina, B. annulata*, and *B. flaviflora, B. panchtharensis, B. palmata, B. sikkimensis* and *B. roxburghii* were tall height Begonias.

4.2.1.2 Plant spread (cm)

Data presented in the table 4.1. showed marked variation in plant spread of *Begonia* species, maximum plant spread was found in the *B. roxburghii* (59.22±2.92cm) followed

by *B. sikkimensis* (53.71±2.31 cm) and minimum was observed in *B. picta* (12.67±0.65 cm), followed by *B. satrapis* (18.44±1.07 cm) and *B. hatacoa* (18.54±2.26 cm).

4.2.1.3 Length of midrib (cm)

The data obtained for length of midrib of studied species was in range of 8.56±0.44 cm to 26.52±1.18 cm which has been presented in table 4.1. Among the collected species, highest length of midrib was found in *B. roxburghii* (26.52±1.18 cm) followed by *B. panchtharensis* (23.65±1.25 cm) and *B. annulata* (23.26±1.95 cm). Minimum length of midrib was collected from the *B. satrapis* (8.56±0.44 cm).

4.2.1.4 Leaf length (cm)

The result of the experiment showed that the leaf length of different wild *Begonia* species was in the range of 10.46±0.45 cm to 31.56±1.63 cm. Among the collected *Begonia* species (Table 4.1), maximum leaf length was found in *B. roxburghii* (31.56±1.63 cm), followed by *B. annulata* (28.40±1.66 cm), *B. flaviflora* (26.98±1.19 cm) and *B. panchtharensis* (26.10±2.12). Whereas, minimum leaf length was observed in *B. satrapis* (10.46±0.45 cm).

4.2.1.5 Leaf blade width (cm)

The observation on leaf blade width of analyzed *Begonia* species shows the range of 5.70±0.39 cm to 25.42±1.63 cm (Table 4.1). Maximum leaf width was found in *B. roxburghii* (25.42±1.63 cm) followed by *B. panchtharensis* (23.42±2.09 cm) and *B. flaviflora* (21.36±1.11 cm). whereas, lowest leaf blade width was found in *B. hatacoa* (5.70±0.39 cm).

Table 4.1. Vegetative morphological characters of wild *Begonia* species from Sikkim Himalayas

Species	PH (cm)	PS (cm)	LM (cm)	LL (cm)	LW (cm)	PTL (cm)	SL (cm)	PL (cm)	NL/P
B. xanthina	42.60±2.86	33.80±1.62	19.50±1.411	23.96±1.62	18.02±0.91	19.48±2.32	1.70±0.18	18.70±1.37	4.40±0.40
B. annulata	45.60±4.10	35.88±2.95	23.26±1.95	28.40±1.66	19.80±0.61	14.84±1.14	1.40±0.05	14.44±1.22	4.80±0.49
B. megaptera	38.60±1.75	40.21±2.20	18.60±1.14	20.62±1.33	14.60±0.89	18.32±1.88	1.60±0.09	12.60±1.50	6.20±0.66
B. palmata	71.38±7.08	38.06±1.06	17.16±0.83	20.92±1.85	16.94±0.84	15.92±1.68	2.10±0.11	13.64±1.00	8.40±1.17
B. josephi	37.54±2.83	26.10±2.79	20.84±1.78	25.28±2.08	17.90±2.14	27.62±2.00	0.70±0.07	25.20±0.76	1.60±0.24
B. cathcartii	37.82±2.89	30.60±2.36	15.66±2.47	19.34±2.46	13.40±2.19	18.20±1.45	1.20±0.08	8.40±0.64	4.40±0.40
B. sikkimensis	73.52±3.42	53.71±2.31	22.72±1.30	25.22±2.27	19.18±1.20	9.16±1.37	2.10±0.17	7.12±0.84	6.40±0.51
B. roxburghii	114.40±8.24	59.22±2.92	26.52±1.18	31.56±1.63	25.42±1.63	11.52±0.57	1.70±0.13	2.22±0.28	11.20±1.02
B. picta	18.66±0.79	12.67±0.65	13.34±0.97	15.02±1.02	11.32±0.88	4.94±0.38	0.80±0.07	16.28±0.41	2.60±0.51
B. hatacoa	35.02±1.93	18.54±2.26	12.24±1.13	15.08±1.70	5.70±0.39	6.06±0.62	1.20±0.11	13.60±0.73	10.40±1.17
B. panchtharensis	69.78±2.80	42.56±2.44	23.65±1.25	26.10±2.12	23.42±2.09	17.72±2.23	2.00±0.14	19.30±1.41	3.60±0.24
B. flaviflora	62.38±6.08	45.38±1.75	20.26±0.84	26.98±1.19	21.36±1.11	15.18±0.95	1.60±0.11	9.10±0.75	6.00±0.63
B. satrapis	23.92±0.97	18.44±1.07	8.56±0.44	10.46±0.45	8.14±0.57	8.84±1.01	0.60±0.05	21.30±1.37	2.60±0.40
CD at 5%	**12.06**	**6.29**	**4.09**	**4.98**	**3.85**	**3.85**	**.31**	**2.97**	**1.92**
CV	**18.37**	**14.14**	**17.25**	**17.64**	**18.29**	**20.95**	**17.47**	**16.69**	**27.16**
SEM	**4.2**	**2.21**	**1.43**	**1.75**	**1.35**	**1.35**	**0.11**	**1.04**	**0.67**

4.2.1.6 Petiole Length(cm)

The petiole length among thirteen different *Begonia* species of Sikkim Himalayas varied between 4.94±0.38 cm to 27.62±2.00 cm (Table 4.1). Petiole length for *B. xanthina, B. megaptera, B. cathcartii* and *B. panchtharensis* were found to be at par with each other. The highest petiole length was recorded in *B. josephi* (27.62±2.00 cm) whereas, lowest petiole length was found in *B. picta* (4.94±0.38 cm).

From the results it was revealed that the petiole length of *Begonia* species is one of the main identical characters for morphological characterization of different species. Each species showed distinct petiole length.

4.2.1.7 Stipule length (cm)

Table 4.1. indicates stipule length observed in thirteen different wild *Begonia* species found in Sikkim Himalayas. The stipule length in all the *Begonia* species under study was recorded in the range 0.60±0.05 cm to 2.10±0.17 cm. *B. sikkimensis* and *B. palmata* showed maximum stipule length (2.10±0.17 cm. and 2.10±0.11 cm, respectively). Whereas, *B. satrapis* has been observed minimum stipule length (0.60±0.05 cm) followed by *B. josephi* (0.70±0.07 cm) and *B. picta* (0.80±0.07 cm).

4.2.1.8 Peduncle length (cm)

The length of peduncle of thirteen different *Begonia* species under the study was recorded and presented in table 4.1. Among the tested species, the peduncle length was found in the range of 2.22±0.28 cm to 25.20±0.76 cm. The peduncle length of *B. josephi* (21.30±1.37 cm) showed maximum followed by *B. satrapis* (21.30±1.37 cm). The

minimum peduncle length was recorded in *B. roxburghii* (2.22±0.28 cm) followed by *B. sikkimensis* (7.12±0.84 cm), *B. cathcartii* (8.40±0.64 cm) and *B. flaviflora* (9.10±0.75 cm.).

4.2.1.9 Number of leaves per plant

The data presented in the table 4.1 pertaining to number of leaves per plant varied between 2.60±0.51 to 11.20±1.02. Among the tested species of wild *Begonia* in this study, the number of leaves per plant was recorded maximum in *B. roxburghii* (11.20±1.02) followed by *B. hatacoa* (10.40±1.17) and *B. palmata* (8.40±1.17). Whereas, minimum number of leaves per plant was observed in *B. picta* and *B. satrapis* (2.60±0.51 and 2.60±0.40, respectively).

4.2.1.10 Number of female flowers per plant

The experimental data presented in the table 4.2 revealed that the number of female flowers per plant in the collected wild *Begonia* species varied between 1.80±0.49 to 34.40±2.04. Highest number of female flowers per plant was recorded in *B. roxburghii* (34.40±2.04) followed by *B. annulata* (16.40±0.93) and *B. megapetra* (16.20±0.58). in contrary, lowest number of female flowers per plant was recorded in *B. satrapis* (1.80±0.49). Most of the *Begonia* species were characterized as monoecious plant, in this study, only *B. roxburghii* is dioecious plant and recorded highest number of female flowers per plant.

4.2.1.11 Number of male flowers per plant

The data presented in the table 4.2 reveals that the number of male flowers per plant was in the range from 4.80±0.49 to 28.40±0.75. Among the thirteen collected wild *Begonia* species, highest number of male flowers per plant was found in *B. roxburghii* (28.40±0.75)

followed by *B. annulata* (28.00±2.45) and *B. megaptera* (21.00±1.82), whereas, lowest number was observed in *B. satrapis* (4.80±0.49).

4.2.1.12 Male flower length (cm)

The data obtained for length of male flower of thirteen different wild *Begonia* species were found to be in the range of 2.62±0.09 to 4.66±0.06 cm (Table 4.2). Among the different *Begonia* species, highest length of male flowers was noted in *B. megaptera* (4.66±0.06 cm), while other tested species in this study were also at par with *B. megaptera* and the lowest was recorded in *B. roxburghii* (2.62±0.09 cm).

4.2.1.13 Male flower width (cm)

In the present experiment, width of male flower in respect of the different *Begonia* species was ranging from 2.00±0.03 to 4.18±0.19 cm (Table 4.2), while comparing the variations among the species, male flower width was found maximum in *B. cathcartii* (4.18±0.19 cm), while other species were found to be at par with each other. Whereas, minimum width of male flower was found in *B. roxburghii* (2.00±0.03 cm).

4.2.1.14 Female flower length (cm)

The observation of the experiment reported that the female flower length of thirteen *Begonia* species was at the range of 2.52 ± 0.04 cm to 4.18 ± 0.13 cm (Table 4.2). Among the different species, maximum female flower length was found in *B. panchtharensis* (4.18±0.13 cm) which is at par with *B. satrapis* (4.14±0.04 cm) and the minimum female flower length was recorded in *B. roxburghii* (2.52±0.04 cm) which is at par with *B. josephi* (2.66±0.09 cm).

Table 4.2. Flowering morphological characters of wild *Begonia* species from Sikkim Himalayas

Species	NFF	NMF	MFL(cm)	MFW(cm)	FFL(cm)	FFW(cm)	FBD(cm)	BL(cm)	WL(cm)	FD(cm)
B. xanthina	12.40±1.29	14.00±0.45	4.08±0.17	3.26±0.054	3.24±0.22	3.12±0.14	17.60±1.17	2.40±0.24	4.40±0.24	46.60±1.54
B. annulata	16.40±0.93	28.00±2.45	4.56±0.13	3.70±0.12	3.42±0.17	3.34±0.08	16.20±0.86	4.20±0.37	4.60±0.51	58.40±1.63
B. megaptera	16.20±0.58	21.00±1.82	4.66±0.06	3.62±0.11	3.72±0.15	3.66±0.14	17.80±0.58	3.60±0.24	5.20±0.37	56.40±2.54
B. palmata	13.40±0.93	14.00±1.41	4.46±0.05	3.84±0.14	3.54±0.05	3.42±0.06	18.40±0.93	4.40±0.24	5.00±0.32	46.20±2.31
B. josephi	5.80±1.69	12.60±0.60	2.90±0.14	2.62±0.15	2.66±0.09	2.42±0.13	21.20±1.28	2.60±0.40	4.80±0.37	37.80±1.80
B. cathcartii	11.20±0.86	14.40±1.03	4.62±0.12	4.18±0.19	3.70±0.17	3.58±0.14	22.60±1.08	3.80±0.37	3.60±0.40	36.80±3.25
B. sikkimensis	12.00±1.41	15.00±1.05	4.52±0.10	3.60±0.17	3.82±0.14	3.62±0.17	23.20±0.92	5.80±0.37	5.40±0.51	52.40±3.53
B. roxburghii	34.40±2.04	28.40±0.75	2.62±0.09	2.00±0.03	2.52±0.04	2.50±0.03	24.80±1.07	6.20±0.49	5.80±0.48	64.80±3.06
B. picta	6.40±0.75	8.20±0.66	2.92±0.17	3.22±0.11	3.42±0.14	3.20±0.14	14.80±0.37	3.40±0.24	2.60±0.24	34.40±2.94
B. hatacoa	12.40±1.17	14.60±0.81	4.62±0.13	2.92±0.11	3.22±0.14	3.10±0.07	15.20±0.37	4.80±0.37	3.80±0.37	47.80±3.28
B. panchtharensis	13.20±1.07	14.00±0.95	4.52±0.09	3.88±0.20	4.18±0.13	3.82±0.13	23.20±0.97	4.00±0.32	4.20±0.37	36.60±2.46
B. flaviflora	6.21±0.66	12.80±1.02	4.60±0.11	4.08±0.12	3.62±0.16	3.58±0.13	16.20±0.80	3.40±0.24	3.40±0.24	36.20±3.97
B. satrapis	1.80±0.49	4.80±0.49	4.18±0.08	3.38±0.37	4.14±0.04	4.12±0.04	13.20±0.37	3.20±0.37	2.40±0.24	33.60±2.18
CD at 5%	**3.19**	**3.41**	**12.06**	**6.29**	**4.09**	**4.98**	**3.85**	**3.85**	**.31**	**2.97**
CV	**20.21**	**17.27**	**18.37**	**14.14**	**17.25**	**17.64**	**18.29**	**20.95**	**17.47**	**16.69**
SEM	**1.12**	**1.19**	**4.2**	**2.21**	**1.43**	**1.75**	**1.35**	**1.35**	**.11**	**1.04**

4.2.1.15 Female flower width (cm)

The data presented in table 4.2. revealed that the female flower length of wild *Begonia* species of Sikkim Himalayas was ranging from 2.42±0.13 cm to 4.12±0.04 cm. The maximum width of female flower was reported in *B. satrapis* (4.12±0.04 cm), whereas, minimum width of female flower was recorded in *B. roxburghii* (2.42±0.13 cm).

4.2.1.16 Flower bud development (days)

The experimental data presented in the table 4.2. showed variation in days required for flower bud development of *Begonia* species, which is in the range of 13.20±0.37 to 23.20±0.92 days. the maximum days required for flower bud development was recorded in *B. roxburghii* (24.80±1.07 days) followed by *B. sikkimensis* (23.20±0.92 days), *B. panchtharensis* (23.20±0.97 days) and *B. cathcartii* (22.60±1.08 days). Whereas, minimum days required for flower bud development was noted in *B. satrapis* (13.20±0.37 days) followed by *B. picta* (14.80±0.37 days).

4.2.1.17 Days taken for anthesis

The observation of the results (table 4.2) revealed that the days taken for anthesis from the flower bud development were at the range of 2.40±0.24 to 6.20±0.49 days. Among the tested species of *Begonia*, the maximum days taken for anthesis was noted in *B. roxburghii* (6.20±0.49 days) and the minimum days taken for anthesis was recorded in *B. xanthina* (2.40±0.24 days).

4.2.1.18 Days taken for flower wilting

The data obtained for days taken for flower wilting (Table 4.2) of thirteen different *Begonia* species was found in the range of 2.40±0.24 to 5.80±0.48 days. Among the tested species, maximum days taken for flower wilting was recorded in *B. roxburghii* (5.80±0.48 days), whereas, minimum days taken for flower wilting was noted in *B. satrapis* (2.40±0.24 days) which is at par with *B. picta* (2.60±0.24 days).

4.2.1.19 Flowering durations (days)

The results obtained from the experiment revealed that the flowering durations (Table 4.2) of different *Begonia* species was in the range of 33.60±2.18 to 64.80±3.06 days. Among the tested species, maximum flowering durations was found in *B. roxburghii* (64.80±3.06 days) followed by *B. annulata* (58.40±1.63 days) and *B. megaptera* (56.40±2.54 days). Minimum flowering days among the species was noted in *B. satrapis* (33.60±2.18 days) followed by *B. picta* (34.40±2.94 days).

4.2.2 Qualitative traits

4.2.2.1 Plant Habit

According to the results obtained from the table 4.3., tuberous and rhizomatous plant habit of wild *Begonia* species were found. In total collected thirteen species, three species were tuberous habit which includes *B. picta, B. josephi* and *B. satrapis,* rest of all other ten species were rhizomatous habit (*B. xanthina, B. annulata, B. megaptera, B. palmata, B. cathcartii, B. flaviflora, B. sikkimensis, B. panchtharensis, B. roxburghii* and *B. hatacoa).*

Table 4.3. Description of Plant habit, Plant form, Leaf lamina, Leaf Margin and Leaf base of wild *Begonia* from Sikkim Himalayas.

Species	Plant Habit	Plant Form	Leaf Lamina	Leaf Margin	Leaf Base
B. xanthina	Rhizomatous	Dioecious	Ovate to broadly ovate	Entire to undulate	Cordate
B. annulata	Rhizomatous	Dioecious	Ovate	Undulate to dentate	Cordate
B. megaptera	Rhizomatous	Dioecious	Ovate	Undulate to dentate	Unequally cordate
B. palmata	Rhizomatous	Dioecious	Narrowly to broadly ovate to orbicular	Acute lobes with denticulate	Cordate
B. josephi	Tuberous	Dioecious	Oblong ovate to broadly ovate, peltate	Acute lobes with denticulate	Rounded
B. cathcartii	Rhizomatous	Dioecious	Ovate to broadly ovate	Serrated to denticulate	Cordate
B. sikkimensis	Rhizomatous	Dioecious	Orbicular to ovate	Denticulate to Lobed	Shallowly cordate
B. roxburghii	Rhizomatous	Monoecious	Ovate to broadly ovate	Entire to denticulate	Cordate
B. picta	Tuberous	Dioecious	Ovate to orbicular	Dentate to denticulate	Cordate
B. hatacoa	Rhizomatous	Dioecious	Ovate to lanceolate	Subentire to denticulate	Cuneate
B. panchtharensis	Rhizomatous	Dioecious	Orbicular to deeply lobed	Palmately lobed	Deeply cordate
B. flaviflora	Rhizomatous	Dioecious	Ovate	Acutely deeply lobed, dentate with finer serration	Cordate
B. satrapis	Tuberous	Dioecious	Ovate	Dentate to denticulate	Cordate

4.2.2.2 Plant Form

It was evident from the results (Table 4.3) that only one species of *Begonia* (*B. roxburghii*) was dioecious whereas, other species were characterized as monoecious (*B. xanthina, B. annulata, B. megaptera, B. palmata, B. cathcartii, B. flaviflora, B. sikkimensis, B. panchtharensis, B. picta, B. josephi, B. satrapis* and *B. hatacoa*).

4.2.2.3 Leaf Lamina

According to table 4.3, leaf lamina of thirteen *Begonia* species was evaluated; variations were recorded for each species. The leaf lamina of the *Begonia* species was found in the range of ovate to lanceolate to linear. The leaf lamina of four *Begonia* species (*B. annulata, B. megaptera, B. flaviflora,* and *B. satrapis)* was found ovate. Similarly, three species viz. *B. xanthina, B. cathcartii,* and *B. roxburghii* were found ovate to broadly ovate leaf lamina. Likewise, leaf lamina of three species (*B. sikkimensis, B. picta,* and *B. palmata*) was found ovate to orbicular. Whereas, *B. josephi* showed distinct from all the other species by having oblong ovate to broadly ovate and peltate leaf lamina, *B. hatacoa* also shows distinct leaf margin of ovate to lanceolate and *B. panchtharensis* showed orbicular to deeply lobed leaf lamina.

4.2.2.4 Leaf Margin

Based on leaf margin the thirteen collected *Begonia* species show distinct characteristics (Table 4.3). Three species having acute lobes with denticulate leaf margin was found (*B. palmata, B. josephi* and *B. flaviflora*). *B. annulata* and *B. megaptera* was found to be undulate to dentate leaf margin. Likewise, *B. picta* and *B. satrapis* shows dentate to denticulate leaf margin, *B. xanthina* was characterized under entire to denticulate

leaf margin. Whereas, *B. cathcartii* with serrated to denticulate, *B. sikkimensis* with denticulate to lobed, *B. hatacoa* having subentire to denticulate, *B. roxburghii* having entire to denticulate and *B. panchtharensis* with palmately lobed leaf margin.

4.2.2.5 Leaf Base

The data presented in table 4.3. show a distinct variation of leaf base among the thirteen *Begonia* species. Out of thirteen collected species, six species shown cordate leaf base (*B. xanthina, B. annulata, B. cathcartii, B. roxburghii, B. flaviflora, B. satrapis, B. palmata* and *B. picta).* Whereas, *B. megaptera* have unequally cordate leaf base, *B. sikkimensis* shown shallowly cordate and *B. panchtharensis* having deeply cordate leaf base. The leaf base of *B. josephi* was found rounded and cuneate to rounded leaf base was recorded in *B. hatacoa.*

4.2.2.6 Leaf Apex

A marked difference in the leaf apex was recorded between the thirteen wild *Begonia* species (Table 4.4). For leaf apex, majority of the species showed acuminate leaf apex, which includes (*B. megaptera, B. cathcartii, B. sikkimensis, B. roxburghii, B. hatacoa* and *B. panchtharensis*), while three species showed acute to acuminate leaf apex (*B. picta, B. satrapis* and *B. josephi),* two species has been found acute leaf apex (*B. flaviflora* and *B. annulata), B. xanthina* showed acuminate to cuspidate leaf apex, whereas, *B. palmata* exhibits acutely toothed or lobed leaf apex.

Table 4.4. Description of Leaf apex, Inflorescence, leaf surface upper side, leaf surface lower side and leaf pubescent of wild *Begonia* from Sikkim Himalayas.

Species	Leaf Apex	Inflorescence	Leaf surface upper side	Leaf Surface lower side	Leaf Pubescent
B. xanthina	Acuminate to cuspidate	Cymose	Green with pale green and greyish spots	Maroon red with green	Rare
B. annulata	Acute	Cymose	Green with white lining	Green with red blotches between the veins	Absent
B. megaptera	Acuminate	Cymose	Green	Light Green	Absent
B. palmata	Acutely toothed or lobed	Cymose	Green with white lining/ Green	Reddish green/ Green	Present
B. josephi	Acute to acuminate	Cymose	Green/Dark green	Light Green/ maroon red	Present
B. cathcartii	Acuminate	Cymose	Green	Green	Dense
B. sikkimensis	Acuminate	Cymose	Green	Green	Rare
B. roxburghii	Acuminate	Cymose	Green	Green	Absent
B. picta	Acute to acuminate	Cymose	Dark green to maroon red/Green	Reddish purple and green blotches/ Light Green	Dense
B. hatacoa	Acuminate	Cymose	Green/Dark green	Light Green/ Maroon red	Rare
B. panchtharensis	Acuminate	Cymose	Green	Light Green	Rare
B. flaviflora	Acute	Cymose	Green with red blotches	Reddish green	Present
B. satrapis	Acute to acuminate	Cymose	Green	Light Green	Present

4.2.2.7 Inflorescence

All the collected thirteen *Begonia* species have Cymose type of inflorescence. There was no variation in terms of inflorescence as presented in the table 4.4.

4.2.2.8 Leaf Colour Upper Surface

In case of leaf colour of the upper surface, green colour dominates among all the species (Table 4.4). The only green colour was observed in eight species (*B. megaptera, B. josephi, B. cathcartii, B. sikkimensis, B. roxburghii, B. hatacoa, B. panchtharensis* and *B. satrapis,* green with while colour lining in the upper surface of leaf was found in *B. annulata* and *B. palmata.* Whereas, leaf colour of the upper surface in *B. xanthina* has found pale green with greyish spots, *B. picta* having dark green to marron red and *B. flaviflora* have green with red blotches in upper surface of the leaf.

Results revealed that the *Begonia* species shows a diverse range of leaf colour, most importantly, *B. jsoephi, B. hatacoa, B. picta* and *B. palmata* showed different characteristics, their upper surface somewhat differed within the species level, two phenotypically different variants of the same species was found. In case of *B. josephi* and *B. hatacoa,* the upper surface of leaf was divided into two phenotypical characters, one is considered as dark green and another green in colour. Whereas, *B. picta* showed dark green to marron reddish colour and green colour, likewise *B. palmata* also showed two distinct phenotypical characters in upper surface of leaves.

4.2.2.9 Leaf Colour Lower Surface

Leaf colour of the lower surface of *Begonia* species was recorded and presented in table (4.4.). The characters showed marked variation among the species. Light green colour on

the lower surface was recorded in five species (*B. satrapis, B. panchtharensis, B. hatacoa, B. josephi, B. picta,* and *B. megaptera).* Green colouration on lower surface was found in three species (*B. cathcartii, B. sikkimensis* and *B. roxburghii).* Whereas, other species showed a mixture of colour on the lower surface of the leaf, which includes reddish green (*B. flaviflora* and *B. palmata),* maroon red with green, in *B. xanthina,* green with red blotches between the vein, in *B. annulata,* reddish purple and green blotches in *B. picta.*

Apart from this, *B. palmata, B. picta, B. hatacoa* and *B. josephi* showed distinct phenotypical characters based on lower surface leaf colour within the species level. Two morphologically different leaf colour was found in *B. palmata,* one having reddish green and another has been found in green colour. Whereas, *B. picta* showed reddish purple and green blotches in one phenotype and light green colouration on lower surface of the leaf in another phenotype. Likewise, in both the species of *B. josephi* and *B. hatacoa* showed light green and marron red phenotypes.

4.2.2.10 Leaf Pubescent

According to the results depicted in table 4.4. leaf pubescent was characterized as absent, rare, present, and dense. Out of thirteen collected species, rare leaf pubescent was found in the leaf of *B. hatacoa, B. sikkimensis, B. panchtharensis* and *B. xanthina.* Leaf pubescent was present in four species (*B. palmata, B. josephi, B. flaviflora* and *B. satrapis*). Dense leaf pubescent was recorded in the leaf of *B. cathcartii* and *B. picta.*

Table: 4.5. Description of Stipules' colour, Petioles colour, Petioles Hair, Flower colour, Flower pubescent of wild *Begonia* species from Sikkim Himalayas.

Species	Stipules' colour	Petioles colour	Petioles hair	Flower colour	Flower pubescent
B. xanthina	Yellowish green	Reddish	Present	Yellow	Absent
B. annulata	Reddish	Green	Dense	Pinkish white	Absent
B. megaptera	Light Pink	Green	Absent	Pinkish white	Absent
B. palmata	Green	Dark Green	Present	Pinkish white	Present
B. josephi	Brown	Greenish brown	Rarely present	Pinkish white	Present
B. cathcartii	Reddish	Green	Dense	Pinkish white	Dense
B. sikkimensis	Green	Dark Green	Absent	Red	Absent
B. roxburghii	Brown	Green	Absent	White	Absent
B. picta	Green	Reddish	Dense	Pink	Present
B. hatacoa	Brown	Greenish brown	Rarely present	Pinkish white	Absent
B. panchtharensis	Green	Green with red dots	Absent	Pinkish white	Present
B. flaviflora	Brown	Reddish	Present	Yellow	Absent
B. satrapis	Light green	Reddish	Present	Pink	Present

4.2.2.11 Stipules' Colour

Stipules' colour of thirteen wild *Begonia* species was characterized and details were presented in table 4.5. green colour stipules were found in *B. palmata, B. sikkimensis, B. picta, B. panchtharensis* and *B. cathcartii.* Likewise, brown colour stipules were recorded in *B. josephi, B. roxburghii,* and *B. hatacoa.* Whereas, *B. annulata* and *B. cathcartii* showed reddish stipules' colour.

4.2.2.12 Petioles Colour

As per the data presented in table 4.5, reddish colour petiole was found in *B. xanthina, B. flaviflora, B. picta,* and *B. satrapis.* Green colour petiole was found in *B. annulata, B. megaptera, B. cathcartii* and *B. roxburghii.* Whereas, dark green petiole colour were observed in *B. palmata* and *B. sikkimensis.* Results showed that greenish brown colour of petiole were found in *B. josephi* and *B. hatacoa. B. panchtharensis* showed green colour with red dotted in petiole.

4.2.2.13 Petiole hair

According to the morphological data presented in the table (4.5) showed marked variation in case of petiole hair present or not in thirteen *Begonia* species. Petiole hairs were absent in *B. megaptera, B. sikkimensis, B. roxburghii* and *B. panchtharensis,* rarely present in *B. josephi* and *B. hatacoa.* Whereas, petioles were present in *B. xanthina, B. palmata, B. flaviflora,* and *B. satrapis,* densely present in *B. annulata, B. cathcartii,* and *B. picta.*

Table 4.6. Description of Capsule colour, Capsule hair, Peduncle colour and Peduncle hair of wild *Begonia* species from Sikkim Himalayas.

Species	Capsule colour	Capsule hair	Peduncle colour	Peduncle hair
B. xanthina	Yellowish green	Absent	Red	Absent
B. annulata	Red	Absent	Reddish green	Absent
B. megaptera	Pink	Absent	Light green	Absent
B. palmata	Green	Present	Green	Present
B. josephi	Dark brown	Absent	Red	Absent
B. cathcartii	Green	Present	Green	Present
B. sikkimensis	Red	Absent	Reddish green	Absent
B. roxburghii	Red	Absent	Red	Absent
B. picta	Green	Present	Green	Present
B. hatacoa	Red /Green	Absent	Reddish green	Absent
B. panchtharensis	Green	Absent	Reddish green	Absent
B. flaviflora	Yellowish Green	Absent	Red	Absent
B. satrapis	Green	Present	Red	Present

4.2.2.14 Flower Colour

Results revealed a marked variation in the flower colour of collected thirteen *Begonia* species (Table 4.5). Pinkish white flower colour dominant the most which includes *B. annulata, B. hatacoa, B. palmata, B. josephi, B. cathcartii* and *B. panchtharensis.* The pink colour flower was found in *B. megaptera, B. picta,* and *B. satrapis,* whereas, the yellow colour flower was recorded in *B. xanthina* and *B. flaviflora*. White colour flower was found in *B. roxburghii* and red colour flower was noted in *B. sikkimensis.*

4.2.2.15 Flower pubescent

According to the data presented in table 4.5, variation in the flower pubescent was observed, species of *B. cathcartii* showed densely flower pubescent, while *B. palmata, B. josephi, B. picta, B. panchtharensis,* and *B. satrapis* noted flower pubescent (present) and *B. xanthina, B. annulata, B. megaptera, B. sikkimensis, B. roxburghii, B. hatacoa*, and *B. flaviflora* were categorized as non-flower pubescent group (absent).

4.2.2.16 Capsule Colour

For capsule colour of thirteen wild *Begonia* species, six species shown green colour capsule (*B. cathcartii, B. palmata, B. hatacoa, B. picta, B. panchtharensis* and *B. satrapis*), three species showed red colour capsule (*B. annulata, B. roxburghii* and *B. sikkimensis*), yellowish green colour capsule was found in two species (*B. flaviflora* and *B. xanthina)* and one species having pink colour capsule (*B. megaptera).* Whereas, one species showed two distinct capsule colour, in *B. hatacoa*, some phenotypes showed a red colour capsule and in some green colouration of capsule was noted (Table 4.6).

4.2.2.17 Capsule Hair

In case of capsule hair, nine species out of thirteen wild *Begonia* species do not possess any capsule hair (*B. xanthina, B. annulata, B. megaptera, B. josephi, B. sikkimensis, B. roxburghii, B. hatacoa, B. panchtharensis* and *B. flaviflora*), whereas, other five species have been found capsule hair (*B. palmata, B. cathcartii, B. picta* and *B. satrapis*) (Table 4.6).

4.2.2.18 Peduncle Colour

According to the results depicted in table 4.6, red colour peduncle was noted in five wild *Begonia* species (*B. xanthina, B. josephi, B. roxburghii, B. flaviflora* and *B. satrapis*), reddish green peduncle colour was found in four species (*B. annulata, B. sikkimensis, B. panchtharensis* and *B. hatacoa),* green colour peduncle was noted in *B. palmata, B. cathcartii* and *B. picta* whereas, light green peduncle colour was found in *B. megaptera.*

4.2.2.19 Peduncle Hair

Peduncle hair of all the thirteen collected *Begonia* species was categorized as absent and present, and the data presented in table 4.6. Nine species in case of peduncle hair was found absent (*B. xanthina, B. annulata, B. megaptera, B. josephi, B. hatacoa, B. roxburghii, B. panchthrensis* and *B. flaviflora*). Four out of thirteen species had peduncle hair (*B. palmata, B. cathcartii, B. picta* and *B. satrapis*).

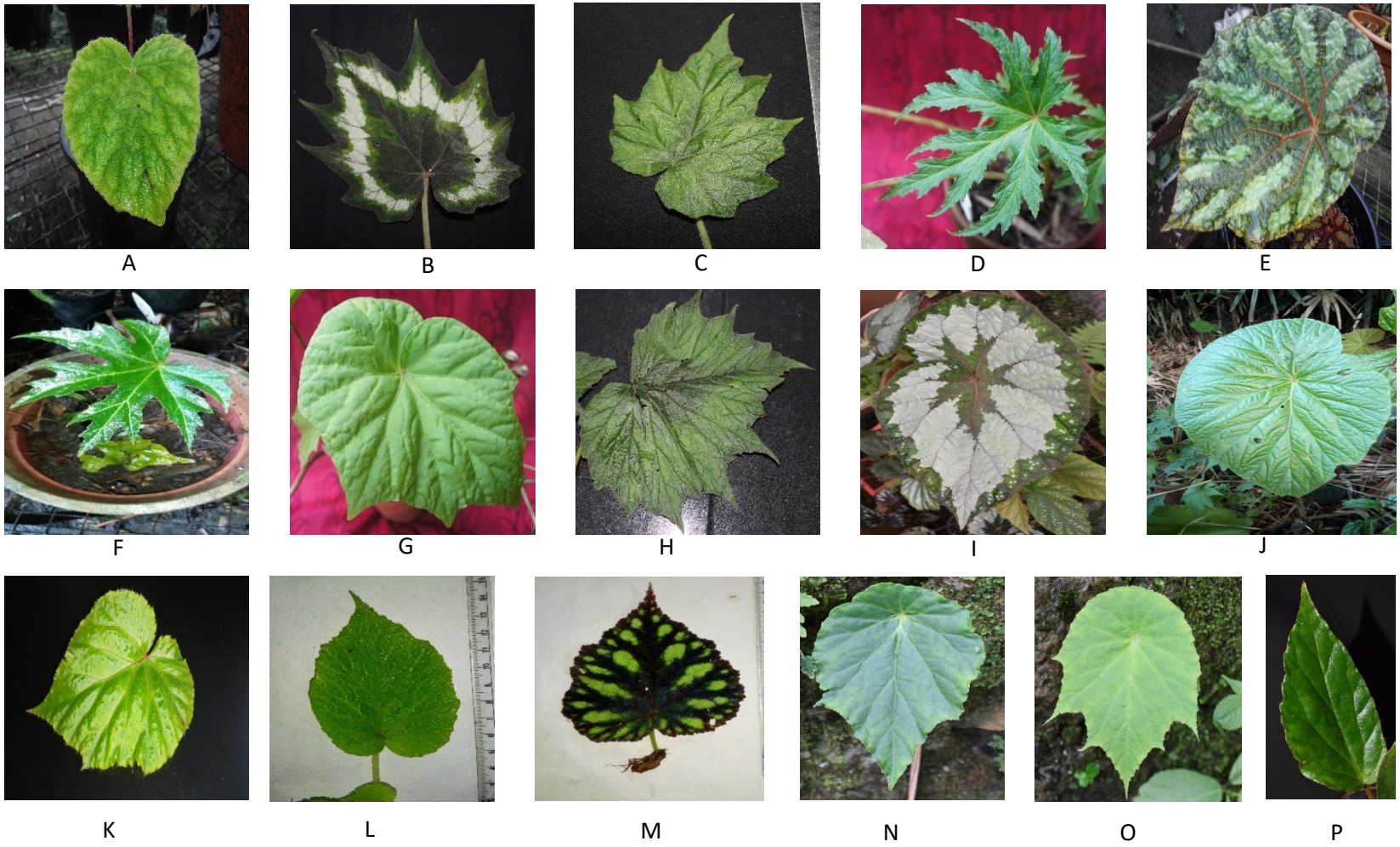

Plate 1. Leaf morphology of wild *Begonia* from Sikkim Himalayas. A-) *B. satrapis*, B- C) *B. palmata*, D) *B. panchtharensis*, E) *B. xanthina* , F) *B. sikkimensis*, G) *B. megaptera*, H) *B. flaviflora*, I) *B. annulata*, J) *B. roxburghii*, K) *B. cathcartii*, L-M) *B. picta*, N-O) *B. josephi*, *and* P) *B. hatacoa.*

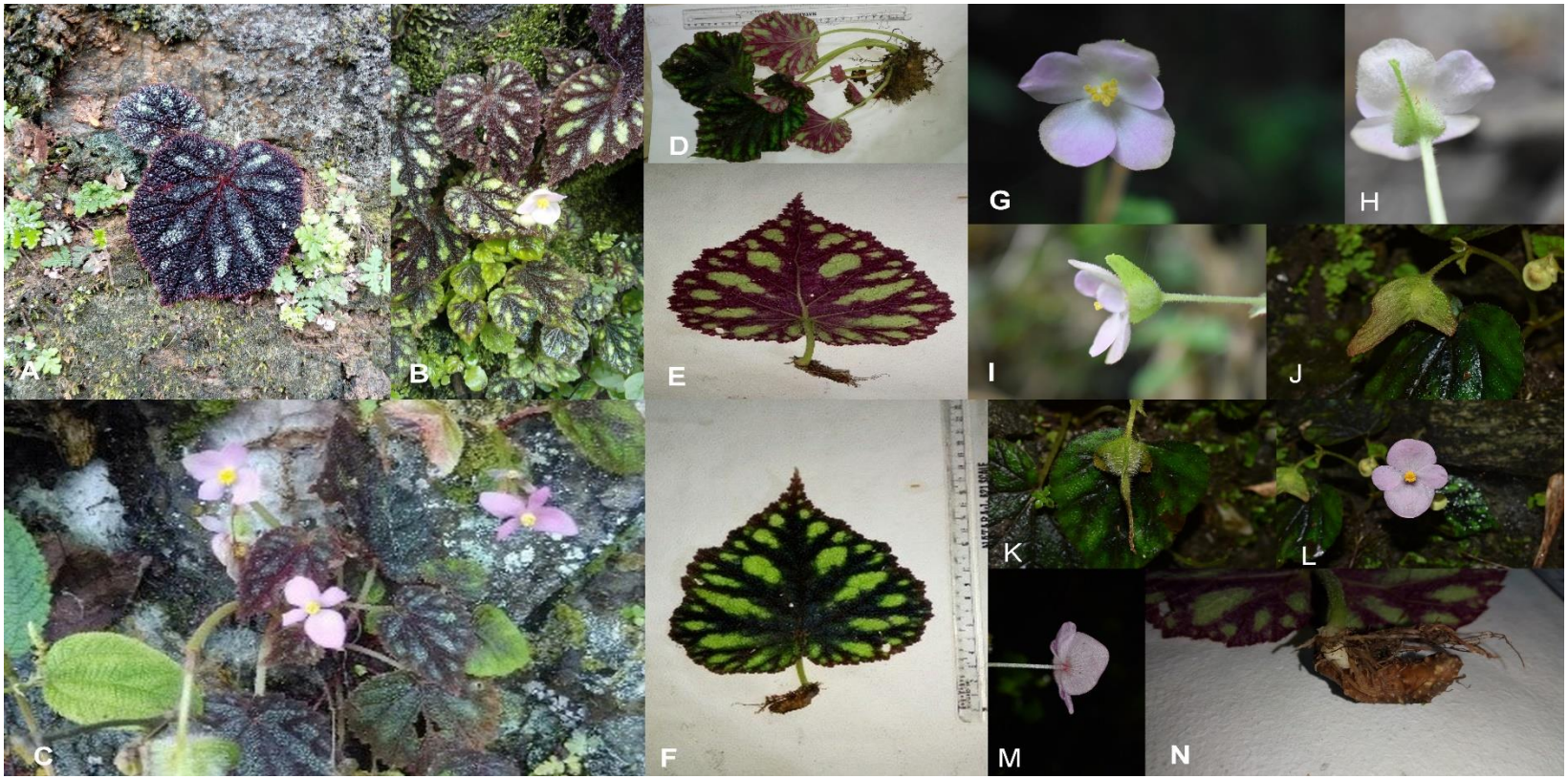

Plate 2. *Begonia picta* Sm. (Red Phenotypes): A-C) Habit, D) Whole Plant, E) Abaxial leaf surface, F) Adaxial leaf surface, G-I) Female flower, J-K) Capsule, L-M) Male flowers and N) Tuber.

