DIVERSITY ANALYSIS AND REPRODUCTIVE BIOLOGY OF MILK YAM (*Ipomoea digitata* L.)

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled **"DIVERSITY ANALYSIS AND REPRODUCTIVE BIOLOGY OF MILK YAM (*Ipomoea digitata* L.)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"DIVERSITY ANALYSIS AND REPRODUCTIVE BIOLOGY OF MILK YAM (*Ipomoea digitata* L.)"** is a record of research work done independently by Mrs. Vidya, K. M (2013-22-103) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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LISTOF ABBREVIATIONS

%	Per cent
µm	Micrometer
^{0}C	Degree Celsius
AChE	Acetylcholinesterase
AMSL	Above mean sea level
CHO	Carbohydrate
cm	Centimeter
DAP	Days after planting
DPPH	2,2-diphenyl-1-picrylhydrazyl
Fig	Figure
g	Gram
HDL	High density Lipoprotein
HPLC	High performance liquid chromatography
HPTLC	High performance thin layer chromatography
IKI	Iodine potassium iodide
kg	Kilogram
m	Meter
MAP	Months after planting
mg	Milligram
NS	Non significant
OTC	Over the counter
ppm	Parts per million
Rf	Retardation factor
Rt	Retention time
SEM	Scanning Electron Microscopy
TLC	Thin layer chromatography
TTC	2,3,5-Triphenyl-2H-tetrazolium chloride
TZ	Tetrazolium chloride
viz	Namely
YAP	Year after planting

Introduction

INTRODUCTION

India has a very long history of usage of many herbal drugs in the indigenous systems of medicine *viz.*, Ayurveda, Unani, Siddha, Homeopathy and Naturopathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems (Gautham *et al.*, 2003). Now-a-days, world-wide shift towards use of herbal preparations over synthetic pharmaceuticals has been realized. The importance of focused research in medicinal plants and herbs are staging a comeback and herbal 'renaissance' is happening all over the globe (Kumar *et al.*, 2004).

Medicinal plants are the primary life-supporting system for rural and tribal communities and over 8000 species of plants have been estimated to be used in indigenous health system. *Ipomoea digitata* L. (Family- Convolvulaceae; Syn.: *Ipomoea mauritiana* Jacq.) has been traditionally used as a medicine in India and parts of Southeast Asia as a general tonic, to treat diseases of the spleen and liver and to prevent fat accumulation in the body. It is commonly known as Milk yam in English or Kshirvidari and Vidarikanda in local language in India. The early Sanskrit writers mentioned it under the names 'Vidari' and 'Bhumi-Kushmanda'. In the Nighantas, it has several synonyms (*Convolvulus paniculatus*, *Ipomoea mauritiana*, *Ipomoea eriosperma*, *Ipomoea paniculata*). The species is distributed throughout India (Bihar, Odisha, West Bengal, Assam, and the west coast from Konkan to Kerala) in deciduous and evergreen forests and coastal tracts and widely naturalized in tropical parts of the world (Warrier *et al.*, 1995). It grows mostly in moist areas, monsoon forests and in coastal tracts. In Kerala, it is found in Kasaragod, Cannanore, Calicut, Malappuram, Trichur, Kottayam, Pathanamthitta and Trivandrum Districts (almost throughout the State), in the low and midlands but its commercial exploitation is still very much limited.

The commercial drug consists of its tubers which has medicinal value. The pharmacological activity of the tuberous roots is attributed to the presence of secondary metabolites and phytoconstituents such as taraxerol, taraxerol acetate, β-sitosterol, scopoletin, 7-O-β- D glycopyranosyl scopoletin (Khan *et al*., 2009) and t-Cinnamic acid, Umbelliferone, ocetadecyl (E-P-coumarete), N–butyl- ß-D-fructopyranoside, Scoparone (Madhavi *et al.*, 2010).

'*Vidari*' is a component of about 50 ayurvedic formulations including Chyavanaprash. The annual industrial requirement of 'Vidari' is about 500–1000 MT(Ved and Goraya, 2008). '*Vidari*' is used as aphrodisiac, cardiotonic, demulcent, diuretic, refrigerant, and galactagogue (Chopra *et al.*, 1992). Many of the ayurvedic industries use '*Vidari*' in popular ayurvedic nutraceutical products (Venkatasubramanian *et al*., 2009).

Many versatile folkloric uses have been associated with milk yam. Tubers are eaten raw in Midnapure district of West Bengal. Raw tuber is also taken to treat blood dysentery and used as astringent (Singh and Panda, 2005). Sun dried root power, boiled in sugar and butter is administrated to promote weight gain and to promote menstrual discharge. Tubers are also been used for treatment of debility, spermatorrhoea, fever, bronchitis, scorpion strings and menorrhagia (Behera *et al.*, 2006). Juice of tubers with one glass of cow milk is given for 7 days to increase lactation by *Kandha* tribe of Orissa (Jain, 2011). *Yaogika chikitsa* and *dravya guna* has mentioned its usefulness in patients with hypertension and heart disease. A teaspoon full of powdered tuber taken twice a day with honey is beneficial for the patients of high blood pressure and heart disease (Sarkar, 1991). In India, the bitter, tuberous roots are used as tonic, alterative and demulcent. In the Konkan region of India, root is peeled, cut in small pieces, and dried for use as aphrodisiac.

The plant has been marked as one of the threatened medicinal plant species deserving priority in advance research (Islam and Bari, 2013). For the purpose of

conservation and improvement programs, there is a need to study the biology of reproduction and genetic variation in this valuable plant. The study of phenology, floral characteristics, pollen-pistil interaction, hybridization technique, cause and nature of variation prevailing between and within population, association of chemo-agronomic characteristics, diversity of population and their interaction with environments are important for developing genetic improvement and conservation strategies. The wild growing population of milk yam has a great measure of variation in morphological and qualitative characters, but the literature concerning the reproductive biology of *I. digitata* is not extensive and are incidental to investigations of taxonomy and classification (Warrier *et al.*, 2007)). There are no published experimental studies that address breeding system and pollination ecology, including observation of pollinator behaviour, experimental manipulation to assess the degree of selfing and out crossing, mean seed set, or a seed germination protocol. Hence, an investigation was under taken to study the diversity analysis and reproductive biology of milk yam with the following objectives:

1. Estimating the magnitude of genetic variation in morphological and biochemical traits in milk yam accessions.
2. Identifying superior accessions with high tuber yield and active ingredient content.
3. A detailed study of the reproductive biology of milk yam
4. Investigations on regional variation and influence of climatic and soil factors on phytochemical content in milk yam.

Review of Literature

2. REVIEW OF LITERATURE

In this chapter an earnest effort was made to review the relevant and updated literature having direct or indirect bearing on the study. The present study is concerned with Diversity analysis and reproductive biology of Milk yam (*Ipomoea digitata* L.). The chapter reviews the literature on;

1. Systematic of *Ipomoea digitata* L.
2. Traditional nutraceutical uses
3. Pharmacological properties
4. Morphology
5. Anatomical characters
6. Reproductive biology
7. Phytoconstituents
8. Chromatography
9. Diversity analysis
10. Variability components

SYSTEMATIC OF *I. digitata* L.

Ipomoea digitata L. is a member of the family convolvulaceae and called bilai-kand, bhui-khola, ksheervidari, payasvinee, bhumi-kumar, bhumi-kushmanda in various languages (Anonymous, 1989.)

Scientific classification:

Kingdom: Plantae

Division: Eudicots

Class: Asterids

Order: Solanales

Family: Convolvulaceae

Genus: *Ipomoea*

Species: *digitata*

Binomial name

Ipomoea digitata L.

Ipomoea mauritiana Jacq.

Convolvulus paniculatus

Ipomoea eriosperma,

Ipomoea paniculata

Synonyms: (Anonymous, 1889)

Sanskrit : Vidari

Malayalam: Palmuthukku

Bengali : Shimiya, Shimiabatraji, Bhui Kumdo

English : Indian Kudju

Gujrati : Khakharvel, Vidaree, Vidareekand

Hindi : Vidareekand, Bilaikand, Sural, Patal kand

Marathi : Bendriya bel, Bindree, Vendrichavel

Punjabi : Siali

Tamil : Nilpushni Kezhugu

Telugu : Nelagummudu

TRADITIONAL NUTRACEUTICAL USES

The genus *Ipomoea* since time immemorial have been in continuous use for different purposes, such as, nutritional, medicinal, ritual and agricultural which includes *I. batatas, I aquatica, I. alba, I. albivenia, I. involucrata* and *I. leptophylla.*

Various species of *Ipomoea* have been used extensively, in many countries, in the traditional medicine for the treatment of several diseases. The most common use of the roots of *Ipomoea* species is to treat constipation (Miranda and Bah, 2003).

Due to the presence of ergot type alkaloids, several species of *Ipomoea* are used as hallucinogenic. Some of them were used in pre-columbian times by ancient people to attain a state of mind suitable for divination during religious ceremonies and magical healing practices (Taber *et al.,* 1963; Dalo and Moussatche, 1978).

Yogika chikitsa and *dravya guna* has mentioned the usefulness of *I. digitata* in treating the patients with hypertension and heart disease. In India, the bitter tuberous roots are used as tonic, alterative and demulcent. In the Konkan region of India, root is peeled, cut into small pieces, and dried for use as aphrodisiac. It is also used as remedy for uterine pain, sexual debility, infertility, lactation, hepato-splenomegaly, gastric ulcer and ulcerative colitis (www.ayurvedacalifornia.com). A teaspoon full of powdered tuber if given twice a day with honey is beneficial for the patients of high blood pressure and heart disease (Sarkar, 1991). The powdered roots of *I. digitata* are used for emaciation of children and also as tonic, alterative, aphrodisiac, demulcent, lactogogue, and cholagogue. Whereas, the decoctions of root were also used against constipation as reported by Singh *et al.* (2004). Tubers of *I. digitata* are eaten raw in Midnapure district of West Bengal and also are used in treating blood dysentery (Singh and Panda, 2005). Sun dried root power, boiled in sugar and butter is administrated to promote weight gain and to promote menstrual discharge and are also been used for treatment of debility, spermatorrhoea, fever, bronchitis, scorpion strings and menorrhagia as reported by Behera *et al.* (2006).The powdered tuber with a glass of cow milk if given for seven days is known to increase milk production in lactating mothers as reported by Jain (2011) in *Kandha* tribe of Orissa. As galactagogue, powdered root-stock is administered with wine. Asha *et al.* (2013) has reported that the powdered tuber with honey is used for high blood pressure and heart disease and also acts as cholagogue for splenic and liver enlargement.

Pharmacological Properties

Ipomoea digitata L. is a medicinal plant belonging to the family Convolvulaceae and can be used as aphrodisiac, cardiotonic, demulcent, diuretic, refrigerant and galactogogue as reported by Chopra *et al.* (1992) and it is one of the source plants of „*Vidari*", an Ayurvedic drug which is a component of about 50 Ayurvedic formulations including Chyavanaprash (Sulaiman *et al.*, 2014).

The study on ether-sol fraction of *I. digitata* showed hypotensive and muscle relaxant activity when tested in frogs, dogs, rats and rabbit (Tewati and Mishra, 1965). The methanolic extract of tuberous root of *I. digitata* were known to possess definite cardio protective activity against hyperlipidemia (Muthu *et al.*, 2007). Ono *et al.* (2009) reported that the tuberous roots of the plant *I. digitata* are brittle, mucilagenous and bitter to taste and they contain a resin which is medicinal as tonic, alterative, demulcent, lactagogue, cholagogue, etc. They also reported that dried and powdered roots of the plant are curative of spleen and liver complaints, debility, fat accumulation. The antidiabetic effects of various fractions of *I. digitata* were studied on alloxan induced diabetic in rats by Minaz *et al.* (2010). The study concluded with results demonstrating the antidiabetic potential of fractions of *I. digitata* suggesting that the plant may have therapeutic value in diabetes. The lipid profile and lipoprotein levels were significantly reduced by administration of methanolic extract of *I. digitata* has studied by Vasagam *et al.* (2010).