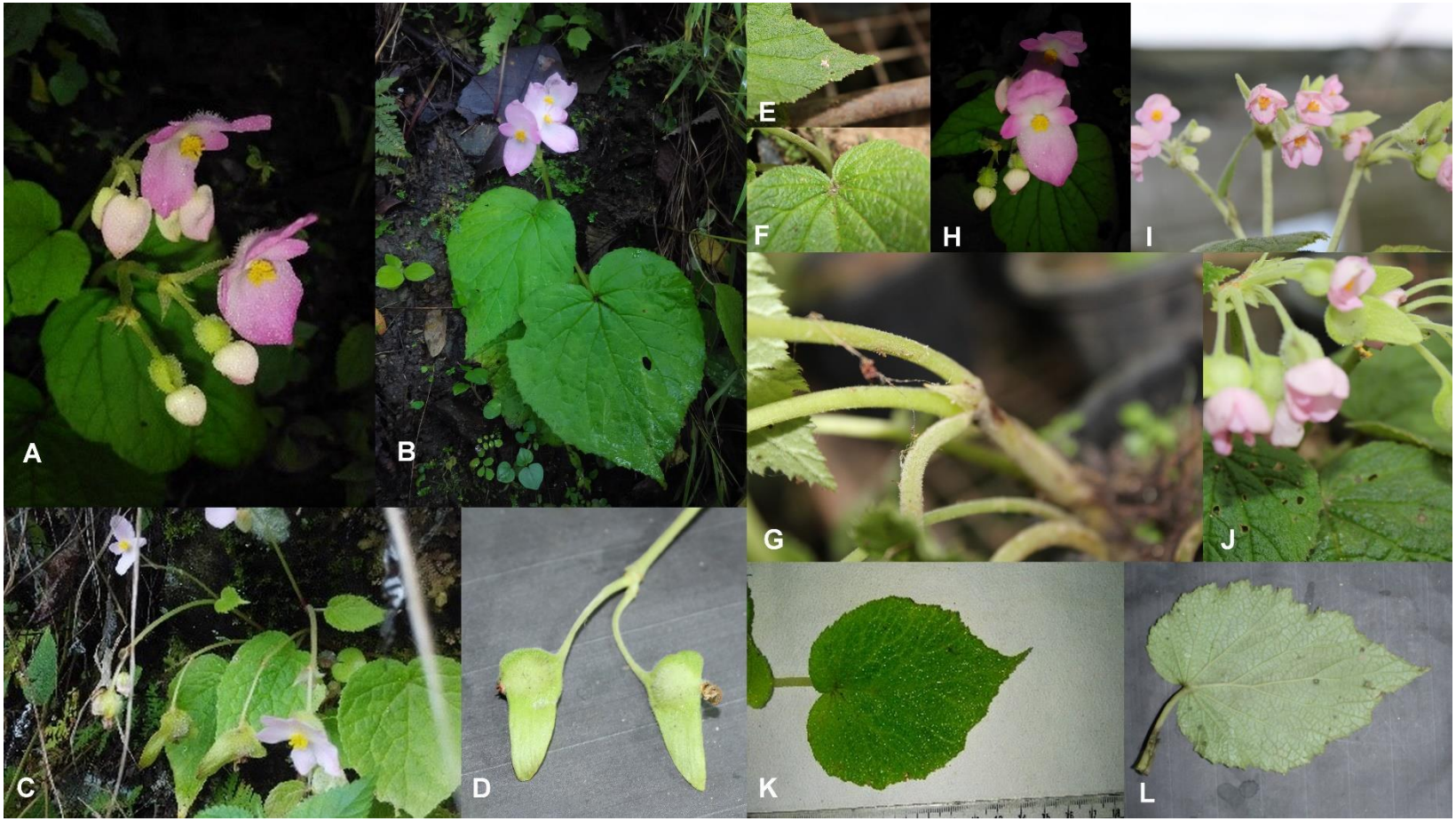

Plate 3. *Begonia picta* Sm. (Green Phenotypes): A-C) Habit, D) Capsule, E) Leaf tip, F) Leaf base, G) Stipules, H) Male flower, I-J) Female flowers, K) Adaxial leaf surface and L) Abaxial leaf surface.

Plate 4. *Begonia josephi* A.D.C (Red Phenotypes): A) & B) Habit, C) Tuber, D) Abaxial leaf surface, E) Adaxial leaf surface, F) back side of petals, G) Front view of petals, H) leaf base and I) leaf apex.

Plate 5. ***Begonia josephi*** **A.D.C (Green Phenotypes):** A) Habit, B) Leaf petioles directly arise from the tuber, C) Leaf apex, D) Leaf base, E) tuber, F) Abaxial leaf surface, G) Adaxial leaf surface, H) Front view of flower petals and I) back side of petals.

Plate 6. *Begonia hatacoa* Buch.-Ham. Ex D.Don (Red Phenotypes): A-C) Habit, D) Rhizome E) Stipules F) Leaf with flower buds, G) Abaxail leaf surface, H) Adaxial leaf surface, I) Male flower and J) Capsule.

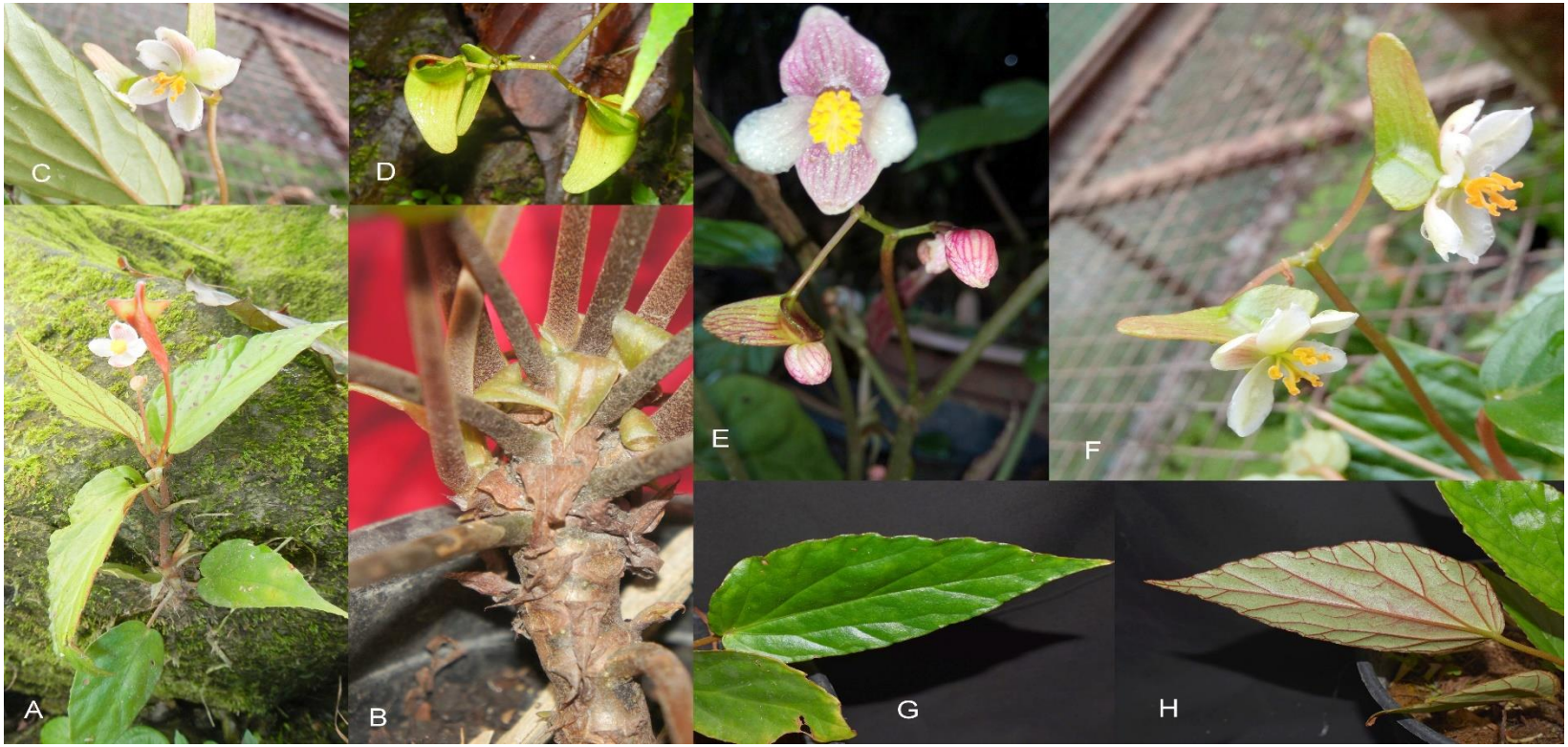

Plate 7. *Begonia hatacoa* Buch.-Ham. Ex D.Don (Green Phenotypes): A) Habit, B) Stem, Petioles arising from stem and stipules, C) & F female flowers, D) Capsule, E) Male flower, G) Adaxial leaf surface, and H) Abaxial leaf surface.

Plate 8. ***Begonia xanthina*** **Hook. F**. A- Whole plant and habitat, B- Stipules and petioles arising from rhizome, C- Leaf base, D- Leaf apex, E -G- Staminate flower, H-I- Carpellate flower and capsule.

Plate 9. *Begonia megaptera* D. Don: A and B- Habit and whole plant, C- Stipule and petioles arising from the rhizome, D: Carpellate flower, E- Leaf apex, F- Stipules, G and H- Staminate flower, and I- Rhizome and roots.

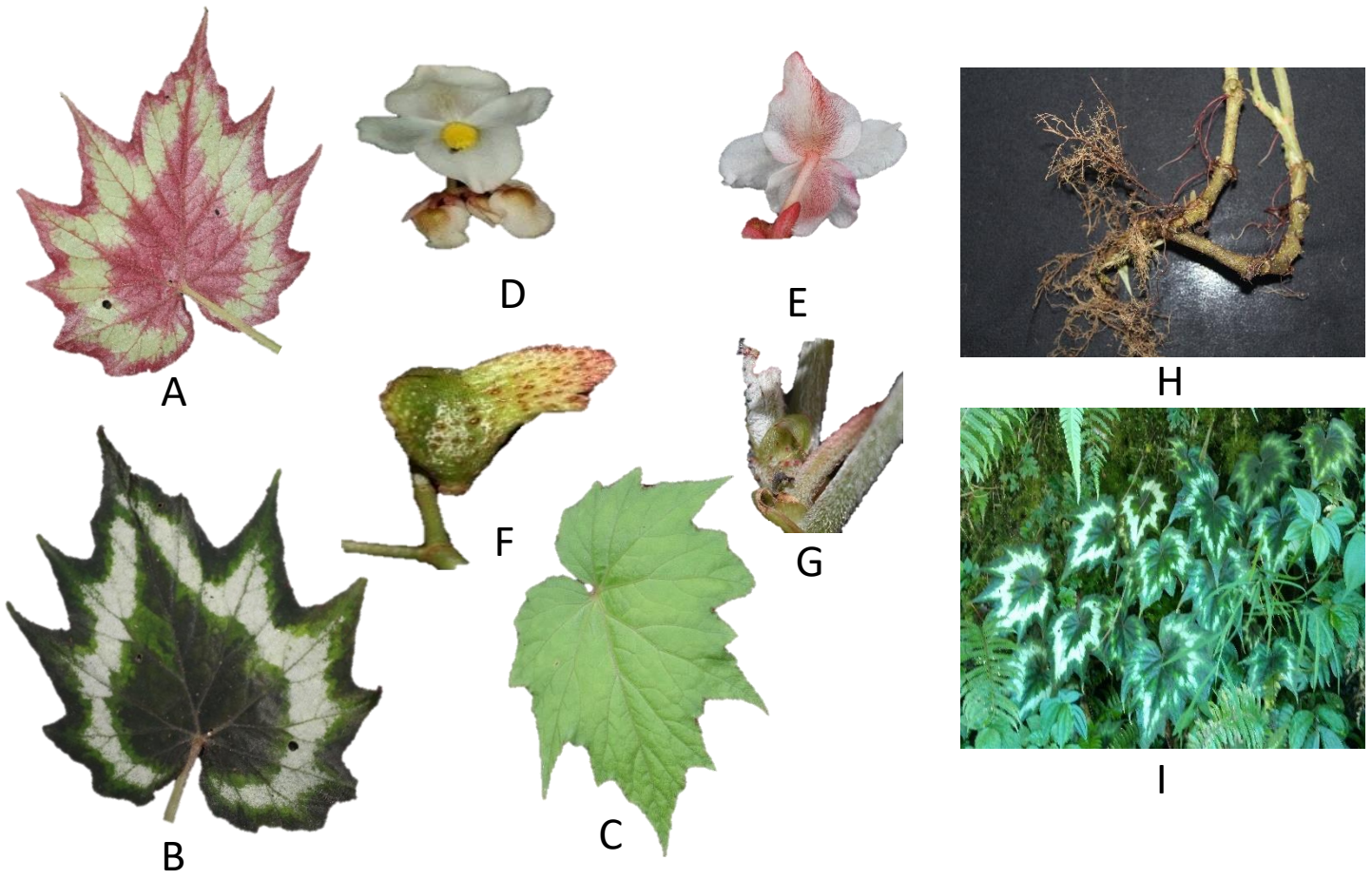

Plate 10. *Begonia palmata* D. Don. Abaxial leaf surface, B) Adaxial leaf surface, C) Green phenotypic variant of *B. palmata*, D-E) Flower, F) Capsule, G) Stipule, H-Root and stem, I) plant habit.

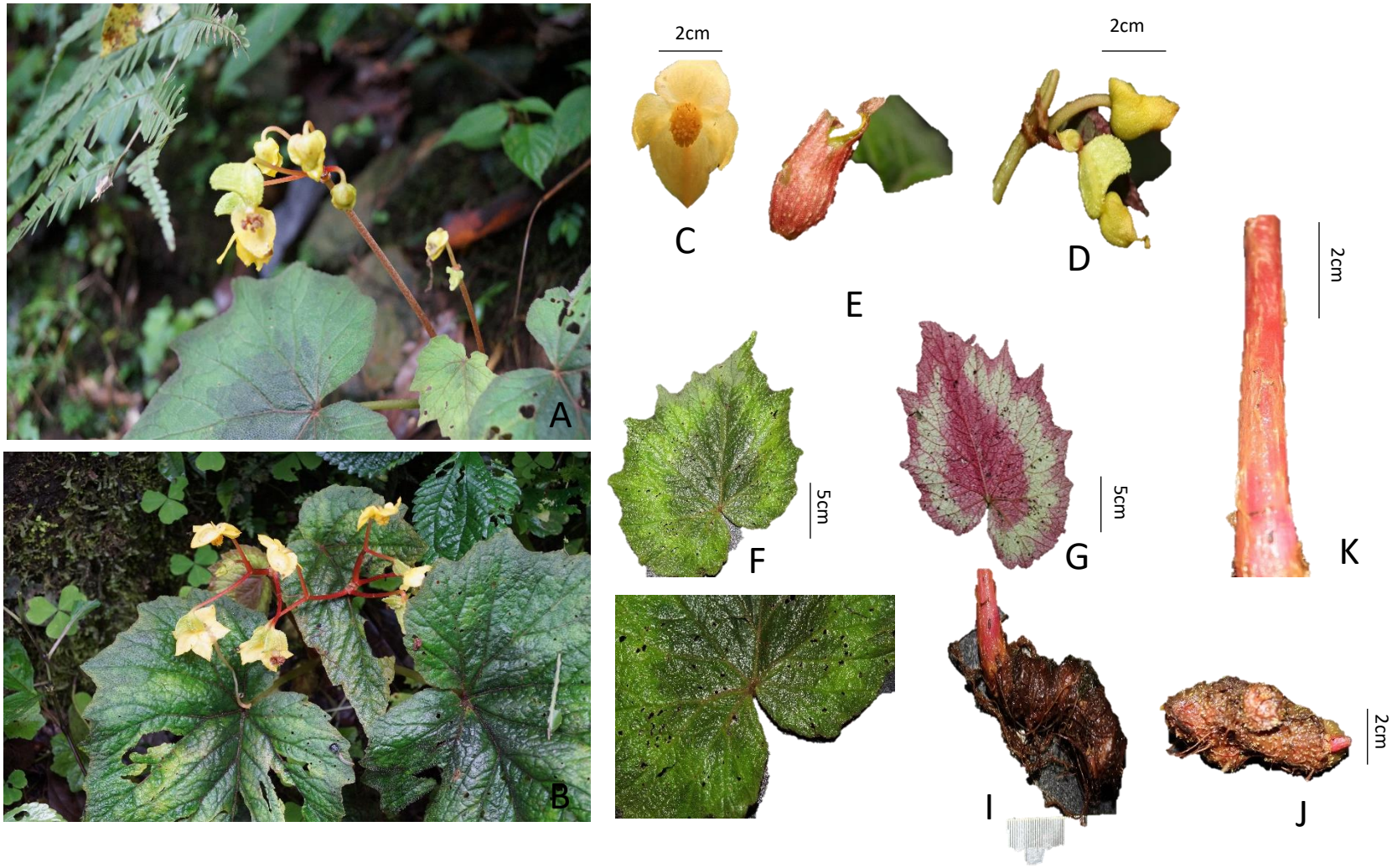

Plate 11. *Begonia flaviflora* (Clarke) Hara, A-B) Plant, C-E Flower, capsule and inflorescence, F) adaxaial leaf surface,G) Abaxial leaf surface, H) Leaf base, I-J) Root and rhizome, K) Stem.

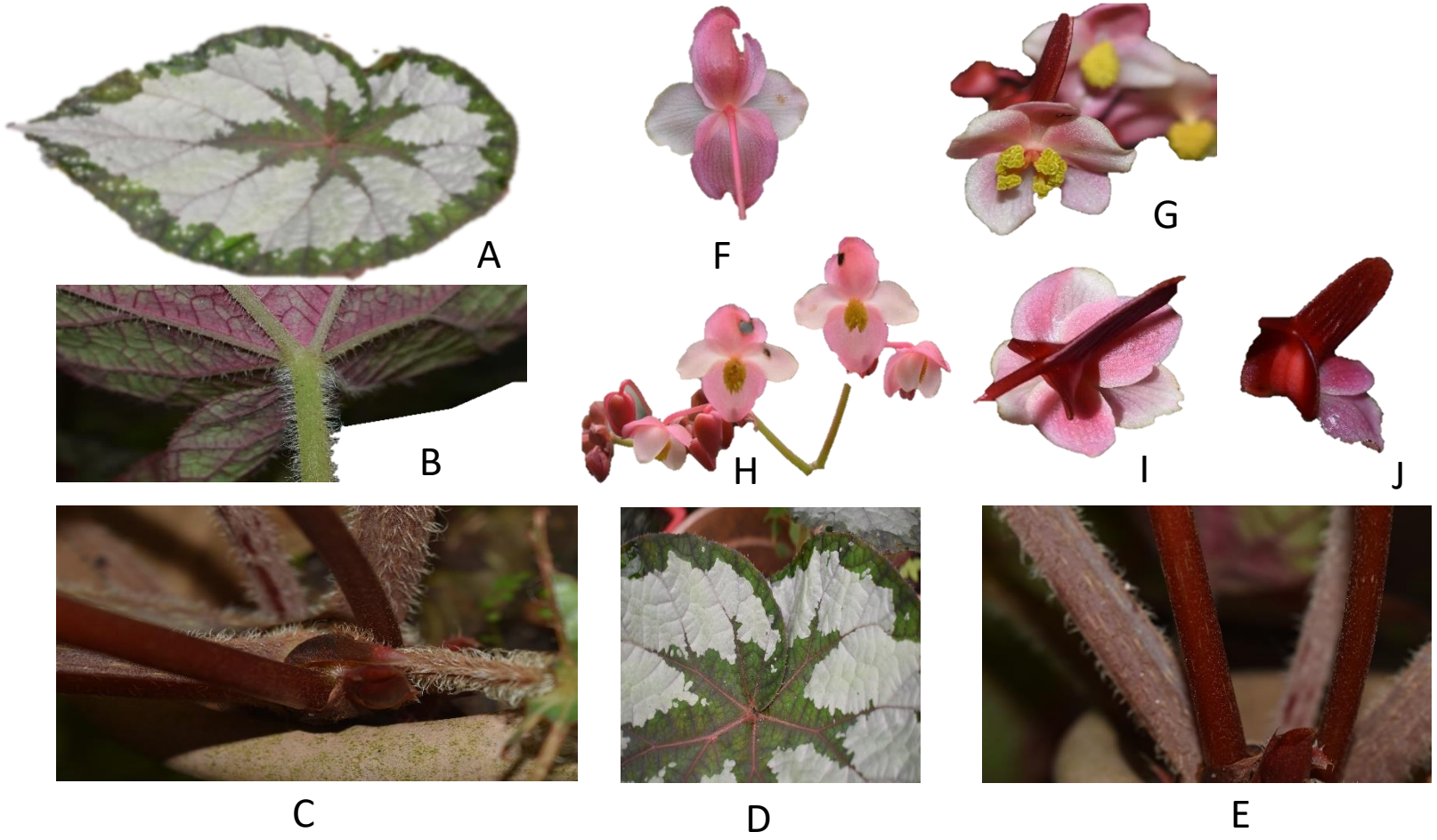

Plate 12. ***Begonia annulata*** **K. Koch. A)** Leaf blade, B) Petioles and Abaxial surface of leaf, C) Petiole, D) Leaf base, E) Peduncle, F-H) Male flowers, G-I) Female flower and J) Capsule.

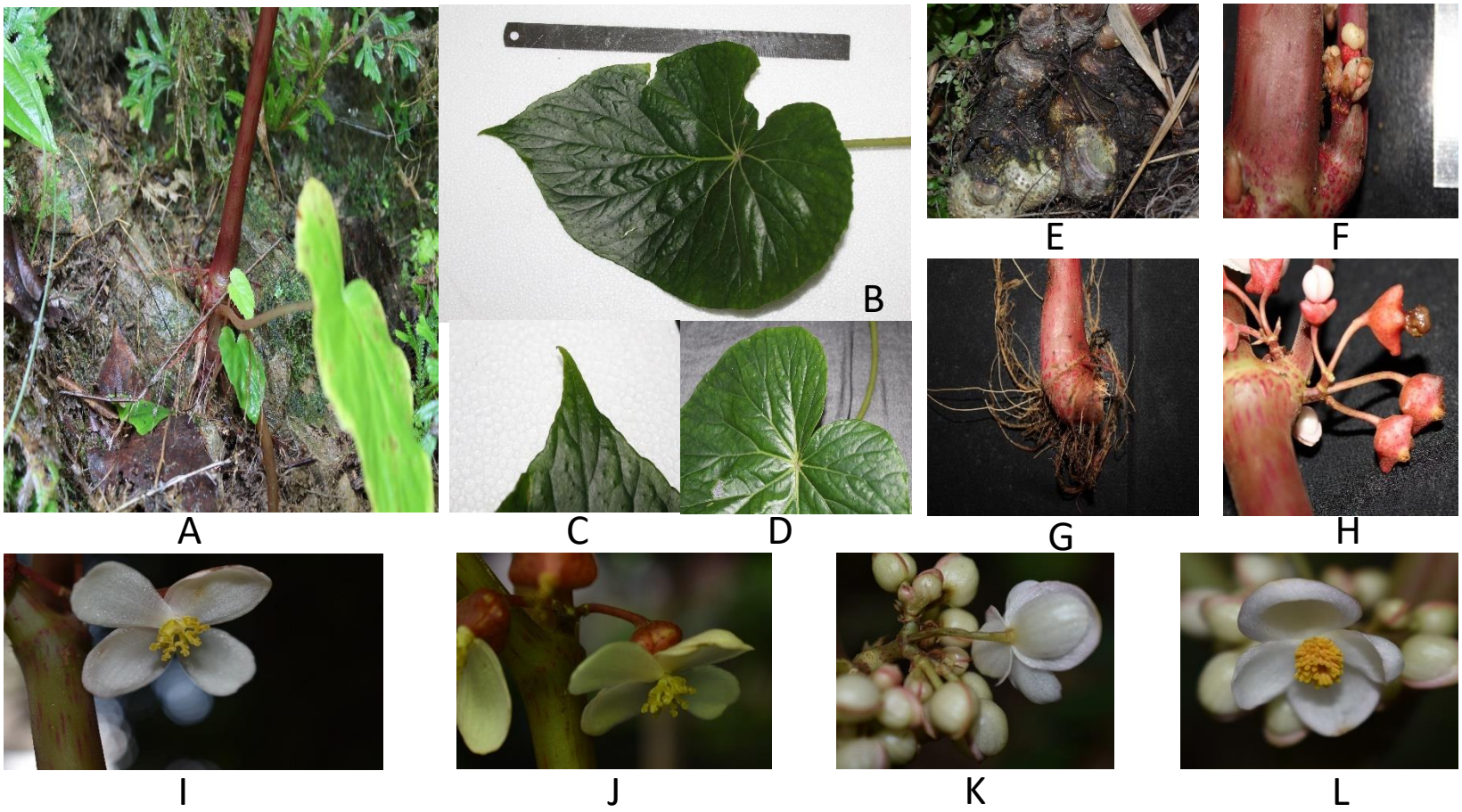

Plate 13. *Begonia roxburghii* A. DC. A- Plant habit, B-Leaf Blade, C-Leaf apex, D- Leaf base, E-Rhizome, F- Stipules, G-Roots, H- Capsule, Iand J- Female flower, and K and L- Male flowers.

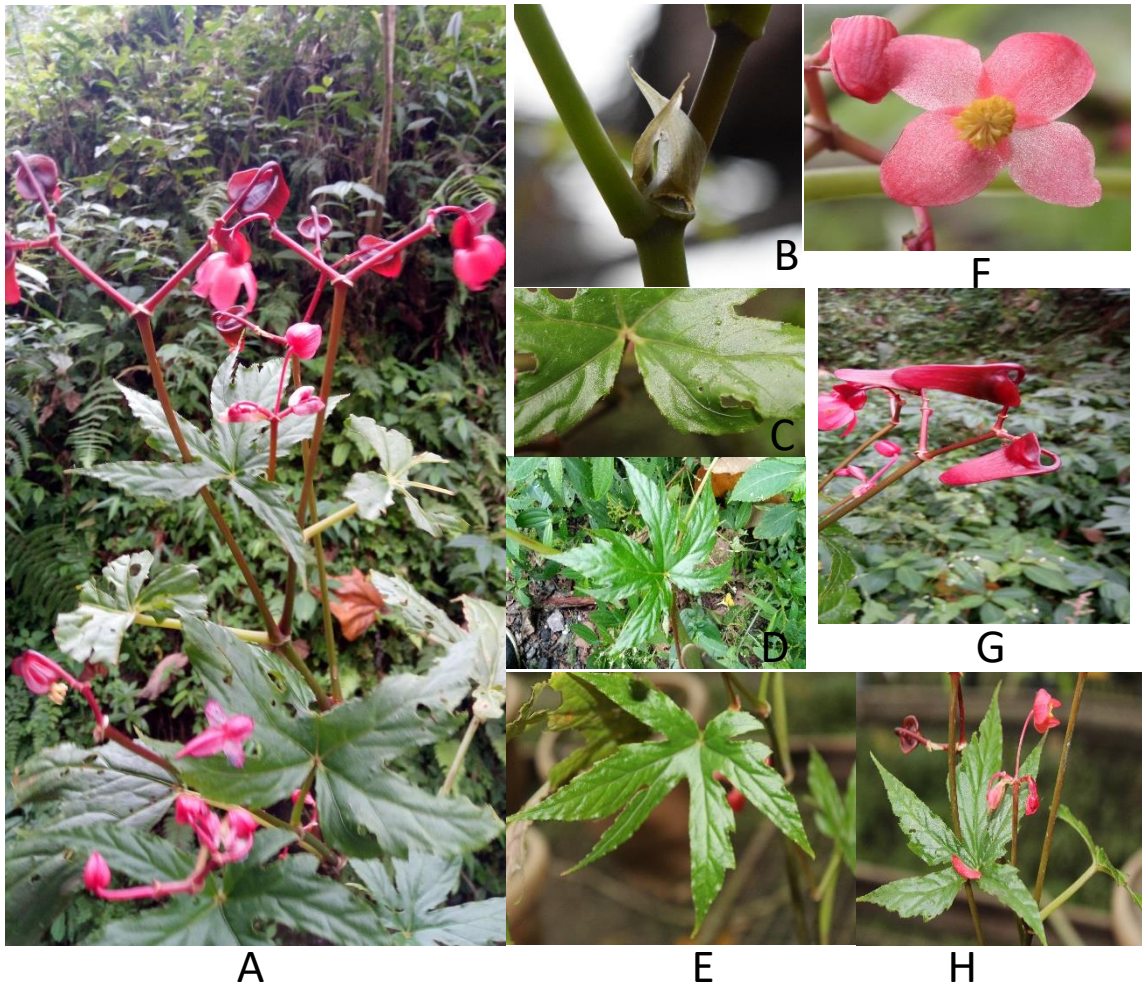

Plate 14. ***Begonia sikkimensis*. A. DC.** A) Whole plant, B) Stipules, C) Leaf base, E) Leaf blade, F) Flower, G-H) capsule and Peduncle.

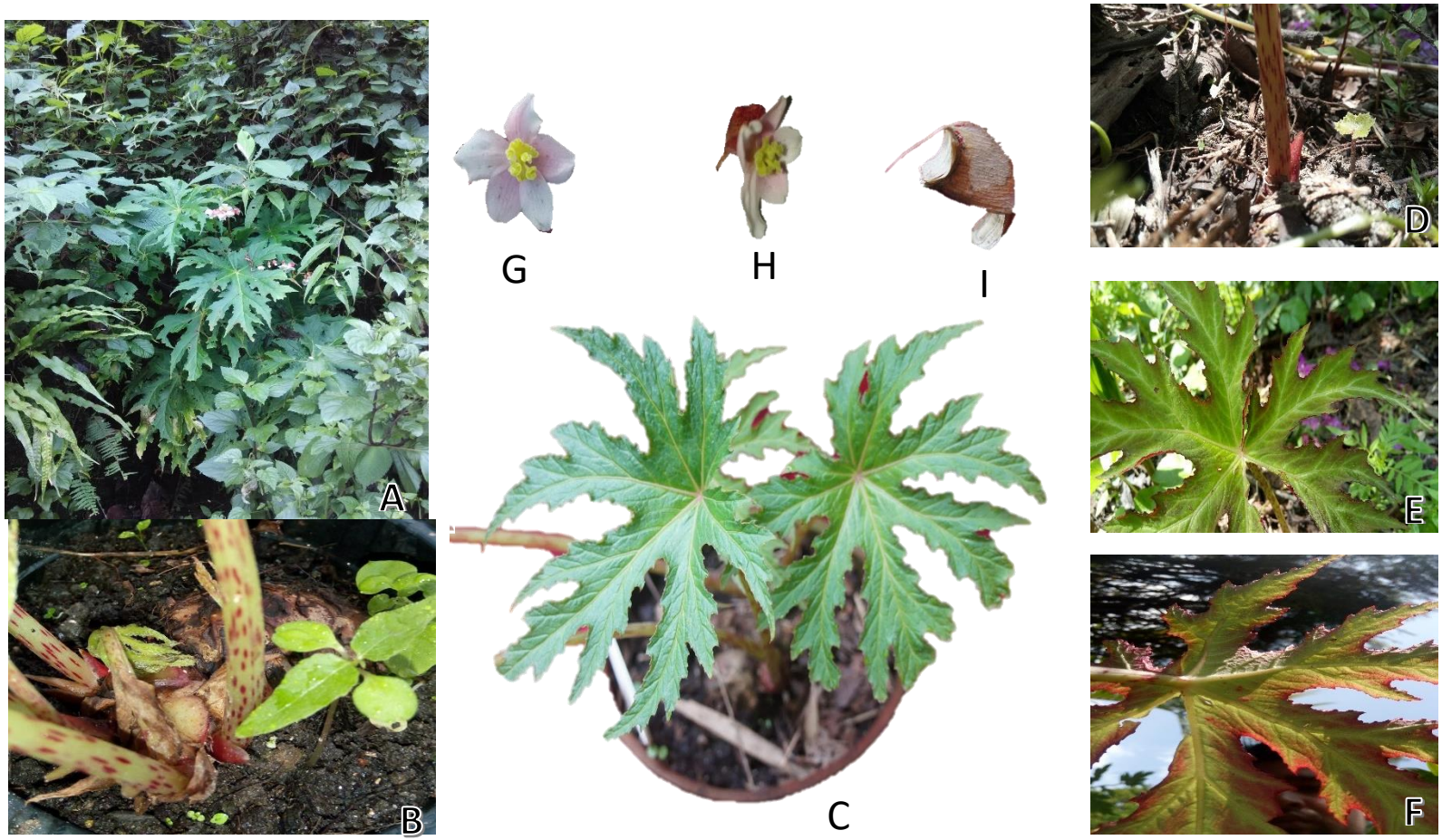

Plate 15. ***Begonia panchtharensis*** **Rajbhandary.** A. Plant habit, B) Petioles arising from rhizome, C. Whole plant, D) Petioles and Stipules, E-F) Leaves, G-I) Flower and capsule.

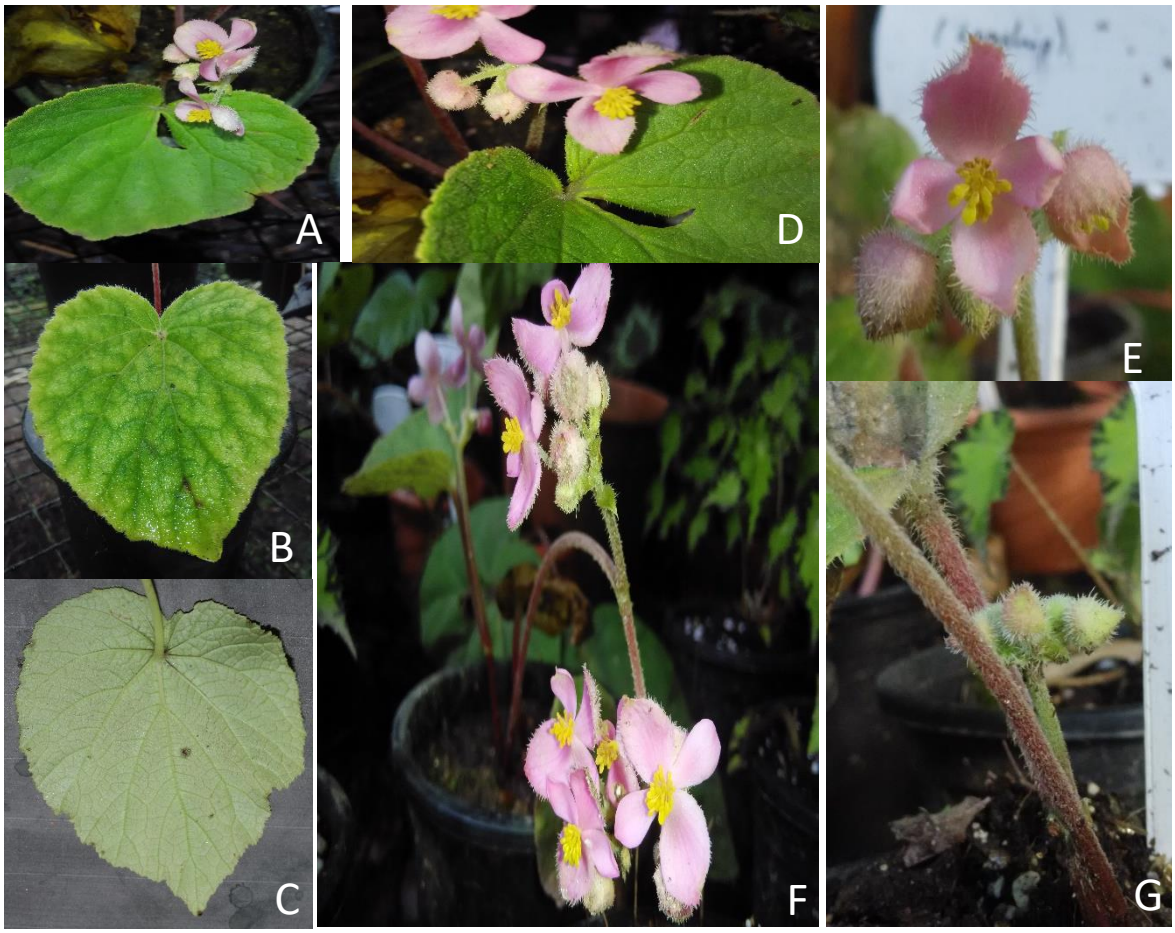

Plate 16. ***Begonia satrapis*** **Clarke.** A) Whole plant with flowers, B) Adaxial Leaf surface, C) Abaxial leaf surface, D-E) Flower, F) Whole plant with inflorescence, G) Leaf Petiole

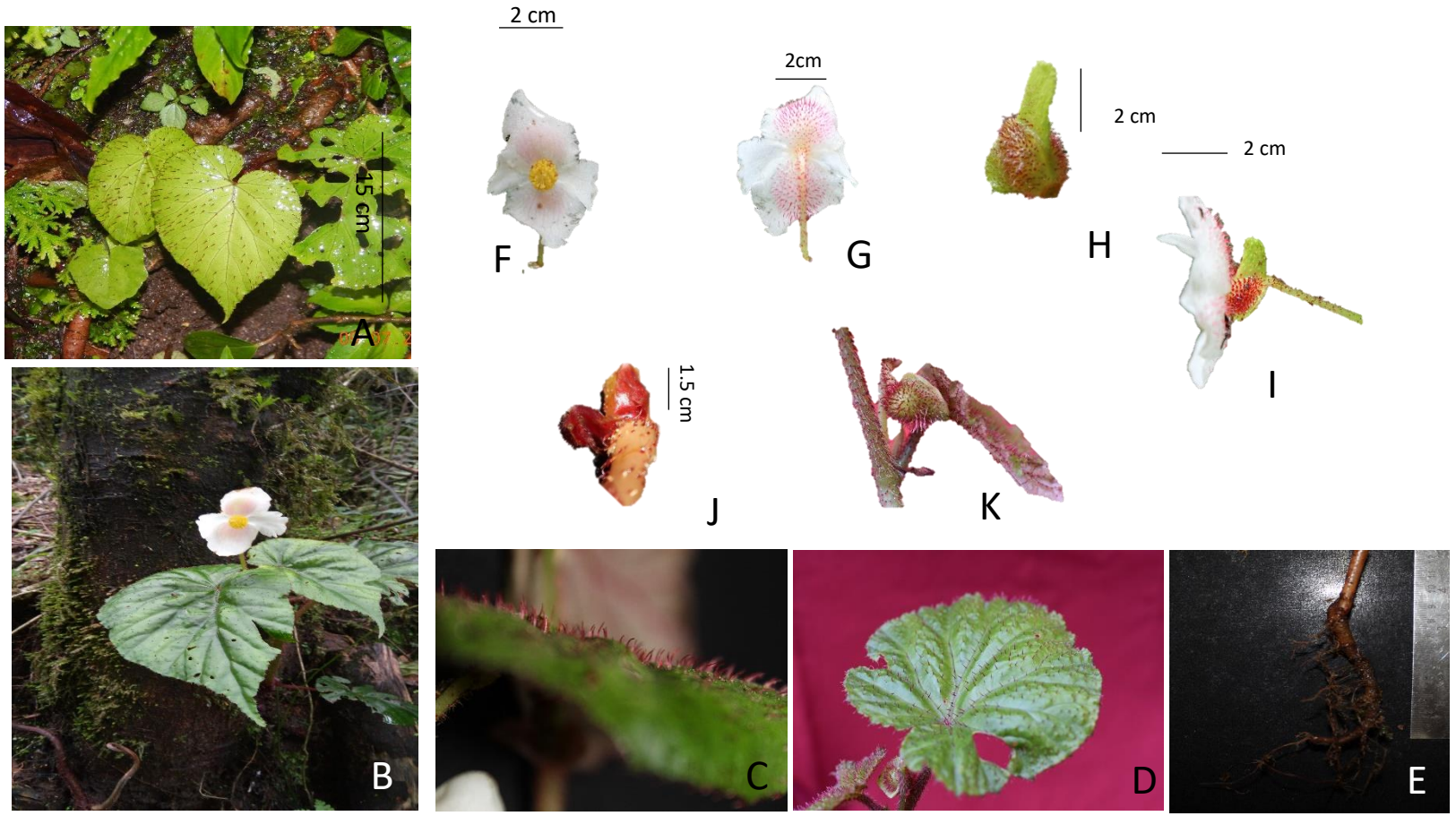

Plate 17. *Begonia cathcartii* Hook. F. A-B) Plant habit, C) Leaf pubescent, D) Leaf blade, E) Rhizome, F-G) Mae flower, H-I) Capsule and Female Flowers, J- Stipules, K- Flower bud.

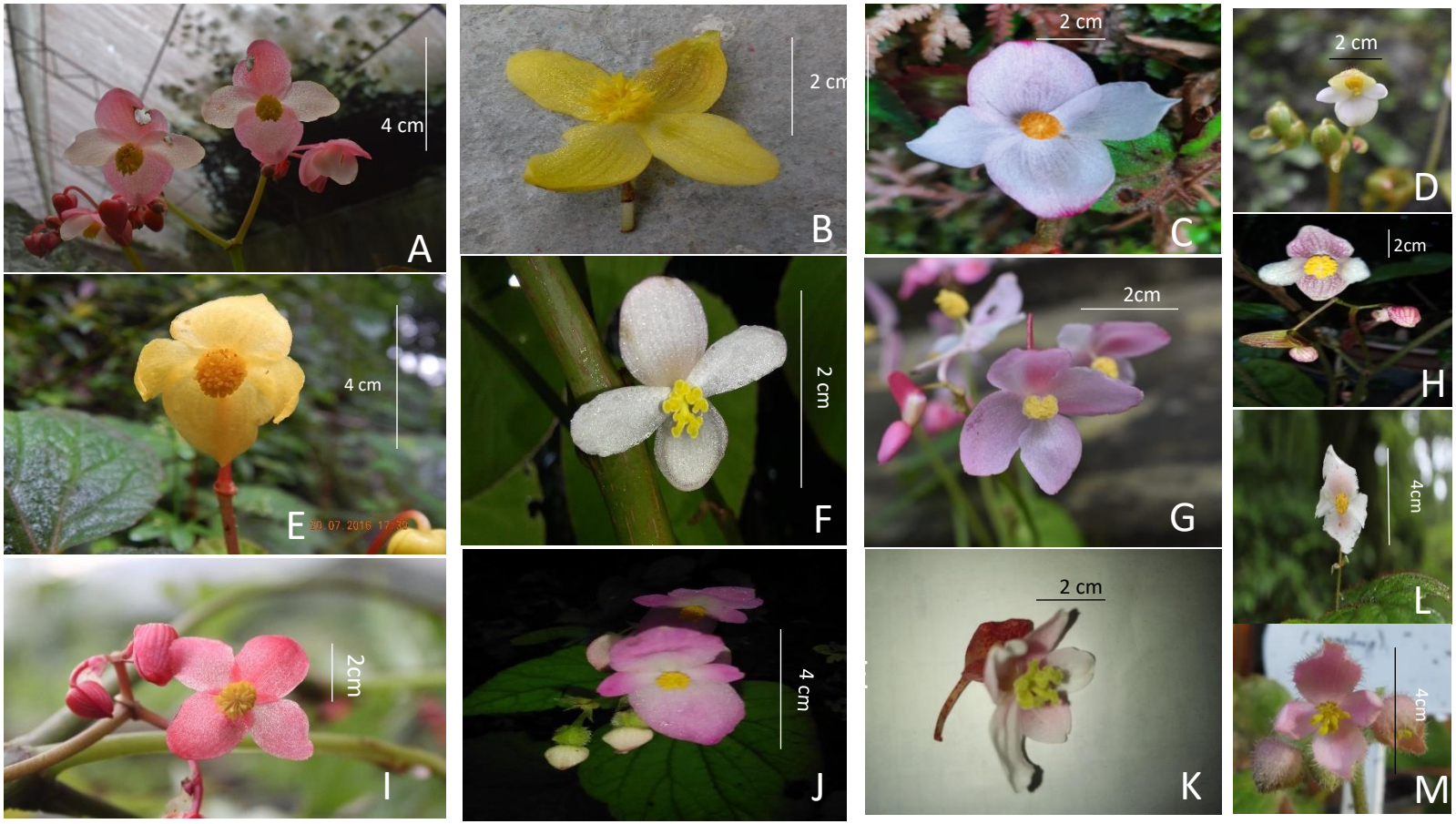

Plate 18. Flower morphology of thirteen wild *Begonia* species. A) *B. annulata*, B) *B. xanthina*, C) *B. palmata*, D) *B. josephi*, E) *B. flaviflora*, *F) B. roxburghii*, G) *B. megaptera*, H) *B. hatacoa*, I) *B. sikkimensis*, J) *B. picta*, K) *B. panchtharensis L) B. cathcartii* and M) *B. satrapis.*

4.3 Biochemical parameters

Selected species of each accession were subjected to tests for biochemical studies which include total soluble sugar, starch, total soluble protein, ascorbic acid, anthocyanin content, total phenol content, and total flavonoid content during the active growth stage of each plant.

4.3.1 Total Protein content

Data depicted in table 4.7, maximum protein content (35.04±0.71 mg g^{-1}) of *Begonia* species was recovered from the *B. josephi* green phenotypes and minimum was found in *B. palmata* Red phenotypes (20.10±0.34 mg g^{-1}) from the leaf sample. Likewise, the maximum content of protein from stem and tuber/rhizome extract of *Begonia* was recorded in *B. josephi* Green (25.97±0.39 mg g^{-1} and 21.89±0.42 mg g^{-1}, respectively). While comparing the data in table 4.7, it was noted that the highest protein content was found in the leaf of *Begonia* species and the maximum was obtained from green phenotypes. Minimum protein content was obtained from leaf, stem, and tuber/rhizome extract of *B. palmata* (20.10±0.34 mg g^{-1}, 13.60±0.52 mg g^{-1} and 12.12±0.28 mg g^{-1}), respectively.

4.3.2 Ascorbic acid

Among the tested species of *Begonia* from Sikkim Himalayas for evaluation of ascorbic acid content in their leaves, stem and rhizome/tuber, it was evident that the data presented in table 4.7, shows highest content of ascorbic acid was found in stem extract of *Begonia josephi* green and red phenotypes with 10.42±0.417 mg $g^{-100}$and 10.00±0.000 mg g^{-100}

Table 4.7. Protein (mg g^{-1}) and ascorbic acid content (mg g^{-100}) of *Begonia* species

Species	Protein (mg g^{-1})			Ascorbic acid (mg g^{-100})		
	Leaf	**Stem**	**Rhizome/Tuber**	**Leaf**	**Stem**	**Rhizome/Tuber**
B. xanthina	20.42±0.42	16.01±0.37	13.89±0.41	7.92±0.417	6.25±0.417	4.58±0.417
B. annulata	22.27±0.55	16.32±0.31	14.02±0.68	7.08±0.417	7.92±0.417	4.17±0.417
B. megaptera	33.04±0.46	21.14±0.74	18.47±0.84	9.58±0.417	7.08±0.417	4.58±0.417
B. palmata Red	20.10±0.34	13.60±0.52	12.12±0.28	6.67±0.417	5.42±0.417	4.17±0.417
B. palmata Green	31.49±0.71	23.01±0.42	20.18±0.74	6.25±0.000	5.83±0.417	3.75±0.000
B. josephi Red	20.82±0.47	15.36±0.67	12.80±0.86	8.75±0.722	10.00±0.000	6.67±0.417
B. josephi Green	35.04±0.71	25.97±0.39	21.89±0.42	9.58±0.417	10.42±0.417	6.25±0.000
B. cathcartii	22.85±0.80	16.87±0.51	14.55±0.37	7.08±0.417	6.67±0.417	4.58±0.417
B. sikkimensis	22.71±0.98	16.66±0.40	14.30±0.26	6.67±0.417	5.42±0.417	4.17±0.417
B. roxburghii	32.40±0.66	23.06±0.26	20.70±0.48	9.58±0.417	6.67±0.417	3.75±0.000
B. picta Red	22.25±1.04	16.26±0.48	13.98±0.21	6.67±0.417	7.08±0.417	4.17±0.417
B. picta Green	34.09±0.44	24.85±0.26	21.25±0.26	6.67±0.417	7.50±0.417	4.17±0.417
B. hatacoa Red	23.20±0.88	17.14±0.36	14.76±0.35	7.92±0.417	7.92±0.417	3.75±0.000
B. hatacoa Green	33.81±0.70	24.19±0.26	20.94±0.38	8.75±0.722	8.75±0.417	3.75±0.000
B. panchtharensis	28.30±0.64	20.07±0.87	17.25±0.13	9.58±0.417	5.83±0.417	3.75±0.000
B. flaviflora	21.62±0.97	15.73±0.14	13.22±0.19	7.92±0.417	7.08±0.417	4.17±0.417
B. satrapis	25.11±0.72	17.81±1.15	15.02±0.33	5.83±0.417	7.50±0.000	5.83±0.417
CD 5%	**2.03**	**1.54**	**1.37**	**1.39**	**1.38**	**1.07**
CV	**4.63**	**4.54**	**4.59**	**10.32**	**11.77**	**28.72**
Sem	**0.71**	**0.54**	**0.48**	**0.46**	**0.48**	**0.37**

Table 4.8. Total sugar (mg g^{-1}) and starch content (mg g^{-1}) of *Begonia* species

Species	Total sugar content (mg g^{-1})		Starch content (mg g^{-1})	
	Leaf	Rhizome/Tuber	Leaf	Rhizome/Tuber
B. xanthina	14.15±0.81	21.30±0.42	18.28±0.52	7.84±0.24
B. annulata	16.75±0.30	23.37±0.46	20.26±0.64	10.31±0.56
B. megaptera	25.93±0.26	33.82±0.85	30.83±0.36	21.07±0.44
B. palmata Red	24.85±0.41	33.02±0.44	29.36±0.49	19.43±0.55
B. palmata Green	12.84±0.30	22.49±0.55	18.11±0.34	7.40±0.42
B. josephi Red	27.22±0.86	34.86±0.71	33.04±0.76	22.92±0.66
B. josephi Green	13.38±0.52	21.24±0.79	18.52±0.56	8.20±0.15
B. cathcartii	14.91±0.91	23.72±0.87	20.88±0.88	11.35±0.63
B. sikkimensis	15.59±1.03	23.85±0.74	20.84±0.87	10.89±0.84
B. roxburghii	25.99±0.38	33.59±0.47	30.52±0.61	20.45±0.59
B. picta Red	26.96±0.31	34.12±0.78	32.16±0.46	22.13±0.29
B. picta Green	13.39±0.65	23.05±0.78	20.36±1.02	10.42±0.43
B. hatacoa Red	26.25±0.93	31.71±0.80	31.71±0.80	21.96±0.66
B. hatacoa Green	15.97±0.39	23.65±1.00	21.23±0.82	11.66±0.37
B. panchtharensis	21.82±0.86	24.18±0.64	24.18±0.64	14.00±0.65
B. flaviflora	16.51±0.67	23.22±0.44	19.85±0.91	10.00±0.81
B. satrapis	14.82±0.88	24.50±0.72	22.65±0.70	12.76±0.74
CD 5%	**1.92**	**2.00**	**2.00**	**1.62**
CV	**6.06**	**4.48**	**5.05**	**7.14**
Sem	**0.67**	**0.70**	**0.98**	**0.57**

respectively, while lowest content was recorded in *B. palmata, B. roxburghii, B. hatacoa, B. panchtharensis* (3.75±0.000 mg g^{-100}).

4.3.3 Total sugar content

Among the tested *Begonia* species found in Sikkim Himalayas, maximum sugar content from leaf extract was recorded in *B. josephi* red phenotypes (27.22±0.86 mg g^{-1}) and lowest from *B. palmata* green phenotypes (12.84±0.30 mg g^{-1}). Whereas, red phenotype of *B. josephi* of tuber/rhizome extract was found maximum sugar content (34.86±0.71 mg g^{-1}) and lowest was recorded in *B. josephi* green phenotypes (21.24±0.79 mg g^{-1}) (Table 4.8).

4.3.4 Total starch content

It was evident from the table 4.8, that the maximum starch content in leaf extract of *Begonia* species was varied from 18.11±0.34 mg g^{-1} to 33.04±0.76 mg g^{-1} and rhizome or tuber extract were varied from 7.40±0.42 mg g^{-1} to 22.92±0.66 mg g^{-1}. The maximum starch content was found in leaf extract of *B. josephi* red phenotypes (33.04±0.76 mg g^{-1}) whereas the lowest was observed in tuber/rhizome extract of *B. palmata* green phenotypes (7.40±0.42 mg g^{-1}).

4.3.5 Anthocyanin content

As per the data in table 4.9, anthocyanin content in the leaf sample of *Begonia* species was found maximum in the red phenotypes of *B. picta* (185.33±1.45 mg L^{-1}) and lowest was found in *B. megaptera* (26.27±0.89 mg L^{-1}). While stem extract of *Begonia* species was also tested and the highest content of anthocyanin in the stem was observed in the red phenotype of *B. josephi* (131.15±2.75 mg L^{-1}) and the minimum was obtained from the

stem extract of *B. palmata* green phenotypes (26.15±2.89 mg L^{-1}). In the case of rhizome/tuber extract of *Begonia* species of Sikkim Himalayas, maximum anthocyanin was found in the tuber of *B. josephi* red phenotypes (34.82±0.73 mg L^{-1}) and lowest content in the rhizome of *B. sikkimensis* (11.78±0.60 mg L^{-1}). Result reveals that the maximum anthocyanin content was found in the leaf surface of *Begonia*, it was due to heavy accumulation of bio colour in the leaf as compared to stem and rhizome/tuber. While red phenotypic species possess greater anthocyanin content as compare to green phenotypes.

4.3.6 Total Phenol content

Experimental data presented in table 4.9, revealed that the total phenol content of leaf extract of *Begonia* was found maximum in the *B. picta* Red phenotypes (55.03±2.85 mg GAE g^{-1}), whereas the minimum was observed in *B. cathcartii* (2.17±0.39 mg GAE g^{-1}). Stem extracts of *Begonia* species also showed marked variation, maximum was obtained from the *B. hatacoa* Red phenotypes (21.26±0.68 mg GAE g^{-1}) and minimum was found in the *B. megaptera* (0.67±0.21 mg GAE g^{-1}). From the tubers/rhizome extract of *Begonia* species, maximum total phenol content was found in *B. picta* red phenotypes (28.9±0.617 mg GAE g^{-1}) followed by *B. picta* green phenotypes (27.55±0.849 mg GAE g^{-1}) and *B. josephi* red phenotypes (26.89±1.155 mg GAE g^{-1}) and minimum was obtained from *B. cathcartii* (0.37±0.092 mg GAE g^{-1}).

Table 4.9. Anthocyanin (mg L^{-1}), total phenol (mg GAE g^{-1}) and flavonoid content (mg QE g^{-1}) of *Begonia* species

Species	Anthocyanin (mg L^{-1})			Total Phenol (mg GAE g^{-1})			Total flavonoids (mg QE g^{-1})		
	Leaf	Stem	Rhizome/Tuber	Leaf	Stem	Rhizome/Tuber	Leaf	Stem	Rhizome/Tuber
B. xanthina	174.33±1.86	125.67±1.53	20.67±1.36	5.61±0.34	2.14±0.56	1.28±0.455	5.48±0.345	2.12±0.972	6.28±0.278
B. annulata	163.71±1.60	115.82±0.92	15.82±0.92	4.26±0.22	1.67±0.30	1.07±0.231	5.28±0.244	1.98±0.969	5.73±0.359
B. megaptera	26.27±0.89	30.70±2.06	13.35±0.63	7.21±0.30	0.67±0.21	0.84±0.138	4.98±0.434	0.65±1.272	1.46±0.218
B. palmata Red	168.26±0.90	124.11±0.70	14.64±0.71	24±0.58	6.78±0.53	4.98±0.377	10.76±1.076	4.25±1.882	6.79±0.310
B. palmata Green	35.00±0.73	26.15±2.89	13.08±0.87	20.14±0.88	2.06±0.59	4.6±0.372	4.78±0.189	1.56±0.930	3.23±0.332
B. josephi Red	163.89±0.87	131.15±2.75	34.82±0.73	32.36±1.19	8.5±0.38	26.89±1.155	9.76±0.797	3.4±1.849	13.56±1.199
B. josephi Green	31.83±0.72	27.95±0.22	32.82±1.27	13.76±0.26	6.43±0.62	25.61±0.733	5.23±0.135	1.19±1.195	8.46±0.554
B. cathcartii	35.37±2.32	33.11±0.81	15.66±0.42	2.17±0.39	0.91±0.20	0.37±0.092	3.27±0.677	0.61±0.775	1.11±0.191
B. sikkimensis	41.94±0.39	34.32±1.45	11.78±0.60	3.67±0.12	1.12±0.47	0.79±0.104	4.93±0.451	0.45±1.296	6.88±0.714
B. roxburghii	27.91±0.15	28.03±1.30	12.33±1.72	5.61±0.46	6.8±0.48	1.18±0.187	3.51±0.355	0.18±0.961	4.79±0.725
B. picta Red	185.33±1.45	127.02±1.02	27.02±1.02	55.03±2.85	13.45±1.22	28.9±0.617	35.19±1.142	7.21±8.097	39. 42±0.558
B. picta Green	36.12±0.44	30.73±2.30	25.19±1.66	23.82±0.66	8.44±0.72	27.55±0.849	16.58±0.979	2.13±4.177	31.24±1.247
B. hatacoa Red	152.97±0.91	121.41±2.64	22.41±1.33	19.2±1.52	21.26±0.68	1.25±0.133	8.47±0.746	1.14±2.124	6.76±0.568
B. hatacoa Green	33.50±1.71	33.24±0.77	22.46±0.51	9.35±1.16	12.39±1.70	0.94±0.088	3.44±0.293	0.38±0.887	5.79±0.159
B. panchtharensis	76.14±4.64	99.14±2.38	24.09±0.90	23.63±1.25	4.12±0.16	1.67±0.254	3.92±0.364	2.22±0.692	3.6±0.344
B. flaviflora	167.20±0.98	126.01±0.40	26.01±0.40	30.19±1.16	9.11±1.17	34.62±0.907	5.36±0.425	0.4251.003	7.74±0.625
B. satrapis	34.29±0.91	128.03±1.51	26.62±1.01	18.95±1.10	4.96±0.64	21.57±1.291	3.21±0.094	0.094±0.531	6.83±0.267
CD 5%	**4.64**	**4.95**	**2.94**	**3.08**	**2.12**	**1.73**	**1.74**	**1.00**	**1.72**
CV	**3.66**	**5.22**	**8.55**	**10.19**	**26.49**	**20.30**	**13.37**	**31.62**	**13.89**
SEm	**1.61**	**1.72**	**1.02**	**1.07**	**0.74**	**0.60**	**0.61**	**0.35**	**0.60**

4.3.7 Total flavonoids content

Results pertaining to table 4.9, it was evident that the total flavonoid content of leaf, stem, and tuber/rhizome extract of *Begonia* species were found maximum in *B. picta* red phenotypes (35.19±1.142 mg QE g^{-1}, 7.21±8.097 mg QE g^{-1} and 39. 42±0.558 mg QE g^{-1}, respectively) and minimum was found in leaf extract of *B. cathcartii* (3.27±0.677 mg QE g^{-1}), stem extract of *B. roxburghii* (0.18±0.961 mg QE g^{-1}) and rhizome/tuber extract of *B. cathcartii* (1.11±0.191 mg QE g^{-1}).

4.4. Evaluation of ornamental value of wild *Begonia* species

On the basis of morphological characters, different *Begonia* species were selected for the evaluation of consumer preference by preparing questionnaires and total of 100 respondents were randomly selected and interviewed. Data obtained from the consumers were presented below:

4.4.1 Demographics of the respondents

Upon collecting all the respondents' answers, customers were categorized in various age groups and gender. In terms of age group, the respondents were ranging from below 21 to above 61 years of age, as per the figure 4.1, the maximum percentage of respondents (36%) were in the range of age group between 41-50 followed by (27%) in between the age group of 31-40. The survey did not attract any respondents in the age group the below 20 and the smallest group of respondents accounted for 6% and was from the age group of above 61 years of age.

As per figure 4.2, male gender dominates the total percentage share by the surveyor (53%) and whereas, the female gender percentage covered was 47% totaling to 100 respondents in total.

4.4.2 Education Qualification of the respondents

From figure 4.3, maximum education qualification percentage of the respondents were from Senior School (28%), followed by Junior School (25%) and Primary School (21%). The minimum percentage was 2 % from Post Graduates, none (6%) and Graduates were 18 %.

4.4.3 Flower grower respondents

To obtained the respondents' interest for the flower cultivation, a question was set to know their interest in gardening and categorized as Hobby, Commercial, Decorative purposes, Amateur growers and others (figure 4.4). Maximum respondents grow their flowers for only decorative purposes (36%) followed by Hobby or passion for flower lovers (26 %), whereas, amateur growers were (15 %), commercial growers were (13 %) and do not grow flowers or they either did not garden (10 %).

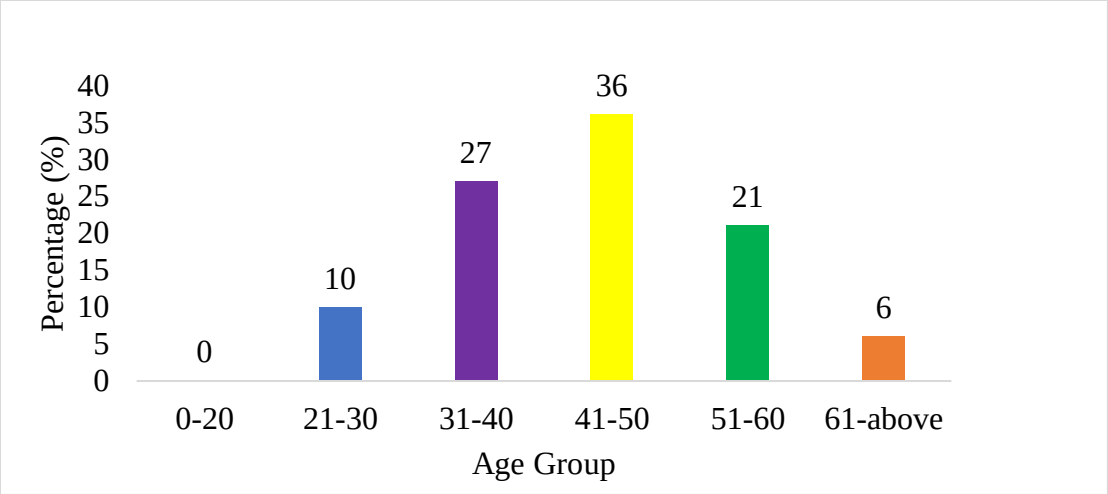

Figure 4.1 Demographics of the respondents (Age Group)

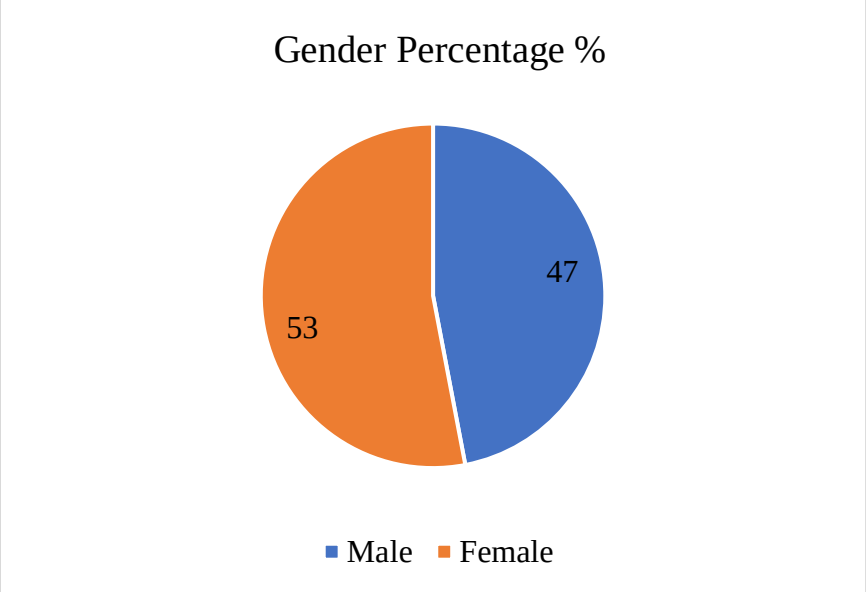

Figure 4.2 Demographics of the respondents (Gender)

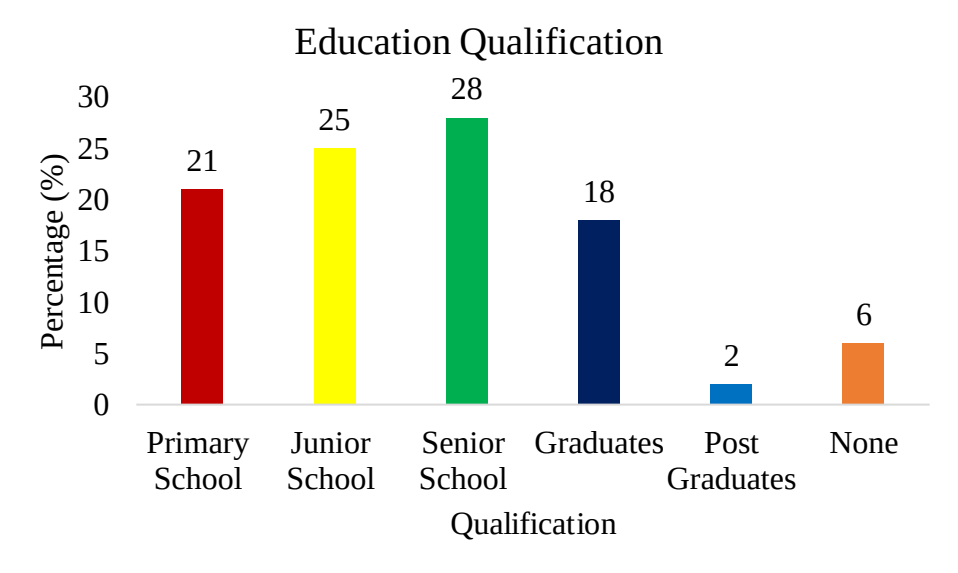

Figure 4.3 Showing percentage of Education qualification of the respondents

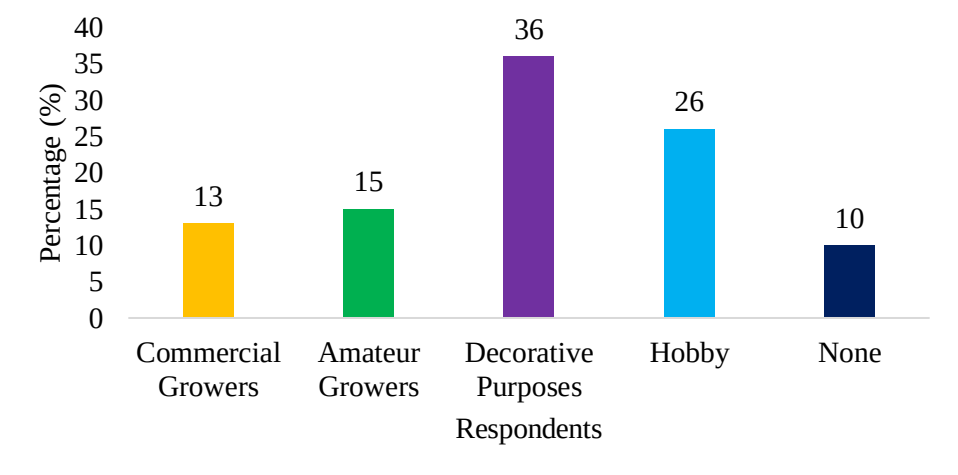

Figure 4.4 Flower grower percentage of the respondents

4.4.4 Flower preference by the respondents

To obtain the flower preference by the respondents, question was asked to each respondent about their choice of interest. From the figure 4.5, it was evident that, people prefer orchids (33%) more than other flower crops which were followed by Rose (16 %), Lilium (10%), Anthurium (9%), Gerbera (8%), Foliage Plants (7%), Gladiolus and Marigold (6%) and Alstroemeria (5%).

4.4.5 *Begonia* species preference by the respondents

To know the respondent's choice of interest to buy or grow wild *Begonia* species, questions were asked by displaying the photographs of thirteen different species of wild *Begonia* found in Sikkim Himalayas. Data pertinent in figure 4.6 showed that *B. xanthina* was preferred by the 22 % of the respondents, followed by *B. palmata* with 16% and *B. annulata* and *B. megaptera* with 14% each. Least preference was given to *B. roxburghii* (0%) and *B. cathcartii* (1%).

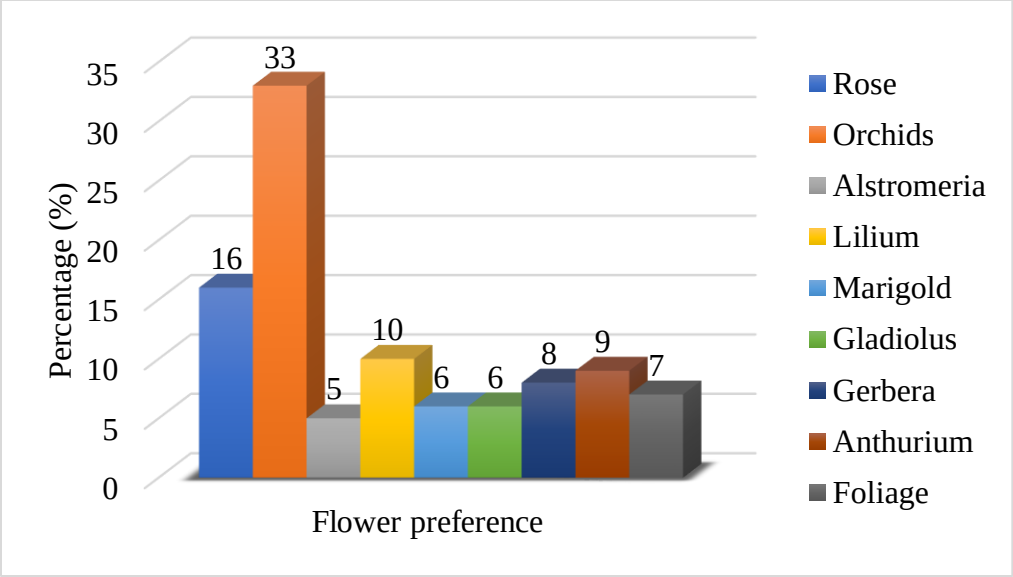

Figure 4.5 Flower preference by the respondents

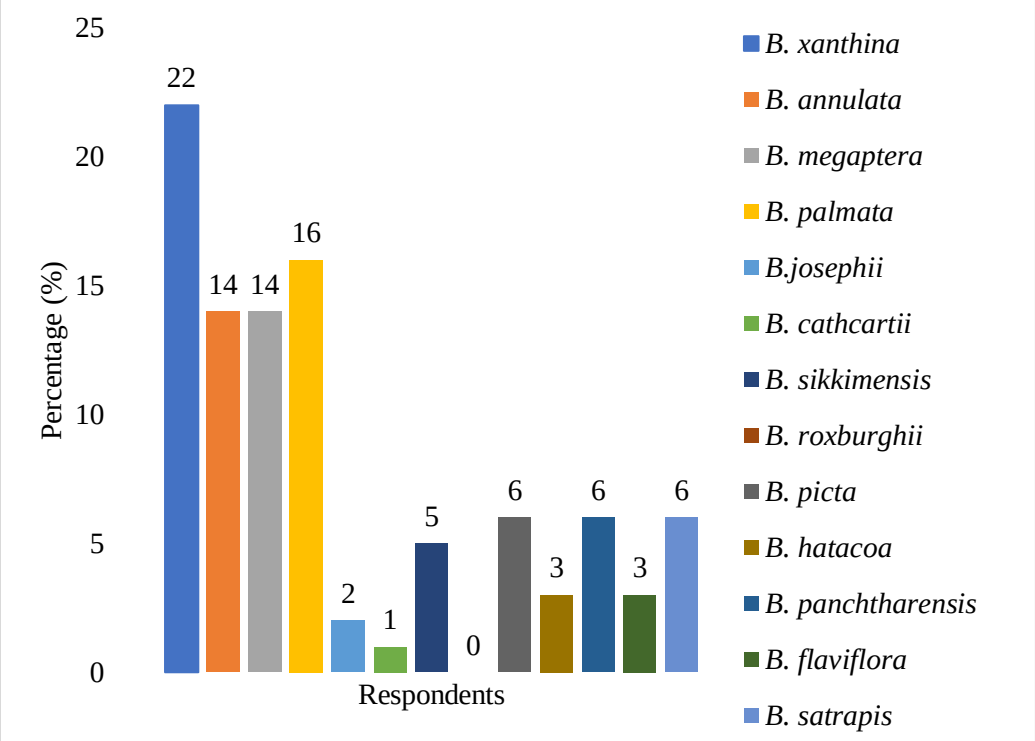

Figure 4.6 Showing percentage of *Begonia* species prefer by the respondents

4.4.6 Overall Assessment of *Begonia* Species

To know the people's perception regarding the wild *Begonia* species, the questionnaire was set for each individual, and asked the same on the basis of their liking, foliage pattern, flower arrangement, suitability, edibility, attractiveness and overall appearance of wild *Begonia* species. Photographs measuring 10 cm x 15 cm were printed for thirteen *Begonia* species showing a single plant with leaves and flowers. Each photograph was mounted on a gray card for display. Results obtained from the respondents were recorded in table 4.10 and presented below:

4.4.6.1 Liking

Data reveals that the maximum satisfactory rate on the basis of the liking of wild *Begonia* species was found in *B. xanthina* (6.77%), followed by *B. palmata* (6.62%), *B. picta* (6.46%) and *B. annulata* (6.15%). Whereas, the minimum satisfactory rate was recorded in *B. roxburghii* (6.46 %) and *B. josephi* (6.23%).

4.4.6.2 Foliage Pattern

On the basis of foliage pattern of wild *Begonia* species (table 4.10), maximum satisfactory results were obtained from the wild Begonia species of *B. picta* (6.92%), followed by *B. xanthina* (6.38%), *B. annulata* (6.15%) and *B. palmata* (5.92%). Maximum not satisfactory rate for the foliage pattern of wild *Begonia* species by the respondents were found in *B. roxburghii* (6.69%) followed by *B. josephi* (4.38 %).

Table: 4.10. Consumers/ Respondent perception on ornamental value of the collected wild *Begonia* species

Species	Liking%		Foliage pattern %		Flower arrangement %		Suitability %		Edibility %		Attractiveness %		Overall Appearances %		Total % (Average)	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
B. xanthina	6.77	0.92	6.38	1.31	5.15	2.54	6.77	0.92	0.00	7.69	6.54	1.15	6.54	1.15	5.45	2.24
B. annulata	6.15	1.54	6.15	1.54	5.15	2.54	6.23	1.46	0.31	7.38	6.46	1.23	6.31	1.38	5.25	2.44
B. megaptera	5.00	2.69	3.62	4.08	5.38	2.31	5.85	1.85	0.00	7.69	6.38	1.31	5.77	1.92	4.57	3.12
B. palmata	6.62	1.08	5.92	1.77	3.69	4.00	6.00	1.69	0.69	7.00	6.31	1.38	5.92	1.77	5.02	2.67
B. josephi	1.46	6.23	3.31	4.38	3.46	4.23	0.77	6.92	4.69	3.00	2.00	5.69	1.92	5.77	2.52	5.17
B. cathcartii	4.54	3.15	5.23	2.46	2.69	5.00	3.54	4.15	0.23	7.46	3.08	4.62	2.77	4.92	3.15	4.54
B. sikkimensis	3.54	4.15	5.08	2.62	5.00	2.69	2.00	5.69	1.92	5.77	3.54	4.15	4.00	3.69	3.58	4.11
B. roxburghii	1.23	6.46	1.00	6.69	1.85	5.85	1.46	6.23	0.08	7.62	0.38	7.31	1.46	6.23	1.07	6.63
B. picta	6.46	1.23	6.92	0.77	4.69	3.00	6.77	0.92	0.85	6.85	6.15	1.54	6.08	1.62	5.42	2.28
B. hatacoa	4.77	2.92	4.46	3.23	4.38	3.31	5.00	2.69	2.08	5.62	5.38	2.31	5.31	2.38	4.48	3.21
B. panchtharensis	3.92	3.77	4.62	3.08	1.92	5.77	5.69	2.00	0.15	7.54	4.92	2.77	4.69	3.00	3.70	3.99
B. flaviflora	2.92	4.77	4.08	3.62	5.46	2.23	3.46	4.23	1.23	6.46	4.69	3.00	4.00	3.69	3.69	4.00
B. satrapis	4.62	3.08	3.46	4.23	4.85	3.31	2.54	5.15	0.46	7.23	4.46	3.23	4.23	3.46	3.52	4.24

 # S is referred as Satisfactory
#NS is referred as Not Satisfactory

4.4.6.3 Flower Arrangement

Data pertinent to table 4.10 showed that the maximum satisfactory on the basis of flower arrangement of wild *Begonia* species was found in *B. flaviflora* (5.46 %) *followed by B. megaptera* (5.38 %), *B. xanthina* (5.15 %), *B. annulata* (5.15 %), and *B. sikkimensis* (5.00%), whereas, not satisfactory was found maximum in *B. roxburghii* (5.85 %), followed by *B. panchtharensis* (5.77 %) and *B. cathcartii* (5.00 %).