Ipomoea digitata L. is used in the treatments of hypolipodemic, hypogycemic, for debility, to increase secretion of milk, poor digestion, tuberculosis, enlarged liver etc. It was also found to have alterative, aphrodisiac, cholagogue, demulcent, diuretic, rejuvenative actions as reported by Chandira and Jaykar (2010). They also reported that a solid pharmaceutical dosage formulation using various excipients were statically significant as anti-diabetic. The isolation and characterization of a-sitosterol, t-cinnamic acid, an unknown coumarin and a lignan type resin glycoside

from the tuberous roots of *I. digitata* was studied by Madhavi *et al.* (2010). Importantly, one of the compounds obtained from the study exhibited significant antibacterial activity against *Pseudomonas aeruginosa* and *E. coli*. The isolated resin glycosides from the tuberous roots of *I. digitata* posed purgative properties which were of therapeutic importance. Hydroxyl radical scavenging activity of methanolic extract of *I. digtata* (Vasagam, 2010) revealed that the plant is an excellent source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Mali *et al.* (2011) reported that the hydroethanolic extract of *I. digitata* L. Conferred significant hepatoprotective activity in rat.

Ethnomedicinal Uses of Different *Ipomoea spp.*

The powdered roots of *I. aquatica* are used for treatment of diabetes (Jayaweera, 1982; Malalavidhane *et al.,* 2001) as emetic, diuretic, purgative, to treating debility, liver complaints, ringworm, leucoderma, leprosy, fever (Ghani, 1989; Mamun, *et al.,* 2003), against nose bleed and high blood pressure (Prasad *et al.,* 2005b). Scorpion venom antidote (Uawonggul, *et al.,* 2006). *I. asarifolia* is used against itch (Silva, 2002).

I. batatas is used for treatment of tumours of the mouth and throat. Leaves decotions are used as alterative, aphrodisiac, astringent, bactericide, demulcent, fungicide, laxative and tonic. Sweet potato is used to treating asthma, bug bites, burns, catarrh, ciguatera, convalescence, diarrhea, dyslactea, fever, nausea, renosis, splenosis, stomach distress, tumours, and whitlows (Duke and Wain, 1981). In region of Kagawa, Japan, a variety of white sweet potato has been eaten raw to treating anaemia, hypertension and diabetes (Ludvik *et al.,* 2004).

I.campanulata Antidote to snake poison (Singh *et al.,* 2003). *I.cairica* in the treatment of rheumatism and inflammations (Ferreira *et al.,* 2006).

I. carnea against acquired Immuno deficiency Syndrome (AIDS) (Woradulayapinij *et al.*, 2005) and to treat hypertension (Lamidi *et al.*, 2000).

The smoke of burned the roots of *I.leptophylla* is used in treatment of nervousness (Gilmore, 1977). The root for stomach distress and tonic (Barnes *et al.*, 2003).

I. muricata to treating several types of skin ailments such as chronic and gangrenous wounds, cuts and blisters due to burns. Glycerol preparations of the crude drug of *I. muricata* are used for the treatment of pharyngitis and an otic preparation for the treatment of otitis externa (Ysrael, 2003).

The smoke from the burned tree of *I.murucoides* is used against mosquitoes. Infusions of the leaves, bark and flowers to treat inflammations and against scorpion bites (Leon *et al.*, 2005).

I. nil is used for treatments against cancer (Ko *et al.*, 2004). *I. orizabensis* as purgative (Miranda, 1995), antihelmintic and to treat abdominal fever, dysentery, epilepsy, hydrocephaly, meningitis and tumours (Martinez, 1990).

I. pes-caprae in treatment of inflammatory and algesic processes (Souza *et al.*, 2000). Heated leaves are used to treating wound, skin infections, inflamed sores and stings from poisonous fish, manta-ray and insects (Infusions have been recommended for treating hypertension, kidney ailments and decoctions to treat digestive disorders, colic, internal and external pain, dysentery, inflammations, fatigue, strain, arthritis and rheumatism. The roots are used in diuretic disorders and in constipation (Diaz, 1976; Martinez, 1998; Miranda *et al.*, 2000 and Lorenzi and Matos, 2002).

2.3 MORPHOLOGY

I. digitata L.popularly called Vidarikanda is the perennial climber belonging to family Convoluaceae. Plant bears bisexual flowers, which is a long peduculate

axillary cyme, generally purple in color. Fruit is a ovoid capsule, four-celled, four-seeded with woolly seeds (Sivarajan and Balachandran, 1994; Warrier *et al.*, 1995). The plant produces tuberous roots, up to 60cm long and 30cm thick, which weighs from 5-10kg. Leaves are found to be simple, alternate, long-petioled and palmately lobed (Ashajyothi *et al.*, 2013).

Hernandez *et al.* (1965; 1967) observed the skin colour of sweet potato as a quantitative character which was controlled by several genes in complimentary action. The immature leaf colour among the entire accessions shown green colour indicating that green colour may be an incompletely dominant trait.

According to Ahmed and his co-workers in their studies on sweet potato (1996), about 48.36 per cent of the accessions were found to be semi-compact types followed by compact types (29.20%) and only 1.88 per cent of extremely spreading types by following the descriptor data.

Saraswati and Prabawardani (2007) reported that the predominant green colour of vine was due to the influence of nutrient supply (N and K), exposure to sunlight and availability of water at critical stages of crop growth.

I. digitata L. is an extensive perennial climber with large, ovoid, and tuberous roots. Leaves are large, ovate, lanceolate, acute or acuminate, palmately lobed, glabrous with prominent nerves beneath. Widely campanulate flowers born on the axillary corymbose inflorescence. Petals are purple and campanulate infundibuli form. Ovary is four celled. Capsules are small ovoid and four seeded in each fruit which is black and wooly. (www.la-mediccaindiapvt.com, 2007). The stem and leaves of the plants of *I. digitata* make fodder and the leaves also contain carotene at the rate of 6.3 mg 100 g^{-1} as studied by Ono *et al.* (2009).

Fongod *et al.* (2012) reported that 55 per cent of sweet potato leaves were slightly lobed, followed by deep lobed (30%) and very deep lobed (20%). Where, 55

per cent of leaves are single lobed followed by 20 per cent plants with 3 lobes, 15 per cent plants with 5 and 7 lobes, respectively. Further, he reported that petiole pigmentation varying from green, green with purple near leaf, green with purple near stem, green with purple spots throughout petiole, some petiole purple, others green and totally or mostly purple.

Ashajyothi *et al.* (2013) reported that dried pieces of *I. digitata* were 3 to 5 cm long, 2 to 4 cm broad and fibrous. According to them the outer surface and epidermis looked light brown in color and the epidermis was with transverse warts and ridges. They also reported that the cut surface looked creamy, fleshy, and transverse small warts and ridges were found on the surface. They observed the texture to be smooth, sweet in taste, and with no particular smell.

Qian (2013) reported that the leaves of *I. digitata* are lobed with long petioles which are heart shaped at the base. It is 5-15 cm long and lanceolate. Flowers are 3-5 beard on a stock in the leaf axil. Sepals are ovate concave and generally 1cm long. The broadly bell shaped corolla is generally pink or purple measuring about 6 cm long.

Kareem (2013) reported that medium sized plants with height ranging from 140-180 cm and medium sized nodal length (14-15cm) gave the best yield of sweet potato. He also observed genotypes with large leaf size can easily trap sunlight and hence carry out better photosynthesis required for carbohydrates synthesis than those with small leaf size.

Yooyongwech *et al.* (2014) stated that vine diameter is a genetic character and may differ from genotype to genotype under similar soil and environmental conditions. He also reported that yield potentiality of sweet potato depends on the lower length to girth ratio of tubers, better size of the tubers, more number of tubers per plant and high dry tuber yield per plant.

Mesenbet (2015) reported that leaf size varied from 5.27 cm to 11.29 cm with the mean of 9.05 cm, petiole length of *I. batatas* ranged from 6.62 to 20.79 cm with total mean of 12.6 cm, Vine internode length ranging between 2.09 and 4.97cm with the overall mean of 3.94 cm, storage root stalk length of the genotypes varying from 7.46 to 11.33 cm with the overall mean of 9.23 cm and arrangement of storage root on underground stem of the genotypes varied from open cluster (40 %), disperse (12 %) to very disperse (44%) root formations in sweet potato.

2.4 ANATOMICAL CHARACTERS

Ashajyothi *et al.* (2013) reported that the *I. digitata* L. root tubers exhibit a slightly wavy outline in its transverse section and the epidermis was reported not discernible with 3 to 4 layers of cork cells followed by 5 to 7 layers of parenchymatous cells. Cork cambium was observed to be brown in colour and 2 or 3 cells thick. Epidermis was well developed. According to them the pericycle was fibrous followed by 2 layers of stone cells which were filled with sandy crystals.

The phloem consisted of sieve tubes, companion cells, patches of bast fibres and phloem parenchyma. Xylem was observed to be pentarch in young roots which consisted of vessels with scalariform cross perforation, tracheids, xylem fibres and parenchyma. They also observed medullary rays to be broad and parenchymatous. The medullary rays in phloem cells were filled with starch grains which were polygonal with a diameter of 2 to 5 µm. It was simple or compound (two to many), hilum was usually observed to be indistinct, occasionally a central cleft, lamellae was also indistinct. In macerated preparation, crystal fibres were observed to be multicellular, articulated, each cell carried a crystal of calcium oxalate (Ashajyothi *et al.*, 2013). They also reported that a few of the articulated fibres were swollen in the middle like a bulb pipette. The powder was greyish-brown in color without any characteristic odour. It was reported to be bitter in taste. Starch filled parenchymatous cells was also observed. Septate fibers were present in the form of

crystals fibers as well as shaped bulb like pipette. Vessels were observed with perforation plates which was simple and scalariform which carried stone cells, and starch.

2.5 REPRODUCTIVE BIOLOGY

2.5.1 Phenology

Phenology is the timing of biological events which involves the study of flushing, flower initiation, development, anthesis, fruiting etc. in relation to seasons and climatic factors. (Armstrong and Drummond, 1984; Bawa *et al.*, 1985; Morgan *et al.*, 2005; Solomon Raju *et al.*, 2007; Solomon Raju and Venkataramana, 2007).

Seasonal duration of leafing, flowering, fruiting etc., are mainly determined by phenological behaviour of tropical plants. Phenological variation was observed within a species at different levels like geographical races, ecotypes and among individuals (Mandal *et al.*, 1994). Consequently, studies of flowering phenology have been used to address ecological and evolutionary questions concerning intra- and interspecific competition, community structure, keystone relationships, co-evolution, animal foraging behaviour, phylogenetic constraints etc. (Stiles, 1977; Primack, 1980; Crepet, 1983; Gross and Werner, 1983; Hamrick and Murawski, 1990; Dieringer, 1991; Ollerton, 1996; Ollerton and Lack, 1998; Fuchs *et al.*, 2003).

Varying phenological episodes within the populations may affect the distribution of the species in space and time (Loveless and Hamrick, 1984). Natural selection should produce a regular sequence of flowering times, in order to minimize competition between plant species for pollinators or to minimize interspecific hybridization (Poole and Rathcke, 1979; Cole, 1981). Newstrom *et al.* (1994) proposed a new classification for plant phenology based on flowering patterns that included frequency time series and subsidiary classes based on other quantitative descriptors such as regularity, duration, amplitude, date, synchrony and the

conceptual framework that separates patterns at each level of analysis. An analysis of the proximate controls of flowering in tropical forest species indicates that the timing of vegetative phenology strongly determines the flowering periods and hence, flowering at least depends indirectly on environmental periodicity (Rivera *et al.*, 2002).

High light intensity is clearly known to enhance flower production in moist and dry deciduous forest tracts (Nanda, 1962). Environmental factors such as light intensity, temperature, moisture, soil nutrient levels, photoperiod etc. are known to affect flowering (Sun *et al.*, 2009). In case of semi-evergreen plants, they are known to increase their plasticity through phenological asynchronicity among individuals and species (Williams *et al.*, 1997). The variation in flowering and fruiting patterns may affect the degree of genetic variability in the ensuring offspring of each species therefore, observing phenological event is a crucial step in reproductive biological studies.

Jones 1980 stated that sweet potato flowers opened soon after daybreak and generally were faded by noon depending on the environmental conditions. Reynoso *et al.* (1999) stated that sweet potato flowers were bisexual and within the species, a wide variation was generally observed in the flowering habits of different genotypes. Similar observations was made by Rossel *et al.* (2008) who reported, under normal sowing conditions, some genotypes did not flower or had scanty flowering and others flowered profusely.