4.4.6.4 Suitability

On the basis of suitability of wild *Begonia* species, maximum satisfactory results were obtained from the wild *Begonia* species of *B. picta* (6.77 %) and *B. xanthina* (6.77 %), followed by *B. annulata* (6.23 %), *B. palmata* (6.00 %) and *B. megaptera* (5.85 %). The maximum not satisfactory rate for the suitability of wild *Begonia* species by the respondents was found in *B. josephi* (6.92%).

4.4.6.5 Edibility

Data pertinent to table 4.10, showed that the maximum satisfactory on the basis of edibility of wild *Begonia* species was found in *B. josephi* (4.69%) whereas, not satisfactory was found maximum in *B. xanthina* (7.69 %), *B. megaptera* (7.69 %) followed by *B. roxburghii* (7.62 %) and *B. panchtharensis* (7.54 %)

4.4.6.6 Attractiveness

Data reveals that the attractiveness of wild *Begonia* species was found maximum in *B. xanthina* (6.54 %), followed by *B. annulata* (6.46 %), *B. megaptera* (6.38 %) and *B. palmata*

(6.31 %), while, not satisfactory for attractiveness was found maximum in *B. roxburghii* (7.31 %).

4.4.6.7 Overall Appearance

As per the data on table 4.10, on the basis of overall preference of wild *Begonia* species by the respondents, a maximum satisfactory rate (6.54%) was given to *B. xanthina* whereas 1.15% of respondents found unsatisfactory for the same species. Likewise, 6.31 % of respondents rated satisfactory for *B. annulata*, 6.08 % for *B. picta*, 5.92 % for *B. palmata*, and 5.77 % for *B. megaptera*. Whereas, the maximum percentage in case of not satisfactory rate by the consumers were found in *B. roxburghii* (6.23 %) followed by *B. josephi* (5.77 %) and *B. cathcartii* (4.92 %).

It was obtained from the consumer preference of thirteen wild *Begonia* species, the consumer prefers maximum for *B. xanthina* (5.45%) followed by *B. picta* (5.42%), *B. annulata* (5.25%), *B. palmata* (5.02%), *B. megaptera* (4.57%), *B. hatacoa* (4.48), whereas maximum not satisfactory percentage was given for *B. roxburghii* (6.63%) and *B. josephi* (5.17%).

4. 5. Micropropagation

Three *Begonia* species were selected for the development of micropropagation protocol. Several treatments and culture condition with combination of plant growth regulators, pH, and sucrose for promoting efficient plant regeneration *in vitro* were tested and result obtained presented as below:

The initial process of micropropagation of *Begonia* involves the selection of explant and induction, development of micro adventitious shoots, and plantlets production. Plant growth regulators play an important role in *in vitro* tissue culture for inducing regeneration. Leaf disc, petiole and immature bud were used as an explant for *Begonia* regeneration in this experiment. Different combinations of BAP and NAA were selected.

4.5.1. *In-vitro* regeneration of *Begonia palmata*

The direct organogenesis of *B. palmata* was attempted from the leaf disc, petiole and immature bud explants using MS media supplemented with various concentration and combination of BAP and NAA. The various parameters like survival rate, days required for shoot initiation, number of shoots per explant, days required for root initiation, number of plantlets per explant, shoot length and root length were observed and recorded during the course of the investigation.

4.5.1.1 Survival rate

As shown in table 4.11 and figure 4.7 the data on survival rate showed significantly affected by the growth regulators in all three different explants. The maximum survival rate of leaf disc explant (84.00 ± 7.48 %) was recorded in treatment T_{17} *i.e.* MS media supplemented with BAP (1.5 mg l^{-1}) in combination with NAA (0.5 mg l^{-1}) which is at par with the treatments T_{16}, T_{18} and T_{24}, (80.00 ± 6.32%, 80.00 ±6.32 % and 80.00 ± 8.94% respectively). Incase of petiole explant, maximum survival rate (84.00 ± 7.48 % and 84.00 ± 7.48 %) was recorded in both the treatments T_{17} and T_{18} *i.e.* MS media supplemented with BAP (1.5 mg l^{-1}) with NAA (0.5 mg l^{-1}) and BAP (2.0 mg l^{-1}) in combination with NAA (0.5 mg l^{-1}), respectively.

Highest survival rate (88.00 ± 8.00 %) was recorded in immature bud explant with the treatment T_{17} *i.e.* MS media supplemented with BAP (1.5 mg l^{-1}) in addition to NAA (0.5 mg l^{-1}) which is at par with treatment T_{16} (84.00 ±7.48 %), T_{18} (84.00 ± 4.00%) and T_{24} (80.00 ± 4.00%). Comparing all the explants' maximum survival rate was noted in immature bud explants with 88.00 ± 8.00 % in treatments T_{17}. Whereas, in all the three explants, the least survival rate (8.00 ± 4.90 %, 12.00 ± 4.90 % and 12.00 ± 4.90 % in leaf, petiole, and immature bud, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

Results depicted in table 4.11 and figure 4.7 showed that medium survival rate was obtained from the combination of BAP and NAA, a high concentration of cytokinin with a low concentration of auxin as compared to control (without plant growth regulators) or cytokinin and auxin alone combination.

4.5.1.2 Shoot initiation

As per the data presented in table 4.11 and figure 4.8, application of different concentrations of growth regulators significantly influenced the days required for shoot initiation. The average minimum days required for shoot initiation in leaf disc explant of *B. palmata* was recorded in treatment T_{16} (25.80 ± 1.20 days) *i.e.* MS media supplemented with BAP (1.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}) which was at par (29.80 ± 1.74 days) with treatment T_{17} *i.e.* MS media supplemented with BAP (1.5 mg l^{-1}) in combination with NAA (0.5 mg l^{-1}) and treatment T_{18} (32.40 ± 0.24 days) *i.e.* MS media supplemented with BAP (2.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}).

Table 4.11. Survival rate (%), days required for shoot initiation and number of shoots per explant of *in vitro* micropropagation of *Begonia palmata* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Survival rate %			Shoot initiation (days)			Number of shoots per explant		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	8.00±4.90	12.00±4.90	12.00±4.90	89.60±2.93	119.40±10.16	104.80±6.34	8.60±0.40	5.80±0.80	5.80±.49
T2	12.00±8.00	12.00±8.00	16.00±4.00	78.40±2.64	93.80±7.60	99.40±4.62	15.40±0.75	11.60±1.12	8.60±0.87
T3	24.00±4.00	24.00±4.00	28.00±4.00	71.40±3.17	76.20±5.27	86.60±0.93	17.60±1.86	10.60±1.21	8.00±1.22
T4	20.00±6.32	28.00±4.90	32.00±4.90	56.80±2.13	53.40±2.14	71.60±5.45	26.40±2.73	19.80±2.27	16.80±1.36
T5	28.00±4.90	32.00±8.00	24.00±4.00	58.20±2.91	54.60±3.23	67.80±3.46	24.60±2.42	21.20±3.01	15.80±1.46
T6	32.00±8.00	36.00±7.48	40.00±6.32	49.00±1.30	48.80±1.59	53.20±1.53	29.20±2.44	22.80±1.43	17.40±2.50
T7	32.00±4.90	36.00±4.00	36.00±7.48	65.20±3.17	71.80±2.27	73.40±3.54	15.20±1.56	10.80±1.53	4.60±0.40
T8	44.00±11.66	48.00±14.97	44.00±11.66	57.20±2.11	57.60±3.94	60.20±3.15	22.40±1.17	12.60±1.86	9.20±0.86
T9	40.00±10.95	36.00±7.48	40.00±6.32	52.40±4.28	54.60±2.77	56.60±3.71	26.20±2.22	22.40±1.33	8.60±0.98
T10	64.00±9.80	64.00±7.48	68.00±8.00	49.40±2.11	50.40±1.69	51.20±3.28	38.80±5.27	30.60±2.04	15.20±0.97
T11	56.00±7.48	64.00±7.48	76.00±7.48	48.40±2.11	49.40±2.50	47.80±2.22	38.60±2.14	29.20±1.83	14.40±1.03
T12	78.00±4.90	80.00±4.47	60.00±10.95	33.80±0.86	34.20±0.97	37.20±1.46	113.20±13.94	72.20±3.75	43.40±3.89
T13	36.00±7.48	44.00±7.48	40.00±6.32	62.80±2.85	57.20±2.63	71.60±5.45	21.00±1.48	17.60±2.44	12.80±1.69
T14	36.00±4.00	48.00±4.90	52.00±13.56	52.60±2.94	52.40±2.58	54.20±0.66	24.60±2.11	20.20±1.32	13.40±1.57
T15	24.00±4.00	32.00±4.90	28.00±4.90	49.20±3.20	49.60±2.42	53.20±2.22	26.40±2.11	23.20±2.42	17.80±1.07
T16	80.00±6.32	76.00±7.48	84.00±7.48	25.80±1.20	30.20±1.43	30.80±1.98	62.20±3.51	38.80±2.69	35.80±1.62
T17	84.00±7.48	84.00±7.48	88.00±7.48	29.80±1.74	30.60±1.36	32.20±1.24	64.80±12.48	53.60±5.26	33.60±3.11
T18	80.00±6.32	84.00±7.48	84.00±4.00	32.40±0.24	32.80±0.97	33.80±0.58	132.40±3.50	50.40±8.97	37.60±6.49
T19	44.00±7.48	48.00±10.20	28.00±4.00	63.40±3.33	61.20±3.89	69.80±2.20	21.40±1.03	13.60±1.57	11.20±1.16
T20	64.00±7.48	68.00±8.00	72.00±10.20	50.60±2.68	51.60±2.32	52.20±3.31	26.80±2.71	18.00±2.02	16.80±1.98
T21	68.00±8.00	64.00±7.48	64.00±7.48	49.60±2.71	46.40±2.52	47.40±3.59	29.80±2.35	23.60±1.60	19.80±1.59
T22	36.00±7.48	32.00±4.90	28.00±4.90	44.20±0.86	46.80±2.52	39.80±2.49	45.40±2.82	29.20±2.35	21.20±1.62
T23	28.00±.90	48.00±10.20	40.00±10.95	34.40±2.98	37.40±3.04	36.80±2.08	65.20±6.52	33.40±3.85	19.80±4.00
T24	80.00±8.94	76.00±7.48	84.00±4.00	33.40±0.81	34.60±0.75	34.20±0.80	80.60±5.10	68.80±7.04	38.40±2.48
CD 5%	**20.24**	**21.10**	**21.01**	**7.02**	**10.12**	**8.89**	**13.32**	**9.18**	**6.41**
SEm	**7.21**	**7.52**	**7.48**	**2.50**	**3.61**	**3.17**	**4.75**	**3.27**	**2.29**
SEd	**10.20**	**10.63**	**10.58**	**3.54**	**5.10**	**4.48**	**6.71**	**4.62**	**3.23**

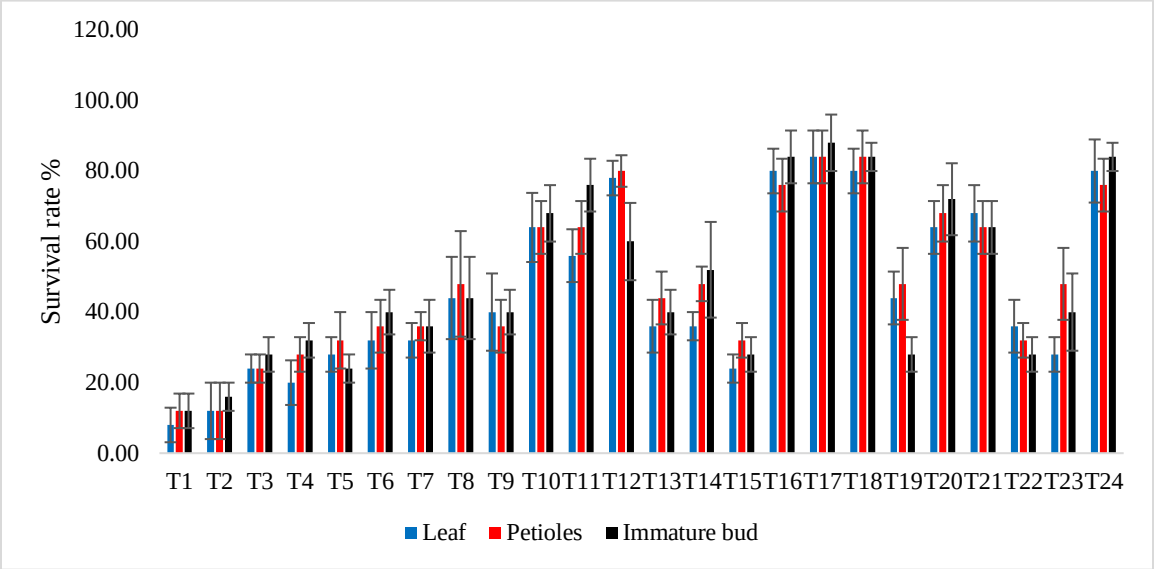

Figure 4.7 Survival rate of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA.

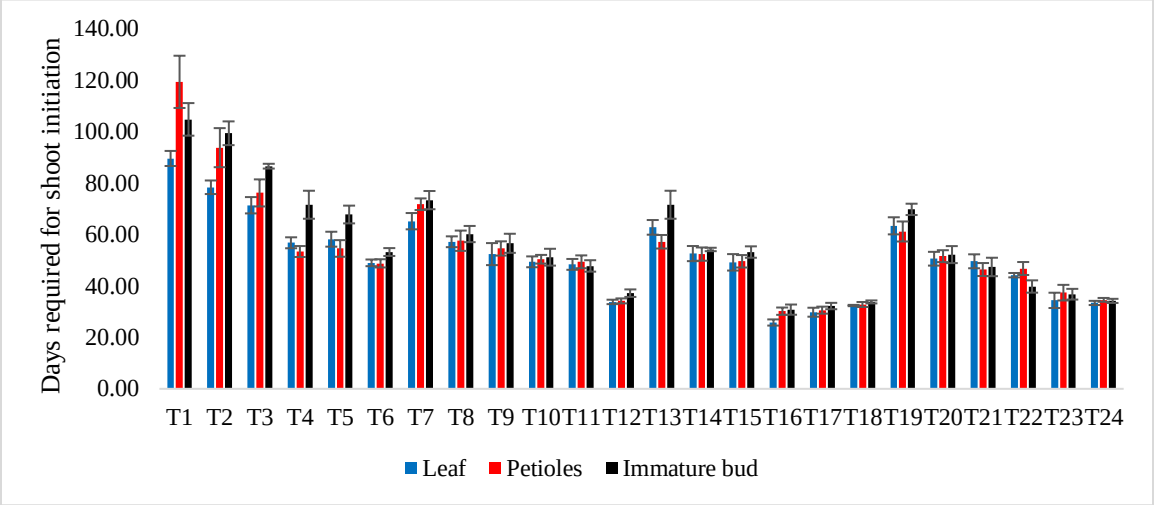

Figure 4.8 Days required for shoot initiation of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA.

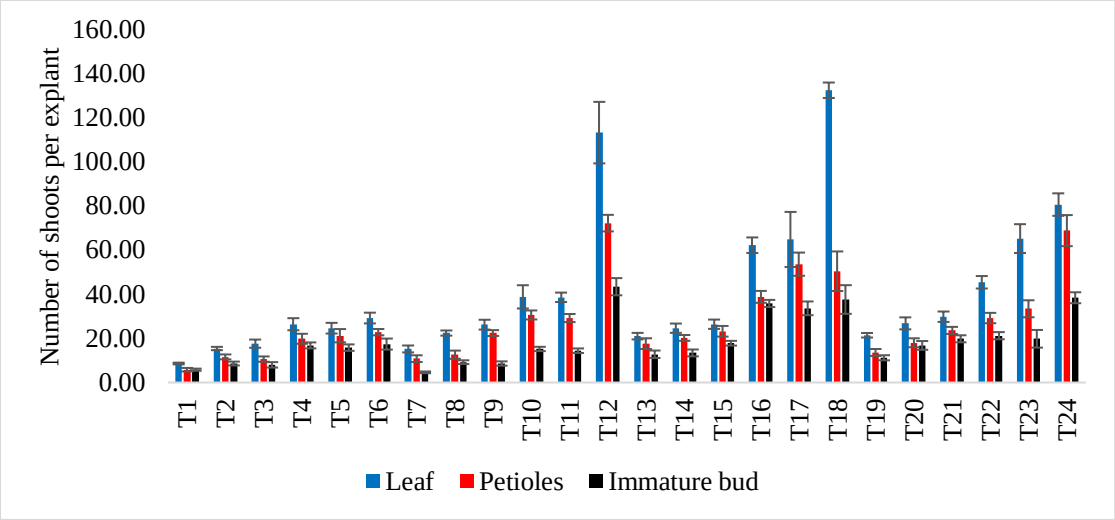

Figure 4.9 Number of shoots per explant of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA.

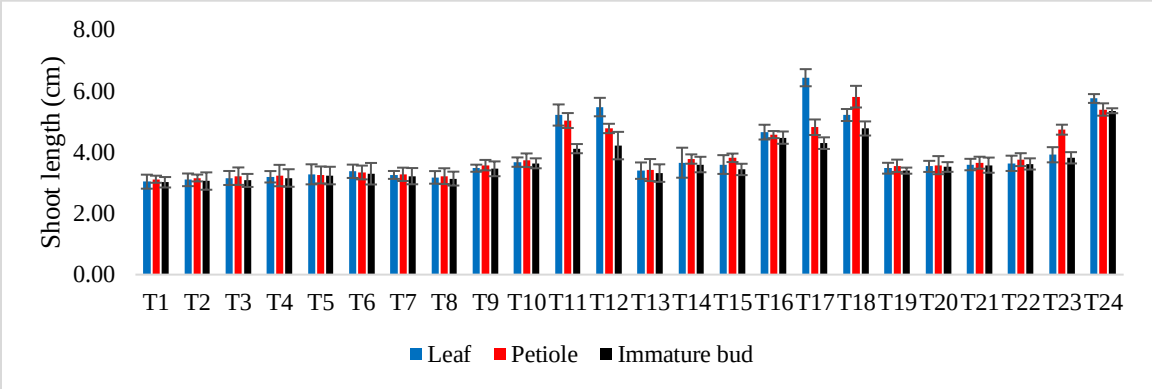

Figure 4.10 Shoot length (cm) *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA.

In case of petiole explant culture, the average minimum days required for shoot initiation (30.20 ± 1.43 days) was noted in treatment T_{16} *i.e.* MS media supplemented with BAP (1.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}), followed by (32.20 ± 1.36 days) treatment T_{17} *i.e*. MS media supplemented with BAP (1.5 mg l^{-1}) in combination with NAA (0.5 mg l^{-1}). In immature bud explant culture, the average minimum days required for shoot initiation (30.80 ± 1.98 days) was observed in treatment T_{16} *i.e*. MS media supplemented with BAP (1.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}) followed by (32.20 ± 1.24 days) treatment T_{17}.

While comparing all three explants, the average minimum days required for shoot initiation was recorded in leaf disc explant of *B. palmata*, followed by petiole and immature bud explant culture. Whereas, in all the three explants, the average maximum days required for shoot initiation (119.40 ± 10.16 days, 104.80 ± 6.34 days and 89.60 ± 2.93 days in the petiole, immature bud and leaf disc explants, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.1.3 Number of shoots per explant

The data presented in the table 4.11 and figure 4.9 showed a significant effect of plant growth regulators on the average number of shoots per explant. In leaf disc explant culture, the maximum average number of shoots per explant (132.40 ± 3.50) was found in treatment T_{18} (MS + BAP 2.0 mg l^{-1} + 0.5 mg l^{-1} NAA), followed by (113.20 ± 13.94) treatment T_{12} (MS + 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA). In case of petiole explant culture, the maximum number of shoots per explant (72.20 ± 3.75) was recorded in treatment T_{12} *i.e.* MS media supplemented with BAP (2.0 mg l^{-1}) in addition of NAA (0.1 mg l^{-1}) followed by (68.80 ±

7.04) treatment T_{24} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). In case of immature bud explant culture, the highest average number of shoots per explant (43.40 ± 3.89) was recorded in treatment T_{12} (MS + 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA) which is at par with treatment T_{24} and T_{18} (38.40 ± 2.48 and 37.60 ± 6.49, respectively).

Comparing all the tested explant the highest number of shoots per explant was recorded in leaf disc explant of *B. palmata*. Whereas, the minimum number of shoots per explant was recorded in petiole and immature bud explant respectively (5.80 ± 0.80 and 5.80 ± 0.49 in petiole and immature bud explant, respectively), followed by leaf disc (8.60 ± 0.40) in T_1 *i.e.* MS media without any growth regulators. The number of shoots per explant was maximum when a higher concentration of BAP in combination of low concentration of NAA used.

4.5.1.4 Shoot length

The data presented in table 4.12 and figure 4.10 clearly revealed that the effect of plant growth regulator concentration of different treatments was significant on the length gain by the adventitious shoot raised by different explant culture. In leaf disc explant, significantly the maximum shoot length (6.44 ± 0.28 cm) was recorded in treatment T_{17} (MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) which is at par with the treatment T_{24} (5.76 ± 0.14 cm) *i.e.* MS media supplemented with 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA.

In case of petiole explant culture, the average highest shoot length (5.82 ± 0.35 cm) was recorded in treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) followed by (5.40 ± 0.20 cm) in treatment T_{24} (MS + 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA).

Table 4.12. Shoot length (cm) and number of plantlets per explant of in vitro micropropagation *Begonia palmata* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Shoot length (days)			Number of plantlets per explant		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	3.04±0.23	3.12±0.12	3.02±0.17	5.20±0.37	3.40±0.51	2.80±0.20
T2	3.10±0.21	3.16±0.11	3.06±0.28	12.60±2.48	7.80±0.58	6.40±0.93
T3	3.16±0.23	3.22±0.29	3.08±0.21	12.40±1.21	7.60±1.08	6.20±0.66
T4	3.20±0.19	3.24±0.35	3.16±0.29	21.20±1.69	12.80±1.71	9.80±0.73
T5	3.28±0.33	3.26±0.28	3.24±0.29	19.60±1.89	12.40±0.51	8.60±1.29
T6	3.38±0.22	3.34±0.23	3.30±0.35	17.80±2.15	11.20±0.66	8.40±1.29
T7	3.26±0.13	3.28±0.22	3.22±0.27	8.80±0.66	5.60±0.81	4.60±0.51
T8	3.18±0.21	3.22±0.26	3.14±0.23	11.60±0.81	7.20±1.20	7.20±0.66
T9	3.48±0.12	3.58±0.17	3.46±0.24	18.20±1.85	12.20±1.20	6.60±0.87
T10	3.68±0.15	3.74±0.22	3.64±0.16	21.20±2.48	18.20±1.62	9.40±1.81
T11	5.22±0.35	5.04±0.24	4.12±0.15	23.40±3.25	13.80±0.58	6.80±0.86
T12	5.48±0.30	4.78±0.15	4.22±0.45	38.40±2.73	26.60±2.62	15.80±1.46
T13	3.40±0.27	3.42±0.36	3.32±0.29	14.40±1.29	10.40±0.68	7.80±1.07
T14	3.66±0.49	3.78±0.15	3.60±0.25	18.20±1.02	14.80±1.07	8.60±0.51
T15	3.60±0.31	3.82±0.14	3.44±0.19	19.20±2.03	16.80±1.80	10.20±1.07
T16	4.66±0.24	4.58±0.12	4.48±0.20	52.40±6.07	29.20±1.66	24.80±2.37
T17	6.44±0.28	4.82±0.25	4.30±0.19	55.20±2.33	31.60±2.46	16.60±0.87
T18	5.22±0.20	5.82±0.35	4.78±0.23	69.40±5.04	34.00±1.41	26.40±1.89
T19	3.48±0.18	3.56±0.20	3.40±0.10	16.60±1.75	11.80±1.24	9.60±1.94
T20	3.54±0.18	3.58±0.30	3.52±0.16	19.80±1.59	15.20±1.36	12.20±1.02
T21	3.60±0.19	3.66±0.20	3.58±0.25	20.60±1.33	16.40±2.01	13.20±1.07
T22	3.64±0.25	3.76±0.21	3.62±0.18	22.60±1.03	13.40±0.75	10.40±1.25
T23	3.92±0.25	4.74±0.16	3.82±0.19	26.00±3.16	15.60±1.29	14.40±1.17
T24	5.76±0.14	5.40±0.20	5.36±0.08	55.60±3.20	43.20±3.15	26.20±4.95
CD 5%	**0.70**	**0.65**	**0.67**	**5.20**	**3.40**	**2.80**
SEm	**0.25**	**0.23**	**0.24**	**12.60**	**7.80**	**6.40**
SEd	**0.35**	**0.33**	**0.34**	**12.40**	**7.60**	**6.20**

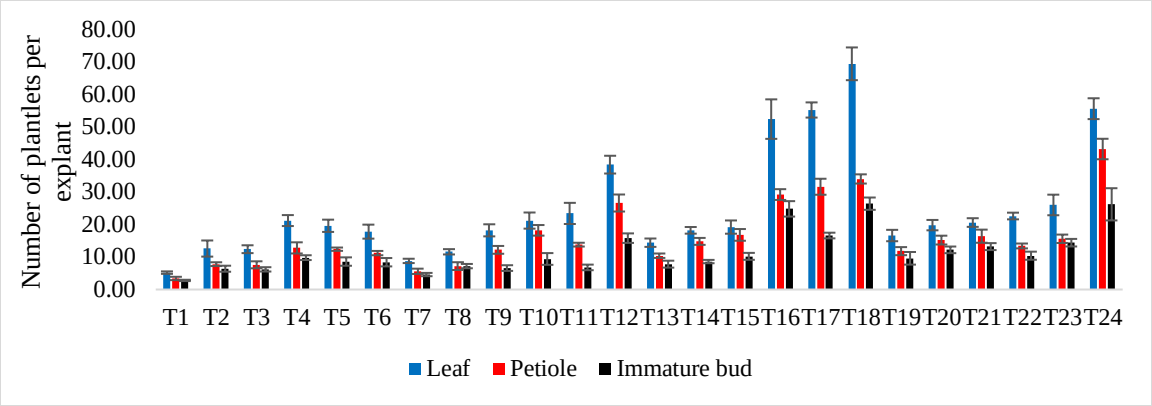

Figure 4.11 Number of plantlets per explant of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA

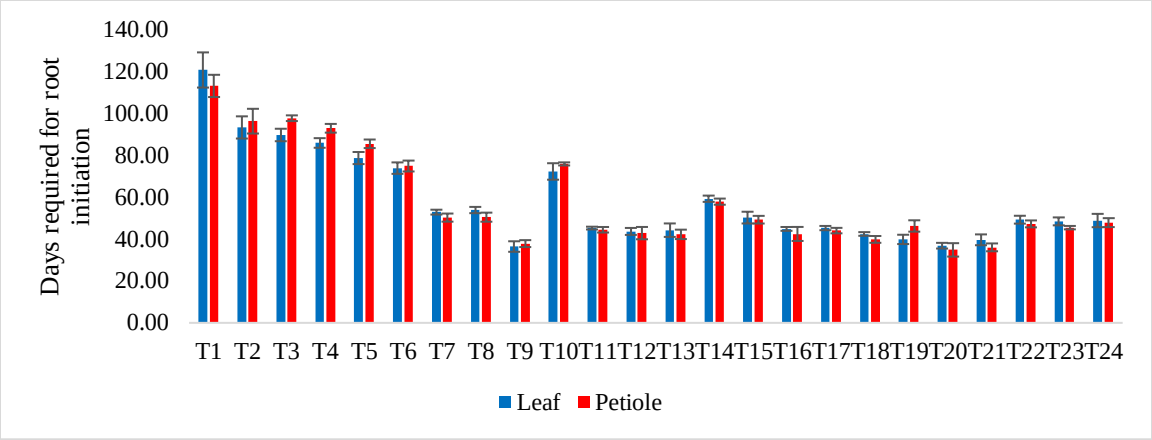

Figure 4.12 Days required for root initiation of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA

Whereas, in immature bud explant, highest shoot length (5.36 ± 0.08 cm) was recorded in treatment T_{24} (MS +2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) followed by (4.78 ± 0.23 cm) in treatment T_{18}. While, the minimum shoot length (3.02 ± 0.17 cm, 3.04 ± 0.23 cm and 3.12 ± 0.12 cm) was observed in immature bud, leaf disc and petiole explant respectively, with MS media containing no plant growth regulators.

4.5.1.5 Number of plantlets per explant

The pursual of data presented in table 4.12 and figure 4.11 showed significant variation in respect of number of plantlets per explant in *B. palmata*. The average number of plantlets per explant developed from leaf disc explant (69.40 ± 5.04) was observed highest in treatment T_{18} (MS +2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) followed by (55.60 ± 3.20) treatment T_{24} (MS +2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA).

Incase of petiole explant culture, the highest number of plantlets per explant (43.20 ± 3.15) was recorded in treatment T_{24} (MS +2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA), followed by (34.00 ± 1.41) treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). The highest average number of plantlets per explant in immature bud explant (26.40 ± 1.89) was recorded in treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) followed by (26.20 ± 4.95) treatment T_{24} (MS media + 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA).

While comparing the data presented in the table 4.12 and figure 4.11, the lowest number of plantlets per explant was recorded in T_1 *i.e.* MS media containing no plant growth regulators in all the three explants culture.

4.5.1.6 Root initiation

The data presented in table 4.13 and figure 4.12, showed significant effect of growth regulators on days required for root initiation in different tested explants. The minimum days required for root initiation of leaf disc explant (36.40 ± 2.50 days) was recorded in treatment T_9 (MS media + 0.5 mgl^{-1} BAP + 0.1 mg l^{-1} NAA) which was at par with (36.80 ± 1.36 days) the treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA). In case of petiole explant culture, the average minimum days required for root initiation (34.80 ± 3.20 days) was recorded in treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) followed by (36.00 ± 1.87 days) treatment T_{21} *i.e.* MS media supplemented BAP (0.5 mg l^{-1}) in addition of NAA (1.0 mg l^{-1}). While, incase of immature bud explant culture no root initiation was observed.

When comparing all the tested explant culture, the average maximum days required for root initiation was noted in leaf disc explants whereas, average minimum days required for root initiation in petiole explants.

Overall, the maximum days taken for root initiation (120.60 ± 8.39 and 113.00 ± 5.30 days in leaf disc and petiole explant, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators among all the tested media combinations.

Table 4.13 Days required for root initiation and root length (cm) of in vitro micropropagation *Begonia palmata* in MS media supplemented with different combination of BAP and NAA, (average values ± SEm)

Parameters/ Treatments	Root initiation (cm)			Root length (cm)		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	120.60±8.39	113.00±5.30	NR	0.72 ±0.12	0.84±0.09	NR
T2	93.20±5.29	96.20±5.89	NR	1.22±0.09	1.12±0.09	NR
T3	89.60±2.98	97.60±1.36	NR	1.34±0.07	1.22±0.34	NR
T4	85.80±2.29	92.80±2.08	NR	1.38±0.11	1.36±0.09	NR
T5	78.60±2.89	85.40±2.04	NR	1.42±0.20	1.46±0.45	NR
T6	73.80±2.73	74.80±2.60	NR	1.44±0.10	1.52±0.15	NR
T7	52.80±1.16	50.20±1.93	NR	1.58±0.21	1.70±0.53	NR
T8	53.80±1.50	50.40±2.16	NR	2.20±0.51	2.04±0.23	NR
T9	36.40±2.50	37.80±1.66	NR	3.32±0.23	3.26±0.27	NR
T10	72.20±3.93	75.80±0.73	NR	3.96±0.57	4.08±0.56	NR
T11	45.20±0.66	44.40±1.33	NR	3.22±0.17	3.52±0.27	NR
T12	43.60±1.66	42.80±2.94	NR	4.28±0.37	4.70±0.28	NR
T13	44.20±3.22	42.20±2.24	NR	4.02±0.14	3.86±0.21	NR
T14	59.20±1.50	57.80±1.46	NR	3.68±0.47	3.32±0.25	NR
T15	50.20±2.80	49.20±1.83	NR	3.06±0.43	3.08±0.41	NR
T16	44.80±0.92	42.40±3.34	NR	3.18±0.33	2.94±0.27	NR
T17	45.20±1.02	44.00±1.30	NR	3.26±0.09	3.66±0.28	NR
T18	42.40±0.87	39.80±1.62	NR	3.48±0.32	3.98±0.36	NR
T19	39.80±2.22	46.20±2.71	NR	2.92±0.47	3.88±0.24	NR
T20	36.80±1.36	34.80±3.20	NR	5.04±0.36	5.44±0.42	NR
T21	39.60±2.58	36.00±1.87	NR	4.58±0.20	4.90±0.15	NR
T22	49.20±1.88	47.20±1.66	NR	3.84±0.14	4.12±0.30	NR
T23	48.40±1.89	45.40±0.81	NR	3.98±0.30	4.86±0.49	NR
T24	48.80±3.18	47.80±2.13	NR	4.28±0.33	4.88±0.22	NR
CD 5%	**8.31**	**7.18**	**-**	**0.85**	**0.89**	**-**
SEm	**2.96**	**2.56**	**-**	**0.30**	**0.32**	**-**
SEd	**4.19**	**3.62**	**-**	**0.43**	**0.45**	**-**

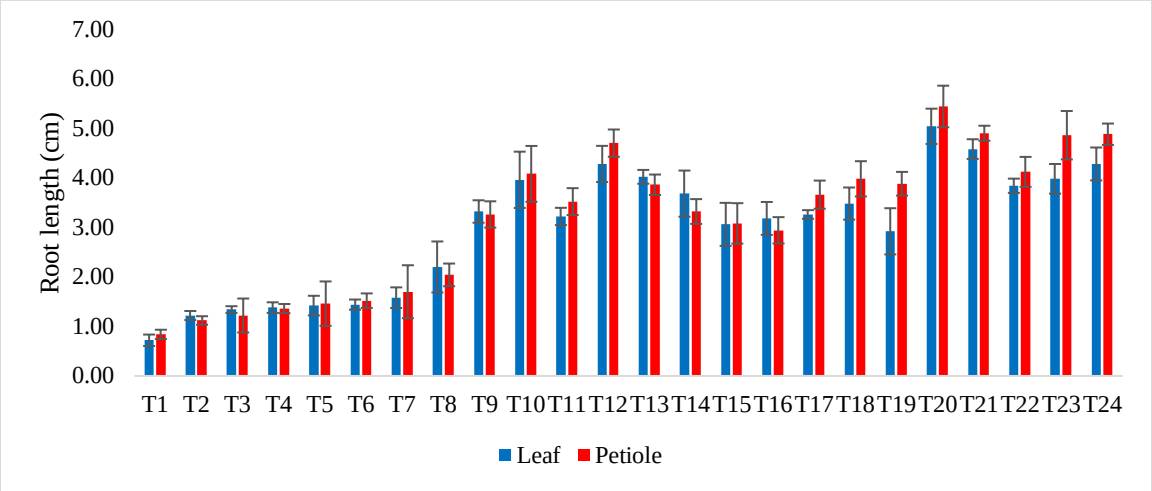

Figure 4.13 Root length (cm) of *in vitro* culture of *B. palmata* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA

4.5.1.7 Root length

It was evident from the data presented in table 4.13 and figure 4.13, significant differences with regard to root length produced among the treatments from different tested explant culture. In case of leaf disc explant, the maximum root length (5.04 ± 0.36 cm) was produced in treatment T_{20} (MS + 0.1 mg l^{-1} BAP+ 1.0 mg l^{-1} NAA) which was at par with (4.58 ± 0.20 cm) treatment T_{21} (MS + 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA), while the treatment T_1 produced minimum root length.

In petiole explant culture, the maximum root length (5.44 ± 0.42 cm) was recorded in treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) which was at par with (4.90 ± 0.15 cm) treatment T_{21} (MS + 0.5 mg l^{-1} BAP+ 1.0 mg l^{-1} NAA). Immature bud explant culture fails to produce roots in any combination of the treatments as well as in control. Treatment T_1 of leaf disc and petiole segments produced minimum root length as compared to all other tested treatments.

4.5.2 *In-vitro* regeneration of *Begonia hatacoa*

The direct organogenesis of *B. hatacoa* were attempted from the leaf disc, petiole and immature bud explants using MS media supplemented with various concentration and combination of BAP and NAA. The various parameters like survival rate, days required for shoot initiation, number of shoots per explant, days required for root initiation, number of plantlets per explant, shoot length and root length were observed and recorded during the course of the investigation

4.5.2.1 Survival rate

As per the data shown in table 4.14 and figure 4.14, a significant effect of growth regulators on survival rate percentage in three different explants was observed. The maximum survival rate of leaf disc explant (84.00 ± 7.48 %) was recorded in treatment T_{18} *i.e.* MS media supplemented with BAP (2.0 mg l^{-1}) incorporation with NAA (0.5 mg l^{-1}) which was at par with the treatments T_{16}, T_{17} and T_{24}, (80.00 ± 6.32 %, 80.00 ± 6.32 % and 80.00 ± 10.95 % respectively). Incase of petiole explant culture, maximum survival rate was recorded in both the treatments T_{17} and T_{18} (96.00 ± 4.00 % and 96.00 ± 4.00%, respectively).

In immature bud explant culture also highest survival rate was recorded in treatment T_{17} and T_{18} (92.00 ± 4.90 % and 92.00 ± 4.90%, respectively) which was at par with treatment T_{16} (88.00 ± 4.90 %) and T_{11} (80.00 ± 6.32 %). Among comparing all the explant's culture, a maximum survival rate was noted in immature bud explants with 96.00 ± 4.00 % under treatments T_{17} and T_{18}. Whereas, the minimum survival rate (8.00 ± 4.90 %, 12.00 ± 4.90 % and 16.00 ± 4.00 % in leaf disc, petiole and immature bud explants, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.2.2 Shoot initiation

It is evident from the data presented in table 4.14 and figure 4.15 that the application of different concentrations of growth regulators significantly influenced the days required for shoot initiation. The average minimum days (28.20 ± 0.86 days) required for shoot initiation in leaf disc explant was recorded in treatment T_{16} (MS + 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) which was at par with treatments T_{18}, T_{12} and T_{24}.

Table 4.14 Survival rate (%), days required for shoot initiation and number of shoots per explant of in vitro micropropagation *Begonia hatacoa* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Survival Rate (%)			Shoot Initiation (days)			Number of Shoots per explant		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	8.00±4.90	12.00±4.90	16.00±4.00	94.60±3.93	104.20±7.81	113.20±6.37	9.80±0.58	5.60±0.51	4.60±0.51
T2	12.00±4.90	16.00±4.00	16.00±7.48	88.80±2.69	87.80±2.06	104.40±3.66	14.00±0.71	9.20±0.73	6.40±0.68
T3	24.00±4.00	28.00±10.20	20.00±6.32	78.20±2.71	75.20±3.95	85.20±3.26	14.40±0.93	9.20±0.80	8.40±0.81
T4	24.00±4.00	32.00±8.00	36.00±7.48	55.40±3.03	58.40±2.44	69.40±2.50	27.40±0.60	20.80±1.07	16.40±1.78
T5	24.00±4.00	36.00±7.48	40.00±6.32	59.60±3.43	59.40±2.16	62.60±5.43	27.20±2.08	19.40±2.01	15.80±1.39
T6	32.00±4.90	48.00±10.20	36.00±7.48	50.40±2.69	53.40±4.50	50.60±3.19	29.80±1.98	23.80±0.97	17.20±1.32
T7	36.00±7.48	40.00±10.95	24.00±4.00	67.80±1.77	68.40±2.93	72.80±1.28	16.80±1.46	10.80±1.07	5.20±0.37
T8	40.00±6.32	40.00±6.32	36.00±7.48	55.40±2.04	52.60±3.59	58.20±3.06	21.20±2.54	15.40±1.29	8.40±1.03
T9	36.00±7.48	44.00±9.80	48.00±10.20	52.80±1.98	49.20±3.95	50.20±2.71	51.40±5.32	32.80±3.07	10.80±1.46
T10	52.00±10.20	60.00±14.14	68.00±8.00	35.80±1.53	42.40±1.33	45.20±2.94	31.60±1.44	28.80±2.08	12.40±1.03
T11	76.00±7.48	80.00±6.32	80.00±6.32	35.60±1.75	44.80±1.16	43.40±2.54	30.60±1.91	24.80±2.20	16.40±1.33
T12	68.00±10.20	60.00±8.94	52.00±8.00	30.00±1.61	33.20±0.73	35.20±0.97	89.20±11.21	64.60±5.82	45.40±2.62
T13	36.00±7.48	44.00±7.48	52.00±10.20	73.60±3.17	73.40±3.56	78.80±3.76	19.80±1.53	16.60±1.75	8.20±1.11
T14	44.00±11.66	52.00±8.00	56.00±7.48	55.20±3.18	56.80±2.22	61.60±4.08	22.20±2.01	17.40±1.57	9.40±1.33
T15	24.00±4.00	36.00±4.00	44.00±4.00	53.40±3.44	52.80±3.47	54.80±3.14	24.80±2.60	19.20±3.09	16.80±2.50
T16	80.00±6.32	88.00±4.90	88.00±4.90	28.20±0.86	31.20±1.93	29.40±0.98	68.60±6.65	46.20±3.90	44.60±4.83
T17	80.00±6.32	96.00±4.00	92.00±4.90	32.40±0.93	33.80±0.86	30.20±1.11	62.80±3.10	44.60±4.89	26.20±1.85
T18	84.00±7.48	96.00±4.00	92.00±4.90	29.00±0.95	31.80±0.58	31.60±1.03	105.40±8.60	59.80±4.35	39.80±1.77
T19	52.00±8.00	56.00±7.48	32.00±4.90	71.80±1.83	71.20±2.20	76.40±2.73	18.80±1.39	15.20±2.13	11.40±1.44
T20	68.00±10.20	72.00±10.20	68.00±10.20	46.20±3.73	46.40±2.27	49.80±3.76	25.20±2.29	23.40±1.57	18.60±2.14
T21	40.00±8.94	68.00±4.90	72.00±10.20	44.60±2.14	45.00±2.28	48.40±3.14	32.80±0.73	26.80±1.46	21.80±2.44
T22	28.00±4.90	32.00±8.00	44.00±11.66	40.20±3.12	39.40±3.09	43.60±2.20	56.60±3.44	31.80±2.13	24.40±2.25
T23	40.00±8.94	52.00±10.20	40.00±6.32	34.80±1.39	36.40±1.33	37.20±2.27	40.20±2.35	29.60±4.38	20.20±0.86
T24	80.00±10.95	76.00±7.48	76.00±9.80	31.60±0.51	32.20±0.49	31.80±2.08	83.00±7.52	56.60±3.33	42.20±3.81
CD 5%	**21.07**	**22.52**	**21.13**	**6.92**	**8.38**	**8.78**	**11.48**	**7.69**	**5.54**
SEm	**7.51**	**8.02**	**7.53**	**2.46**	**2.98**	**3.13**	**4.09**	**2.74**	**1.97**
SEd	**10.61**	**11.34**	**10.65**	**3.49**	**4.22**	**4.42**	**5.78**	**3.87**	**2.79**

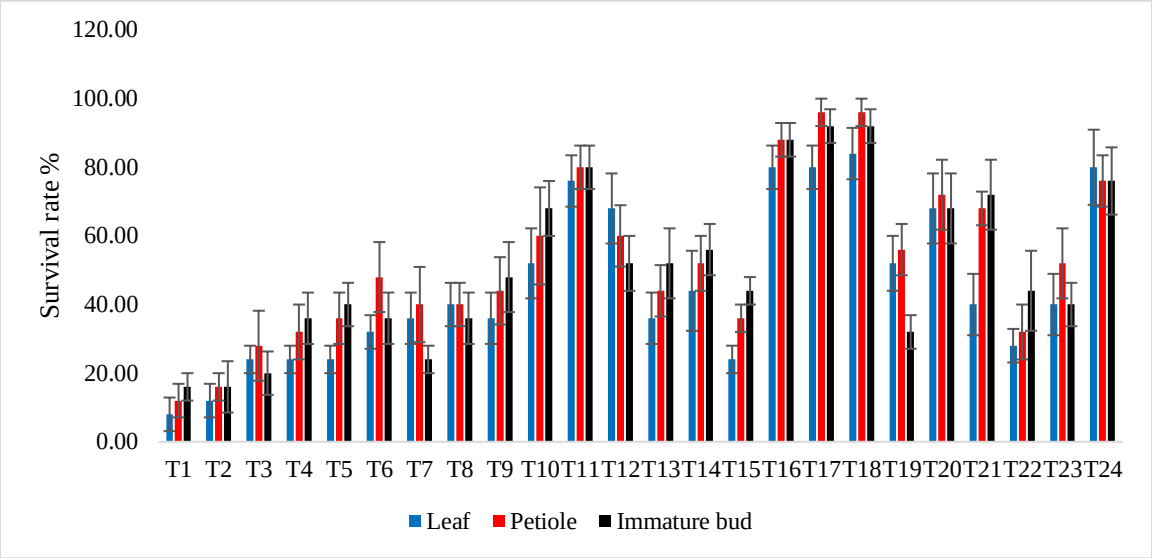

Figure 4.14 Survival rate percentage of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

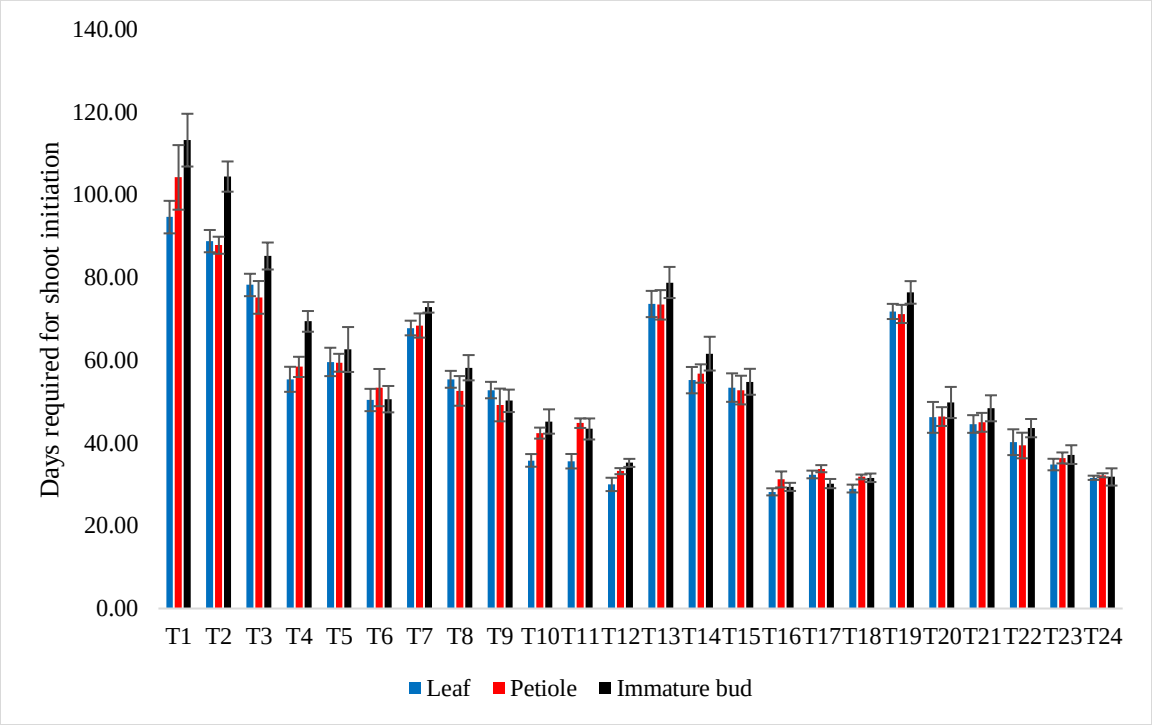

Figure 4.15 Days required for shoot initiation of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

In case of petiole explant culture, the average minimum days required for shoot initiation (31.20 ± 1.93 days) was noted in treatment T_{16} *i.e.* MS media supplemented with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, followed by treatment T_{18} and T_{24} with 31.80 ± 0.58 days and 32.20 ± 0.49 days, respectively. Whereas for immature bud explant culture, the average minimum days required for shoot initiation (29.40 ± 0.98 days) was observed in treatment T_{16} *i.e.* MS media supplemented with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA followed by treatment T_{17} and T_{18} (30.20 ± 1.11 days and 31.60 ± 1.03 days, respectively).

While comparing all the explant culture, the average minimum days required for shoot initiation was recorded in leaf disc explant of *B. hatacoa*, followed by immature bud and petiole explant. Whereas the average maximum days required for shoot initiation (94.60 ± 3.93 days, 104.20 ± 7.81 days and 113.20 ± 6.37 days in leaf disc, petiole, and immature bud, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.2.3 Number of shoots per explant

The data in table 4.14 and figure 4.16 showed significant effect of plant growth regulators on average number of adventitious shoots per explant. The number of shoots per explant were maximum when higher concentration of BAP in combination of low concentration of NAA used. In leaf disc explant, the maximum average number of shoots per explant (105.40 ± 8.60) was found in treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA), followed by treatment T_{12} and T_{24}. For petiole explant culture, the maximum number of shoots per explant (64.20 ± 5.82) was recorded in treatment T_{12} (MS + 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA) followed by treatment T_{18} (59.80 ± 4.35). And under immature

bud explant culture, the highest average number of shoots per explant (45.40 ± 2.62) was recorded in treatment T_{12}, which was at par with treatment T_{16} and T_{24} (44.60 ± 4.83 and 42.20 ± 3.81, respectively).

Leaf disc culture recorded the highest average number of shoots per explant among all three explants tested. Whereas, the minimum number of shoots per explant (4.60 ± 0.51) was recorded in immature bud explant of *B. hatacoa*, followed by petiole (5.60 ± 0.51) and leaf disc explant (9.80 ± 0.58) in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.2.4 Shoot length

The data presented in table 4.15 and figure 4.17 clearly revealed a significant effect of different treatments combination of plant growth regulator on adventitious shoot length.

For shoot length, treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) has recorded average highest shoot length in all three explants culture with 5.74 ± 0.22 cm in leaf disc culture, 5.42 ± 0.28 cm in petiole explant culture, and 4.92 ± 0.19 cm in immature bud explant, which was at par with the treatment T_{17} (MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) with 5.22 ± 0.19 cm, 4.56 ± 0.25 cm, and 4.42 ± 0.07 cm shoot length from leaf disc, petiole, and immature bud explant, respectively.

While average minimum shoot length (2.98 ± 0.34 cm, 3.00 ± 0.33 cm and 3.04 ± 0.22 cm) was observed in immature bud, leaf and petiole explant respectively, with MS media containing no plant growth regulators *i.e.* treatment T_1.

Table 4.15. Shoot length (cm) and number of plantlets per explant of in vitro micropropagation *Begonia hatacoa* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Shoot length (cm)			Number of plantlets per explant		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	3.00±0.33	3.04±0.22	2.98±0.34	4.80±0.37	3.80±0.37	2.60±0.24
T2	3.38±0.23	3.22±0.12	3.12±0.28	11.20±1.53	6.40±0.75	4.80±0.66
T3	3.50±0.27	3.46±0.43	3.22±0.34	11.60±0.98	6.60±1.66	4.20±1.32
T4	3.52±0.32	3.48±0.35	3.26±0.31	23.40±0.93	13.40±1.50	8.20±1.02
T5	3.72±0.25	3.54±0.44	3.48±0.19	20.20±2.03	10.40±0.81	9.60±1.08
T6	4.12±0.23	3.48±0.12	3.52±0.43	17.80±2.01	10.80±0.80	9.20±0.49
T7	3.40±0.26	3.36±0.12	3.36±0.22	9.20±0.49	5.40±0.51	5.20±0.66
T8	3.12±0.20	3.18±0.27	3.10±0.36	12.80±0.58	7.40±0.51	5.60±0.51
T9	3.98±0.15	3.76±0.25	3.72±0.21	17.40±2.42	12.40±0.68	7.40±1.17
T10	4.10±0.21	4.04±0.25	3.78±0.32	19.60±2.48	16.80±2.24	9.20±0.86
T11	4.16±0.26	4.46±0.53	4.08±0.42	21.60±2.42	14.20±0.58	8.20±1.16
T12	4.34±0.53	4.32±0.24	4.18±0.36	36.20±2.52	21.40±0.93	14.60±1.44
T13	3.34±0.56	3.14±0.43	3.10±0.46	12.80±1.39	13.20±1.53	6.80±0.97
T14	3.68±0.39	3.56±0.20	3.50±0.25	14.80±0.80	9.80±0.58	7.40±0.51
T15	3.48±0.31	3.32±0.33	3.24±0.33	18.20±2.40	12.40±2.77	9.40±0.68
T16	4.54±0.25	4.48±0.16	4.22±0.24	50.40±5.32	33.40±2.34	27.40±1.75
T17	5.22±0.19	4.56±0.25	4.42±0.07	54.40±2.06	32.20±3.89	18.40±3.91
T18	5.74±0.22	5.42±0.28	4.92±0.19	57.60±2.84	41.20±1.66	25.40±2.38
T19	3.32±0.36	3.16±0.41	3.14±0.35	14.40±1.03	13.20±1.56	9.80±1.16
T20	3.20±0.32	3.18±0.41	3.12±0.31	19.20±1.46	15.40±1.29	11.80±1.46
T21	3.52±0.37	3.32±0.25	3.18±0.35	24.60±1.86	19.00±2.47	13.40±1.69
T22	3.62±0.39	3.60±0.30	3.42±0.38	20.80±2.08	15.60±0.75	12.40±1.21
T23	4.66±0.28	4.02±0.17	3.92±0.12	17.60±1.47	13.20±0.73	13.60±2.94
T24	4.94±0.24	4.42±0.52	4.28±0.12	49.40±4.55	38.80±5.34	27.40±4.49
CD 5%	**0.88**	**0.89**	**0.86**	**6.28**	**5.37**	**4.90**
SEm	**0.31**	**0.32**	**0.31**	**2.24**	**1.91**	**1.75**
SEd	**0.44**	**0.46**	**0.43**	**3.16**	**2.70**	**2.47**

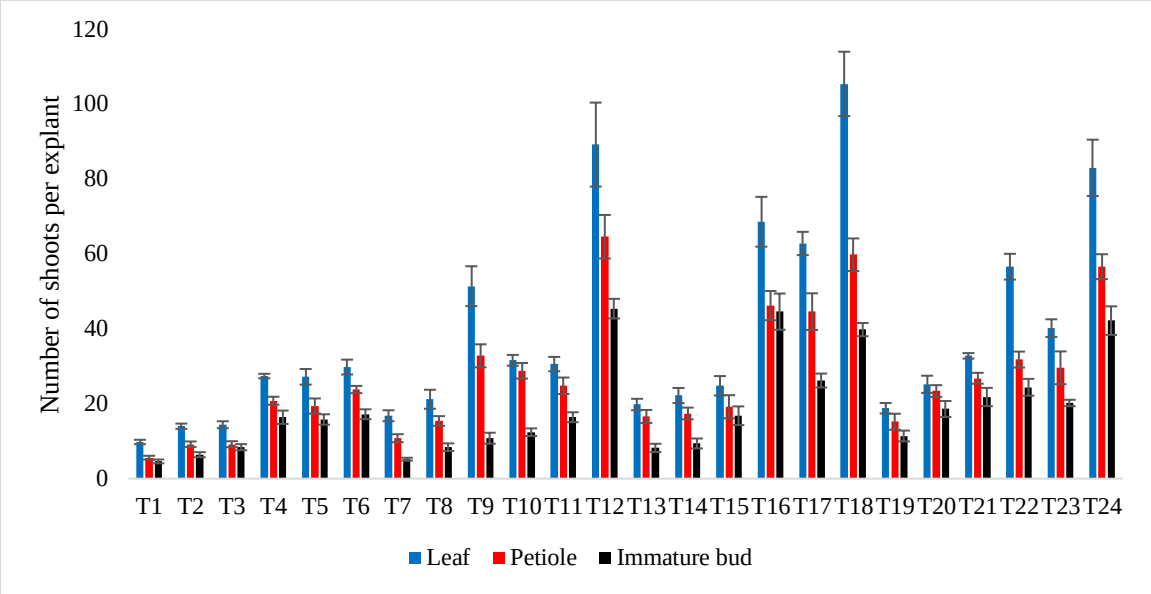

Figure 4.16 Number of shoots per explant of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

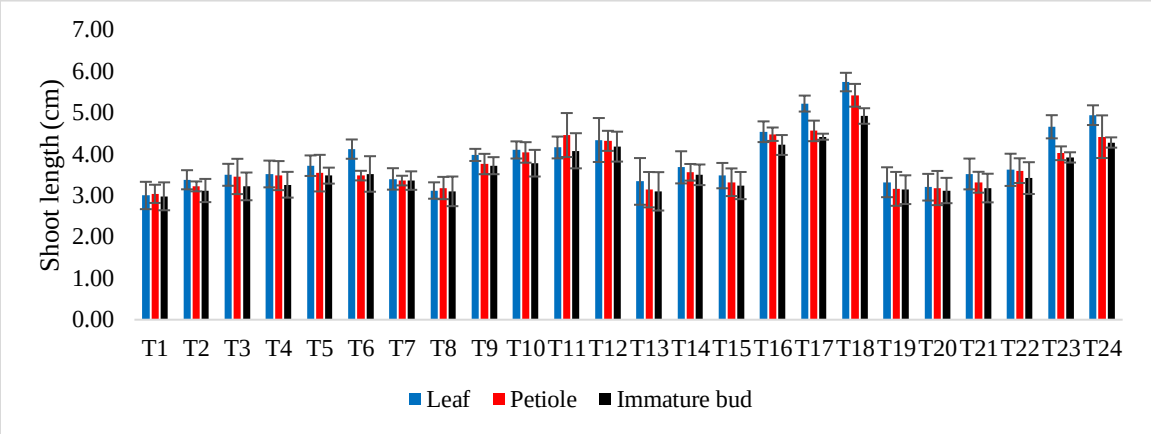

Figure 4.17 Shoot length (cm) of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

4.5.2.5 Number of plantlets per explant

The average number of plantlets per explant in leaf disc explant (57.60 ± 2.84) was observed highest in treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA), followed by (54.40 ± 2.06) treatment T_{17} (MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) and (50.40 ± 5.32) T_{16}(MS + 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). And in petiole explant culture, the highest number of plantlets per explant (41.20 ± 1.66) was observed in treatment T_{18}, followed by (38.80 ± 5.34) treatment T_{24} (MS + 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) and (33.40 ± 3.89) in treatment T_{17} (MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). Whereas, the highest number of plantlets per explant in immature bud explant (27.40 ± 1.75 and 27.40 ± 4.49) was recorded in treatment T_{16} and T_{24}, respectively.

While comparing the data presented in table 4.15 and figure 4.18 showed that the lowest number of plantlets per explant was recorded in T_1 *i.e.* MS media containing no plant growth regulators in all the three explants tested. The average number of shoots per culture was found correlated with the higher concentration of growth regulators which also produce maximum number of plantlets per explant.

4.5.2.6 Root initiation

The data presented in table 4.16 and figure 4.19 showed significant effect of growth regulators on days required for root initiation in different explants tested. The average minimum days required for root initiation of leaf disc explant (31.80 ± 2.13 days) was recorded in treatment T_{21} (MS + 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) which was at par with (32.20 ± 1.83 days) the treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA). And for petiole explant of *B. hatacoa*, the average minimum days required for root initiation (29.60

± 2.42 days) was recorded in treatment T_{21}, followed by (33.20 ± 1.59 days) in treatment T_{20}. While, incase of immature bud explant root initiation was not observed.

Comparing all the tested explants culture, the maximum days required for root initiation was noted in immature bud explant and minimum days required for root initiation in petiole explant. Whereas, in all the three explants, the maximum days required for root initiation (119.60 ± 3.63 and 117.60 ± 3.41 days in leaf disc and petiole explant, respectively), which was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.2.7 Root length

It is evident from the data presented in table 4.16 and figure 4.20 there was significant differences with regard to root length produced among the treatments from different explants tested. In case of leaf disc explant culture, the maximum root length (4.78 ± 0.25 cm) were produced in treatment T_{21} in MS media supplemented with 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA which was at par with (4.28 ± 0.39 cm) treatment T_{13} *i.e.* MS media having 0.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, while the treatment T_1 produced minimum root length.

In petiole explant culture, the maximum root length (4.34 ± 0.19 cm) was recorded in treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA) which was at par with (4.30 ± 0.25 cm) treatment T_{13} (MS + 0.0 mg l^{-1} BAP+ 0.5 mg l^{-1} NAA) and (4.24 ± 0.17 cm) treatment T_{14} (MS + 0.1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). There was no root initiation found in immature bud explants. The treatment T_1 recorded to produced minimum root length as compared to all the tested treatments which is without growth regulators.

Table 4.16 Days required for root initiation and root length (cm) of in vitro micropropagation *Begonia hatacoa* in MS media supplemented with different combination of BAP and NAA, (average values ± SEm)

Parameters/ Treatments	Root initiation (days)			Root Length (cm)		
	Leaf disc	Petiole	Immature bud	Leaf disc	Petiole	Immature bud
T1	119.60±3.63	117.60±3.41	NR	1.08±0.05	1.18±0.10	NR
T2	94.40±4.09	90.80±2.85	NR	1.22±0.10	1.16±0.07	NR
T3	93.20±2.44	93.20±3.98	NR	1.80±0.10	1.46±0.09	NR
T4	85.40±1.63	87.60±2.94	NR	1.30±0.09	1.62±0.12	NR
T5	76.80±2.48	81.40±2.11	NR	1.62±0.52	1.52±0.48	NR
T6	73.20±3.06	69.40±2.73	NR	1.70±0.18	1.46±0.09	NR
T7	58.20±1.85	56.60±3.33	NR	1.90±0.60	1.42±0.30	NR
T8	53.40±2.50	46.40±2.52	NR	1.60±0.22	1.84±0.20	NR
T9	38.60±1.91	37.80±2.20	NR	2.60±0.21	2.30±0.13	NR
T10	69.40±2.62	71.20±2.35	NR	3.96±0.57	4.08±0.56	NR
T11	61.80±1.83	58.80±1.83	NR	2.88±0.18	3.20±0.17	NR
T12	49.60±2.06	50.80±2.63	NR	3.82±0.34	3.42±0.51	NR
T13	46.40±2.11	48.40±2.58	NR	4.28±0.39	4.30±0.25	NR
T14	52.80±2.27	52.20±2.13	NR	3.68±0.15	4.24±0.17	NR
T15	52.80±1.39	47.40±2.79	NR	2.98±0.21	3.28±0.10	NR
T16	44.40±2.87	43.60±3.08	NR	3.10±0.16	3.18±0.27	NR
T17	47.20±3.46	35.40±7.63	NR	2.80±0.16	2.94±0.16	NR
T18	46.20±2.71	45.60±6.77	NR	3.76±0.37	4.04±0.36	NR
T19	39.20±4.09	44.40±3.19	NR	2.88±0.21	3.66±0.21	NR
T20	32.20±1.83	33.20±1.59	NR	4.24±0.28	4.34±0.19	NR
T21	31.80±2.13	29.60±2.42	NR	4.78±0.25	3.74±0.16	NR
T22	50.60±2.80	49.40±2.20	NR	3.44±0.30	3.72±0.19	NR
T23	45.60±1.08	47.20±2.75	NR	3.44±0.12	3.94±0.30	NR
T24	49.40±2.29	46.20±1.46	NR	3.12±0.26	3.86±0.32	NR
CD 5%	**7.24**	**9.24**	**-**	**0.82**	**0.75**	**-**
SEm	**2.58**	**3.29**	**-**	**0.29**	**0.27**	**-**
SEd	**3.65**	**4.65**	**-**	**0.41**	**0.38**	**-**

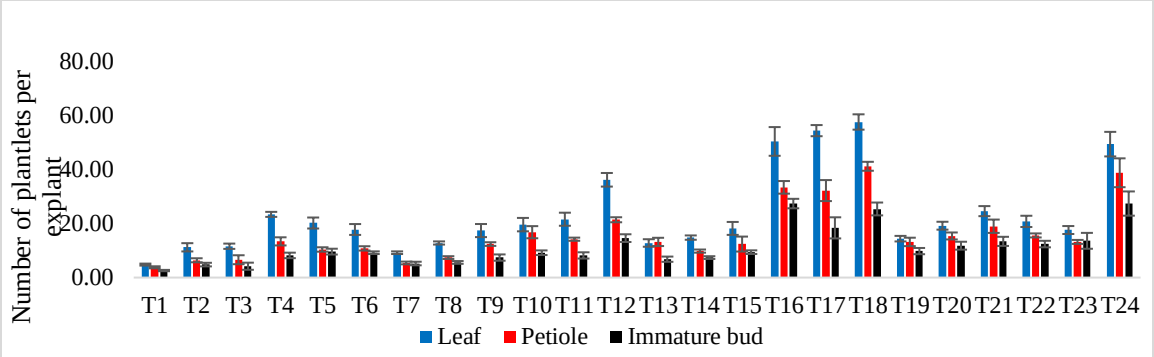

Figure 4.18 Number of plantlets per explant of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

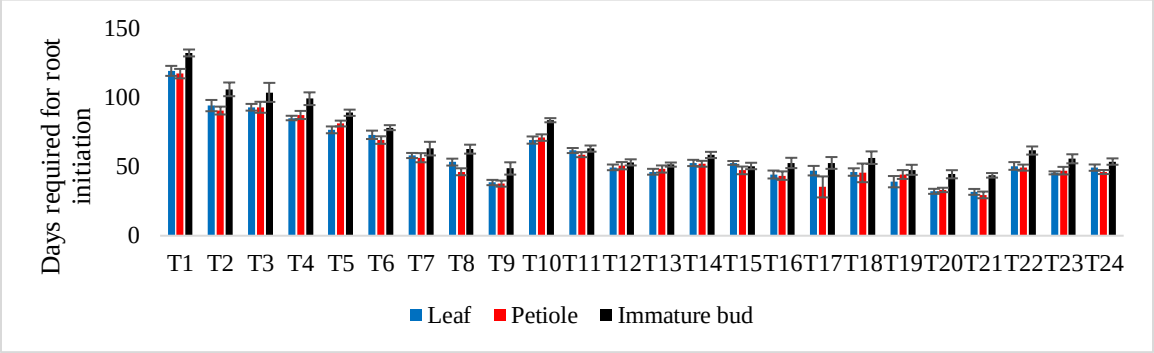

Figure 4.19 Days required for root initiation of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

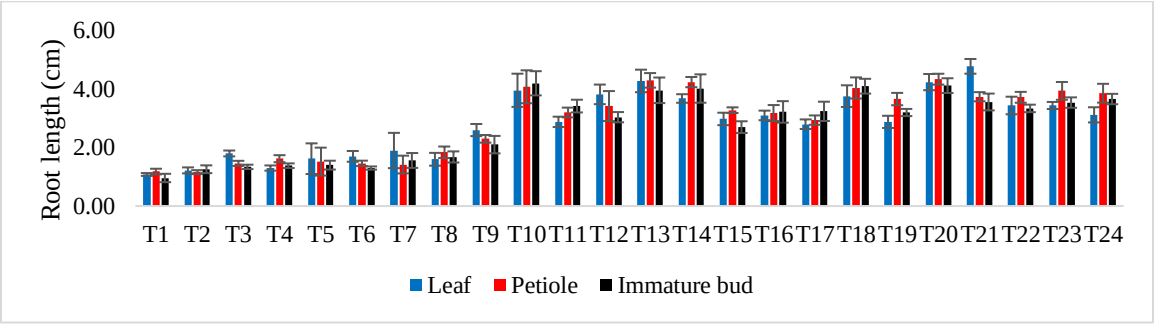

Figure 4.20 Root length (cm) of *in vitro* culture of *B. hatacoa* (leaf disc, petiole and immature bud explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

4.5.3 *In-vitro* regeneration of *Begonia xanthina*

The direct organogenesis of *B. xanthina* were attempted from the leaf disc and petiole explants using MS media supplemented with various concentration and combination of BAP and NAA. The various parameters like survival rate, days required for shoot initiation, number of shoots per explant, days required for root initiation, number of plantlets per explant, shoot length and root length were observed and recorded during the course of the investigation.

4.5.3.1 Survival rate

As per the data depicted in table 4.17 and figure 4.21 significant effect of growth regulators on survival rate percentage in different explants tested was observed. The maximum survival rate of leaf disc explant culture (88.00 ± 4.90 %) was recorded in treatment T_{18} *i.e.* MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA at par with the treatments T_{24}, and T_{17} (88.00 ± 4.90 % and 84.00 ± 4.00 %, respectively).

Petiole explant culture of *B. xanthina* showed maximum survival rate (92.00 ± 4.90 %) in treatments T_{18} *i.e.* MS media supplemented with BAP (2.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}) followed by (88.00 ± 4.90) in treatment T_{17} (MS + 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). Comparing all the tested explants culture, the maximum survival rate was noted in petiole explant. Whereas, the least survival rate (12.00 ± 4.00% and 16.00 ± 4.90% in petiole and leaf disc, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

Table 4.17 Survival rate (%), days required for shoot initiation, number of shoots per explant and days required for root initiation of in vitro micropropagation *Begonia xanthina* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Survival rate (%)		Shoot Initiation (days)		Number of shoots per explant		Shoot length (cm)	
	Leaf disc	Petiole	Leaf disc	Petiole	Leaf disc	Petiole	Leaf disc	Petiole
T1	12.00±4.90	16.00±4.00	97.40±6.03	110.80±4.58	8.60±0.98	5.40±0.24	2.90±0.20	2.80±0.15
T2	16.00±9.80	12.00±4.90	87.60±1.96	100.40±4.15	14.20±0.37	6.60±0.51	3.06±0.25	3.00±0.21
T3	20.00±6.32	20.00±6.32	75.60±5.01	75.80±3.12	15.20±0.86	9.80±0.73	3.18±0.34	3.10±0.10
T4	24.00±4.00	28.00±4.90	60.20±3.60	65.60±3.12	26.80±1.77	16.60±1.44	3.20±0.12	3.14±0.20
T5	28.00±4.90	36.00±7.48	62.20±3.44	65.80±2.75	29.20±2.82	22.40±1.60	3.36±0.14	3.16±0.26
T6	56.00±11.66	32.00±8.00	54.60±2.66	55.20±2.76	28.60±1.86	19.20±1.07	3.56±0.31	3.30±0.20
T7	36.00±7.48	32.00±4.90	68.40±1.57	71.40±2.73	14.20±0.92	9.60±0.81	3.06±0.22	2.98±0.27
T8	36.00±7.48	36.00±7.48	51.40±2.11	55.40±0.98	24.80±2.15	7.80±1.07	3.30±0.23	3.10±0.07
T9	40.00±6.32	44.00±11.66	54.80±2.15	57.20±1.71	25.60±1.60	11.60±1.21	3.64±0.18	3.52±0.22
T10	56.00±11.66	60.00±8.94	48.40±1.75	49.80±2.31	38.00±4.94	22.80±2.75	3.92±0.20	3.60±0.28
T11	72.00±4.90	76.00±9.80	39.60±3.44	40.60±0.93	31.40±2.62	29.40±1.89	4.24±0.25	4.12±0.24
T12	64.00±9.80	68.00±12.00	31.60±0.87	32.20±0.73	61.20±7.21	49.40±4.84	4.30±0.25	4.20±0.27
T13	68.00±12.00	56.00±14.70	71.80±2.31	68.60±3.91	23.20±2.63	15.80±1.07	3.16±0.33	3.04±0.28
T14	56.00±7.48	64.00±11.66	54.00±0.89	51.80±2.85	25.20±1.59	17.20±1.69	3.34±0.65	3.20±0.35
T15	32.00±4.90	36.00±4.00	49.80±2.63	50.40±2.20	23.40±2.27	16.80±1.46	3.78±0.21	3.66±0.36
T16	68.00±12.00	84.00±7.48	26.80±1.66	30.20±2.01	86.40±3.28	68.80±2.54	4.68±0.23	4.18±0.14
T17	84.00±4.00	88.00±4.90	33.80±1.16	32.60±1.29	50.80±2.40	35.40±7.63	5.14±0.18	4.62±0.23
T18	88.00±4.90	92.00±4.90	34.60±0.68	35.40±1.12	102.00±8.46	52.80±5.41	4.72±0.23	4.46±0.24
T19	24.00±4.00	32.00±4.90	69.60±2.73	67.40±3.59	16.80±1.24	12.40±1.63	3.24±0.09	3.20±0.27
T20	64.00±7.48	52.00±8.00	48.80±3.06	51.40±1.91	24.20±2.13	15.80±1.85	3.24±0.15	3.18±0.17
T21	64.00±7.48	68.00±10.20	47.00±2.17	48.60±2.58	30.40±2.94	21.40±3.50	3.80±0.23	3.38±0.27
T22	44.00±11.66	40.00±6.32	42.20±1.93	40.80±4.12	49.20±2.46	28.60±2.23	4.04±0.18	3.86±0.24
T23	32.00±8.00	40.00±6.32	36.40±2.50	36.80±2.18	34.80±3.04	31.60±3.88	4.14±0.30	4.04±0.16
T24	88.00±4.90	72.00±10.20	27.60±0.93	31.80±1.59	78.80±5.36	50.80±5.89	4.24±0.16	4.00±0.13
CD 5%	**22.22**	**22.98**	**7.57**	**7.55**	**9.40**	**8.45**	**0.72**	**0.65**
SEm	**7.92**	**8.19**	**2.70**	**2.69**	**3.35**	**3.01**	**0.26**	**0.23**
SEd	**11.20**	**11.58**	**3.81**	**3.80**	**4.73**	**4.26**	**0.36**	**0.33**

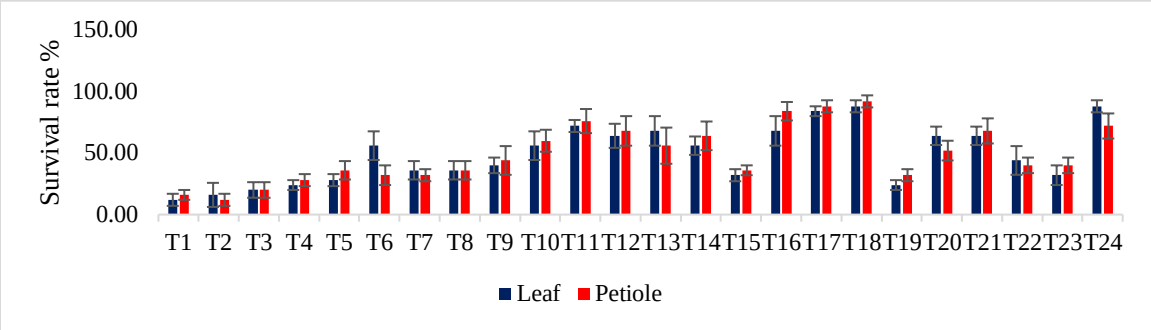

Figure 4.21 Survival rate percentage of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

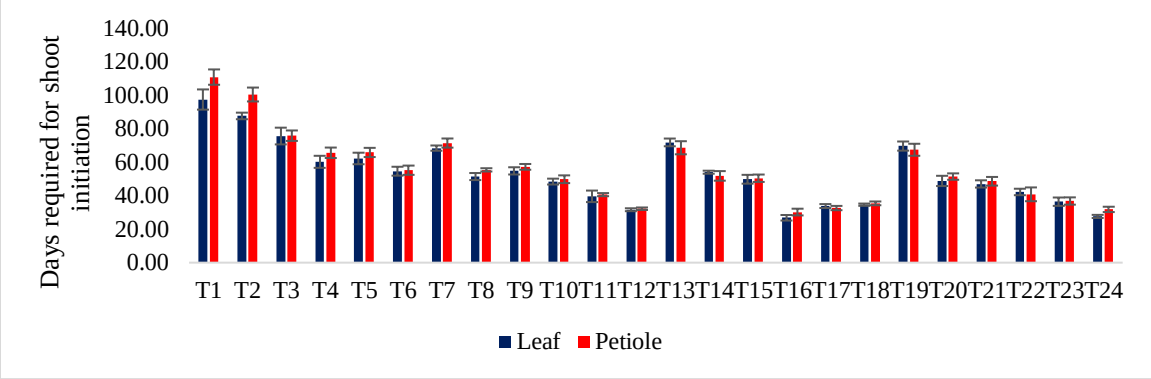

Figure 4.22 Days required for shoot initiation of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

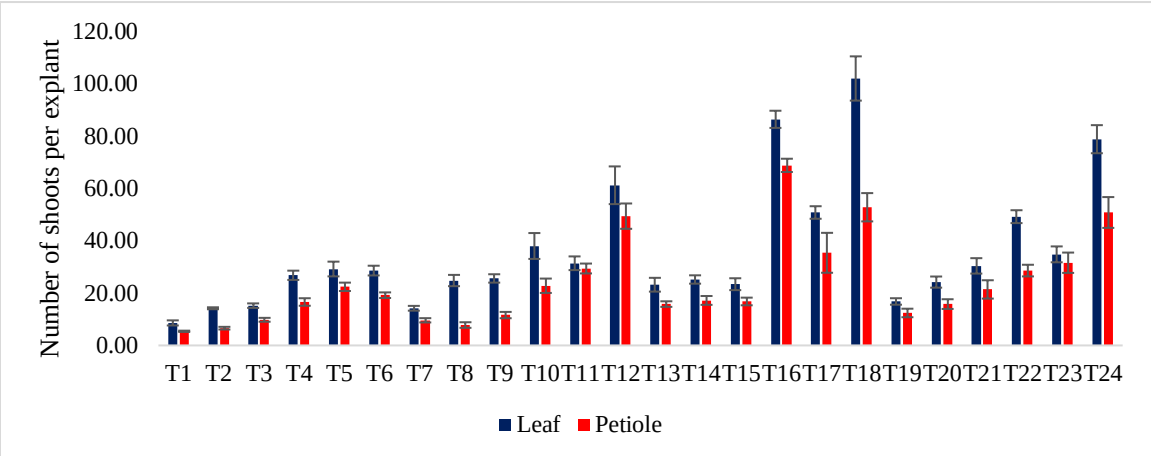

Figure 4.23 Number of shoots per explant of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

4.5.3.2 Shoot initiation

The data presented in table 4.17 and figure 4.22 showed that the application of different concentrations of growth regulators was found significant in days required for shoot initiation. The average minimum days required for shoot initiation (26.80 ± 1.66 days) in leaf disc explant was recorded in treatment T_{16} MS media fortified with BAP (1.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}) which was at par (27.60 ± 0.93 days) with treatment T_{24} (MS +2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA). In case of petiole explant, the average minimum days required for shoot initiation (30.20 ± 2.01 days) was noted in treatment T_{16}, followed by (31.80 ± 1.59 days) treatment $T_{24.}$

In comparison of all the explant, the average minimum days required for shoot initiation was recorded in leaf disc explant of *B. xanthina*, followed by petiole explant. Whereas, the average maximum days required for shoot initiation (110.00 ± 4.58 days, and 97.40 ± 6.03 days in petiole and leaf disc explant, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.3.3 Number of shoots per explant

The data shown in table 4.17 and figure 4.23 recorded significant effect of plant growth regulators on average number of shoots per explant of *B. xanthina*. In leaf disc explant, the maximum average number of shoots per explant (102.00 ± 8.46) was found in treatment T_{18} *i.e.* MS media fortified with BAP (2.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}), followed by (86.40 ± 3.28) treatment T_{16} *i.e.*(MS media + 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). In petiole explant culture, the maximum average number of shoots per explant (68.80 ± 2.54) was recorded in treatment T_{16} followed by treatment T_{24} (50.80 ± 5.89).