Galetto and Bernardello (2004) in their study on floral nectaries in *Ipomoea species* reported flowers of Ipomoea lasted less than half a day: from 8–9 h in *I. hieronymi,* approximately 10 h in *I. cairica, I. purpurea* and *I. rubriflora* to approximately 12 h in *I. alba* and *I. indica*. With the exception of *I. alba*, whose flowers opened at twilight and faded at sunset or exceptionally at midday, the remaining species had diurnal anthesis, lasting from early morning to the afternoon.

Flowers lasted less than half a day: from 8-9h in *I. hieronymi*, approximately 10h in *I. cairica, I. purpurea* and *I. rubriflora* to approx. 12h in *I. alba* and *I. indica*. With the exception of *I. alba,* whose flowers opened at twilight and faded at sunset or exceptionally at midday, the remaining species had diurnal anthesis, lasting from early morning to the afternoon (Galetto and Bernardello, 2004). Terada *et al.* (2005) carried out research to evaluate the floral biology of *Ipomoea acuminata, I. batata, I. cairica and I. quamoclit*. They reported that *I. quamoclit* had their flowers opening at 6:00am. and their closing at 2:00pm.

Rodella *et al.* (2007) while studying the floral biology of three *Ipomoea species* reported that *Ipomoea cairica* bloomed all-round the year since the flowering amplitude was not uniform. In the rainy season, whereas *I. cairica* had a flowering peak which lasted from October to January, and in the dry season, the number of flowers decreased considerably. This flowering behavior might be associated to the avoidance of competition by pollinators. The three *Ipomoea* flowers were ephemeral, lasting only one day. Anthesis started around 05:00am and lasted mostly until afternoon, when the corollas wilted and fell off. At the opening time, for all species, the stigma was receptive and the anthers were dehisced.

Rossel *et al.* (2008) pointed out that flowering and fruit set were highest with the temperature of 20-25°C and at relative humidity above 75 per cent. They concluded that because of this condition the pollinations were more successful in the early morning. Raju *et al.* (2014) on their study of floral biology of *Ipomoea pes-caprae* reported the matured flower buds opened daily over a fairly short period of time from 07:00–08:00 h. Anther dehiscence took place by longitudinal slits within 30 min after anthesis. They also reported the pollen grains were white, ellipsoidal, spinous, sticky, and were 86.3 ±10.4 μm in size and a large circular nectary disc located at the base of the ovary secretes nectar throughout the morning until noon.

2.5.2 Floral biology

Angiosperm flowers are very diverse in shapes and colours. Functionally, the flower is a compound organ in which all its structural complexities are presumably adapted to sexual reproduction (Dafni and Firmage, 2000).

Floral biology is often correlated with the pollinator mechanisms like nectar and pollen rewards, temporal separation of male and female phases and the arrangement of floral parts which may influence pollen deposition and carry over (Endler, 1979). Floral morphology is a factor closely related to breeding system since autogamy only occurs in hermaphroditic or monoecious plants while dioecious and heterostylous plants are always out crossed (Loveless and Hamrick, 1984). Each part of the flower may have a special role in one or more events during production and dispersal of gametes and seeds. Flower is a morphological term but the bloom is a pollination unit and thus become an ecological entity (Faegri and Pijl, 1980). It is usually assumed that every floral organ has a more or less definite role in pollination but quite often replacement functions are also known (Galen, 1999; Dafni and Firmage, 2000). Each species has its own calendar of events to perform these cyclic developmental changes. A basic understanding of floral structure, phenology and pollination systems is a prerequisite for studies on reproductive biology (Dafni and Firmage, 2000).

Morning glories flowers occur in different size, shape, and color as said by Hsia and Ching-Kuan (1956); Jones (1980). They also examined that the flowers occurred in axillary inflorescences of 1 to 22 buds which opened singularly or in groups of two or more. Jones (1980) reported that in sweet potato the corolla was funnel shaped mostly with white limbs and lavender to light purple throats. His study also revealed that calyx consisted of 5 sepals, 2 outer and 3 inner, that stayed attached to the floral axle after the petals dried up and fallen down. *Ipomoea* (whose species are commonly known as „morning glory") is a cosmopolitan climbing genus from

warm and pantropical regions with approximately 650 species (Austin and Huaman, 1996) with large showy flowers that are easy to manipulate. It was postulated by Reese (1997) that in some morning glory flowering can be stimulated at 24-30°C. Its members have trumpet shaped flowers of different colours mainly white, purple, blue, pink, and red (Cronquist, 1981). Huaman (1999) reported that the androecium of *Ipomoea spp.* had five stamens with filaments that were covered with glandular hairs and were partly fused to the corolla. He also reported on the filament length, which was variable in relation to the position of the stigma and the anthers were whitish, yellow or pink, with a longitudinal dehiscence.

2.5.2.1 *cairica* (subgen. Quamoclit) with violet-pink flowers, *I. hieronymi* var. hieronymi (subgen. Eriospermum) with pink flowers, *I. indica* (subgen. Ipomoea) with blue flowers and *I. purpurea* (subgen. Ipomoea) with pink, white, or purple flowers, all bee-pollinated (Real, 1981; Rodella *et al.*, 1982; Pinheiro and Schlindwein, 1998; Galetto *et al.*, 2002).

In *I. acuminata*, the medium height of the style was 27.245 ± 2.431 mm and the medium height of the filament was 26.439 ± 3.147 mm. In *I. batatas*, the medium height of the style was 19.77 mm ± 1.956 mm that is higher than the medium height of the filament. The medium height of the style in *I. cairica* was 19.827 mm and the medium height of the filament was 17.952 mm. In *I. quamoclit* the medium height of the style and the medium height of the filament had both bigger and smaller height variations of the style (Terada *et al.*, 2005).

Elena *et al.* (2008) in their study on floral biology of the morning glory observed that twelve species of visiting insects were captured during the observation period. Of these, a halictid bee (Hymenoptera: Halictidae), a carpenter bee of the genus *Ceratina* (Hymenoptera: Anthophoridae), and a stingless bee of the genus *Trigona,* the bumble bee *Bombus atratus* and the honey bee *Apis mellifera* (all these last three insects Hymenoptera: Apidae) were regarded as effective pollinators

according to their behavior on the flowers, the distribution of pollen carried on their bodies, and contact with the stigmas. The study also showed that the beetle *Strigoderma sp.* (Coleoptera: Scarabaeidae) and two species of flies (Diptera) were only occasional visitors and ant *Conomyrma sp.* (Hymenoptera: Formicidae) was occasionally found on flowers of *Merremia macrocalyx*. Finally, two wasps (Hymenoptera: Vespidae) and the butterfly *Leodonta dysoni* (Lepidoptera: Pieridae) visited flowers to collect nectar but they did not touch pollen or stigma and they might be regarded as nectar robbers.

Angiosperms have specialized floral structures that are adaptive in promoting insect pollination. The basic adaptive strategy of insect pollinated flowering plants has been the evolution of an attractant and a reward. The attractant facilitates to enter the animal (ants, beetles, butterflies, birds etc.) to the flower either by vision (colour) or by fragrance. A visual attractant is usually a showy perianth (corolla or calyx) that may be brightly coloured or otherwise contrasting with the external environment *i.e*, white perianth at night (Simpson, 2006). Many species of flowering plants have evolved structures or exudates that act as a reward, ensuring that the pollinator will consistently return to transport pollens. The most common floral reward is nectar, a fluid rich in sugars secreted by specialized organs of the flowers called nectaries.

Raju *et al.* (2014) while studying the pollinators of *Ipomoea pes-caprae* (Convolvulaceae) revealed that the funnel-shaped flowers, with spacious corolla tube towards its rim, expose the stamens and stigma to foragers which were foraged consistently during day time from 07:00–15:00 h. and the foragers included hymenopterans, *Apis dorsata*, *Apis cerana*, *A. florea*, *Trigona iridipennis*, *Xylocopa latipes*, *X. pubescens*, *Ceratina* sp. (bees – Apidae), *Scolia* sp. (wasp – Scoliidae) and *Euplecta subdecussata* (snail – Ariophantidae: Neogastropoda). Of these, *Apis*, *Trigona* and *Ceratina* bees gathered both pollen and nectar while *Xylocopa* bees and the *Scolia* wasp collected only nectar.

Raju *et al.* (2014) experimented on melittophily and malacophily in *Ipomoea pes-caprae* (Convolvulaceae) and reported that flowers were pedicellate, large (70 mm long and 68 mm wide), showy, bisexual, and actinomorphic. The calyx was green with five ovate to elliptic sepals (20 mm long), these were united at the base and free at the distal end. its colour ranged from pink to red-purple or violet and the colour was darker at the inside base with stamens five, white, epipetalous, arising at the base of the corolla, and unequal in length; the filaments of all stamens were hairy with dithecous, introse and basifixed anthers, style was white with 12 mm long, and terminating by a bilobed, wet stigma.

2.5.3 Pollen Biology

Contribution to the pollen morphology of the family Convolvulaceae have been made by Gamble (1923), Sayeed- Uddin *et al.* (1942); Erdtman (1952); Natarajan (1957); Nair and Rehman (1963). In the family Convolvulaceae, on the basis of pollen morphology the plants may be separated into two major groups (Nair and Rehman, 1963). The basic types of pollen grain in this family are the pantoporate and spinose type from which the other morphotypes have evolved as outlined by Erdtman in his Scandinavian pollen flora (Nair and Rehman, 1963).

Pollen characters have been considered by Gamble (1923) in grouping the genera of Convolvulaceae of South India into two groups, spinulose and non-spinulose where in the species *I. digitata* belongs to spinulose group. Nair and Sharma (1962) suggested that pollen keys can be used to categorize plants of a family at various taxonomic levels. Hooker (2010) included the genus Calonyction as a sub genus of Ipomoea. Based on the nature of pollen grains, Hallier (1893) has grouped the genera with Ipomoea type of pollen grains under the Echinoconiae and all others in Psiloconiae, Ipomoea is under Echinoconiae.

In his studies on the Himalayan species *of I. purpurea*, Nair, (1965) suggested that the pollen of I. *purpurea*, differ from the rest of the species of Ipomoea, in

having large sized pollen. Variation in exine structure, sculpture, variation in the number, position and complexities of apertures in the pollen grain wall gives the morphological diversity of Convolvulaceae have been made by Gamble, (1921); Sayeed-Uddin *et al.,* (1942); Erdtman , (1952); Natarajan, (1957); Nair and Rehman, (1963); Rao and Ong, (1974) and Vij and Sachdeva (1974).

Telleria and Daners (2003) also studied the pollen of 75 species of eleven genera, and three main pollen types were described some subtypes were recognized in *I. digitata*. The maximum spine length was recorded in *Ipomoea involucrate* (8.3-9.6µm) and minimum was recorded in *Ipomoea hederifolia* (3.3-4.0µm) as reported by Jayeola and Oladunjoye (2012). Highest length in styles was recorded in *Ipomoea hederifolia* (37.0-38.5 mm) while the minimum was recorded in *Ipomoea vagans* (16.5-19.0 mm as reported by Jayeola and Oladunjoye (2012).

2.5.4 Pollen viability, Fertility, Germination and Stigma Receptivity

Pollen viability refers to the ability of the pollen to the successful completion of post pollination events on a receptive stigma to deliver functional male gametes to the embryo sac (Shivanna *et al.,* 1991). Comparative studies carried out on 2 and 3 celled pollen (Brewbaker, 1959; Hoekstra and Bruinsma, 1975; Johri and Shivanna, 1977; Hoekstra, 1979) have demonstrated a broad correlation between the cytology of pollen and their viability. Assessment of pollen viability is a critical factor for the study of pollen-stigma interaction, crop improvement and breeding programmes (Stanley and Linskens, 1974; Dafni, 1992; Mulugeta *et al.,* 1994).

Germination of pollen is the first morphogeneric event in fulfilling its function of transport and discharge of sperm cells into the embryo sac. Since pollen grains of large number of species readily germinate *in-vitro* on a simple medium it has been extensively used in studies on structural and physiological details of germination and tube growth.

Stigma receptivity

Stigma receptivity refers to the ability of the stigma to support germination and tube growth of viable and compatible pollen. Pollen viability and stigma receptivity are essential for the effective initiation of pollen-pistil interaction. Stigma surface plays a vital role in pollen recognition and screening process in relation to compatibility and incompatibility reactions (Bhattacharya and Mandel, 2003; Huang *et al.*, 2004). In general, the stigma becomes receptive by the time the flowers open and the pollen is shed or transferred. However, in self pollinated and protogynous species, the receptivity of stigma is advanced. In protandrous species, it is delayed by one day or few days in relation to flower opening (Williams *et al.*, 1991) to facilitate cross-pollination.