When comparing the explant culture, the highest number of shoots per explant was recorded in leaf disc explant (102.00 ± 8.46) in treatment T_{16} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) and the minimum number of shoots per explant (5.40 ± 0.24) was recorded in petiole explant of *B. xanthina* in T_1 *i.e.* MS media without any growth regulators.

4.5.3.4 Shoot length

The pursual of data presented in table 4.17 and figure 4.24 revealed that the effect of different plant growth regulator treatments on shoot length of the in vitro raised plantlet was significant. In leaf disc explants culture, the maximum average shoot length (5.14 ± 0.18 cm) was recorded in treatment T_{17} *i.e.* MS media fortified with BAP (1.5 mg l^{-1}) in addition of NAA (0.5 mg l^{-1} NAA), followed by (4.72 ± 0.23 cm) the treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). In case of petiole explant culture, the mean highest shoot length (4.62 ± 0.23 cm) was recorded in treatment T_{17} (MS +1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) followed by (4.46 ± 0.24 cm) treatment T_{18} (MS + 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA). Whereas, the minimum shoot length (2.80 ± 0.15 cm and 2.90 ± 0.20 cm) was recorded in petiole and leaf disc explant respectively, with MS media containing no plant growth regulators.

4.5.3.5 Number of plantlets per explant

The average number of plantlets per explants in case of leaf disc explants culture as presented in table 4.18 and figure 4.25, was observed highest (53.20 ± 2.20) in treatment T_{18} *i.e.* MS media fortified with BAP (2.0 mg l^{-1}) in addition of NAA (0.5 mg l^{-1}), followed by (48.40 ± 2.42) in treatment T_{24} (MS + 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA). Incase of petiole explant culture, the highest number of plantlets per explant (35.20 ± 2.73) was

observed in treatment T_{24}, followed by (29.40 ± 3.57) treatment T_{16} (MS +1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA) and treatment T_{18} (27.80 ± 4.88).

While comparing the data presented in the table 4.18 and figure 4.25 showed that the lowest number of plantlets per explant was recorded in T_1 *i.e.* MS media containing no plant growth regulators in petiole (3.20 ± 0.37) and leaf disc explants (4.40 ± 0.60). The number of shoots also correlated with the higher concentration of growth regulators which gave the maximum number of plantlets per explant.

4.5.3.6 Root initiation

As per the data depicted in table 4.18 and figure 4.26 showed the significant effect of growth regulators on days required for root initiation in different explants tested. The minimum days required for root initiation of leaf disc explant (33.80 ± 2.56 days) was recorded in treatment T_{21} *i.e.* MS media fortified with BAP (0.5 mg l^{-1}) in addition of NAA (1.0 mg l^{-1}) which is at par with (34. 40 ± 3.04 days) the treatment T_{20} (MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA). Petiole explant of *B. xanthina* showed minimum days required for root initiation (32.20 ± 1.36 days) in treatments T_{21}, followed by (32.80 ± 1.66 days) treatment T_{20}.

Table 4.18 Number of plantlets per explant, shoot length (cm) and root length (cm) of in vitro micropropagation of *Begonia xanthina* in MS media supplemented with different combination of BAP and NAA (average values ± SEm).

Parameters/ Treatments	Number of plantlets per explant		Root initiation (days)		Root Length (cm)	
	Leaf disc	Petiole	Leaf disc	Petiole	Leaf disc	Petiole
T1	4.40±0.60	3.20±0.37	118.40±5.56	112.60±5.05	1.18±0.11	1.20±0.11
T2	10.80±1.66	5.20±0.37	94.20±3.53	89.60±2.32	1.36±0.15	1.20±0.08
T3	10.60±1.44	5.80±0.58	89.60±2.77	87.60±3.36	1.48±0.10	1.50±0.09
T4	23.20±1.24	7.80±0.58	85.80±2.13	88.40±3.61	1.42±0.07	1.32±0.26
T5	21.20±2.48	10.20±0.80	76.60±3.09	79.80±2.65	1.24±0.10	1.80±0.25
T6	18.20±2.13	10.40±1.25	70.60±1.47	71.20±2.94	1.46±0.11	1.30±0.06
T7	9.80±0.73	6.20±0.80	66.20±3.34	58.80±1.46	1.82±0.40	1.74±0.35
T8	14.20±0.66	4.40±0.60	61.60±3.31	56.20±3.34	1.40±0.11	1.50±0.12
T9	19.60±2.62	7.60±1.44	39.60±2.50	36.40±2.40	2.80±0.31	2.42±0.14
T10	20.80±2.56	11.60±1.25	73.20±2.27	70.20±4.79	2.20±0.35	2.62±0.17
T11	21.20±3.31	12.20±1.07	59.40±2.73	65.40±2.96	3.34±0.39	3.30±0.33
T12	38.60±2.77	17.60±2.06	41.60±1.91	39.40±2.66	3.12±0.31	2.94±0.38
T13	14.40±1.63	7.60±0.75	40.80±2.37	43.00±1.22	4.54±0.45	4.50±0.34
T14	15.20±1.02	10.20±0.97	54.40±1.21	53.40±3.36	4.04±0.49	4.02±0.42
T15	18.40±2.94	13.40±2.58	51.80±3.25	50.80±2.44	3.60±0.59	3.82±0.59
T16	38.60±1.33	29.40±3.57	48.40±2.52	45.60±3.23	2.34±0.37	2.44±0.06
T17	41.40±3.63	21.60±1.03	47.20±1.11	44.20±1.91	2.88±0.18	3.02±0.15
T18	53.20±2.20	27.80±4.88	41.80±0.86	39.20±2.01	3.40±0.26	3.28±0.34
T19	13.40±2.06	8.80±1.16	43.80±2.42	41.20±1.91	3.44±0.27	3.70±0.47
T20	18.80±2.18	9.80±0.97	34.40±3.04	32.80±1.66	4.48±0.11	4.06±0.14
T21	21.20±2.08	14.20±1.50	33.80±2.56	32.20±1.36	3.28±0.07	3.50±0.19
T22	21.20±1.36	12.20±0.37	51.40±2.46	56.80±2.67	2.94±0.66	3.32±0.51
T23	19.60±2.16	17.80±2.42	47.40±2.58	52.80±2.22	3.26±0.30	3.06±0.09
T24	48.40±2.42	35.20±2.73	49.60±2.23	47.80±1.46	3.40±0.44	4.36±0.20
CD 5%	**5.96**	**5.03**	**7.63**	**7.85**	**0.91**	**0.80**
SEm	**2.12**	**1.79**	**2.72**	**2.80**	**0.33**	**0.29**
SEd	**3.00**	**2.53**	**3.84**	**3.96**	**0.46**	**0.40**

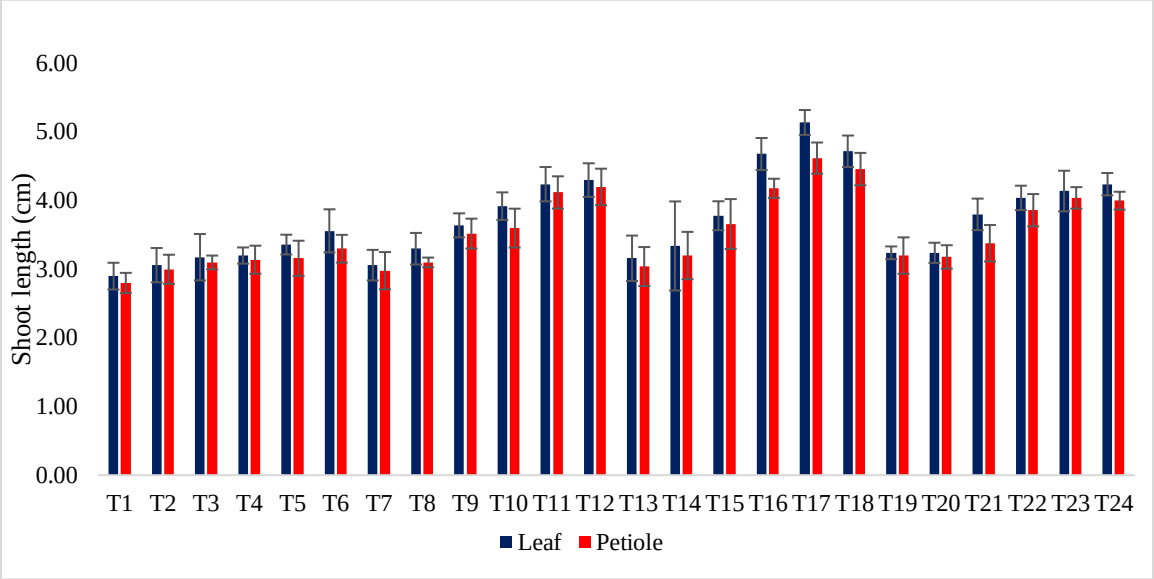

Figure 4.24 Shoot length (cm) of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

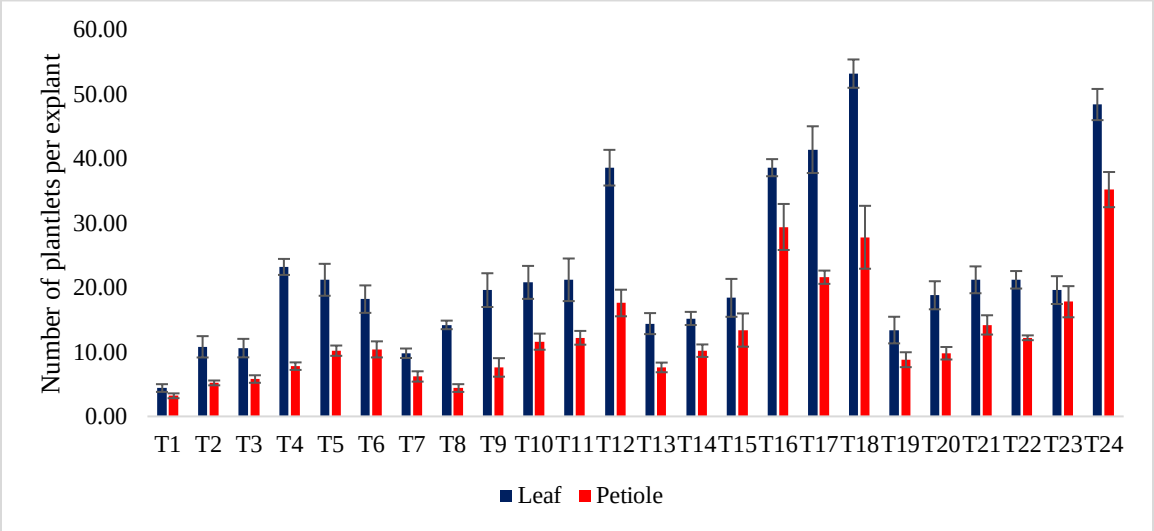

Figure 4.25 Number of plantlets per explant of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

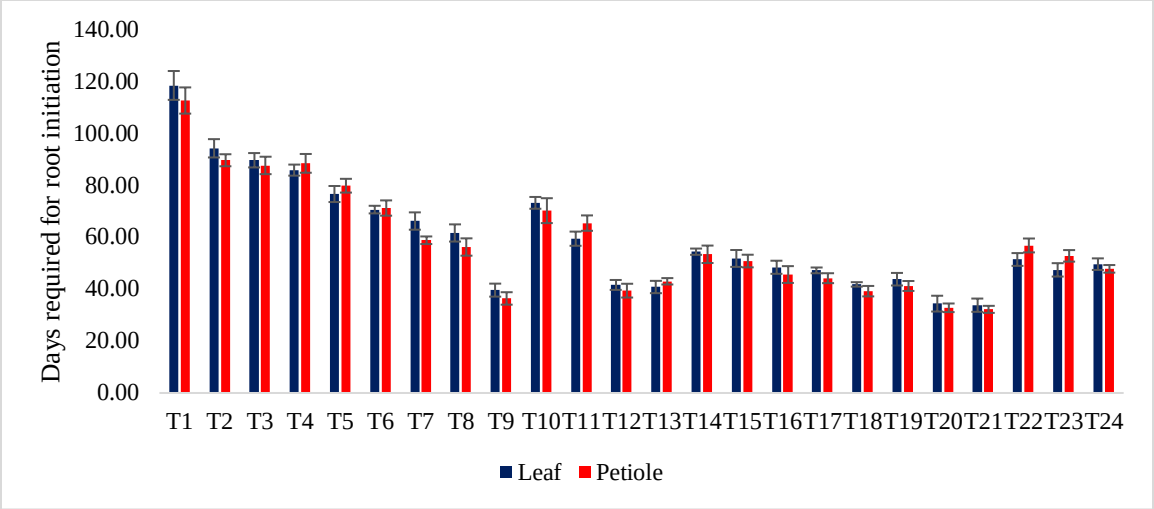

Figure 4.26 Days required for root initiation of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

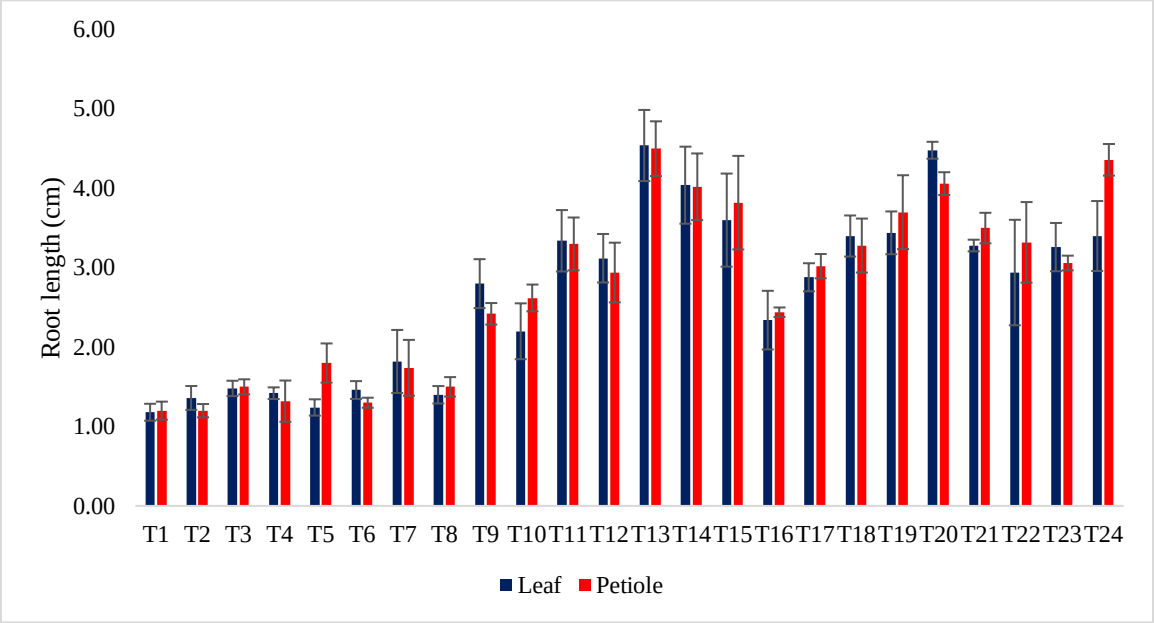

Figure 4.27 Root length (cm) of *in vitro* culture of *B. xanthina* (leaf disc and petiole explants) in MS media supplemented with different concentration of BAP with NAA with average values ±SEm

Comparing all the tested explants, the maximum days required for root initiation were noted in leaf disc explant, and minimum days required for root initiation in petiole explant. Whereas, in all the two explants, the maximum days required for root initiation (118.40 ± 5.56 days and 112.60 ± 5.05 days in leaf and petiole explant, respectively) was observed in treatment T_1 *i.e.* MS media without any growth regulators.

4.5.3.7 Root length

The data presented in table 4.18 and figure 4.27 shows significant differences concerning to root length produced among the treatments from different explants tested. In case of leaf disc explant, the maximum root length (4.54 ± 0.45 cm) was produced in treatment T_{13} *i.e.* MS media supplemented with 0.0 mg l^{-1} BAP+ 0.5 mg l^{-1} NAA which is at par with the value (4.48 ± 0.11 cm) of treatment T_{20} *i.e.* MS + 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA, while the treatment T_1 produced minimum root length which is without growth regulators.

In petiole explant, the maximum root length (4.50 ± 0.34 cm) was recorded in treatment T_{13} which is at par with (4.36 ± 0.20 cm) treatment T_{24}. In comparison to all the treatments, the minimum root length was recorded in T_1 *i.e.* MS media containing no plant growth regulators in leaf disc (1.18 ± 0.11 cm) and petiole explants (1.20 ± 0.11 cm).

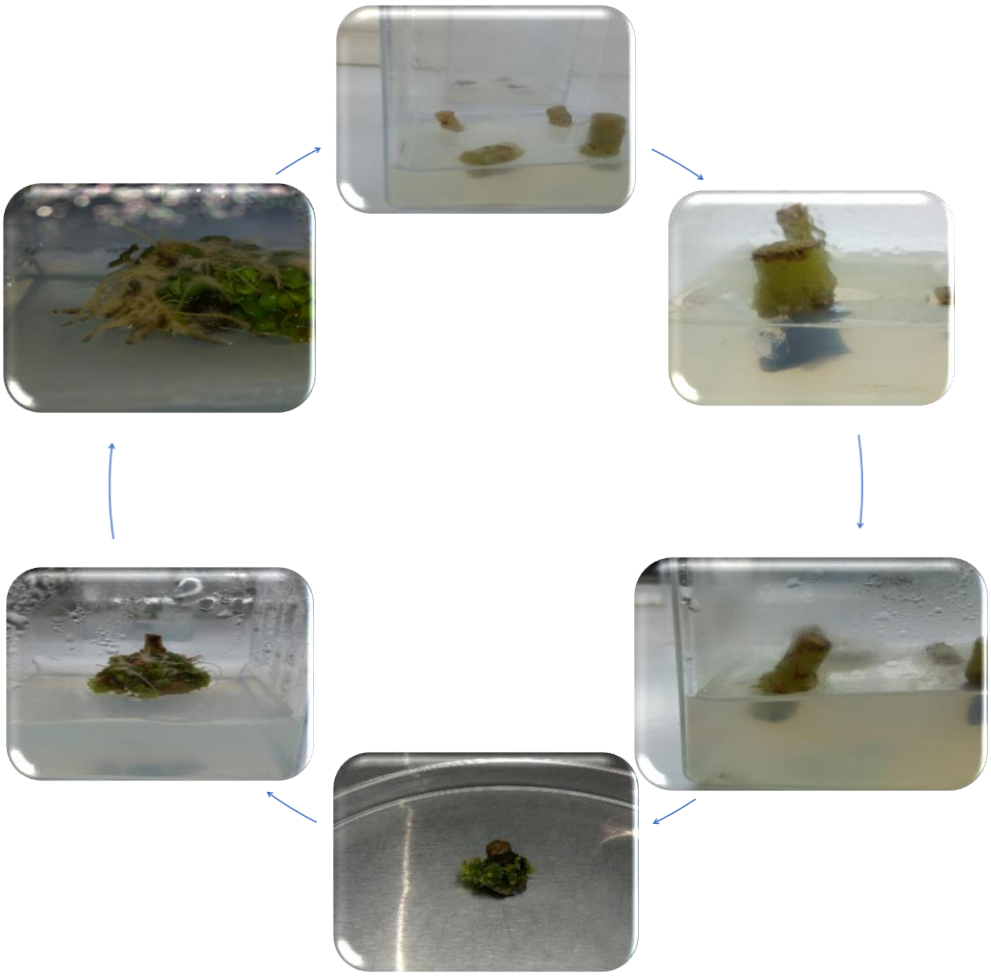

Plate 19. *In vitro* regeneration of *Begonia* through petiole explants

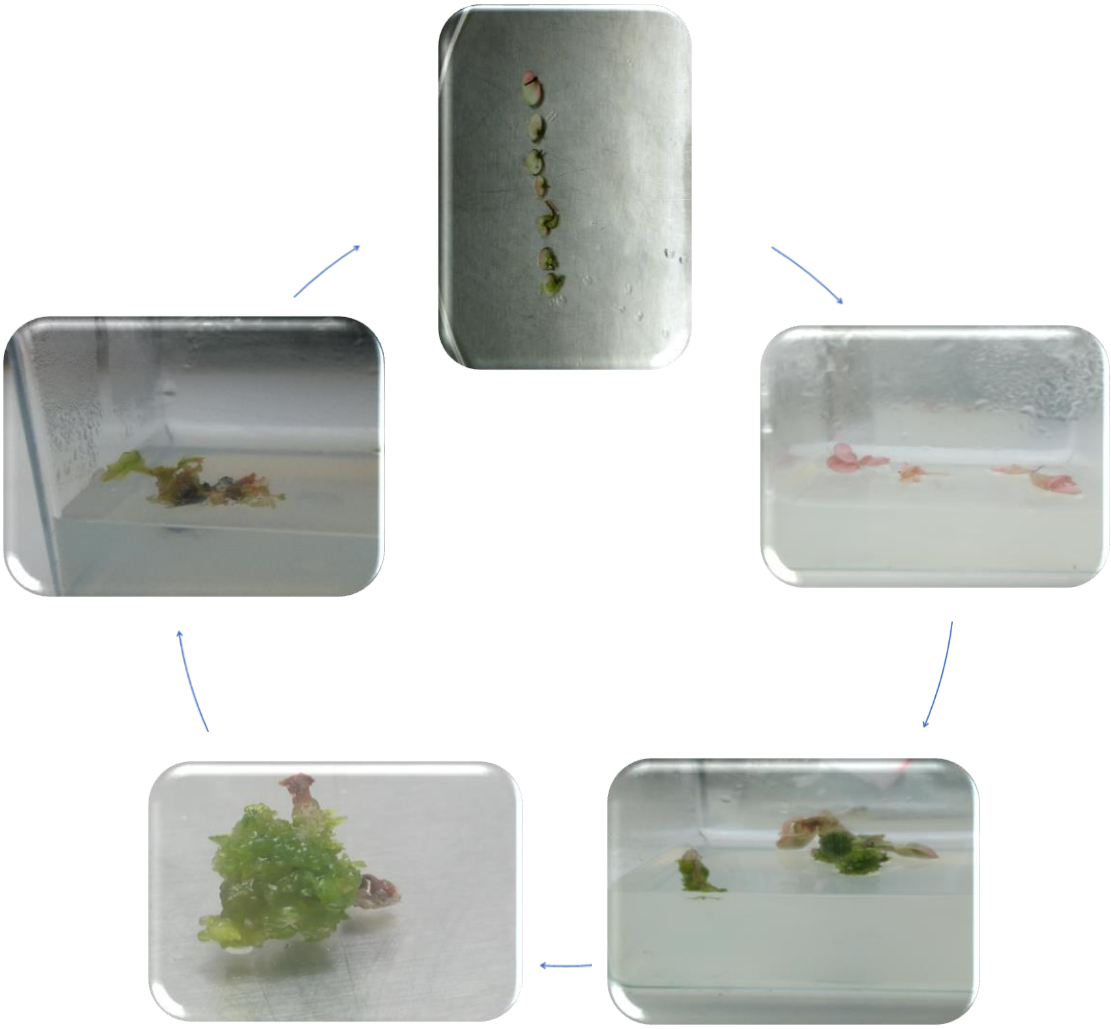

Plate 20. Flow cycle of immature bud explant of *Begonia in vitro* regeneration.

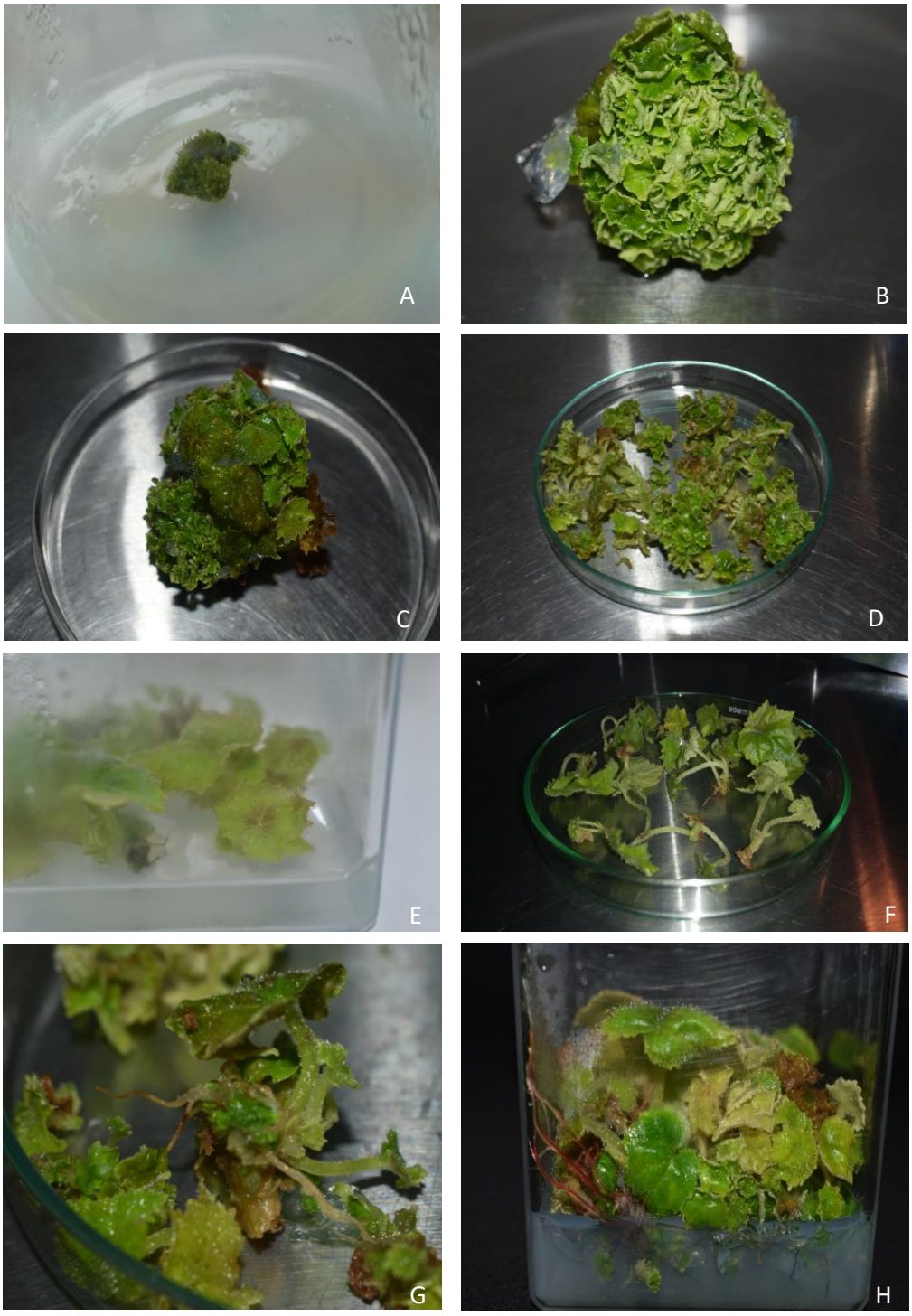

Plate 21. *In vitro* shoot and root organogenesis of *Begonia palmata:* A) *In vitro* shoot initiation, B) formation of *in vitro* shoots and developed leaves, C-F) Subculture of developed shoots and G-H) *In vitro* root formation.

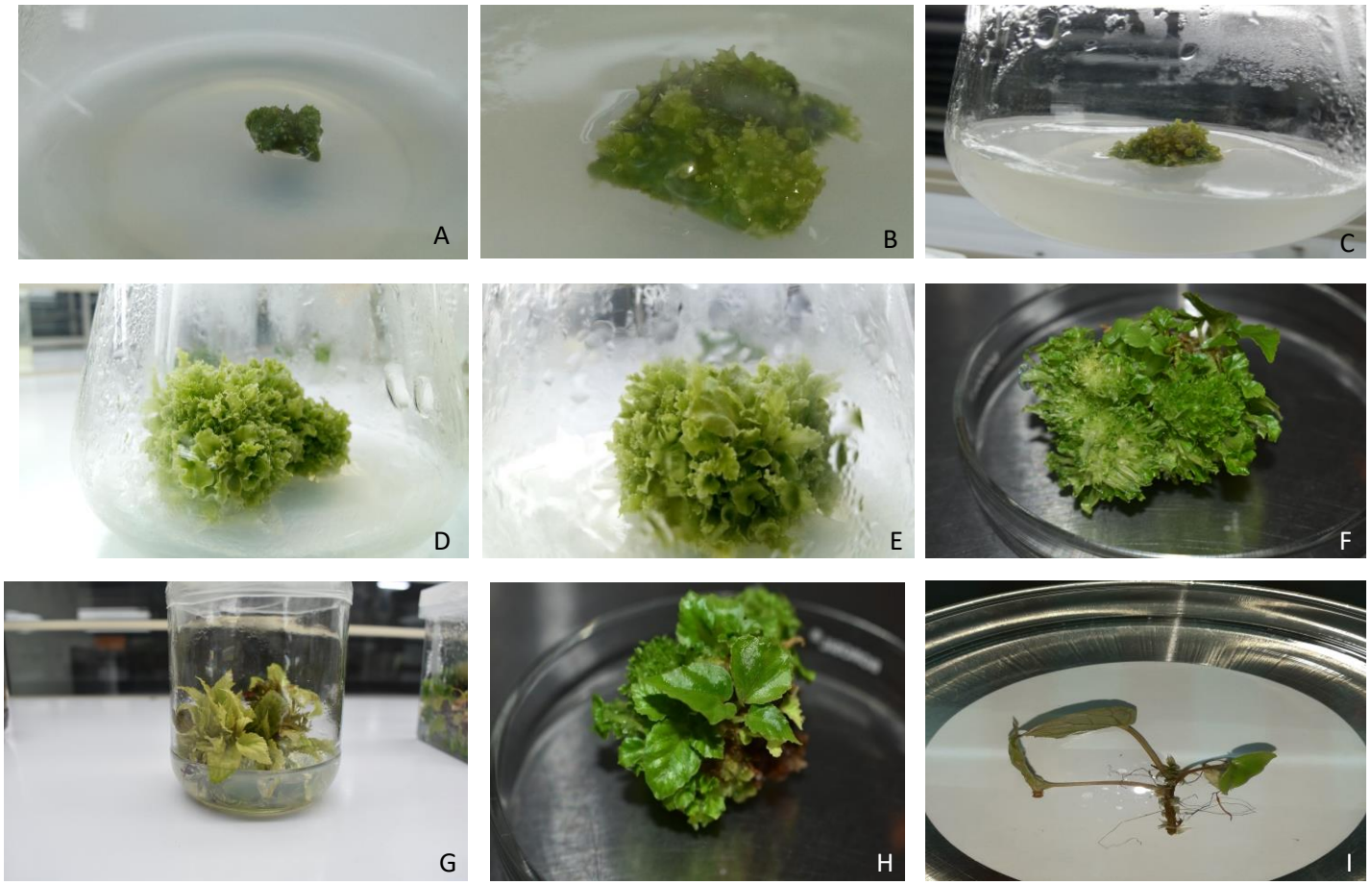

Plate 22. *In vitro* shoot and root organogenesis of *Begonia hatacoa:* A-C) *In vitro* shoot initiation, D-F) *In vitro* shoot multiplication, G-H) Developed plantlets, I) *In vitro* root formation.

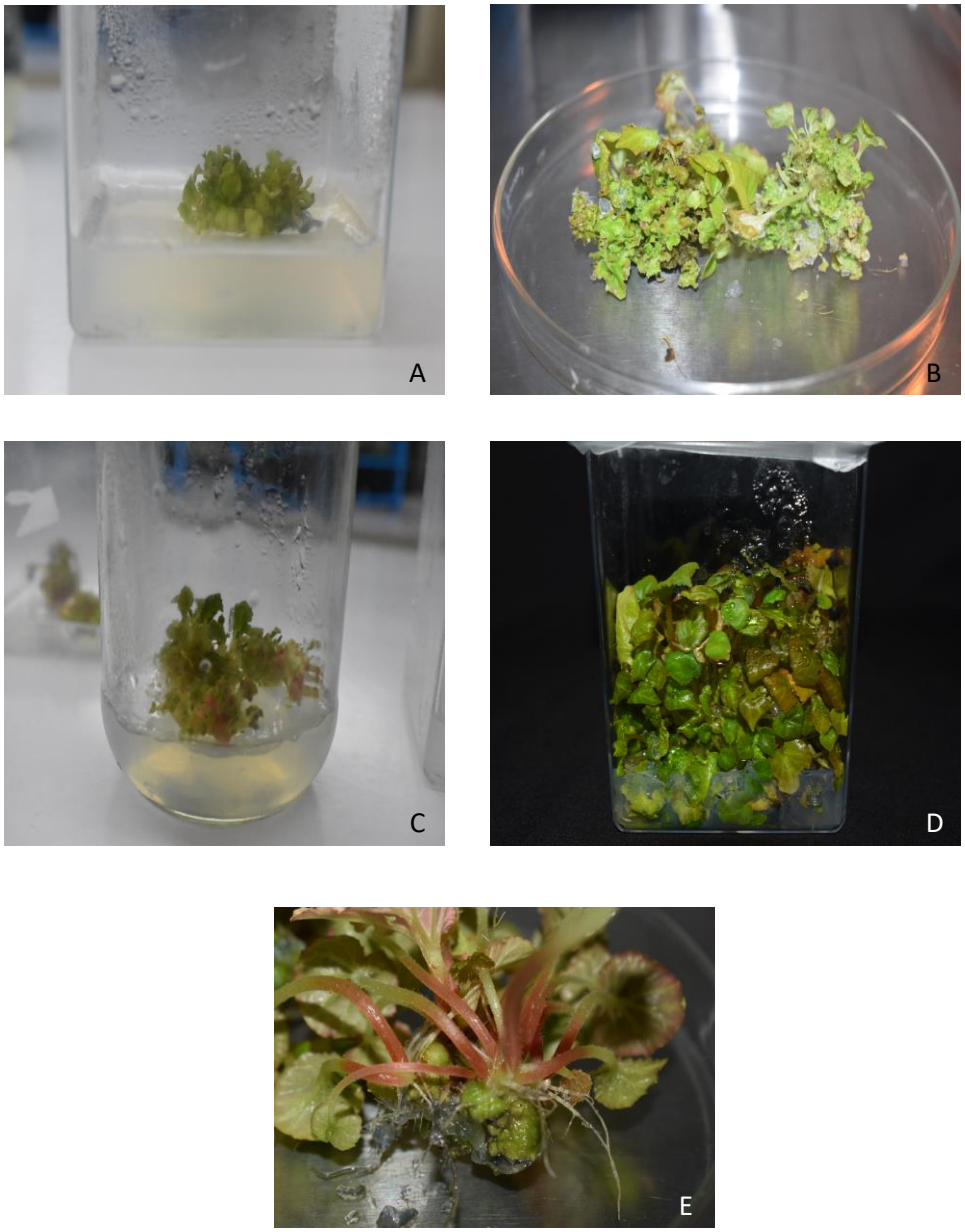

Plate 23. *In vitro* shoot and root organogenesis *of Begonia xanthina:* A) *In vitro* shoot initiation, B) Subculture of *in vitro* shoots, D) *In vitro* shoot multiplication and E) *In vitro* shoot and root formation.

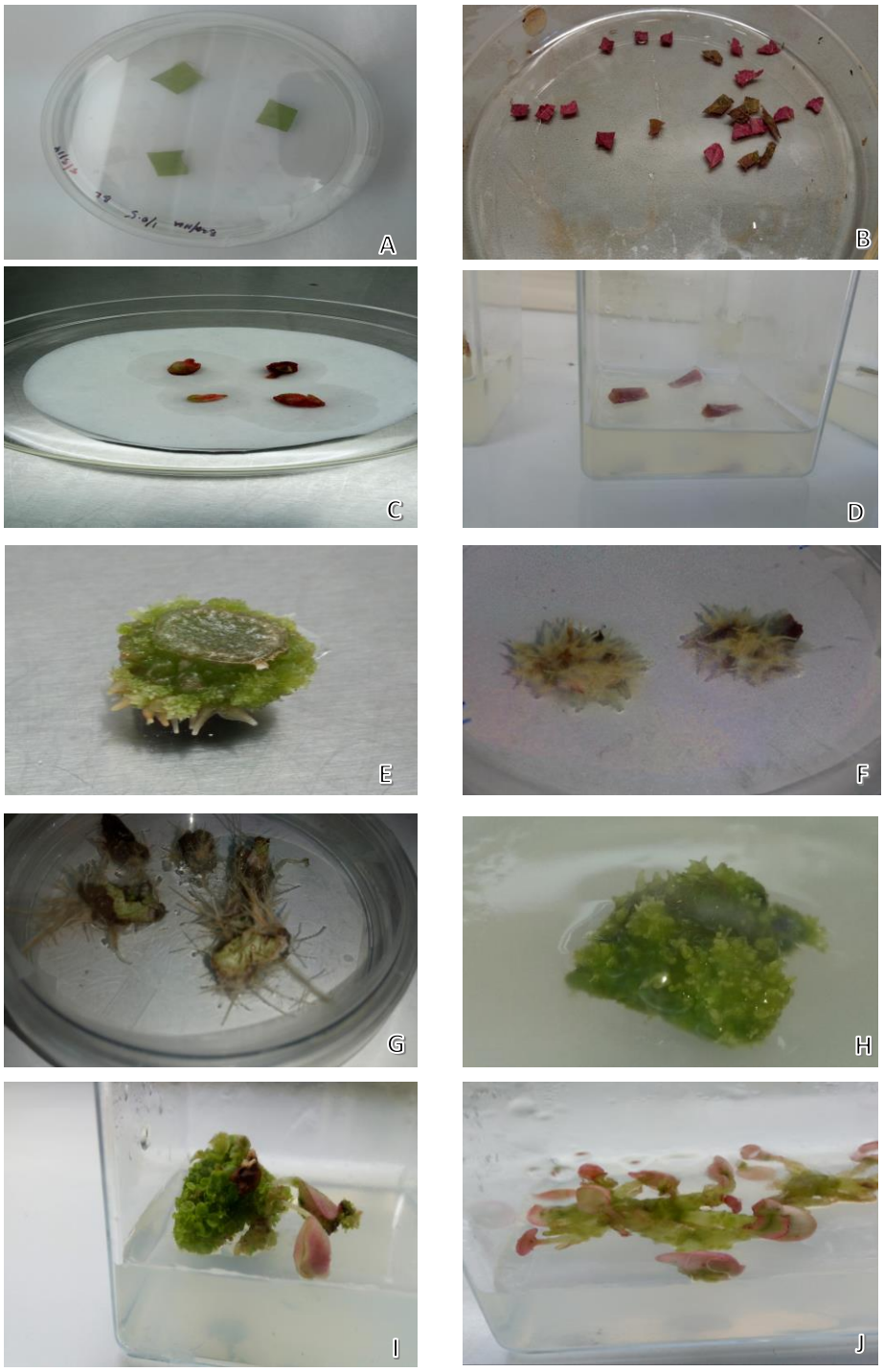

Plate 24. Explants used for *in vitro* regeneration of *Begonia* species. A-B) Leaf disc explants, C) Immature bud explant, D) Petiole explant, E) *In vitro* shoot emergence from petiole explant, F-G) *In vitro* root formation from leaf disc explant, H) *In vitro* shoot emergence from leaf disc, and I-J) *In vitro* shoot emergence from immature bud explant.

CHAPTER 5

DISCUSSION

The results obtained from the present study on **"Collection, Characterization and Evaluation of Selected *Begonia* species of Sikkim Himalayas for its ornamental value"** are discussed in this chapter and are justified with possible scientific causes and support of available literature under cited:

5.1 Morphological characterization of *Begonia* species

For any crop improvement program, the availability or the variability of the gene pool plays an important role to make it a success. Morphological characterization and evaluation are regarded as the initial step in the description and classification of any germplasm. Data on the various morphological traits will help to classify, identify, naming, and documentation of the wild relatives or germplasm of the given species (Sharma *et al.*, 2019; Hajjar and Hodgkin, 2007, Teso *et al.*, 2018). Singh and Abhilash (2018a) also suggested that the wild species are the treasure house of genetic resources for crop improvement.

The data of quantitative traits of morphological characters were subjected to analysis of variance (ANOVA) for randomized block design (RBD). The average values for these morphological characters for the thirteen collected *Begonia* species were found significant, thereby indicating a substantial amount of variability among the species (table 4.1 and to 4.2). The average values were found significantly for almost all the characters under study namely, plant height (cm), plant spread (cm), length of midrib (cm), leaf length (cm), leaf

width (cm), petiole length (cm), stipule length (cm), peduncle length (cm), number of leaves per plant, number of female flowers per plant, number of male flowers per plant, male flower length (cm), male flower width (cm), female flower length (cm), female flower width (cm), flower bud development (days), blooming (days), wilting of flowers (days) and flowering durations per plant (days), which indicates the diversity existed among the species and can contribute to crop improvement of the crop.

The results of evaluation of the quantitative morphological traits of thirteen wild *Begonia* species revealed a marked variation in these qualitative morphological traits, which showsthat these species can be of importance in breeding programs of the *Begonia*. For ornamental purpose/utilization, quantitative traits like plant height, number of leaves per plant, flower number, flower size, and flowering durations play a pivotal role. Species of *B. roxburghii* showed maximum plant height (114.40 ± 8.24 cm) and minimum plant height was obtained in *B. picta* (18.66±0.79 cm). After characterizing the plant height of all the analyzed *Begonia* species, *B. picta* and *B. satrapis* were found short heighted plant, under medium heighted *B. hatacoa, B. josephi, B. megaptera, B. cathcartii, B. xanthina, B. annulata* were characterized, whereas, *B. flaviflora, B. panchtharensis, B. palmata, B. sikkimensis,* and *B. roxburghii* tall heighted species. Medium height *Begonia* species can be utilized for the bedding purposes, or potted plant, whereas, short and tall height *Begonia* species may be used for crop improvement program. The maximum number of leaves per plant (11.20±1.02) was recorded in in *B. roxburghii,* followed by *B. hatacoa* (10.40±1.17) and *B. palmata* (8.40±1.17), which correlate with the plant height obtained for the same species. Data presented in table 4.2 showed a significant difference in respect of the number of female flowers and the number of male flowers per plant. The maximum number of

female flowers and male flowers per plant was found in *B. roxburghii* (34.40±2.04 and 28.40±0.75) followed by *B. annulata* (16.40±0.93 and 28.00±2.45), *B. megaptera* (16.20±0.58 and 21.00±1.82) and *B. palmata* (13.40±0.93 and 14.00±1.41) female flowers and male flowers per plant respectively. In respect of flower size of thirteen *Begonia* species, maximum male flower length was found in *B. megaptera* (4.66±0.06 cm) followed by *B. cathcartii* (4.62±0.12 cm), *B. hatacoa* (4.62±0.13 cm) *B. flaviflora* (4.60±0.11 cm) and *B. annulata* (4.56±0.13 cm). Whereas, female flower length was found maximum in *B. panchtharensis* (4.18±0.13 cm), *B. satrapis* (4.14±0.04 cm), *B. sikkimensis* (3.82±0.14 cm), *B. megaptera* (3.72±0.15 cm) and *B. cathcartii* (3.70±0.17 cm). Likewise, *B. roxburghii* recorded maximum flowering durations (64.80±3.06 days), followed by *B. annulata* (58.40±1.63 days), *B. megaptera* (56.40±2.54 days), and *B. sikkimensis* (52.40±3.53 days).

The above results showed quite a variability among the species which may be due to both genetic makeup of the species and environmental condition under which species were developed.Similar results were found by Grela *et al.* (2010), who suggested that the variation in quantitative characters like plant height, plant form was highly and significantly affected by the environmental conditions.

Data pertaining to the qualitative morphological traits among the thirteen collected *Begonia* species showed distinct variation in plant habit, plant form, leaf lamina, leaf margin, leaf base, leaf apex, inflorescence, leaf colour upper surface, leaf colour lower surface, leaf pubescent, stipules colour, petioles colour, petioles hair, flower colour, flower pubescent, capsule colour, capsule hair, peduncle colour, and peduncle hair. Qualitative

morphological characters results presented in table 4.3 to 4.6, revealed diversity among the morphological traits between the species and also within the species.

Results revealed that out of thirteen collected *Begonia* species, ten species were noted to have rhizomatous plant habit and three species were recognized as a tuberous habit. Whereas, plant form was categorized into dioecious and monoecious, under which only one species showed dioecious plant form, rest were of monoecious nature. Diverse form of leaf lamina was observed in thirteen wild *Begonia* species and results were shown in the previous chapter (table 4.3.). Leaf lamina ranges from ovate to lanceolate to peltate to deeply lobed, which reflects the diverse *Begonia* germplasm present in Sikkim Himalayas. Likewise, main interesting qualitative traits for evaluating the ornamental value among the others traits is leaf colour. Results revealed that the collected *Begonia* species showed a diverse range of leaf colour, most importantly, *B. josephi, B. hatacoa, B. picta* and *B. palmata* showed different characteristics, the upper surface of leaves colour differs within the species level, two phenotypically different variants of the same species was found. In the case of *B. josephi* and *B. hatacoa,* the upper surface of leaf was divided into two phenotypical characters, one is considered as dark green and another green in colour. Whereas, *B. picta* showed dark green to marron reddish colour and green colour, likewise *B. palmata* also showed two distinct phenotypical characters in the upper surface of leaves colour.

The results depicted in table 4.4. based on leaves colour of the lower surface of *Begonia* species, showed marked variation among the species. Results showed that light green colour on the lower surface of leaves was recorded in five species *i.e. B. satrapis, B. panchtharensis, B. hatacoa, B. josephi, B. picta* and *B. megaptera.* Green colourationon

lower surface of leaves was found in three species *i.e. B. cathcartii, B. sikkimensis* and *B. roxburghii.* Whereas, reddish green on the lower surface of leaf was noted in *B. flaviflora* and *B. palmata,* maroon red with green in *B. xanthina,* green with red blotches between the vein in *B. annulata* and reddish purple and green blotches in *B. picta.*

Apart from this, *B. palmata, B. picta, B. hatacoa* and *B. josephi* showed distinct phenotypical characters on the basis of lower surface leaf colour within the species level. Two morphologically different leaf colour was found in *B. palmata*, one having reddish green and another has been found in green colour. Whereas, *B. picta* showed reddish purple and green blotches in one phenotype and light green colouration on lower surface of leaf in another phenotype.

Results revealed a marked variation in the flower colour of thirteen collected *Begonia* species (Table 4.5). Pinkish white flower colour dominant the most which includes *B. annulata, B. hatacoa, B. palmata, B. josephi, B. cathcartii* and *B. panchtharensis.* Pink colour flower was found in *B. megaptera, B. picta* and *B. satrapis,* whereas, yellow colour flower was recorded in *B. xanthina* and *B. flaviflora*. White colour flower was found in *B. roxburghii* and red colour flower was noted in *B. sikkimensis.* Diverse form of flower morphology was due to varied species, most of the *Begonia* flower was in the range of pinkish white in colour. Among the collected species, red and pink colour flowering species can be introduced in the floriculture industry for their ornamental value. Yellow colour flowering species may be used in the breeding program (Guan *et al.,* 2008).

The quantitative, as well as the qualitative morphological traits of thirteen collected *Begonia* species were found in close conformity with the findings of Flora of China, Flora of Sikkim (Hajra and Verma, 1996), Flora of British India (Hooker, 1854), Morris (2006),

Morris (2010), Rajbhandary *et al.* (2010a), Rajbhandary *et al.* (2010b), Camfield and Hughes (2018); Bhattarai and Rana (2020); Morris (2011a); Morris (2011b); Nautiyal *et al.* (2009); Hughes (2008); Morris (2016); Tebbit (2003); Tebbit (2005); Uddin (2007); Hooker (1859); Hooker (1830); Hooker (1854); Hooker (1852); Clarke (1879); Clarke (1881).

Morphological traits variation between phenotypes in a single plant having different leaf types or different plants of the same species which exhibits phenotypic or ecotypic differentiation were observed by Wang *et al.* (2016) in red and green phenotypes of *Begonia fimbristipula*, Deng *et al.* (2012) in double petal and multi petal *Jasminum sambac*, and Leonid *et al.* (2012) in sun and shade ecotype of *Stellaria longipes*, such differentiation of these traits may be induced by environmental change. Alterations in phenotypic characters of same species allow the plant to modify its physiological characteristics and pigment content when habitat conditions change (Dalling *et al.*, 2001). It may also be due to the gene expression or hormonal regulation when the plant receives appropriate environmental signals (Sultan 2000; Schlichting and Smith 2002). Sheue *et al.* (2012) also suggested that *Begonia* species possess natural foliar variegation patterns, resulting in diverse phenotypic characters. Morphological results of *Begonia palmata, Begonia xanthina* and *Begonia megaptera* are in close conformity with the findings of Bhattrarai and Rana (2020).

In the present study, all the quantitative characters were important for distinguishing between the collected *Begonia* species were in accordance to Dorrenbos *et al.* (2008) who also conducted similar experiment on the *Begonia* species, Tebbit (2003); Rajbhandary (2010b); Camfield and Hughes (2018).

Guan *et al.* (2008) reported that 50 % of wild *Begonia* of the Chinese Begonias might possess ornamental value and author also suggest that many wild species may directly develop as ornamental plants, whereas, some may potential for the breeding program and possesses traditional uses of *Begonia* in China (Guan *et al.*, 2007).

Similar morphological characterization work was undertaken by the Setiawan *et al.* (2016) in *Portulaca* species, Arrazate *et al.* (2017) in wild *Heliconia* species, Poverene and Cantamutto (2010) in wild *Helianthus* species, marigold genotypes by Srinivas and Rajasekharam (2020). The present results are in close agreement with the results reported Bhattarai and Rana (2020). Mladenovic *et al.* (2012) also describe the variation of Cucurbita species within 23 germplasm accession for its use in horticulture. Souza *et al.* (2011) evaluated 31 accessions of banana with the use of 32 morphological descriptors for identification of accessions with great ornamental potential, and are grouped into landscape plants, cut flower, potted plants, and male inflorescence mini fruits. Duyen *et al.* (2017) evaluated ten morning glory accessions (*Ipomoea nil* sp.) and found the variation in morphological traits among the accessions. Carver *et al.* (2016) also found the variations in vegetative and floral characteristics of potential commercial values in four native species of Texas and identified the important traits within each species.

5.2. Biochemical analysis of *Begonia* species

5.2.1 Total Protein content

Total protein is essential for human body growth and also useful for plant steady stable state of the plant. Data depicted in table 4.7 shows that the maximum protein content among the collected *Begonia* species was recovered from the *B. josephi* green phenotypes

(35.04±0.71mg g^{-1}) and the minimum was found in *B. palmata* red (20.10±0.34mg g^{-1}) from the leaf sample. Likewise, green phenotypes of *Begonia* species exhibit more protein content as compare to red one in respect to all the parts under study i.*e.* leaf, stem, and tuber extracts. Protein estimation also helps to understand the plants under stress or not. Maximum content was found in leaf as compared to stem and tuber/rhizome. This may be since protein accumulated more in leaves rather than the stem or tuber/rhizome in *Begonia* species. Similar kind of findings was reported by Das *et al.* (2014) where *Costus specious* extract of leaves showed the highest protein content. The present findings are in close agreement with the findings of Teso *et al.* (2016).

5.2.2 Ascorbic acid

Among the tested species of *Begonia* from Sikkim Himalayas for evaluation of ascorbic acid content in their leaves, stem and rhizome/tuber, as presented in table 4.7, highest content of ascorbic acid was found in stem extract of *B. josehii* green and red phenotypes with 10.42±0.417 mg g^{-100} and 10.00±0.000 mg g^{-100} respectively, while lowest content was recorded in *B. palmata, B. roxburghii, B. hatacoa, B. panchtharensis* (3.75±0.000 mg g^{-100}). As a key molecule in plant metabolism ascorbic acid has been recognized to play a crucial role in several physiological processes such as photosynthesis, photo-protection, cell division, plant development, stress responses, regeneration of other essential molecules (Cavaiuolo *et al.*, 2013; Gallie, 2013). Barth *et al.* (2004) suggested that ascorbic acid is important in the regulation of development senescence and plant defense against pathogens. In flowers, polyphenols, carotenoids, and vitamin C represent essential compounds with antioxidant activity and anti-inflammatory properties (Mleck and Rop, 2011).

5.2.3 Total Sugar content

Sugar is a source of carbohydrate and energy. The data depicted in the table 4.8 showed maximum sugar content from leaf extract was recorded in *B. josephi* red phenotypes (27.22±0.86 mg g^{-1}) and lowest from *B. palmata* green phenotypes (12.84±0.30 mg g^{-1}). Whereas, from tuber/rhizome extract, red phenotype of *B. josephi* was found to have maximum sugar content (34.86±0.71 mg g^{-1}) and lowest was recorded in *B. josephi* green phenotypes (21.24±0.79 mg g^{-1}). As per the Sood (2006) the content of reducing sugar found to be lower in younger petals and increased rapidly with the age in *Rosa damascena* Mill and *R. bourboniana* Desport. The role of sugars in flower development have multifunction such as they act as an energy source (Moalem-Beno *et al.*, 1997), osmotic regulators (Bieleski, 1993), and as precursors for metabolic processes. Zhang *et al.* (2018) experimented in *Begonia semperflorens* for estimation of sugar. From their study it was evident that the anthocyanin content has correlation with the sugar content in the leaves.

5.2.4 Total Starch content

Starch is made of glucose and reducing sugar and provide energy to living cells. Starch is the predominantly non mobile storage polysaccharides in plants. It was evident from the table 4.8, that the maximum starch content in leaf extract of *Begonia* species were varied from 18.11±0.34 mg g^{-1} to 33.04±0.76 mg g^{-1} and rhizome or tuber extract were varied from 7.40±0.42 mg g^{-1} to 22.92±0.66 mg g^{-1}. The maximum starch content was found in leaf extract of *B. josephi* red phenotypes (33.04±0.76 mg g^{-1}) whereas, lowest was observed in tuber/rhizome extract of *B. palmata* green phenotypes the (7.40±0.42 mg g^{-1}). Samariya *et al.* (2015) also found similar results, where concentration was found highest in leaf in

comparision to rhizome of *Cyperus rotundus.* Results obtained by Nissar (2017) in *Nicotiana plumbaginifolia,* Zhang *et al.* (2018) in *Begonia semperflorens,* Tirosh and Mayak (1988) in Carnation reported that changes in starch content in different parts of the plant.

5.2.5 Anthocyanin content

As per the data in the table 4.9, anthocyanin content in the leaf sample of the collected *Begonia* species was found maximum in the red phenotype of *B. picta* (185.33±1.45 mg L^{-1}) and the lowest was found in *B. megaptera* (26.27±0.89 mg L^{-1}). Stem extract of *Begonia* species was also tested for anthocyanin content and highest value for anthocyanin in the stem was observed in red phenotype of *B. josephi* (131.15±2.75 mg L^{-1}) and the minimum in *B. palmata* green phenotypes (26.15±2.89 mg L^{-1}). In the case of rhizome/tuber extract of *Begonia* species of Sikkim Himalayas, maximum anthocyanin was found in the tuber of *B. josephi* red phenotype (34.82±0.73 mg L^{-1}) and lowest content in the rhizome of *B. sikkimensis* (11.78±0.60 mg L^{-1}). Result reveals that the maximum anthocyanin content was found in the leaf surface of *Begonia*, it may be due to heavy accumulation of pigment in the leaf as compared to stem and rhizome/tuber. While red phenotypic species possess greater anthocyanin content as compare to green phenotypes.

Previous reports on anthocyanins study suggested that it can significantly affect plant response to environmental stress, protect organs and substances involve in photosynthesis processes, relieve photo-oxidation damage to leaves (Lee and Collins, 2001; Lee, 2002; Wang *et al*., 2016). Anthocyanins may act as an effective antioxidant and can greatly improve the viability and resistance to plants (Lee, 2002; Wang *et al.,* 2016). This

preventive effectiveness of anthocyanin may be related to the existence of a relationship between the content of anthocyanin to the antioxidant activity resulting in cellular defenses. The extracts of *Begonia* may have excellent potential as functional ingredients representing a potential source of natural antioxidants. These results are highly correlated with the previous reports on the *Begonia* plants (Bhattarai and Rana, 2020; Diengdoh 2017; Awasthy *et al.*, 2016; Awasthy and Murungan, 2015; Ambhujaksi *et al.*, 2018).

5.2.6 Total phenol content

Phenolic compounds are considered to be the most important class of antioxidants. Phenolic compounds can donate hydrogen atoms to free radicals and possess ideal structural properties for free radical scavenging properties. Phenolic compounds commonly found in edible and medicinal plants have various biological effects including antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities. A synthetic antioxidant like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) butyl hydroxy quinone, propyl gallate and gallic acid esters are commercially available but their usages have been limited due to their adverse effects to human health due to their toxicity (Kahl, 1984; Kahl and Kappus, 1993). Hence, consumer concern regarding their safety has motivated the food industry to look for a natural source of antioxidants (Bravo, 1998). Consumption of wild edible plants may provide a natural source of antioxidant to be best against synthetic antioxidant and are harmless too (Anubudhasan *et al.*, 2014; Barlow 1990). In the present study, the total phenol content of leaf extract of *Begonia* was found maximum in the *B. picta* red phenotypes (55.03±2.85 mg GAE g^{-1}), whereas minimum was observed in *B. cathcartii* (2.17±0.39 mg GAE g^{-1}). From stem extracts of *Begonia* species also showed marked

variation, maximum phenol content was obtained from the *B. hatacoa* red phenotypes (21.26±0.68 mg GAE g^{-1}) and minimum was found in the *B. megaptera* (0.67±0.21 mg GAE g^{-1}). In tubers/rhizome extract of *Begonia* species, maximum total phenol content was found in *B. picta* red phenotypes (28.9±0.617 mg GAE g^{-1}) followed by *B. picta* green phenotypes (27.55±0.849 mg GAE g^{-1}) and *B. josephi* red phenotypes (26.89±1.155 mg GAE g^{-1}) and minimum was obtained from *B. cathcartii* (0.37±0.092 mg GAE g^{-1}). Similar results were also obtained by various author countering phenolic content in the *Begonia* species (Bhattarai and Rana, 2020; Han *et al.*, 2013; Geetha *et al.*, 2016; Shrestha *et al.*, 2016; Isaivani *et al.*, 2014; Deinghdoh, 2017; Jose *et al*., 2016). It has also confirmed that the pharmacological effect of phenol is correlating with their antioxidant activities.

5.2.7 Total flavonoid content

From the results pertaining in the table 4.9, it was evident that among the collected *Begonia* species the total flavonoid content from leaf, stem, and tuber/rhizome extract were found maximum in *B. picta* red phenotypes (35.19±1.142 mg QE g^{-1}, 7.21±8.097 mg QE g^{-1} and 39. 42±0.558 mg QE g^{-1}, respectively) and minimum in leaf extract of *B. cathcartii* (3.27±0.677 mg QE g^{-1}), stem extract of *B. roxburghii* (0.18±0.961 mg QE g^{-1}) and rhizome/tuber extract of *B. cathcartii* (1.11±0.191 mg QE g^{-1}). The result shows that these wild *Begonia* species are excellent sources of phenolic and flavonoid antioxidants. Flavonoids are associated with health promoting effects and are key components in pharmaceutical, medicinal and cosmetics industries; due to their antioxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties (Evans *et al.*, 1995; Cook and Samman, 1996; Steinmetz and Potter, 1996; Panche *et al.*, 2016; Kaurinovic and Vastag, 2019). The present study carried out for flavonoids on the thirteen collected

Begonia species plant extract revealed the presence of medicinally active constituents in these *Begonias*. The previous report also suggests the presence of phenol and flavonoid in the *Begonia* (Ramesh *et al.*, 2002; Solomon-Jeeva, 2012; Jose and Kumar, 2016). The findings of the current study show the plant extracts of *Begonia* species revealed similar results to previous findings. Phytochemical constituents such as alkaloids, flavonoids, phenols, tannins, saponins, and several other organic compounds are secondary metabolites of plants that serve as a defense mechanism against microorganisms and insects.

5.3 Evaluation of ornamental value of wild *Begonia* species

Ornamental plants are meant to beautify living and hold sentimental value for peoples around the world. In every society flower are associated with almost all the social and personal event in a person life. Exploring the relative importance of consumer on flower attributes can enable decision making by flower producers (Rombach *et al.*, 2017). Attributes that can influence the consumers' choice are flower appearance or flower colour (Hudson and Griffin, 2004; Yue and Behe, 2008), foliage pattern, scent (Behe and Wolnick, 1991; Behe *et al.*, 1992), price, packaging and overall appearance. However, these attributes may use for some sections of consumers but these attributes may vary according to individuals' or consumers choices.

To see the probable changes in customer's perception, each respondent was categorized into age group and gender. To understand the nature of participants were asked questions about their gardening habit/experiences. Among the interviewed participants 36 % of respondent grow flowers for decorative purposes, 26 % grow as a hobby, 15% were categorized as amateur growers, and participants who are commercial growers were 13 %,

and the remaining 10 % did not grow flowers or do not have a garden. From the present study it was evident that people prefer orchids (33%) more than other flower crops which was followed by Rose (16 %), Lilium (10%), Anthurium (9%), Gerbera (8%), Foliage Plants (7%), Gladiolus and Marigold (6%) and Alstroemeria (5%). It may be due to the fact that Sikkim is known for its orchid treasure, thus most of the respondents prefer orchids, followed by rose which is the second most famous flower in Sikkim.

For the evaluation of consumer preference for ornamental uses of the collected thirteen *Begonia* species questionnaires was prepared and results regarding the same were presented in chapter 4. Consumer preferences play a crucial role in commercialization of any plant species. Through random sampling respondent were slected and each *Begonia* species was displayed to them, on the basis of their ratings on various aspect results were evaluated. Photographs measuring 10 cm×15 cm were printed for thirteen *Begonia* species showing a single plant with leaves and flowers. Each photograph was mounted on a gray card for display.

Results in the figure 4.6 showed that *B. xanthina* was most preferred species by the respondents 22 %, followed by *B. palmata* (16%) and *B. annulata* and *B. megaptera* with 14% each. Least preference was recorded in *B. roxburghii* (0%) and *B. cathcartii* (1%). This showed that the diverse range of consumers' interest among the different *Begonia* species.

Overall assessment of each *Begonia* species through consumers' perception was also recorded and results were presented in the chapter 4 (table 4.10). On the basis of liking of the species, *B. xanthina* showed maximum satisfactory (6.77%) results followed by *B. palmata* (6.62%) as compared to other wild *Begonia* species, likewise on the basis of

foliage pattern, *B. picta* was rated satisfactory with 6.92 %, followed by *B. xanthina* (6.38%), *B. annulata* (6.15%) and *B. palmata* (5.92%). The percentages of consumers on plant suitability of thirteen collected *Begonia* species, maximum percentage of satisfactory results was with *B. picta* (6.77 %) and *B. xanthina* (6.77 %), followed by *B. annulata* (6.23 %), *B. palmata* (6.00 %) and *B. megaptera* (5.85 %). In case of edibility of species tested *B. josephi* gained 4.69%. Plant attractiveness attributes was found satisfactory in *B. xanthina* (6.54 %), followed by *B. annulata* (6.46 %) *B. megaptera* (6.38 %) and *B. palmata* (6.31 %). Results pertaining to the overall appearance attributes of wild *Begonia* species by the different respondents were found the maximum satisfactory rate in *B. xanthina* with 6.54% whereas, 1.15 % of respondents found unsatisfactory for the same species. Likewise, 6.31 % of respondents rated satisfactory for *B. annulata*, 6.08 % for *B. picta*, 5.92 % for *B. palmata* and 5.77 % for *B. megaptera*. Whereas, maximum percentage in case of not satisfactory rate by the consumers were found in *B. roxburghii* (6.23 %) followed by *B. josephi* (5.77 %) and *B. cathcartii* (4.92 %).

It was noticed that the maximum percentages of consumers prefer *B. xanthina, B. megaptera, B. palmata, B. annulata, B. picta* over other species and only in case of edibility, *B. josephi* was preferred most among other species.

Similar kind of work has been reported by various authors *viz.* Kelley *et al.,* 2001; Kelley *et al.,* 2002 Behe *et al.,* 1999; Barton *et al.,* 1996; Eweida and Sverkel, 2009; Lavanya, 2013; Hinsely *et al.,* 2015; Rombach *et al.,* 2018.

5.4 Micropropagation of *Begonia* species

5.4.1 Survival rate

In the present investigation, maximum survival rate was obtained from an immature bud explant in comparison of leaf disc and petiole explant. Immature bud segment of *B. hatacoa* (96.00%) in MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. Petiole explant of *B. xanthina* showed maximum survival rate (92.00 ± 4.90 %) in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and immature bud explant of *B. palmata* showed highest survival rate (88.00 ± 8.00 %) in MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. Best explant in respect of survival rate for *in vitro* regeneration was obtained from the immature bud. But prolific regeneration was obtained later on from leaf disc and petiole explants. In respect of treatment combination, the maximum survival rate was recorded from MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. The present findings are in close conformity with the findings of various researchers. Nabieva *et al.* (2019) also conducted *in vitro* regeneration of *Begonia sutherlandii* Hook F. through immature flower buds from 1.0 mgl^{-1} BAP with 0.25 mgl^{-1} IBA. Similar results were reported in African violet from MS media in the combination of NAA and BAP by Daud and Taha (2008). Pierik and Tetteroo (1987) also established *in vitro* vegetative regeneration of *Begonia venosa* Skan from inflorescence explants through NAA and BA concentration. Ahmed *et al.* (2012) conducted an *in vitro* regeneration of *Gerbera jamesonii* in which normal adventitious shoots were obtained from petiole explants culture on MS medium supplemented 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA with 94.3 % regeneration rate.

It was also noted that the combination of auxin with cytokinin in regeneration medium increased regeneration efficiency in *Begonia* (Mendi *et al.,* 2009; Fonnesbech, 1974).

5.4.2 Shoot initiation

The findings of the present study showed that after 25.80 ± 1.20 days of culture, explants exhibited adventitious shoot and progressed into the early stage of shoot development. Adventitious shoots directly emerged from the entire surface of explants. Minimum days required for shoot initiation 25.80 ± 1.20 days was recorded in leaf disc explant of *B. palmata*, 26.80 ± 1.66 days in leaf disc explant of *B. xanthina* and 28.20 ± 0.86 days in leaf disc explant of *B. hatacoa* in MS media supplemented with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. Maximum days for shoot initiation was recorded in MS medium supplemented without any growth regulators. The ratio of auxins and cytokinin plays an crucial factors in early shoot initiation. Statistically significant differences between the treatments was recorded (Table 4.11). The present findings are in close conformity with the findings of Nada *et al.* (2011) in *Begonia tuberhybrida*, Burritt and Leung (1996) in *Begonia* × *erythrophylla*, Mikkelsen and Sink (1978) in *Begonia* × *heimalis*, Takayama and Misawa (1981) in *Begonia* × *heimalis.* Rosilah *et al.* (2014) in their study suggested that a low concentration of auxin and higher concentration of cytokinin induce shoot organogenesis in *Begonia pavonina.* Awal *et al.* (2008) reported in their study that the best induction of direct somatic embryogenesis of *Begonia* × *heimalis* was achieved on MS media supplemented with 1.0 mg l^{-1} BAP with the addition of 0.1- 0.5 mg l^{-1} 2,4-D, whereas, Castillo and Smith (1977) noted that 0.5 mgl^{-1} Kinetin was found effective indirect somatic embryogenesis in *Begonia*× *gracilis.* Mendi *et al.* (2009) suggested that

the combination of BA and NAA gives better cell division and regeneration rate as compared to BA and IAA in *Begonia elatior*.

The results corroborate the findings of the experiments conducted by Mendi *et al.* (2009). They observed that the days required for shoot initiation were decreases by increasing the concentration of NAA with lower BAP rate. Moreover, BAP application reduces the time required for shoot initiation and found to be most effective in inducing early shoot initiation. Nakano *et al.* (1999) also reported that a low rate of shoot formation was obtained from leaf explant when BAP alone was used, it was increases when NAA was added. In present study, MS media supplemented with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and MS media supplemented with 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA were found best treatments for shoot initiation. Similar results were also reported by Espino *et al.* (2004) in four different genotypes of *Begonia*.

5.4.3 Number of shoots per explant

The morphological observation of present study showed high percentage of *in vitro* regeneration from the different explant, especially from leaf disc explant which recovers132.40 ± 3.50 number of shoots per explant in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA in leaf disc explant of *B. palmata* followed by 113.20 ± 13.94 number of shoots per explant in MS media supplemented with 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA in leaf disc explant of *B. palmata*, 102.00 ± 8.46 number of shoots per explant was found in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA in leaf disc explant of *B. xanthina* and 64.20 ± 5.82 number of shoots per explant was recorded in MS media

supplemented with 2.0 mg l^{-1} BAP + 0.1 mg l^{-1} NAA in leaf disc explant of *B. hatacoa*. The minimum number of shoots was found in MS media without growth regulators, whereas, maximum shoots were produced when high BAP concentration was added with low NAA concentration. Similar results were reported by Nada *et al.* (2011) they recorded 132 number of shoots per leaf disc explant in MS media supplemented with 1.0 mg l^{-1} NAA and 2.0 mg l^{-1} TDZ, whereas, 33 shoots per explant through petiole explant was recorded from MS media supplemented with 0.5 mg l^{-1} NAA and 2.0 mg l^{-1} TDZ.

Mikkelsen and Sink (1978) also reported 135 shoots per petiole explant in Reigor *Begonia*. Whereas, Fonnesbech 1974 suggested that a lower concentration of BA produced no shoots, the highest concentration of BA yielded more shoots. Kumari *et al.* (2017) also suggested that BAP induced the high number of shoots in leaf explants. Several researchers also reported the effectiveness of both cytokinin and auxin on shoot regeneration.

Shobi and Viswanathan (2017) reported that MS media supplemented with 0.5 mgl^{-1} BAP and 0.5 mgl^{-1} NAA produced 20.8 shoots with 4.0 cm shoot length, whereas, 0.1 mg l^{-1} NAA and 0.5 mg l^{-1} BAP produced maximum number of shoots in *Begonia fallax.*

Kaviani *et al.* (2015) recorded that 0.5 mgl^{-1} BAP and 0.10 mgl^{-1} NAA produced maximum shoot number (43.00 per plant), root number (11.42 per plant), and root length (6.00 cm per plant). They also recorded the largest number of shoots per explant (46.24) in the same concentration of PGRs. In the present study minimum number of shoots per explant was recorded in MS media without growth regulators, these findings were in close conformity with the findings of Shobi and Viswanathan (2017) in *Begonia fallax.*

Kabirnataj *et al.* (2012) reported that highest adventitious shoot regeneration with an average of 41.6 from leaf explant after 5 weeks culture on MS media fortified with 1 mg l^{-1} BAP and 0.5 mg l^{-1} IBA whereas, lowest shoots were observed in petiole explant in MS media supplemented with 0.2 mg l^{-1} BAP and 0.2 mg l^{-1} NAA.

5.4.4 Shoot length

The beneficial effect of PGRs on plant growth has been reported by many authors as PGRs involved in the regulation of various processes of plant growth and development. Nikolic *et al.* (2006) in their study also revealed positive effects of BAP and NAA on shoot length in *Lotus corniculatus*. In current study, the maximum shoot length was recorded 6.44 ± 0.28 cm in MS media supplemented with1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA in leaf disc explant of *B. palmata* followed by 5.82 ± 0.35 cm in MS media supplemented with 2.0 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. palmata*, in case of *B. hatacoa* 5.74 ± 0.22 cm was recorded in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, whereas, in case of leaf disc explant of *B. xanthina* 5.14 ± 0.18 cm shoot length was recorded in MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA.

It has been observed that shoot length increases as rate of BAP increases with increasing rate of NAA and such findings are in close agreement of Naeem *et al*. (2004); Mendi *et al*. (2009); Nada *et al*. (2009); Kumari *et al*. (2017); Lai *et al*. (2018), they showed a significant increase in shoot length on the application of combined of BAP and NAA. Mikkelsen and Sink (1978) reported maximum shoots after four weeks of culture in 0.1 ppm NAA and 0.4 ppm BA. Shobi and Viswanathan (2017) also reported 4.0 cm shoot length from MS media supplemented with 0.5 mgl^{-1} BAP and 0.5 mgl^{-1} NAA in *B. fallax*.

Ramachandra and Khatamian (1989) also observed a higher shoot in higher concentrations of BAP and low concentration of NAA in *B. erythrophylla*. Prolific shoot regeneration was also obtained from MS media supplemented with BA with GA_3 (Mikkelsen and Sink, 1978; Takayama and Misawa, 1981; Kaviani, *et al.,* 2013; Kumari *et al.,* 2017).

5.4.5 Number of plantlets per explant

The number of shoots also correlated with the higher concentration of growth regulators which gave the maximum number of plantlets per explant. There was a significant influence of different concentrations of BAP in combination with NAA found in the number of plantlets per explant. In the present study, 69.40 ± 5.04 number of plantlets per explant was recorded maximum in leaf disc explant of *B. palmata* in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, followed by leaf disc explant of *B. hatacoa* 57.60 ± 2.84 number of plantlets per explant in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and 53.20 ± 2.20 number of plantlets per explant in MS media supplemented with2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA in leaf disc explant of *B. xanthina.* It has been noted that 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA was found best plant growth regulator combination in MS media for producing the maximum number of plantlets per explant. These results were also supported by previous work of several authors (Mendi *et al.,* 2009; Kumaria *et al.,* 2012; Shobi and Viswanathan, 2017; Kumari *et al.,* 2017).

Kim and Kim (2003) also reported that the presence of BA in *in vitro* culture is necessary for shoot formation. It has been noted that the effect of BAP in combination with auxin has been reported for the development of multiple shoot regeneration. Most of the

cases BAP, NAA and IAA were used for the induction of multiple shoots in different ornamental plants (Rasheed, 2013; Yesmin *et al.*, 2014)

5.4.6 Root Initiation

Direct root initiation and development were observed in different treatments of PGRs in MS media. The initiation of roots was visible after 29.60 ± 2.42 days in MS media supplemented with 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. hatacoa* followed by 32.20 ± 1.36 days in MS media supplemented with 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. xanthina* and 34.80 ± 3.20 days in MS media supplemented 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. palmata*. It has been noted that petiole explant of *Begonia* showed higher rooting percentage as compared to leaf disc, but immature bud explant showed necrosis after certain shoot length of explant and did not responds well in case of root initiation, whereas, the best combination for root initiation was obtained from MS media supplemented with 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA. These findings are more or less similar to earlier reports where 100% root induction was reported in the same medium (Yesmin *et al.*, 2014; Karim *et al.*, 2002 Kabirnataj *et al.*, 2012). It was noticed that the time required for root initiation was decreased with the increased concentration of BAP.

Direct root initiation was observed in the treatment containing a high concentration of NAA with a low concentration of BAP. It was noticed that the time required for root initiation was decreased with the increased concentration of BAP. It was also noted in *Plumbago zeylanica* Linn by Dasgupta and Reddy, (2013). Similar results were reported by Simmonds (1984) in *Begonia* × *hiemalis*. Bai *et al.* (2018) also reported direct root

induction in *Trichosanthes cucumerina* var. *cucumerina* from MS medium fortified with 0.5 mgl^{-1} BAP, 0.5 mgl^{-1} 2,4-D, and 1.0mgl^{-1} NAA. Pandey *et al.* (2010) also conducted an experiment in *Rauwolfia serpentina* for direct root induction through leaf explant and found that 97 % of root regeneration was noted in MS media supplemented with BAP and NAA as compared to NAA alone. It has been observed that various plant types exert different effects on root formation and development as a result of auxin treatment. It was also found that a high concentration of auxin leads to the production of adventitious roots (Pandey *et al.*, 2010).