The pollen grains of several species of *Ipomoea* had been studied by Erdtman (1952). Nair (1965) reported the variation in the size of pollen in the 45 species of *Ipomoea* and suggested that the pollen grain of *I. purpurea* were larger, when compared to other species of *Ipomoea*.

Rao and Ong (1974) studied the pollen grains of *I. carnea* and *I. purpurea* which were spheroidal, pantoporate, surface spiniferous, interspinal areas finely reticulate and spines long surrounded basally by baculate.

Walker and Doyle (1975) found that pollen size and shape were relatively less diagnostic as they were not stable characters. A wide range of grain size and shape may occur in the same taxon. Over and above the method of pollen preparation also affected considerably the pollen size and shape.

According to Muller (1979), increase in pollen size primarily caused by increased adaptation to different systems of animal pollination.

Many reports on pollen by Muller (1979); Harder (1998); Sarkissian and Harder (2001); Tejaswini (2002) proved that wind pollinated plants had pollen that

ranged from 17-58μm, animal pollinated plants had pollen that ranged from 5-200μm and plants that had pollen carried in the water current could have possessed linear pollen up to 6mm long.

Muller (1979) reviewed that ecological factors such as climate, altitude, latitude, light intensity, water and nutrient availability also had been shown to influence pollen size; for example, intraspecific pollen size tends to increase with increased light intensity, temperature, water availability, and nutrient availability in some species. Bee grooming might have occurred while still on the flower or during flight between visits (Buchmann and Cane 1989; Harder 1990).

According to Ennos (1981) *I. hederacea* and *I. purpurea* flowers exhibited marked differences in exsertion of reproductive parts, primarily as a result of differences in style length and anther position. Whereas in *I. purpurea* the stigma was generally exerted above the anthers, in *I. hederacea* the stigma was commonly found at the same level as, and tightly surrounded by, the anthers.

The test of stigma receptivity demonstrated that *I. quamoclit* was receptive between 6:00am. and 9:00am. and *I. batata* between 6:35 am. and 1:00 pm. The period of higher viability of the pollen grains in *I. quamoclit* was the same of the stigma receptivity (Terada *et al.*, 2005).

There were highest frequencies of viable pollen grains in *I. batata* between 6:35 a.m. and 7:00 a.m.; the variations in the frequency of viability of the pollen grains keep along the day until the flowers closing (Terada *et al.*, 2005).

2.5.5 Pollination Biology

Pollination is the transfer of pollen grains from the anther to the stigma and is a critical factor for sustainable agriculture and for commercial production of hybrid seeds. It is also a basic force for gene recombination in flowering plants and plays a key role in plant breeding programmes. In angiosperms, the pollination mechanism is

typically developed in 3 phases; the release of pollen from anther, transfer of pollen from anther to stigma and finally successful placement of the pollen on the receptive surface followed by germination of pollen grains which begins the next phase of fertilization. Pollination biology in India started mostly as a descriptive science, aiming to understand plant morphology and anatomy in relation to pollination. Most of the early work on pollination biology was restricted to documenting the kind, number and time of floral visitors on various plant species (Vasudeva and Lokesha, 1993). Among the early workers Rao (1926), Parija and Samal (1936) and Narayana (1937) studied the anatomical feature in relation to pollination. Lopez *et al.* (1999) stated that, the combination of androecium, style and stigma types reflects important adaptations related to pollination.

Floral structural characters such as color, shape and size assume an important role in predicting the pollinator type. Insect pollinated plants have predominantly creamy or dull white, green and yellow flowers. However, bird pollinated species have characteristically scarlet red or bright red flowers and bat pollinated species have variously colored flowers (Solomon Raju, 2007). Insect pollinated flowers are usually having cup, salver, star and brush shaped, while bird pollinated flowers are papillonaceous, tubular and cup shaped. Flower size in most of the species is either large or medium. Small size flowers having cup, salver or star like are exclusively entomophilies.

Breeding system in plants is used as a tool to regulate the component of fecundity and other commercial traits for selection and domestication (Frankel and Galun, 1977). It has been identified as a major factor influencing genetic structure (Baker, 1959). The most obvious way to discover the breeding system of plants starts with the morphological examination of the flowers (Silvertown and Charlesworth, 2001). The breeding system of a given taxon is reflected in its floral structure, advertisement and reward. Plants can be predominantly out breeding, inbreeding or

some mixture of the two. In many flowering plants, specific mechanisms have been evolved to promote one of these systems.

Out-breeding is also known as out-crossing where allogamy or xenogamy involves the transfer of gametes from one individual to another which is genetically different. Out-crossing can be promoted by genetically determined self-incompatibility mechanisms. Self-incompatibility refers to the inability for fertilization between gametes derived from an individual genotype (Simpson, 2006). Inbreeding is referred as selfing and is the union of gametes derived from a single individual. In flowering plants, inbreeding may occur either within the single flower (autogamy) or between the flowers of the same plant (geitonogamy). The disadvantage of inbreeding is that it reduces variations in the populations and can even results in the accumulation of deleterious alleles, a phenomenon known as inbreeding depression.

Elena *et al.* (2008) in their study on reproductive biology of the morning glory showed that its flowers comprised the typical features of the melittophilous pollination syndrome. The study also reveled that the Anthophoridae, Apidae, and Halictidae were its main floral visitors. In their study *Merremia macrocalyx* showed a mixed mating system with features that promoted outcrossing while allowing some autogamy however it was self-compatible and partially autogamous due to the position of anthers above the stigma.

A intensive study conducted by Kowyama *et al.* (2000) on the genetic analysis of diploid *I. trifida,* showed the linear dominance-recessive hierarchy. These characteristic features of *Ipomoea* indicate that its self-incompatibility reactions were evaluated not as quantitative genetic traits but as qualitative genetic traits.

As pre Conley (2009), *Ipomoea habeliana* which was an endemic, night-flowering member of the Galapagos flora reported that the large, white flowers of this species set fruit via open pollination (55%), autonomous autogamy (51%), facilitated

autogamy (91%), cross-pollination (80%), diurnal open pollination (60%) and nocturnal open pollination (60%). Fruit set was pollen-limited. Ants, beetles, crickets and hawk moths regularly visited the flowers. Ants were the most frequent visitors, but hawk moths were the only effective pollinators. Nectar was available throughout the night, but was most abundant early in the evening when hawk moth visits were most frequent.

I. habeliana exhibits facultative xenogamy as studied by Conley (2009). He reported the mean pollen size was 145 ± 16 mm, which is appropriate for insect pollination and greatly outside the range for wind pollination.

Murcia (2016) while studying floral morphology in *Ipomoea trichocarpa* identified several insect taxa visiting *I. trichocarpa* flowers; one hawk moth species (Enyo lugubris) and the bumble bee Bombus pennsylvanicus visited the flower frequently. The bell shaped flowers were too wide with respect to the pollinator's head (twice the head's diameter) and the average corolla and style were significantly longer than the tongue of the major hawk moth visitor, *E. lugubris*. The results concluded that the hawk moths could potentially pollinate *I. trichocarpa* before pollen from later rising bumble bees could send tubes to ovules.

However, in *Ipomoea acuminata* the most frequent visitors are bees, but some Lepidoptera and Coleoptera are also observed. Regarding to Hymenoptera species of the families, Andrenidae, Anthophoridae, Apidae and Halictidae were registered. The most abundant and efficient species in the pollination belong to the family Anthophoridae (Rodella, 1986). In that species the formation of 14 fruits was observed with normal and healthy seeds, happening therefore self-pollination in 56 per cent of the tested flowers (Maimoni- Rodella and Rodella, 2007). These data suggest that the species is autogamic, benefiting also the visit of insects that promote certain proportion of crosspollination. This characteristic group is very frequent to the

family of Convolvulaceae (Stucky, 1984; Maimoni-Rodella and Rodella, 1986, 1986/87).

The floral biology of three weeds, *Ipomoea cairica*, *I. grandifolia* and *I. nil* (Convolvulaceae), was studied by Rodella and Yanagizawa, 2007. The three species are melittophilous, with a varied set of floral visitors, but with some overlapping.

Cluster analysis using Jacquard similarity index indicated a greater similarity among different plant species in the same locality than among the populations at different places, in relation to floral visitor sets (Rodella and Yanagizawa, 2007). This flowering behaviour may be associated to the avoidance of competition by pollinators, as pointed out by Gentry (1974a, 1974b) in relation to bignon species that share pollinators. Considering the amount of flowers produced by these *Ipomoea*, they can be regarded as important sources of nutritional rewards to flower visitors , especially in disturbed environments where habitat may become increasingly fragmented (Aizen, 2002). Such resource offer patterns are generally associated with pollination by different bee species (Frankie, 1976).

The pollen:ovule ratios indicate that *I. cairica* is alogamous and that *I. grandi folia* and *I. nil* are facultative autogamous, according to Cruden (1977). These results were confirmed in the breeding system tests.

Melittophilous pollination performed by several bee species was observed in other *Ipomoea* weeds (Real, 1981; Stucky and Beckmann, 1982; Galetto *et al.*, 2002). Butterflies, beetles and some bees were robbers (Dafni, 1992) visiting the flowers and not performing pollination.

Flower opening and closure are traits of a reproductive syndrome, as it allows pollen removal and pollination.

Compounds such as amino acids, phenols, lipids and antioxidants, are found in flowers of *Ipomoea* but mostly in trace quantities (Baker and Baker, 1975, 1983a).

All these substances often impart a particular taste and odour that may be essential for maintaining certain pollinator groups (Southwick, 1990).

I. species are visited by a diverse array of animals, including bees, hawk moths, beetles, butterflies, long-tongued flies, hummingbirds and bats (Pijl, 1954; Vogel, 1954; Schlising, 1970; Sobreira-Machado and Sazima, 1987; Donald, 1991).

I.alba (subgen. Quamoclit) with long, white, hawk-moth-pollinated flowers (McDonald, 1991), I. rubriflora (subgen. Quamoclit) with mediumsized, red, allegedly hummingbird-pollinated flowers (Wilson, 1960; Austin, 1975).

Hymenopterans were regular visitors of *I. cairica, I. hieronymi, I. indica* and *I. purpurea.* The introduced European bee (*Apis mellifera*) was occasionally observed on the study species, but most visits corresponded to native bees from the families Apidae (*Bombus opifex, B. morio*), Megachilidaem (*Megachile sp.*), Anthophoridae (*Centris sp., Thygater sp.*) and Halictidae (*Anglochloropsis sp.*) as reported by Galetto and Bernardello (2004).

In the populations of *I. cairica* and *I. purpurea* studied, both Bombus species were more frequent visitors than the other bees. On the other hand, hummingbirds were the most frequent visitors of *I. rubriflora* and sphingids (Sphingidae: *Manduca sp.* and *Agrius cingulata*] of *I. alba.* Galetto and Bernardello (2004).

2.5.6 Seed biology

Seeds provide the most natural means of plant reproduction, preservation of genetic variability, transportation and propagation of phanerogamic plants (Vazquez and Rojas, 1996). It represents a unique form of life in the plant kingdom and act as principal mode for propagating most of the species. Seed germination and early growth of seedling is considered as the most important stage in the plants life cycle (Pathak *et al.,* 1980; Akeroyd and Jackson, 1995; Hong and Ellis, 1996). The duration of seed maturity, type and germination in nature vary widely among species. Seed dispersal mechanisms can have a direct impact on the genetic structure of

populations. Species whose seeds are dispersed near the maternal plant (e.g. by gravity or wind dispersal) or species whose seeds are deposited in clumps or patches should have more fine-scale genetic structure than species whose seeds are dispersed by mobile animals (Hamrick *et al.*, 1998).

Huaman, 1999 concluded that sweet potato seeds did not have a dormancy period and could able to maintain their viability for many years.

Jones (1980) experimented that the best sweet potato seed yield could be obtained during a period of minimum daily temperature from 12.8 to 18.6°C, maximum daily temperatures of 23 to 34 °C, relative humidity from 62 to 75 per cent, and a photoperiod of about 14.5 hours and the high seed set was generally related to good flowering. Seed set in sweet potato was reduced when vine growth was rapid and vigorous. (Martin, 1968).

Wang and Burnham, (1968) stated that the sweet potato seeds matured in about one month and its seeds were matured when capsules were completely dried and browned. Capsules contained a maximum of four seeds, but the average was much less, about 1.1 to 1.7.

Martin and Cabanillas, (1968) observed that in sweet potato mature seeds were about half of their maximum green size and the seeds had diameters of 3 to 5mm and were flat on two sides and round on the other.

Rossel *et al.* (2008) found that germination was very irregular in sweet potato unless some means of seed scarification is used because of the hard seed coat.

Moisture content in seeds of morning glory collected at day 10 after blooming had a moisture content of 90.2 percentages. However, the moisture content gradually decreased until 21 days after blooming. The moisture content continued to drop to approximately 14 per cent at 28th day after blooming. From this period until 32 days

after blooming the moisture content was relatively stable (Suwanketnikom and Julakasewee, 2005).

The seeds of small white flower morning glory collected prior to day 18 after blooming could not germinate. The germination percentage of the seeds increased sharply during 18 to 20 days after blooming, and reached a peak of 96 per cent at day 21. The germination percentage of seed collected 24 days or more after blooming decreased sharply until 30 days after blooming when germination decreased to only 6 per cent (Suwanketnikom and Julakasewee, 2005).

A number of un-germinated seeds found could possibly be the result of the impermeability of the seed coat which was observed in many *Ipomoea species* (Elmore *et al.,* 1990). However, this type of dormancy might be referred to as coat-imposed dormancy while the other type of dormancy is influenced by the embryo (Bradbeer, 1988; Kelly *et al.,* 1992).

The appropriate period for collecting seeds should be during 27 to 28 days after blooming which is the field maturity period as noted by the increase in viable seeds to about 80 to 90 per cent and decrease in moisture content to approximately 14 per cent (Suwanketnikom1 and Julakasewee, 2005)

The moisture content in soil and relative humidity during seed development might affect their germination and relative humidity during seed development might affect their germination and dormancy (Morley, 1958).

Moisture content of developing seeds increased from about 70 per cent at day 0 to about 85 per cent at 10 DAP, after which it decreased gradually to about 65 per cent at 26 DAP. Between 26 and 32 DAP, seeds dried rapidly to a moisture content of 13 per cent as studied by Jayasuriya *et al.* (2007) in sweet potato.

Germinability of a portion of the seeds was attained by 20 DAP, whereas 24 DAP resulted in 100 per cent seed germination. Seeds became impermeable by 30 DAP and fail to germinate unless manually scarified (Jayasuriya *et al.*, 2007).

Ipomoea turbinata seeds became impermeable at 8.5 per cent (Chandler *et al.*, 1977), and those of *I. pes-tigridis* dried at 60° C became impermeable at 2.5-10 per cent (Bhati and Sen, 1978).

Seeds of *I. turbinata* (Chandler *et al.*, 1977) germinated to 100 per cent at physiological maturity. However, most crop seeds attain the ability to germinate to 100 per cent well before physiological maturity (Tekrony and Egli, 1995).

Seed, being a biological propagule serves as a connecting link between two generations of the plant and is responsible for maintaining the genetic continuity of the species (Noggle and Fritz, 1977). A seed thus contains the message to reconstitute an entire plant with similar shape structure and function (Van, 1981).

2.6 ROOT AND YIELD CHARACTERS

Siddique *et al.*, (1988) stated that the fresh tuber weight per plant in sweet potato varied widely with different genotypes and fresh weight of stored roots increased with the increasing length and diameter of roots.

Hossain *et al.*, (2000) in their study on genetic variability, correlation and path analysis of yield contributing characters in sweet potato noted that the root yield was positively and significantly correlated with root diameter (r=0.756), average tuber weight (r=0.729) and numbers of tuber per plant (0.635).

Rashid *et al.*, (2002) reported that the length of tuber were between 18.3- 34.3 cm and reported that the length of the tubers is inversely correlated to number of tubers and vice-versa. They also observed diameter of stored roots varied from accession to accession due to varied genetic makeup of the accessions. The

differences of tuber girth are controlled by genetic makeup of the genotypes and it is obviously varied from one genotype to another. Nwauzor *et al.,* (2006) reported that the disease invades sweet potato roots in the region of tissue differentiation and infection in the primary roots cause galling of the roots, secondary roots are deformed and tubers may crack due to nematode infestation.

Tairo *et al.* (2008) reported tubers with circumference of 6.60 to 19.4 3 cm in *Ipomoea batatas*, the accessions which had tubers with more girth produced less number of tubers per plant resulting lesser yield. As the tuber girth increased and tuber length decreased, the tuber girth to length ratio increases and vice versa. Lower the ratio better was the tuber shape and higher weights of tubers were reported contributing for higher yields. Salawu and Mukhtar (2008) in their study on yield characters of *Ipomoea batatus* observed that vine length, fresh tuber weight and tuber dry matter are positively correlated with yield components. Khan *et al.,* (2009) reported that the length of matured sweet potato tubers range between 9-12.2cm and of immature tuber length varies between 4-12.5cm. The girth of mature tubers ranging between 13-23.1cm and immature tubers 6 - 10.1cm. He also reported brownish skin color in both mature and immature tubers.

Vimala and Hariprakash (2011) reported the presence of ovate (28%), round (20.45%) and elliptic (19.6%) tuber shapes and also observed that 58.8 per cent constituted other than white color and only 8.4 per cent constituted white color of the total progenies. Richardson (2012) who reported storage stalk of varied length ranging very short (15%), short (60%) and intermediate (25%) in sweet potato. In the same crop Hammett (1966) reported that tuber shape is controlled by additive gene effect and tuber color is controlled by a few genes with partial dominance.

Yahaya *et al.* (2015) in their study on Growth and Yield Components of Sweet Potato (*Ipomoea batatas* L.) reported that yield was found to be significant ($p<.01$) and positively correlated with number of leaves per plant, number of roots

per plant and average root weight. Vine length was negatively correlated to average root weight and root yield, whereas number of roots per plant was positively correlated to the average root weight and root yield.

Saad *et al.* (2015) in their study on yield parameters of *Ipomoea batatus* noted that the percentage (direct) contribution of number of leaves was 0.1961. Similarly, the contributions of the vine length, number of roots and average root weight were 0.0096, 3.2907 and 59.0114, respectively. This re-emphasize the importance of number of roots and the average root weight in contributing to root yield in sweet potato.

2.7 PHYTOCONSTITUENTS

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to as "secondary metabolites" of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004).

The study by Mishra and Datta (1962) reported the presence of 1.3 per cent fixed oil in *I. digitata* L. tubers which consists of palmitic (8.15 per cent), oleic (60.10 per cent), linoleic (19.38 per cent), and linolenic acids (1.11 percent). Four antimicrobial glycosides were isolated from *Ipomoea bahiensis*. Among which one of these compound revealed significant activity against Sarcoma 180 in mice (Bieber *et al.*, 1986).

A glycoside called paniculatin isolated from the tubers of *I. digitata*, showed a stimulant effect on myocardium and respiration, a vasoconstrictor and bronchoconstrictor effect, a spasmogenic effect on smooth muscles of gut, as well as it elevated the blood pressure and also presented oxytocic activity (Matin *et al.*, 1969).

Indolizidine alkaloids were isolated from *I. alba* which demonstrated non-addictive analgesic properties (Wang and Chu, 1996; Honda *et al.,* 2003). Moreover it showed inhibitory effects on respiratory burst of leukocytes and scavenged oxygen-free radicals (Chen and Chu, 1998).

An indole-type alkaloid called Ipomine A was isolated from the hairy roots of *I. batatas* (Yuan *et al.,* 2004). Other constituents isolated from *I. digitata* are taraxerol, taraxerol acetate, *N*-butyl-β-Dfructopyranoside, octadecyl (*E*)-*p*-coumarate and the coumarins umbelliferone, scopoletin, scopolin (Dai *et al.,* 2000) and scoparone (Rao *et al.,* 1974). Scopoletin and taraxerol inhibited AChE (acetylcholinesterase) activity. These AchE inhibitors were important for the treatment of Alzheimer's disease (Lee *et al.,* 2004). Vitamin C, caffeic acid and flavonoids, such as, rutin, quercetin (Guan *et al.,* 2006), were also found in this species.

Isochlorogenic acids a, b and c isolated from *I. aquatica* showed inhibitory activity of disaccharide-degrading enzyme. They also showed the possibility for the use of these substances as a food additive and a remedy for the prevention and treatment of diabetes and obesity as studied by Okudaira *et al.,* (2005). The dried tuber of *I. digitata* were used for clinical study by Girishthakur and Baghel, (2007) to count the total sperms in patients of oligozoospermia.

From the tuber of *Ipomoea batatas* two saponines were isolated as reported by Dini *et al.* (2009). The study also showed that the tuberous roots of *Ipomoea batatas* contain a large amount of storage protein being sporamin the major (Sun *et al.,* 2009).

The tuberous roots of *I. digitata* showed the presence of Isobutyric, (*S*)-2-Methylbutyric, Tiglic, *n*-decanoic, *n*-dodecanoic and Cinnamic acids and two glycosidic acids, Quamoclinic acid A and Operculinic acid A. Further, a new resin glycoside, named Digitatajalapin was also extracted from *I. digitata* (Ono *et al.,* 2009).

Jayakar and Chandira (2010) in their study on formulation and evaluation of herbal tablets containing *I. digitata* L. extract indicated the presence of alkaloids, carbohydrate, glycoside, saponins, phytosterols, flavanoids and proteins in the extracts of tuberous roots of *I. digitata* L.

Mungole *et al.* (2010) in their study on preliminary phytochemical screening of *Ipomoea obscura* a hepatoprotective medicinal plant reported the presence of alkaloids, steroids, triterpenoids, coumarins, flavonoids, phenolics, cynogenic glycosides, anthraquinones, acubins, irodoids and tannins.

Yadav and Agarwala (2011)"s work on phytochemical analysis of some medicinal plants revealed the presence of proteins, carbohydrates, phenols, tannins, flavonoids, saponins in the plants *Ipomea aquatica.*

Phytochemical screening of the ethonolic extract of *I. digitata* ensured the presence of alkaloids, tannis, steroids, glycosides, gums, carbohydrates and saponins which confirms the use of *I. digitata* as an analgesic agent. (Monjur-Al-Hosin *et al.,* 2013).

Nilofer Sheikh (2013) in his study on phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species confirms the presence of various phytochemicals like flavonoids, saponins, steroids, cardiac glycosides and terpenoids.

Sharma and Bachheti (2013) in their study on review on *Ipomoea carnea* reported the presence of steroids, saponins, xanthoproteins, flavonoids, tannins, glycosides, alkaloids, carbohydrates and phenolic compound.

Kalitha *et al.* (2013) in their study on biochemical estimation of primary metabolites and mineral composition of *Clitoria ternatea* Linn roots revealed highest amount of carbohydrate and crude protein along with the mineral composition of the root of the plant *Clitoria ternatea* L.

Sahu *et al.* (2014) in his study on phytochemical analysis *Cajanus cajan* showed the presence of saponins, tannins, alkaloids flavonoids, anthraquinones and reducing sugars. He reported that the presence of these bioactive compounds like phenol, alkaloids, flavonoids, saponins and tannins in *Cajanus cajan* L. probably contributes to its medicinal properties making it widely used in traditional medicines.

Sahu and Gupta (2014) in their study on medicinal plants of morning glory of Central India reported the presence of flavonoids, gum, alkaloids, saponins, tannins, carbohydrate and steroids.

Khan and Hossain (2015) isolated Scopoletin and β-sitosterol glucoside from ethanol extracts of roots of *I. digitata* which was very much important for its medicinal value.

Devhade *et al.* (2015) in their study on preliminary phytochemical analysis of *Merremia dissecta* (Convolvulaceae) reported the presence of alkaloids, glycosides, phytosterols, saponins, tannins and steroids in both leaf and root extracts.

2.7.1. Quantitative Estimation

Venkata *et al.* (2010) reported 35 per cent of flavanoids and 41 per cent of alkaloids from methanolic extracts of different medicinal plants.