5.4.7 Root length

The highest value of root length may due to enhanced growth of tissue on the application of plant growth hormones because interaction between endogenous and exogenous levels of the hormone might have play important role in plant growth. In present study, maximum root length 5.44 ± 0.42 cm was recorded in MS media supplemented with 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. palmata* followed by 4.78 ± 0.25 cm in MS media supplemented with 0.5 mg l^{-1} BAP+ 1.0 mg l^{-1} NAA in leaf disc explant of *B. hatacoa* and 4.54 ± 0.45 cm in MS media supplemented with 0.0 mg l^{-1} BAP+ 0.5 mg l^{-1} NAA in leaf disc explant of *B. xanthina*. It has been noted that petiole explant produced maximum root length and the best combination of plant growth regulators was 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA and 0.5 mg l^{-1} BAP+ 1.0 mg l^{-1} NAA. In the present study profuse rooting was observed on MS media supplemented with BAP and NAA. This finding is in close agreement with the findings Mendi *et al.* (2009) that reported the maximum values for root length on NAA application with combination of BAP and a similar finding was also reported by Kaviani *et al.* (2015). This may be due to fact that NAA is better for

inducing root growth and there is the tendency of increasing root length with the increase of NAA concentration in the MS medium (Mendi *et al.,* 2009; Simmonds, 1984; Espino *et al.,* 2004; Nakano *et al.,* 1999; Nada *et al.,* 2011). Shobi and Viswanathan (2017) also reported that 0.5 mgl^{-1} NAA was found best for root induction and obtained 2.3 cm root length in *Begonia fallax*.

It was noted that a higher concentration of BAP in combination with low concentration of NAA resulted in profuse microshoots and healthy *in vitro* plants for mass multiplication. *Begonia* was selected to investigate the in vitro regeneration and develop a protocol for high yield. Many researchers also attempted to obtain *in vitro* plants of *Begonia* have been reported by Nada *et al.* (2011) in *Begonia tuberhybrida*, Kumari *et al.* (2017) in *Begonia homoyana*, Burritt and Leung (1996) in *Begonia* × *erythrophylla*, Rosilah *et al.* (2014) in *Begonia pavonina*, Awal *et al.* (2008) in *Begonia* × *heimalis* Fotsch., Kumaria *et al.* (2012) in *Begonia rubroviena var* meisneri, Mendi *et al.* (2009) in *Begonia elatior* cv. Torran Orange, Murugan *et al.* (2016) in *Begonia malabrica* Lam.

CHAPTER 6

SUMMARY AND CONCLUSION

The present investigation titled **"Collection, Characterization and Evaluation of Selected *Begonia* species of Sikkim Himalayas for its ornamental value"** was undertaken to characterize the *Begonia* species collected from Sikkim Himalayas. The objectives of the present study were to collect and evaluate the morphological characters for ornamental purpose, along with to conduct biochemical analysis of the collected *Begonia* species and to develop micropropagation protocol of selected *Begonia* species for the production of elite planting material. The experimental findings of this investigation are being summarized below:

6.1 Summary

Thirteen wild *Begonia* species from Sikkim Himalayas were collected for the present experiment. Collected plant samples were planted in the field of Department of Horticulture, Sikkim University for germplasm establishment and were subjected to study morphological characters (19 quantitative and 19 qualitative traits), biochemical analysis including total soluble sugar, starch, total soluble protein, ascorbic acid, anthocyanin content, total phenol content, and total flavonoid content during the active growth stage of each plant, evaluation of the ornamental value of *Begonia* species through consumer preference and to develop micropropagation protocols of selected *Begonia* species.

The results obtained from morphological characterization revealed that the diverse range of *Begonia* species was found in Sikkim Himalayas. After characterizing the plant height of all the analyzed *Begonia* species, *B. picta,* and *B. satrapis* was found short height species, medium height species were *B. hatacoa, B. joesphi*, *B. megaptera, B. cathcartii, B. xanthina and B. annulata*, tall height species includes *B. flaviflora, B. panchtharensis, B. palmata, B. sikkimensis,* and *B. roxburghii.*

Likewise, all the thirteen collected Begonias were also characterized for qualitative plant characteristics such as leaf and stem colour, leaf shape, plant habit, flower colour, petiole length, leaf colour, and shape can be used to classify a given genus into different species. The results obtained from the present study show diverse morphological traits among the species or within the species. Comparing the morphological traits of thirteen wild Begonias it was found that they contribute diverse in phenotypic traits, results obtained in table 4.1 to 4.6 found that *B. palmata, B. josephi, B. picta* and *B. hatacoa* were having different phenotypic variants among the species level. Leaf margin of collected species were also found distinct from entire to undulate to denticulate to palmately lobed. Flower colour also differs in the respective species, *B. xanthina, B. flaviflora* have yellow colour flowers, *B. palmata, B. josephi, B. megaptera, B. annulata, B. hatacoa, B. cathcartii* and *B. panchtharensis* produced pinkish white flowers, *B. picta* and *B. satrapis* have pink colour flower, and *B. sikkimensis* flowers were red in colour. Details morphological trait which was taken during the flowering time/season at maximum attainable height were evaluated and presented in table 4.1 to 4.6 which shows distinct phenotypic characters for each species.

Morphological characterization is a preliminary and basic requirement for the exploitation of useful traits for the evaluation of wild species for their ornamental values. The objective of this study was, therefore, to assess the diversity of *Begonia* species using quantitative and qualitative morphological traits to identify the best traits of interest that could be used for the further evaluation process.

The biochemical study which includes total soluble sugar, starch, total soluble protein, ascorbic acid, anthocyanin content, total phenol content, and total flavonoid content has been conducted for all thirteen collected *Begonia* species from Sikkim Himalayas.

Results revealed that the maximum protein content of *Begonia* species was recovered from the leaf sample of *B. josephi* green phenotypes (35.04±0.71 mg g^{-1}), whereas maximum content of protein from stem and tuber extract was recorded in *B. josephi* green (25.97±0.39 mg g^{-1} and 21.89±0.42 mg g^{-1}, respectively).

Among the collected species of *Begonia* from Sikkim Himalayas for evaluation of ascorbic acid content in their leaves, stem and rhizome/tuber, the highest content of ascorbic acid was found in stem extract of *B. josephi* green and red phenotypes with 10.42±0.417 mg g^{-100} and 10.00±0.000 mg g^{-100} respectively, while lowest content was recorded in *B. palmata, B. roxburghii, B. hatacoa, B. panchtharensis* (3.75±0.000 mg g^{-100}).

Studies of maximum sugar content showed significant difference among the collected species of wild *Begonia*. Red phenotype of *B. josephi* extract from tuber/rhizome recorded maximum sugar content (34.86±0.71 mg g^{-1}) whereas, the lowest was recorded leaf extract of *B. palmata* green phenotypes (12.84±0.30 mg g^{-1}).

Maximum starch content was found in leaf extract of *B. josephi* red phenotypes (33.04±0.76 mg g^{-1}) whereas the lowest was observed in tuber/rhizome extract of *B. palmata* green phenotypes (7.40±0.42 mg g^{-1}).

Anthocyanin content in the leaf sample of *Begonia* species was found maximum in the red phenotype of *B. picta* (185.33±1.45mg L^{-1}) and the lowest content in the rhizome of *B. sikkimensis* (11.78±0.60 mg L^{-1}).

Results reveal that the total phenol content of leaf, stem and tuber extract of *Begonia* was found significantly differ and the total phenol content was found maximum in the *B. picta* red phenotypes (55.03±2.85 mg GAE g^{-1}), minimum was obtained from *B. cathcartii* (0.37±0.092 mg GAE g^{-1}) in tuber/rhizome extract.

It was evident from the present study that the total flavonoid content of leaf, stem and tuber/rhizome extract of *Begonia* species show significant variation among the species and maximum total flavonoid was found in tuber/rhizome extract of *B. picta* red phenotypes 39. 42±0.558 mg QE g^{-1} stem extract of *B. roxburghii* (0.18±0.961 mg QE g^{-1}).

Based on morphological characters evaluation, different *Begonia* species were subjected to consumer preference study by preparing questionnaires and total of 100 respondents were randomly selected and interviewed. Data reveals that the maximum satisfactory rate on the basis of the liking of the collected *Begonia* species was obtained by *B. xanthina* (6.77%), minimum satisfactory rate was recorded for *B. roxburghii* (6.46 %). Based on foliage pattern of the collected *Begonia* maximum satisfactory results were obtained for *B. picta* (6.92%), and minimum satisfactory rate by the respondents were given to *B. roxburghii* (6.69%). Data pertinent to the flower arrangement of the maximum

satisfactory rate was given to *B. flaviflora* (5.46 %) followed by *B. megaptera* (5.38 whereas, minimum was recorded for *B. roxburghii* (5.85 %).

For suitability of collected wild *Begonia* species, maximum satisfactory results were obtained for *B. picta* (6.77 %) and *B. xanthina* (6.77 %), and minimum satisfactory rate by the respondents was found for *B. josephi* (6.92%). Data pertinent to the edibility of wild *Begonia* species, the maximum satisfactory was found in *B. josephi* (4.69%) whereas, not satisfactory was found maximum in *B. xanthina* (7.69 %), *B. megaptera* (7.69 %) followed by *B. roxburghii* (7.62 %). Data regarding the attractiveness of the collected wild *Begonia* species was maximum for *B. xanthina* (6.54 %), while, minimum attractiveness was for *B. roxburghii* (7.31 %). For overall preference of the collected wild *Begonia* species by the respondents, maximum satisfactory rate (6.54%) was given to *B. xanthina*. Whereas, *B. roxburghii* (6.23 %) score minimum percentage for overall preference by the respondents.

It was observed from the consumer preference of the collected thirteen wild *Begonia* species, the maximum preference was for *B. xanthina* (5.45%) followed by *B. picta* (5.42%), *B. annulata* (5.25%), *B. palmata* (5.02%), *B. megaptera* (4.57%), *B. hatacoa* (4.48), whereas minimum satisfactory percentage was given for *B. roxburghii* (6.63%) and *B. josephi* (5.17%).

To develop micropropagation protocol of *Begonia* species for the production of elite planting material three species were selected which includes *B. xanthina*, *B. hatacoa* and *B. palmata* from the present study. Leaf disc, petiole and immature bud segments were used for explants in different combinations of BAP and NAA supplemented to MS media. The various parameters like survival rate, days required for shoot initiation, number of

shoots per explant, days required for root initiation, number of plantlets per explant, shoot length and root length were observed and recorded during the course of the investigation.

Immature bud explant of *B. hatacoa* (96.00%) in MS media supplemented with 1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA showed maximum survival rate, whereas, minimum survival rate (8.00 ± 4.90 %) was obtained from leaf disc explant of *B. hatacoa* and *B. palmata* in MS media without growth regulators.

The current findings showed that after 25.80 ± 1.20 days of culture, leaf disc explant showed shoot initiation in MS media supplemented with 1.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA whereas, maximum days required for shoot initiation (119.40 ± 10.16 days) in immature bud explant was found in MS media without growth regulators.

Leaf disc explant culture which recovers 132.40 ± 3.50 number of shoots per explant in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA in leaf disc explant of *B. palmata*. A minimum number of shoots was found in MS media without growth regulators.

In the current study, maximum shoot length was recorded 6.44 ± 0.28 cm in MS media supplemented with1.5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA from leaf disc explant of *B. palmata,* whereas, minimum shoot length was recorded in (2.80 ± 015 cm) in petiole explant of *B. xanthina* in MS media without growth regulators.

In the present study, 69.40 ± 5.04 number of plantlets per explant was recorded maximum in leaf disc explant of *B. palmata* in MS media supplemented with 2.0 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, whereas, the minimum number of plantlets per explant (2.60±0.24)

was recorded in in immature bud explant of *B. hatacoa* in MS media without growth regulators

Direct root initiation and development was observed in different treatments of PGRs in MS media. The initiation of roots was visible after 29.60 ± 2.42 days in MS media supplemented with 0.5 mg l^{-1} BAP + 1.0 mg l^{-1} NAA from petiole explants of *B. hatacoa,* whereas, maximum days required for root initiation (120.60±8.39 days) was recorded in leaf disc explants of *B. palmata* in MS media without growth regulators. Immature bud explant showed no root initiation in all the treatments.

In the present study, maximum root length 5.44 ± 0.42 cm was recorded in MS media supplemented with 0.1 mg l^{-1} BAP + 1.0 mg l^{-1} NAA in petiole explant of *B. palmata* and minimum root length (0.72±0.12 cm) was recorded in leaf disc explants of *B. palmata* in MS media without growth regulators.

It was noted that higher concentration of BAP in combination of a low concentration of NAA resulted profuse microshoots and healthy *in vitro* plantlets for mass multiplication. *Begonia* was selected to investigate the in vitro regeneration and develop a protocol for high yield.

6.2 Conclusion

Based on the present findings it can be concluded that the wild *Begonia* species found in Sikkim Himalayas may possess great potential in commercial floriculture with respect to morphological characters, biochemical studies and consumer preference presented in current findings. In case of morphological characters all the thirteen colected wild *Begonia* speceis showed distinct charatcers in terms of qualtitatve amd quantitatvie tratis studied

among the species. It has been observed that the consumer preference for ornamental values of wild *Begonia* and comparing the data from the present experiment, *B. xanthina, B. palmata, B. hatacoa* and *B. megaptera* was highly preferred by the consumers for introducing as ornamental plant for the floriculture industry. It was noticed that other species may also possess enormous amount of potential as some contain high amount of ascorbic acid, starch, protein, anthocyanin content, total flavonoid, and alkaloid content, it may reveal that they can be use as medicinal plants or as indoor plant by plant hobbyist. Results were evaluated by comparing the species availability, adaptability and morphological and biochemicals studies.

After evaluating all the necessary data in this experiment three high value *Begonia* species (*B. xanthina, B. palmata* and *B. hatacoa*) were subjected to develop protocol *in vitro* propagation for direct organogenis for high yield. It was noted that higher concentration of BAP in combination of a low concentration of NAA resulted profuse microshoots and healthy *in vitro* plantlets for mass multiplication.

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Annexure 1

PASSPORT DATA SHEET

Date..... Collector's No.....

Species Name ……Common Name.....

Regions explored…… Village/Block…….....

District..... State.....……

Latitude (E)..... Longitude (N).....

Altitude (M).....

Table of characteristics for *Begonia* evaluation

	Characteristics	Recorded	Remarks
1	Plant height (cm) including flowers		
2	Plant spread (cm) including flowers		
3	Length of midrib (cm)		
4	Leaf Length (cm)		
5	Leaf width (cm)		
6	Petiole length (cm)		
7	Stipule length (cm)		
8	Peduncle length (cm)		
9	Number of leaf/plants		
10	Number of Female flowers/plants		
11	Number of male flowers/plants		
12	Male flower length (cm)		
13	Male flower width (cm)		
14	Female flower length (cm)		
15	Female flower width (cm)		
16	Days required for flower bud development		
17	Days required for blooming		
18	Days required for wilting of flowers		
19	Flowering durations (days)		
20	Plant Habit		
21	Plant Form		
22	Leaf Lamina		
23	Leaf Margin		
24	Leaf Base		

25	Leaf apex		
26	Inflorescence type		
27	Leaf Colour (upper side)		
28	Leaf colour (lower side)		
29	Leaf pubescent		
30	Stipules colour		
31	Petioles colour		
32	Petioles hair		
33	Flower colour		
34	Flower pubescent		
35	Capsule colour		
36	Capsule hair		
37	Peduncle colour		
38	Peduncle hair		

Other characteristics……

Annexure 2

Questionnaire for consumer preferences of wild *Begonia* species

Department of Horticulture, Sikkim University

1. Which village/ward/place do you live in at present?

   ……

2. Gender- (Male/Female)

3. Age-

Sl/No	Age Group	(√ Mark)
A	0-20	
B	21-30	
C	31-40	
D	41-60	
E	61 above	

4. What is your profession?

   ……
   ……

5. What is your highest level of education?

Sl./No	Education	(√ Mark)
A	Primary School	
B	Junior School	
C	Senior School	
D	Graduates	
E	Post graduates	
F	None	

6. Do you own nursery currently?

   ……

7. Do you cultivate flower? (If yes than mark the any one option from the following):

Sl/No	Flower growers	(√ Mark)	Other Remarks
A	Commercial growers		
B	Amateur growers		
C	Decorative purposes		
D	Hobby		
E	None		

8. Which flower do you prefer most to grow in your garden?

……

9. Do you have *Begonia* in your home garden/nursery?

……

10. Which option describes your relationship to *Begonia*?

Sl/No	Source	(√ Mark)	Other Remarks
1.	Don't own *Begonia*		
2.	Own *Begonia* as potted plant		
3.	Own *Begonia* as indoor plant		
4.	Professional grower		
5.	Competition judge		
6.	Researcher		
7.	Other		

11. If you have *Begonia*, from where did you get it?

Sl/No	Source	(√ Mark)	Other Remarks
1.	Not applicable		
2.	Supermarket		
3.	Local market		
4.	Nursery		
5.	Social Media		
6.	Online		
7.	Flower show/ expo		

8.	Gift		
9.	others		

10. Which *Begonia* do you prefer the most and want to cultivate in your garden? If you select not to buy any then select None.

Sl/No	***Begonia* species**	**(√ Mark)**	**Other Remarks**
1.	*B. xanthina*		
2.	*B. annulata*		
3.	*B. megaptera*		
4.	*B. palmata*		
5.	*B. josephi*		
6.	*B. cathcartii*		
7.	*B. sikkimensis*		
8.	*B. roxburghii*		
9.	*B. picta*		
10.	*B. hatacoa*		
11.	*B. panchtharensis*		
12.	*B. flaviflora*		
13.	*B. satrapis*		

14. Give score for each *Begonia* species by looking at the given postcards.

Sl/No	Education	Score
A	Liking	(1), (2), (3), (4), (5)
B	Foliage pattern	(1), (2), (3), (4), (5)
C	Flower arrangement	(1), (2), (3), (4), (5)
D	Suitability	(1), (2), (3), (4), (5)
E	Edibility	(1), (2), (3), (4), (5)
F	Attractiveness	(1), (2), (3), (4), (5)
G	Overall appearance	(1), (2), (3), (4), (5)

If you are interested in the results of the survey, then write your email or postal address. I will send a summary of results to you.

Thank You.

Email……

Contact Number……

Annexure 3

GPS coordinates of wild *Begonia* species of Sikkim Himalayas

Sl. No.	Longitude (N)	Latitude (E)	Altitude (msl)	*Begonia sp*
1.	N 27° 18' 35"	E 88° 28' 557"	1460	*B. palmata*
2.	N 27° 18' 40"	E 88° 28' 665"	1453	*B. palmata*
3.	N 27° 18' 49"	E 88° 29' 58"	1456	*B. palmata*
4.	N 27° 18' 92"	E 88° 29' 799"	1446	*B. hatacoa*
5.	N 27° 16' 83"	E 88° 27' 726"	1376	*B. hatacoa*
6.	N 27° 16' 83"	E 88° 27' 735"	1362	*B. palmata*
7.	N 27° 16' 83"	E 88° 27' 741"	1398	*B. hatacoa*
8.	N 27° 16' 835"	E 88° 27' 752"	1401	*B. palmata*
9.	N 27° 16' 836"	E 88° 27' 779"	1415	*B. hatacoa*
10.	N 27° 16' 827"	E 88° 27' 782"	1405	*B. hatacoa*
11.	N 27° 16' 918"	E 88° 30' 560"	1822	*B. josephi*
12.	N 27° 22' 599"	E 88° 37' 861"	1818	*B. josephi*
13.	N 27° 22' 939"	E 88° 38' 389"	1549	*B. palmata*
14.	N 27° 23' 774"	E 88° 31' 496"	945	*B. roxburghii*
15.	N 27° 23' 707"	E 88° 31' 494"	1042	*B. roxburghii*
16.	N 27° 21' 127"	E 88° 29' 874"	1476	*B. megaptera*
17.	N 27° 6' 801"	E 88° 34' 237"	1699	*B. palmata*
18.	N 27° 10' 49"	E 88° 22' 34"	1786	*B. sikkimensis*
19.	N 27° 13' 36"	E 88° 23' 34"	2078	*B. cathcartii*
20.	N 27° 13' 39"	E 88° 23' 47"	2142	*B cathcartii*
21.	N 27° 14' 36"	E 88° 18' 7"	387	*B. roxburghii*
22.	N 27° 14' 41"	E 88° 18' 2"	439	*B. picta*
23.	N 27° 15' 58"	E 88° 18' 6"	475	*B. hatacoa*
24.	N 27° 16' 31"	E 88° 17' 54"	500	*B. hatacoa*
25.	N 27° 16' 40"	E 88° 17' 49"	492	*B. roxburghii*
26.	N 27° 16' 4"	E 88° 19' 1"	1113	*B. picta*
27.	N 27° 16' 51"	E 88° 18' 35"	1498	*B. picta*
28.	N 27° 16' 50"	E 88° 18' 37"	1492	*B. sikkimensis*
29.	N 27° 16' 47"	E 88° 18' 59"	1575	*B. panchtharensis*
30.	N 27° 14' 51"	E 88° 25' 22"	1158	*B. palmata*
31.	N 27° 18' 29"	E 88° 34' 58"	938	*B. megaptera*
32.	N 27° 15' 15"	E 88° 28' 54"	1053	*B. hatacoa*
33.	N 27° 15' 31"	E 88° 28' 4"	887	*B. roxburghii*
34.	N 27° 7' 21"	E 88° 20' 19"	865	*B. picta*
35.	N 27° 10' 10"	E 88° 11' 49"	1569	*B. palmata*
36.	N 27° 11' 13"	E 88° 23' 14"	1839	*B. cathcartii*
37.	N 27° 12' 16"	E 88° 23' 45"	1957	*B. panchtharenesis*

38.	N 27° 14' 2"	E 88° 23' 57"	2034	*B. panchtharensis*
39.	N 27° 13' 44"	E 88° 24' 48"	1930	*B. cathcartii*
40.	N 27° 20' 40"	E 88° 37' 45"	2143	*B. josephi*
41.	N 27° 11' 36"	E 88° 36' 46"	503	*B. hatacoa*
42.	N 27° 21' 740"	E 88° 33' 965"	2052	*B. sikkimensis*
43.	N 27° 21' 392"	E 88° 33' 538"	2186	*B. cathcartii*
44.	N 27° 14' 54"	E 88° 30' 14"	1210	*B. picta*
45.	N 27° 14' 55"	E 88° 30' 14"	1220	*B. picta*
46.	N 27° 14' 54"	E 88° 30' 15"	1221	*B. picta*
47.	N 27° 14' 56"	E 88° 30' 14"	1224	*B. picta*
48.	N 27° 14' 55"	E 88° 30' 18"	1222	*B. picta*
49.	N 27° 14' 59"	E 88° 30' 59"	1242	*B. annulata*
50.	N 27° 15' 11"	E 88° 30' 1"	1200	*B. hatacoa*
51.	N 27° 15' 27"	E 88° 29' 46"	1355	*B. palmata*
52.	N 27° 15' 22"	E 88° 29' 17"	1245	*B. annulata*
53.	N 27° 16' 52"	E 88° 28' 25"	1263	*B. hatacoa*
54.	N 27° 16' 48"	E 88° 28' 22"	1827	*B. cathcartii*
55.	N 27° 16' 47"	E 88° 28' 22"	1776	*B. palmata*
56.	N 27° 16' 50"	E 88° 28' 21"	1775	*B. panchtharensis*
57.	N 27° 17' 19"	E 88° 28' 29"	1768	*B. flaviflora*
58.	N 27° 17' 19"	E 88° 28' 29"	1864	*B. cathcartii*
59.	N 27° 17' 15"	E 88° 28' 39"	1976	*B. panchtharensis*
60.	N 27° 17' 16"	E 88° 28' 38"	1962	*B. sikkimensis*
61.	N 27° 17' 31"	E 88° 28' 29"	1840	*B. cathcartii*
62.	N 27° 17' 34"	E 88° 28' 29"	1820	*B. sikkimensis*
63.	N 27° 17' 29"	E 88° 28' 29"	1853	*B. flaviflora*
64.	N 27° 17' 47"	E 88° 28' 27"	1704	*B. flaviflora*
65.	N 27° 17' 34"	E 88° 29' 13"	2031	*B. cathcartii*
66.	N 27° 18' 18"	E 88° 30' 7"	1875	*B. panchtharensis*
67.	N 27° 18' 22"	E 88° 30' 3"	1850	*B. flaviflora*
68.	N 27° 18' 42"	E 88° 30' 11"	1618	*B. josephi*
69.	N 27° 18' 41"	E 88° 30' 7"	1644	*B. palmata*
70.	N 27° 20' 30"	E 88° 30' 28"	1571	*B. palmata*
71.	N 27° 22' 51"	E 88° 32' 4"	1736	*B. cathcartii*
72.	N 27° 22' 22"	E 88° 32' 28"	1854	*B. josephi*
73.	N 27° 21' 47"	E 88° 32' 44"	1952	*B. josephi*
74.	N 27° 21' 43"	E 88° 33' 18"	1841	*B. cathcartii*
75.	N 27° 22' 7"	E 88° 33' 39"	1815	*B. flaviflora*
76.	N 27° 22' 15"	E 88° 34' 17"	1873	*B. panchtharensis*
77.	N 27° 22' 11"	E 88° 34' 40"	1956	*B. panchtharensis*
78.	N 27° 20' 39"	E 88° 37' 48"	2141	*B. josephi*

79.	N 27° 20' 38"	E 88° 37' 47"	2141	*B. josephi*
80.	N 27° 20' 43"	E 88° 37' 52"	2125	*B. josephi*
81.	N 27° 20' 46"	E 88° 37' 54"	2111	*B. josephi*
82.	N 27° 21' 25"	E 88° 37' 40"	2040	*B. cathcartii*
83.	N 27° 20' 19"	E 88° 37' 41"	1872	*B. josephi*
84.	N 27° 14' 46"	E 88° 35' 17"	1564	*B. palmata*
85.	N 27° 14' 49"	E 88° 35' 16"	1533	*B. palmata*
86.	N 27° 14' 33"	E 88° 35' 5"	1598	*B. palmata*
87.	N 27° 13' 10"	E 88° 35' 30"	1374	*B. annulata*
88.	N 27° 15' 49"	E 88° 36' 39"	1330	*B. palmata*
89.	N 27° 23' 39"	E 88° 38' 35"	1543	*B. palmata*
90.	N 27° 23' 40"	E 88° 38' 22"	1432	*B. palmata*
91.	N 27° 23' 37"	E 88° 38' 9"	1222	*B. palmata*
92.	N 27° 24' 57"	E 88° 37' 44"	1423	*B. palmata*
93.	N 27° 26' 19"	E 88° 36' 45"	1553	*B. sikkimensis*
94.	N 27° 26' 33"	E 88° 36' 34"	1754	*B. sikkimensis*
95.	N 27° 26' 36"	E 88° 36' 29"	1801	*B. sikkimensis*
96.	N 27° 25' 33"	E 88° 35' 0"	1756	*B. panchtharensis*
97.	N 27° 24' 43"	E 88° 34' 55"	1805	*B. sikkimensis*
98.	N 27° 24' 40"	E 88° 34' 49"	1906	*B. josephi*
99.	N 27° 19' 37"	E 88° 9' 9"	1132	*B. hatacoa*
100.	N 27° 21' 0"	E 88° 53' 53"	683	*B. roxburghii*
101.	N 27° 20' 59"	E 88° 49' 49"	699	*B. roxburghii*
102.	N 27° 21' 41"	E 88° 58' 58"	960	*B. hatacoa*
103.	N 27° 18' 998"	E 88° 30' 551"	1713	*B. palmata*
104.	N 27° 18' 069"	E 88° 28' 252"	1630	*B. palmata*
105.	N 27° 22' 599"	E 88° 37' 861"	1818	*B. cathcartii*
106.	N 27° 22' 939"	E 88° 38' 389"	1827	*B. cathcartii*
107.	N 27° 23' 604"	E 88° 38' 569"	1549	*B. palmata*
108.	N 27° 23' 603"	E 88° 38' 567"	1550	*B. palmata*
109.	N 27° 23' 103"	E 88° 36' 389"	1623	*B. palmata*
110.	N 27° 24' 471"	E 88° 31' 126"	625	*B. hatacoa*
111.	N 27° 25' 120"	E 88° 30' 927"	646	*B. hatacoa*
112.	N 27° 25' 120"	E 88° 30' 927"	647	*B. palmata*
113.	N 27° 23' 886"	E 88° 31' 556"	836	*B. hatacoa*
114.	N 27° 6' 905"	E 88° 35' 268"	1772	*B. flaviflora*
115.	N 27° 6' 806"	E 88° 35' 266"	1767	*B. flaviflora*
116.	N 27° 5' 730"	E 88° 37' 275"	1868	*B. flaviflora*
117.	N 27° 5' 789"	E 88° 37' 274"	1878	*B. xanthina*
118.	N 27° 5' 503"	E 88° 39' 37"	2109	*B. josephi*
119.	N 27° 5' 498"	E 88° 39' 37"	2122	*B. cathcartii*

120.	N 27° 5' 477"	E 88° 39' 46"	2172	*B. cathcartii*
121.	N 27° 5' 471"	E 88° 39' 46"	2175	*B. flaviflora*
122.	N 27° 5' 419"	E 88° 39' 372"	2040	*B. xanthina*
123.	N 27° 10' 26"	E 88° 22' 32"	1665	*B. flaviflora*
124.	N 27° 10' 49"	E 88° 22' 34"	1789	*B. flaviflora*
125.	N 27° 13' 45"	E 88° 23' 34"	2078	*B. cathcartii*
126.	N 27° 13' 45"	E 88° 23' 34"	2075	*B. cathcartii*
127.	N 27° 13' 39"	E 88° 23' 47"	2142	*B. josephi*
128.	N 27° 13' 16"	E 88° 24' 5"	2172	*B. sikkimensis*
129.	N 27° 13' 16"	E 88° 24' 4"	2147	*B. josephi*
130.	N 27° 13' 31"	E 88° 17' 53"	557	*B. hatacoa*
131.	N 27° 12' 38"	E 88° 19' 6"	684	*B. hatacoa*
132.	N 27° 13' 36"	E 88° 18' 7"	307	*B. roxburghii*
133.	N 27° 14' 41"	E 88° 18' 2"	439	*B. roxburghii*
134.	N 27° 14' 58"	E 88° 18' 6"	475	*B. roxburghii*
135.	N 27° 14' 58"	E 88° 18' 6"	492	*B. hatacoa*
136.	N 27° 15' 31"	E 88° 17' 54"	500	*B. roxburghii*
137.	N 27° 15' 40"	E 88° 17' 49"	492	*B. roxburghii*
138.	N 27° 16' 4"	E 88° 19' 1"	1113	*B. hatacoa*
139.	N 27° 16' 51"	E 88° 18' 35"	1498	*B. annulata*
140.	N 27° 16' 50"	E 88° 18' 37"	1492	*B. annulata*
141.	N 27° 16' 47"	E 88° 18' 59"	1575	*B. palmata*
142.	N 27° 17' 52"	E 88° 22' 3"	1985	*B. josephi*
143.	N 27° 16' 36"	E 88° 22' 27"	1811	*B. cathcartii*
144.	N 27° 14' 51"	E 88° 25' 22"	1158	*B. hatacoa*
145.	N 27° 18' 29"	E 88° 34' 58"	938	*B. picta*
146.	N 27° 18' 37"	E 88° 35' 1"	933	*B. picta*
147.	N 27° 15' 54"	E 88° 28' 54"	1053	*B. megaptera*
148.	N 27° 15' 54"	E 88° 28' 50"	1065	*B. megaptera*
149.	N 27° 15' 31"	E 88° 28' 4"	887	*B. roxburghii*
150.	N 27° 15' 18"	E 88° 28' 9"	785	*B. hatacoa*
151.	N 27° 6' 40"	E 88° 21' 17"	779	*B. roxburghii*
152.	N 27° 7' 21"	E 88° 20' 19"	865	*B. megaptera*
153.	N 27° 10' 10"	E 88° 11' 49"	1569	*B. palmata*
154.	N 27° 11' 13"	E 88° 23' 14"	1839	*B. josephi*
155.	N 27° 11' 13"	E 88° 23' 16"	1872	*B. cathcartii*
156.	N 27° 12' 16"	E 88° 23' 45"	1957	*B. panchtharensis*
157.	N 27° 14' 2"	E 88° 23' 57"	2034	*B. josephi*
158.	N 27° 14' 10"	E 88° 23' 59"	2021	*B. josephi*
159.	N 27° 13' 54"	E 88° 24' 5"	2026	*B. cathcartii*
160.	N 27° 13' 44"	E 88° 24' 48"	1930	*B. josephi*

161.	N 27° 20' 40"	E 88° 37' 45"	2143	*B. josephi*
162.	N 27° 20' 46"	E 88° 37' 54"	2088	*B. josephi*
163.	N 27° 19' 238"	E 88° 22' 137"	2379	*B. josephi*
164.	N 27° 14' 84"	E 88° 22' 539"	2126	*B. cathcartii*
165.	N 27° 14' 81"	E 88° 22' 528"	2125	*B. josephi*
166.	N 27° 14' 116"	E 88° 22' 465"	2147	*B. cathcartii*
167.	N 27° 14' 121"	E 88° 22' 465"	2164	*B. josephi*
168.	N 27° 14' 75"	E 88° 22' 554"	2136	*B. panchtharensis*
169.	N 27° 10' 59"	E 88° 21' 312"	1413	*B. palmata*
170.	N 27° 10' 171"	E 88° 22' 314"	1347	*B. palmata*
171.	N 27° 13' 756"	E 88° 23' 679"	2121	*B. josephi*
172.	N 27° 13' 683"	E 88° 23' 770"	2137	*B. panchtharensis*
173.	N 27° 13' 659"	E 88° 23' 806"	2137	*B. sikkimensis*
174.	N 27° 13' 587"	E 88° 23' 865"	2144	*B. flaviflora*
175.	N 27° 13' 586"	E 88° 23' 872"	2148	*B. flaviflora*
176.	N 27° 13' 637"	E 88° 23' 789"	2224	*B. josephi*
177.	N 27° 13' 731"	E 88° 23' 725"	2204	*B. josephi*
178.	N 27° 13' 786"	E 88° 23' 618"	2187	*B. cathcartii*
179.	N 27° 13' 774"	E 88° 23' 656"	2182	*B. josephi*
180.	N 27° 16' 266"	E 88° 35' 807"	824	*B. megaptera*
181.	N 27° 16' 259"	E 88° 35' 804"	814	*B. megaptera*
182.	N 27° 16' 251"	E 88° 35' 794"	844	*B. megaptera*
183.	N 27° 16' 204"	E 88° 35' 770"	842	*B. roxburghii*
184.	N 27° 16' 204"	E 88° 35' 768"	840	*B. megaptera*
185.	N 27° 69' 583"	E 88° 37' 480"	1202	*B. megaptera*
186.	N 27° 18' 7"	E 88° 15' 18"	1973	*B. sikkimensis*
187.	N 27° 18' 11"	E 88° 15' 20"	1958	*B. sikkimensis*
188.	N 27° 17' 53"	E 88° 15' 19"	1836	*B. cathcartii*
189.	N 27° 18' 6"	E 88° 14' 43"	1954	*B. panchtharensis*
190.	N 27° 17' 57"	E 88° 15' 19"	1889	*B. josephi*
191.	N 27° 17' 367"	E 88° 36' 467"	503	*B. roxburghii*
192.	N 27° 16' 16"	E 88° 35' 49"	834	*B. megaptera*
193.	N 27° 16' 43"	E 88° 35' 49"	821	*B. megaptera*
194.	N 27° 16' 19"	E 88° 35' 49"	834	*B. roxburghii*
195.	N 27° 16' 19"	E 88° 35' 51"	836	*B. roxburghii*
196.	N 27° 16' 53"	E 88° 36' 54"	1332	*B. megaptera*
197.	N 27° 11' 17"	E 88° 40' 36"	1550	*B. xanthina*
198.	N 27° 11' 17"	E 88° 40' 36"	1552	*B. xanthina*
199.	N 27° 11' 8"	E 88° 40' 28"	1562	*B. xanthina*
200.	N 27° 11' 17"	E 88° 40' 37"	1518	*B. annulata*
201.	N 27° 11' 17"	E 88° 40' 39"	1504	*B. xanthina*

202.	N 27° 11' 34"	E 88° 41' 42"	1187	*B. picta*
203.	N 27° 11' 22"	E 88° 41' 30"	1189	*B. picta*
204.	N 27° 11' 37"	E 88° 41' 53"	1123	*B. roxburghii*
205.	N 27° 13' 44"	E 88° 18' 39"	856	*B. satrapis*
206.	N 27° 13' 42"	E 88° 18' 39"	569	*B. satrapis*
207.	N 27° 14' 1"	E 88° 18' 43"	951	*B. satrapis*
208.	N 27° 14' 3"	E 88° 18' 44"	995	*B. satrapis*
209.	N 27° 13' 59"	E 88° 18' 39"	985	*B. satrapis*
210.	N 27° 13' 42"	E 88° 19' 27"	1214	*B. roxburghii*
211.	N 27° 21' 30"	E 88° 11' 57"	1398	*B. palmata*
212.	N 27° 21' 30"	E 88° 12' 1"	1365	*B. megaptera*
213.	N 27° 21' 34"	E 88° 11' 59"	1394	*B. megaptera*
214.	N 27° 21' 23"	E 88° 12' 6"	1344	*B. hatacoa*
215.	N 27° 20' 55"	E 88° 12' 18"	1205	*B. hatacoa*
216.	N 27° 21' 40"	E 88° 12' 42"	1433	*B. plamata*
217.	N 27° 22' 27"	E 88° 13' 23"	1754	*B. flaviflora*
218.	N 27° 22' 26"	E 88° 13' 23"	1756	*B. flaviflora*
219.	N 27° 22' 43"	E 88° 13' 28"	1846	*B. panchtharensis*
220.	N 27° 22' 26"	E 88° 13' 52"	1871	*B. cathcartii*
221.	N 27° 10' 51"	E 88° 22' 42"	2075	*B. flaviflora*
222.	N 27° 10' 53"	E 88° 22' 43"	2084	*B. falviflora*
223.	N 27° 10' 55"	E 88° 22' 45"	2091	*B. flaviflora*
224.	N 27° 10' 45"	E 88° 22' 56"	2126	*B. josephi*
225.	N 27° 10' 45"	E 88° 22' 59"	2108	*B. sikkimensis*
226.	N 27° 9' 27"	E 88° 19' 18"	1446	*B. hatacoa*
227.	N 27° 9' 27"	E 88° 19' 19"	1432	*B. hatacoa*
228.	N 27° 11' 57"	E 88° 23' 45"	2008	*B. cathcartii*
229.	N 27° 11' 59"	E 88° 23' 45"	1997	*B. josephi*
230.	N 27° 11' 19"	E 88° 23' 28"	1914	*B. josephi*
231.	N 27° 11' 12"	E 88° 23' 22"	1989	*B. josephi*
232.	N 27° 11' 52"	E 88° 23' 40"	1985	*B. panchtharensis*
233.	N 27° 13' 52"	E 88° 24' 17"	2079	*B. josephi*
234.	N 27° 13' 45"	E 88° 23' 32"	2090	*B. sikkimensis*
235.	N 27° 13' 45"	E 88° 23' 33"	2095	*B. josephi*
236.	N 27° 13' 46"	E 88° 23' 36"	2101	*B. cathcartii*
237.	N 27° 12' 44"	E 88° 20' 21"	738	*B. roxburghii*
238.	N 27° 13' 6"	E 88° 21' 19"	1465	*B. palmata*
239.	N 27° 13' 7"	E 88° 21' 17"	1439	*B. megaptera*
240.	N 27° 12' 51"	E 88° 21' 18"	1381	*B. palmata*