Arvind *et al.* (2010) reported total alkaloid (0.31 mg, 0.11 mg and 0.48 mg g^{-1}), total saponins content (140 mg, 120 mg and 149 mg per g^{-1}) and total flavanoids (2.3 mg, 1.3 mg and 1.8 mg g^{-1}) of sample in leaf, stem and seed of *I. obscura.*

Sutharsingh *et al.* (2011) reported alkaloids (0.86±0.023 mg g^{-1}), flavanoids (0.56±0.037 mg g^{-1}) and saponins (2.86±0.023 mg g^{-1}) from the crude powder of *Naravalia zeylanica.*

Dhar (2012) reported carbohydrate content of 0.83 mg g^{-1} (*I. purpurea*), 0.57 mg g^{-1} (*I. alba*), 0.58 mg g^{-1} (*I. batatas*) and 0.67 mg g^{-1} (*I. staphylina*). Protein

content of 1.05 mg g^{-1} (*I. hederacea*), 0.78 mg g^{-1} (*I. alba*), 0.91 mg g^{-1} (*I. aquatica*), 0.89mg g^{-1} (*I. batatas*) and 0.99 mg g^{-1} (*I. carnea*).

Arockiamary *et al.* (2014) revealed the presence of major phyto-compounds like saponins (23.46±0.31 mg g^{-1}) and flavanoids (3.70±0.32 mg g^{-1}) from the tuber powder of *I. batatas*.

Hemalatha *et al.* (2014) reported the presence of primary metabolites of carbohydrate (0.462±0.012mg g^{-1}), protein (13.29±0.244 mg g^{-1}) and amino acid (2.224±0.160 mg g^{-1}) from *I. staphylina*. The chloroform extract showed the presence of steroids, alkaloids, glycosides. Ethyl acetate extract showed the positive test for steroids, alkaloids, glycosides and flavanoids. The methanolic extract showed the presence of sugars, amino acids, proteins, fats, alkaloids, glycosides, flavanoids, gums and mucilage. The presence of flavanoids in plant extracts were further confirmed by chromatographic technique (TLC).

Devakumar *et al.* (2014) reported flavonoids or bioflavonoids are a class of plant secondary metabolites referred to as Vitamin P with permeability to vascular capillaries. Consumption of flavonoid-rich foods triggered the increased production of uric acid, a potent antioxidant in the blood. He reported total alkaloid content of 0.57-0.88 mg g^{-1}, flavanoid content of 143.19-250.59 µg $g^{-1}$and saponin content of 2.8-4.27 mg g^{-1}among acetone and aqueous extracts of *S. cumini* and *S. jambos*.

Essiett *et al.* (2014) reported proteins from *Ipomoea alba* (16.01%), *Ipomoea nil* (7.0%) and *Ipomoea batatas* (4.0%) of protein, carbohydrate content of *Ipomoea alba* (60%), *Ipomoea nil* (57%) and *Ipomoea batatas* (40%), cyanogenic glycoside 0.001- 0.002 mg $100g^{-1}$ respectively.

Sahu *et al.* (2014) reported alkaloids (2.65%: 2.65%: 2.51%), saponin (5.97%: 6.35%: 4.98%) and (4.77%: 2.11%: 5.44%) of flavonoids from leaves, seeds and stem extracts of *Cajanus cajan*.

Pradeepa *et al.* (2016) reported bioactive phytoconstituents such as, carbohydrates (74 mg g^{-1}dw), protein (41.25 mg g^{-1}dw), chlorophyll (2.2±0.05 mg g^{-1}dw), lipids (0.07 mg g^{-1}dw), tannins (135.3 gm g^{-1}dw), phenolic compounds (123.75 mg g^{-1}dw) and flavonoids (50 mg g^{-1}dw) were found to have higher amount in ethanolic extract followed by acetone, methanol and aqueous extract of *pelargonium graveolens*.

Viji and Paulsamy (2016) reported higher contents of phenolics (285.05mg GAE g^{-1} extract), tannins (190.02mg GAE g^{-1} extract) and flavonoids (174.44mg RE g^{-1} extract) in acetone tuber fraction of *I. mauritiana*.

2.7.2 Principal Constituents of *I. digitata* L.

ß-sistosterol, scoparnone (Tiwari *et al.*, 1964), ß-sistosterol 3-O-ß-D-glucoside, umbelliferone, taraxerol acetate, scoparnone (Mendez, 1979), scoparone (Rao, 1984), N–butyl- ß-D-fructopyranoside, ocetadecyl (Dai *et al.*, 2000), scopoletin, 7-O-ß -D- glycopyranosyl scopilitine (Khan *et al.*, 2009) and sistosterol, t-cinnamic acid (madhavi *et al.*, 2010).

2.8 CHROMATOGRAPHY

Rabah *et al.* (2004) in their study on the extract from baked sweet potato confirmed the potential cancer-preventing effects. They reported the results of chromatography which showed strong radical scavenging effects on DPPH radical coinciding with the high content of total phenolic compounds.

Douglas *et al.* (2004) studied the quantitative analysis of sesquiterpene lactones in *Arnica montana* and the study revealed that the different percentages at different stages of flower maturity and the total content of sesquiterpene lactones increased progressively with maturity of flower (0.512% in buds to 0.943% in withered).

Khan *et al.* (2009) studied the concentration of chemical constituents between mature and immature tubers of *I. digitata* by finding the differences in their phytoconstituents using modern tools like HPTLC and HPLC. The results reflected the quantitative phytochemical difference among mature and immature tubers. The mature tubers contain better concentration of phytoconstituents than the immature source, which concluded the authenticity of traditational recommendation. A comparative study of HPLC and HPTLC method has been developed for the quantitation of umbelliferone from dry tuber powder of *Ipomoea mauritiana* Jacq. The study showed that both the methods are of similar efficiency, sensitivity and can be used for determination umbelliferone from dried tuber powder of *Ipomoea mauritiana* Jacq.

Maurya *et al.* (2009) reported that the centrifugal partition chromatography in the pH-zone-refining mode was successfully applied to the separation of alkaloids, directly from a crude extract of *Ipomoea muricata*. The results showed pH-zone-refining centrifugal partition chromatography produced efficient separations of two clavine alkaloids from gram quantities of *I. muricata* crude alkaloid extract.

Dighe and Adhyapak, (2011) conducted a comparative study of HPLC and HPTLC method for the quantitation of umbelliferone from dry tuber powder of *Ipomoea mauritiana* Jacq. and proved that both the methods are of similar efficiency, sensitivity and can be used for determination of umbelliferone from dried tuber powder.

Reddy *et al.* (2013) reported that methanolic extract of *Ipomoea staphylina* leaves were subjected to column chromatography and two compounds namely Sitosteryl-3- O-β-D-glucoside and chiro deoxy inositol were isolated and characterized by various spectroscopic and chromatographic techniques and these two constituents were found to be reported in *Ipomoea staphylina* for the first time.

2.9 DIVERSITY ANALYSIS

Prakash *et al.* (1995) in their study on analysis of genetic diversity of *Ipomoea batatus* reported the degree of polymorphism in the sweet potato collection was very large, indicating a high level of genetic variability. Their study showed that several accessions clustered together were based on their geographic source and most of the USA cultivars formed a separate cluster in the phenogram, while accessions from Papua New Guinea exhibited the highest genetic diversity. The wild species *Ipomoea triloba* and tetraploid *Ipomoea batatus* formed a group distinct from the cultivated sweet potato.

Singh *et al.* (2013) in their study on genetic diversity analysis of *Nardostachys jatamansi* cluster analysis revealed three distinct clusters: I, II and III. The clustering of these populations was independent of variations in altitude and geographical locations. They reported the genetic variations observed in different populations of Jatamansi might be due to environmental influences (biotic and abiotic), rather than altitude level differences. The also reveled abiotic (geographical or climatic differentiation) and biotic (pollination between population and seed dispersal) factors might be responsible for the genetic variations among these accessions of Jatamansi. The study also showed that minimum genetic similarities (0.38) were exhibited between the accessions collected from cluster II and cluster III whereas, maximum genetic similarity of 0.52 was observed for accessions collected from cluster I and cluster III.

Koussao *et al.* (2014) in their study on diversity analysis of sweet potato (*Ipomoea batatas* [L.] Lam) performed hierarchical cluster analysis and within the 112 sweet potato accessions they reported that the characters such as leaf lobe number, leaf lobe type, petiole pigmentation, vine tip pubescence, predominant flesh colour, storage root formation, storage surface, showed a high polymorphism (0.75).

Koussao *et al.* (2014) in their study on diversity analysis of *Ipomoea batatas* [L.] Lam) in Ghana performed hierarchical cluster analysis and reported identical accessions. They reported that the accessions BF1 and BF3 from two close villages in the central region were found to be identical similarly the accession BF13 from the central south was identical to accession BF62 from the Eastern region, BF18 from the central south and BF61 from the Eastern region BF80 and BF68 from the Hauts-Bassins were also identical as were BF65 and BF63 from the same region.

Zawedde *et al.* (2014) while studying genetic diversity of sweet potato (*Ipomoea batatas*) in Uganda, Kenya, Tanzania, Ghana, Brazil and Peru reported the Ugandan collection showed a large number of distinct landraces and very low (3%) levels of genetic diversity between genotypes obtained from the different agro-ecological zones and there was low (6%) levels of genetic diversity observed between the East African genotypes; however unique alleles were present in collections from the various sources.

Ganie *et al.* (2015) while studying genetic diversity of *Convolvulus pluricaulis* (Convolvulaceae), differentiated two major clusters. First represented a solitary genotype of Jodhpur (Rajasthan) and the second, the genotypes of Kurukshetra (Haryana), Delhi, Bhopal (Madhya Pradesh), Udaipur, Jaipur (Rajasthan) and Lucknow (Uttar Pradesh). The genotypes of Delhi and Bhopal shared highest level of similarity coefficient (0.87). The sample collected from Jodhpur (Rajasthan) had the highest degree of divergence and shared a similarity coefficient of only 0.58 with the rest of the genotypes. The intra-zonal diversity was maximum among Rajasthan populations followed by Haryana populations whereas in other samples, intra-zonal diversity was negligible.

2.10 VARIABILITY COMPONENTS

Sivasubramaniah and Menon (1973) classified coefficient of variations as, PCV and GCV values greater than 20 per cent are regarded as high, values between

10 per cent and 20 per cent to be medium whereas values less than 10 per cent are considered to be low.

Singh *et al.* (1993) reported that, if the heritability of a character is very high around 80 per cent or more, selection for such character is fairly easy. This is because there would be a close correspondence between the genotypic and phenotypic variations due to relatively small contribution of the environment to the phenotype expression of the trait.

Singh (1993) suggested that, if heritability is less than 40 per cent selection may be considerably difficult or virtually impracticable to improve the characters due to the masking effect of the environment on the characteristics of genotype.

Johnson *et al.* (1955) indicated though heritability estimates provide basis for selection on the phenotypic performance, the estimates of heritability and genetic advance should always be considered simultaneously. This is because high heritability is not always associated with high genetic gain.

Nedunzhiyan and Reddy (2000) opined, in general the genotypic correlation coefficients were observed to be higher than the corresponding phenotypic correlation coefficients in magnitude for most of the characters indicating inherent association among most characters. Thus, indicating the suppression of phenotypic expression under the influence of environmental factors and the presence of inherent association between various characters.

Teshome *et al.* (2004) reported positive and highly significant association number of tubers and the number of storage root per plant, fresh tuber yield per plant. He also observed that the characters number of tuber and number of storage root per plant are economical characters directly correlating with yield.

Tsegay *et al.* (2007) reported that if both PCV and GCV estimates were high it indicates high genetic variability for effective selection. The PCV values were

greater than GCV but the differences between the two values were narrow indicating variability due to genetic constituent of the genotypes was less influenced by environmental factors.

Ali *et al.* (2008) suggested that selection is hardly possible to improve traits which exhibited low values both for heritability and genetic advance (or) moderate and low values combinations. This is due to the higher influence of environment on the expression of the characters and limits the scope of improvement by selection due to the presence of non-additive (dominant and/ or epistasis) type of gene action.

Kapinga *et al.* (2011) reported high heritability for proteins, internode length and alkaloids. Traits with moderate heritability values suggested the limited scope of improvement and with low heritability the practically impossibility of improvement of these characters through selection.

Das *et al.* (2012) indicated that traits with low PCV and GCV values suggested the higher influence of environment for their expression and thus the phenotypic basis selection would not be effective for the improvement of the trait.

Thiyagu *et al.* (2013) reported that, high GCV and PCV could be an advantage as they can offer opportunity for selection of superior genotypes with respect to the character of interest. Particularly high GCV is an indication of the less influence of environmental factors in the expression of such traits and the higher possibility to improvements through selection and hybridization.

Wassu *et al.* (2015) reported the lowest PCV and GCV values for leaf length, leaf breadth and internode length. The traits with low PCV and GCV values suggested the higher influence of environment for their expression and thus the phenotypic basis selection would not be effective for the improvement of the trait.

2.11 CLIMATE AND HABITAT

The plant *I. digitata* L. belongs to family convolvulaceae and is found throughout tropical Asia. It"s a large perennial creeper having tubers weighing up to 3kg. it is common in states like Eastern Bihar and Tarai region of Uttar Pradesh (Mishra and datta, 1962).The plant is predominantly found in India in the east including Bihar, Orissa, West Bengal, Assam, and the west coast from Konkan to Kerala. It grows mostly in moist areas, monsoon forests and in coastal tracts. The plant is also grown for ornamental purposes and trained against trellises and pillars. In Kerala, it is found in Kasaragod, Cannanore, Calicut, Malappuram, Trichur, Kottayam, Pathanamthitta and Trivandrum districts. In fact it is found almost throughout the State, in the low and midlands (Mishra and Datta, 1962).

In India the species is generally seen in deciduous and evergreen forests, coastal tracts and widely naturalized in tropical parts of the world. (Sivarajan and Balachandran, 1994).

Climatic conditions, such as time of day, precipitation and outside temperature, have a significant influence on the physical, chemical and biological status of medicinal plants. Sunshine duration, the average height of rainfall, average temperature and thermal amplitude between day and night also influence the physiological and biochemical activity of plants (Endrias, 2006).

Materials and Methods

3. MATERIALS AND METHODS

The research project "Diversity analysis and reproductive biology of milk yam (*Ipomoea digitata* L.)" was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2014-2016. The details of the materials used and methods adopted during the course of investigation are presented below.

3.1 SURVEY AND PHYTOCHEMICAL SCREENING

3.1.1 Survey

An ethno botanical survey was conducted along the natural growing tracts of Kerala and twenty accessions (both tubers and vines) were collected. Information regarding habit, habitat, and ethno-medicinal uses of the accessions were also collected during the survey.

3.1.2 Climate data and soil type

In addition to survey the relevant details on soil type and climatic details *viz.*, geographic location, altitude (meters above MSL), mean temperature (^{0}C), rainfall (mm) and average relative humidity (%) of the places were also recorded.

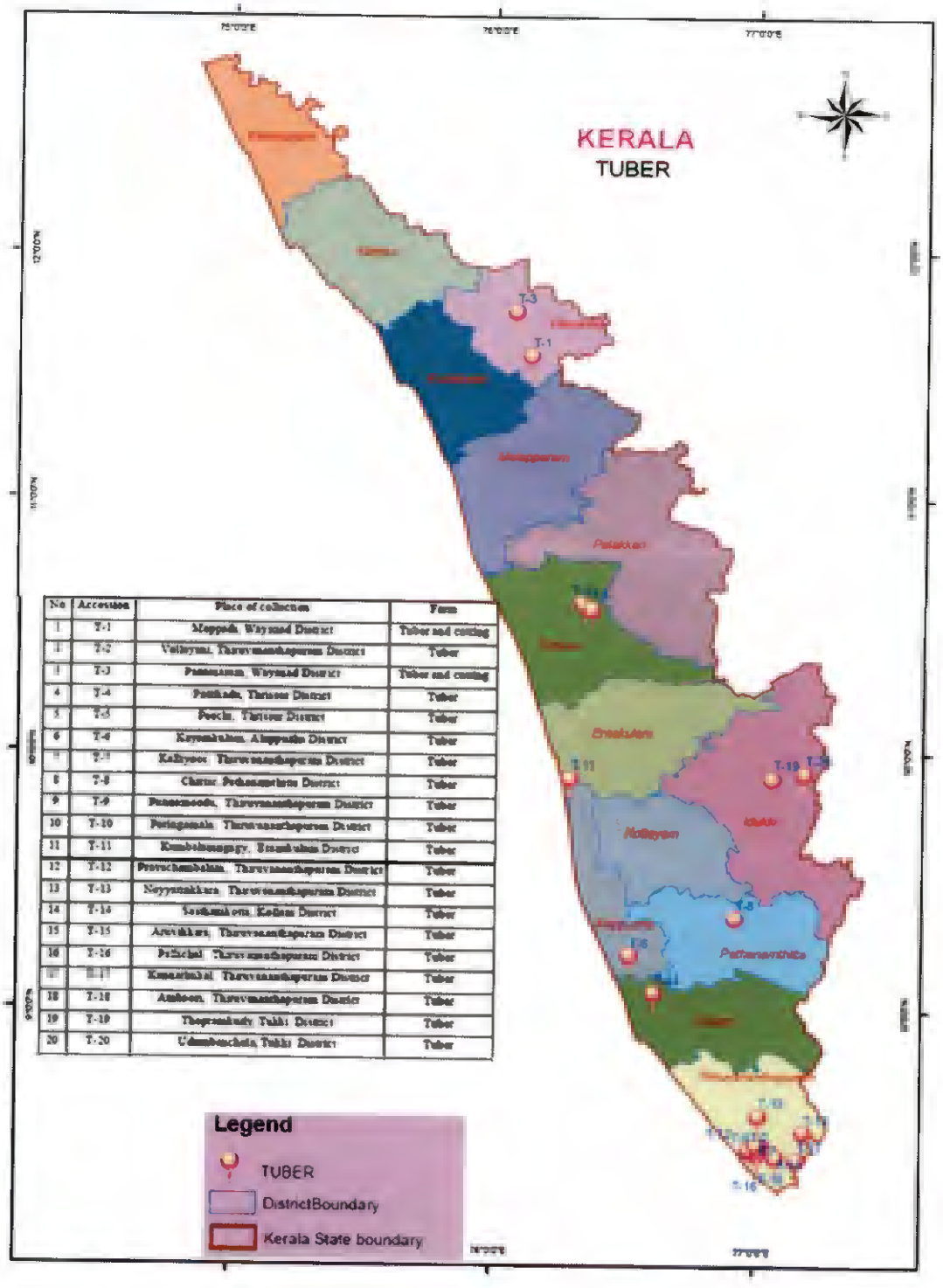
BIOMETRIC OBSERVATIONS

3.2.1`Experimental site

The experiment was carried out in the experimental field at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram District, Kerala during 2014-2016.

3.2.2 Climatic conditions

Geographically Vellayani area (Kalliyoor panchayat, Thiruvananthapuram district) lies in the Zone –III of the Region –II in the agro climatic zone of Kerala. It is situated at 8° 30" North latitude and 476° 54" East longitude and at an altitude



No	Accession	Place of collection	Form
1	T-1	Meppadi, Wayanad District	Tuber and cutting
2	T-2	Vellayani, Thiruvananthapuram District	Tuber
3	T-3	Panamaram, Wayanad District	Tuber and cutting
4	T-4	Pattikadu, Thrissur District	Tuber
5	T-5	Poochi, Thrissur District	Tuber
6	T-6	Kayamkulam, Alappuzha District	Tuber
7	T-7	Kalliyoor, Thiruvananthapuram District	Tuber
8	T-8	Chirayi, Pathanamthitta District	Tuber
9	T-9	Punnamoodu, Thiruvananthapuram District	Tuber
10	T-10	Poovachal, Thiruvananthapuram District	Tuber
11	T-11	Kumbalangapzy, Ernakulam District	Tuber
12	T-12	Poovachalkonam, Thiruvananthapuram District	Tuber
13	T-13	Neyyattinkara, Thiruvananthapuram District	Tuber
14	T-14	Sasthamcotta, Kollam District	Tuber
15	T-15	Aruvikkara, Thiruvananthapuram District	Tuber
16	T-16	Pirappancode, Thiruvananthapuram District	Tuber
17	T-17	Kunnarimalai, Thiruvananthapuram District	Tuber
18	T-18	Amboori, Thiruvananthapuram District	Tuber
19	T-19	Thoppumudy, Taluk, District	Tuber
20	T-20	Udumbanchola, Taluk, District	Tuber

Plate 1. Survey details of 20 *I. digitata* L. accessions collected during the study.

of 29 m above the mean sea level. The field experiment was conducted during January 2014 to December 2015. A warm humid tropical climate is experienced in the area. The data on various weather parameters, *viz.*, weekly rainfall, maximum and minimum temperature, relative humidity and sun shine hours during the period are presented in Appendix II.

3.2.3 Soil Characteristics

The soil of the experimental site is comprised of red loam soil and belongs to Vellayani series which comes under the order Oxisol.

3.2.4 Experimental Details

The field experiment was laid out using accessions of milk yam collected from twenty different places. Tubers of all collected accessions were planted in the field and maintained as mother plants. From these mother plants hard wood single node vine cuttings were prepared and planted in polythene bags and kept under shade. The cuttings sprouted within a week and better sprouting was observed during the month of January. Rooted cuttings were maintained under the shade condition for one month followed by gradual exposure to sun light for hardening. Healthy rooted cuttings with at least four to six leaves were used for transplanting (Plate 2). The field experiment was laid out in RBD with three replications as detailed below:

Crop : *Ipomoea digitata* L.

Design : Randomized Block Design (RBD)

Treatments : Twenty (*I. digitata* L.) accessions.

T1	Meppadi, Wayanad District
T2	Vellayani, Thiruvananthapuram District
T3	Panamaram, Wayanad District
T4	Pattikadu, Thrissur District
T5	Peechi, Thrissur District

(A) Single node cuttings

(B) Rooted cuttings

(C) Nursery

Plate 2. Nursery plot view of *I. digitata* L.

T6	Kayamkulam, Alappuzha District
T7	Kalliyoor, Thiruvananthapuram District
T8	Chittar, Pathanamthitta District
T9	Punnamoodu, Thiruvananthapuram District
T10	Peringamala, Thiruvananthapuram District
T11	Kumbalangy, Ernakulam District
T12	Pravachambalam, Thiruvananthapuram District
T13	Neyyattinkara, Thiruvananthapuram District
T14	Sasthamkotta, Kollam District
T15	Aruvikkara, Thiruvananthapuram District
T16	Pallichal, Thiruvananthapuram District
T17	Kunnathukal, Thiruvananthapuram District
T18	Amboori, Thiruvananthapuram District
T19	Thopramkudy, Idukki District
T20	Udumbanchola, Idukki District

Replications : Three

No of plants per row in each replication: 5

Row planting is used for each treatment with spacing of one meter between rows and 90cm between plants within each row. Morphological observations were recorded from all the five plants.

3.2.5 Cultural operations

The details of various operations carried out during the course of investigation are furnished below.

3.2.5.1 Land Preparation

The experiment field was made to a fine tilth by digging and harrowing. The experimental site was divided into three blocks (three replications) and hundred mounds were formed in twenty rows per block. The treatments were allotted randomly to different rows using table of random numbers.

3.2.5.2 Planting

As a prophylactic measure, the rooted cuttings were treated with Confider (0.2 ml l^{-1}) and Bavistin (2 g l^{-1}) before transplanting. The rooted cuttings were transplanted along with the ball of earth intact at a depth of 10 cm in the mounds taken at a spacing of 0.9 m x 0.9 m. Light irrigation was provided immediately after
planting. View of the experimental plot after planting is presented in Plate 3.

3.2.5.3 Manuring

Farm yard manure @ 2 kg $mound^{-1}$ was applied at the time of planting and 19:19:19 fertilizer mixture was given as foliar feeding at 15 days interval at the rate of two g l^{-1} up to three months of planting.

3.2.5.4 Irrigation and Interculture

Irrigation was provided daily to the rooted cuttings till establishment. Alternate days of irrigation were given after three months of plant establishment except during rainy days. Gap filling was done to replace missing and damaged plants. Two hand weedings were done during each month to keep the crop free of weeds.

3.2.5.5 Plant Protection Measures

Euchiomyia polymina caterpillar (Amitidae) was observed as a serious pest feeding the foliage (Plate 6). The crop was sprayed with neem oil at 2 ml l^{-1} and catch and kill rule was followed by destroying the egg colonies. There were no other serious incidence of pest and disease was observed during the crop growth period.

(A) Mother plot

(B) Field view/Main plot

Plate 3. Field view of *I. digitata* L.

Biometric observations were recorded on various growth and yield characters at three months interval. All the five plants in each row were selected. The selected plants were tagged for recording the observations. Some of the morphological characters of *I. digitata* L. accessions were catalogued based on the descriptor (0-9 scale) developed for sweet potato by IPGRI (1991). The details of all the 33 morphological characters scored using descriptor values are presented in appendix 1.

3.2.6 Vine characters

3.2.6.1 Twining

The ability of the vine to climb the adjacent stakes showing twining characters were recorded as per the descriptor data (Fig 1.) as slightly twining, moderately twining and twining.

3.2.6.2 Length of Main Vein (cm)

The length of the plant was measured from the ground level to the tip of the main vein with the help of scale and was expressed in centimetre (cm) and average value of five plants was computed and recorded as length of main vein at 3^{rd}, 6^{th}, 9^{th} and 12^{th} months. The accessions were categorized in to different growth habits depending on the length of the main stem as erect (< 75 cm), semi-erect (75 - 150cm), spreading (151 – 250 cm) and extremely spreading (> 250 cm) types (IPGRI, 1991).

3.2.6.3 Internode Diameter (cm)

Internode diameter was measured with the help of a thread and scale. Average expression of at least three internodes located in the middle section of the vine was recorded and was expressed in centimetre. Based on the average internodes diameter, the accessions were categorized as very thin (< 4 mm), thin (4 - 6 mm), intermediate (7 - 9 mm), thick (10 - 12 mm) and very thick (>12 mm).

3.2.6.4 Internode Length (cm)

Three internodal lengths in the middle of the main vine were measured. Based on the average internodal length internodes of the accessions were categorized as very short (< 3 cm), short (3 - 5 cm), intermediate (6 - 9 cm), long (10 - 12 cm) and very long (>12cm).

3.2.6.5 Predominant Color of Vine

Predominant color of vine was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7 Leaf characters

3.2.7.1 Type of Leaf Lobes

Type of leaf lobes was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991) and were categorised as slightly lobed, moderately lobed and deeply lobed patterns.

3.2.7.2 Number of Leaf Lobes

Number of leaf lobes was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7.3 Shape of Central Leaf Lobe

Shape of central leaf lobe was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7.4 Matured Leaf Size (cm)

The leaf length of fully developed leaves from leaf base to leaf apex was measured using a meter scale and expressed in centimetre. The breadth was also measured in a same manner across the leaf in mid portion of the leaf and expressed in centimetre.

3.2.7.5 Mature Leaf Color

Mature leaf color was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7.6 Immature Leaf Color

Immature leaf color was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7.7 Petiole Pigmentation

Petiole pigmentation was recorded as per the catalogue based on the descriptor (0-9 scale) (IPGRI, 1991).

3.2.7.8 Petiole Length (cm)

The length of leaf petiole was measured from the base to the insertion point with the help of scale and was expressed in centimetre.

3.2.8 Inflorescence

Inflorescence characters *viz.,* flowering habit, flower size, flower color, shape of limb, sepal shape, sepal apex, sepal pubescence, sepal color, color of stigma, color of style, stigma exertion and seed capsule set were recorded as per the catalogued values based on the descriptor data (0-9 scale) developed for sweet potato by IPGRI (1991).

3.3 REPRODUCTIVE BIOLOGY

3.3.1 Flowering phenology

Fifty healthy plants were randomly selected from the populations in experimental site and daily observations were made on flushing and flowering phenology which included flowering season, flower initiation and development, anthesis and anther dehiscence. Flower openings were noted by tagging ten

flowering vines from different individuals and observing at 30 minutes intervals for 12h (Mathur and Mohan Ram, 1986).

- Emergence of vegetative buds and leaves.
- Number of flowers per inflorescence and number of inflorescence per plant.
- The time of flower opening (anthesis), anther dehiscence and flower longevity.
- Peak period of flowering-the time of maximum number of flowers/ individual and flowering plants in a population.
- Duration of flowering- the number of days flowers are produced in a population.
- Fruit initiation, development, maturation and dehiscence.
- Pest and disease incidence.
- Seed germination and seedling establishment in natural populations.

3.3.2 Days Taken from initiation till opening of flower buds

The date and time of initiation and completion of anthesis (opening) of each tagged flowers was noted and the mean was recorded.

3.3.3 Frequency of flower formation

The time gap between the openings of one flower to another flower which was confined for few days was noted at daily intervals and recorded in days.

3.3.4 Size of fully matured flower bud (cm)

This observation was taken based as length (cm) and breadth (cm) of fully opened flower bud of selectively tagged buds.

3.3.5 Diameter of fully opened flower (cm)

Diameter of fully opened flower was taken with the help of scale and was expressed in centimetre.

3.3.6 Longevity of flower

Flower longevity was recorded based on the length of time the flower remained open and functional, *i.e.* till it faded on each sample plant. The data was expressed in hours.

3.3.7 Time of anthesis and anther dehiscence

Observation regarding time of anthesis was recorded for one week. Matured flower buds were selected and tagged for recording the observation. The data were recorded from 4:30 am to 7:00 am at 30 minutes interval and the opened flowers were removed from the plant after each count, to avoid recounting. Dehiscence of anthers was studied by careful opening of matured flower buds without injuring the stamens. Observations were made under strong incident light. Each observation was based on at least ten matured floral buds.

3.3.8 Stigma receptivity and *in-vivo* pollen germination

The receptivity of the stigma was assessed by hand lens and manual pollination experiments on the stigmas of flowers at different maturity stages (before anthesis to 12 hours after anthesis).

In vivo pollen germination was investigated by observing pollen germination and pollen tube entry into the stigma in manually pollinated pistils (xenogamy) using aniline blue fluorescence method (Shivanna and Rangaswamy, 1992).

According to this technique, flowers at different stages of anthesis i.e., one day prior to anthesis and on the day of anthesis were tagged. The flowers were plucked in every hour gaps for pollinating the flowers. The pollinated pistils fixed for about 24hrs were processed for further studies on pollen germination and

pollen tube growth. The pistils were mounted in 0.005% decolourized aniline blue prepared in 0.05M phosphate buffer at pH 8.2. A drop of 50% glycerine (aqueous) was added in the mounting medium to prevent drying. The tissues were spread by applying gentle pressure on the cover slip. The preparations were observed under a (Leica DME, Germany) microscope and photographs were taken.

3.3.9 *In-vitro* pollen germination

In-vitro pollen germination was conducted to determine the effect of different nutrients like sucrose, boron and calcium nitrate at various concentrations. Different grades of sucrose (5, 10, 15, 20% etc.) were prepared and used individually and also in combination. In addition to this, Brewbaker and Kwack medium (Brewbaker and Kwack, 1963) was also used for *in-vitro* germination test. One or two drops of each medium were placed separately on a clean glass slide. Freshly dehisced, uncontaminated pollen grains were added into the solution and spread thoroughly. The slides were incubated in petridishes lined with moist filter paper for 24 h. After incubation, a drop of cotton blue was added to it and allowed to disperse. Pollen grains which had produced pollen tubes longer than the diameter of the pollen were recorded under a Leica DME light microscope at low magnification (10 X 10) and the same were counted as viable. The percentage of pollen grains and tube elongation were noticed under light microscope and average was calculated.

$$\% \text{ of pollen germination} = \frac{\text{Number of pollen grains germinated}}{\text{Total number of pollen grains observed}} \times 100$$

3.3.10 Pollen fertility and viability

Pollen fertility was assessed by acetocarmine glycerine staining technique. Fresh pollen grains collected from the different flowers were transferred to a clean slide. To this two drops of acetocarmine glycerine mixture (1:1) was added and

mixed thoroughly. After 15 minutes the slides were examined under (Leica DME, Germany) light microscope. The numbers of stained and unstained pollen grains were counted. The stained pollen grains were considered as fertile whereas the unstained, undersized, partially stained and shrivelled pollen grains were counted as sterile. The pollen fertility was calculated as,

$$\text{Pollen fertility} = \frac{\text{No. of well filled and uniformly stained pollen grains}}{\text{Total no of pollen grains}} \times 100$$

3.3.11 Pollen viability by TTC test

0.3% of 2, 3, 5-Triphenyl Tetratzolium Chloride (TTC) in five per cent sucrose (to prevent the bursting of pollen grains) stock solution was prepared. Special precaution has been taken to avoid the photo-oxidation of TTC and stored in amber coloured bottle under refrigeration. A drop of solution was added in a clean micro slide, a small amount of pollen grains was suspended and distributed uniformly. The preparations were incubated in dark humidity chamber (95% RH) under the laboratory temperature for 30-60 min. The dark red colored pollen grains were counted as viable (Shivanna and Rangaswamy, 1992).

3.3.12 Pollen viability by IKI (Iodine + Potassium iodide) test

Potassium iodide (1 g) and 0.5 g iodine were dissolved in 100 ml distilled water for preparing IKI solution. Pollens were placed in IKI solution for five minutes and viability was assessed by counting darkly stained pollens.

3.3.13. Floral morphology

Morphology of the flower and flower parts were studied by using a hand lens and conventional dissecting microscope. The measurements of the floral parts such as length of sepal, petal, stamens and pistils were recorded on ten flowers selected randomly at each population with the help of scale and vernier callipers.

3.3.14 Pollen morphology

For pollen morphological studies, the anthers at the time of anthesis were collected and preserved immediately in 70 per cent ethanol or glacial acetic acid. Pollen preparations were made by the acetolysis method proposed by Erdtman (1952). The preserved materials were transferred to a centrifuge tube and crushed with a glass rod. The dispersion was sieved through a brass mesh of 48 divisions cm^{-2} and was collected in a glass centrifuge tube. After centrifugation, the supernatant was decanted and the pollen grains after washing in glacial acetic acid were treated with acetolysis mixture consisting of acetic anhydride and concentrated $H_2 SO_4$ (9:1) in the centrifuge tube. A glass rod was placed in each tube and was transferred to a water bath at 70-100 °C for three to five minutes, till the medium became brown in colour. After centrifugation, the supernatant was decanted off and glacial acetic acid was added to the sediment and again centrifuged and acid was decanted. The permanent slides of acetolysed pollen grains were made by mounting them in glycerine jelly and sealing the edges with paraffin wax. Photomicrographs of the sample were taken at appropriate magnification using a Leica DME light microscope. The average size of the pollen grains was measured from a random sample of 100 pollen grains from each treatment.

3.3.15 Scanning Electron Microscopy (SEM)

SEM preparations were made to study the ultra structure of pollen. The steps used in the processing for SEM are as follows.

Step I. Fixation

Fresh stigmas were collected during their receptive period and washed thoroughly with 0.015M sodium phosphate buffer (pH 7.2), after which these were fixed in 2 per cent glutaraldyhyde for 4 hrs. Following fixation, the stigmas were washed with 0.015M sodium phosphate buffer (pH 7.2) and dehydrated in an ethanol series (25 %, 50 %, 70 %, 80 %, 90 % v/v and absolute) for 10 minutes in

each grade. They were then passed through a mixture of ethanol and amyl acetate solution for 5 minutes in room temperature.

Step II. Critical point drying

The dehydrated stigmas with pollen grains were dried in a critical point drier (H.C.P-2 Hitachi). The dried specimens were mounted on stubs using double sided adhesive tape and coated with gold palladium in a sputter (model E-101-Hitachi). The coated specimens were then observed under Scanning Electron Microscope (S-2400, Hitachi) at low voltage and photographs were taken. Surface pattern was described following the terminology used by Heslop-Harrison (1990, 1992).

3.3.16 Mode of pollination

Four different pollination experiments were conducted in the field during 2015-2016. The treatments such as autogamous self-bagged, geitonogamy, xenogamy and open (natural) pollination were detailed below. The percentage fruit set of the variously modified flowers was counted and compared with reproductive success under the natural conditions. The pollination experiments were carried out on selected plants at the time of maximum stigma receptivity. A minimum of thirty flowers were chosen for each experiment. In addition, the weight of each seed was also recorded.

3.3.16.1 Autogamous Self-pollination

Autogamous self-pollination (pollens of same flower) was carried out to check, whether the species is self-compatible or incompatible. Artificial self pollination was conducted to test for active autogamy while spontaneous self pollination (passive pollination) was investigated by bagging the intact flowers (one day before anthesis) using pollination bags made of fine bride veil to prevent cross pollen. The pollinated flowers were bagged again and periodically observed for fruit set.

3.3.16.2 Geitonogamous Pollination

For geitonogamous pollination, flower buds of one day before anthesis were emasculated (androecium removed) and bagged. The emasculated flowers were pollinated with the pollen grains from different flowers of the same plant and bagged again. The observation on fruit set, number of days for fruit development and maturation were also observed.

3.3.16.3 Natural /Open Pollination

Flowers of field grown plants were tagged at the time of anthesis. Observations were made on day to day basis on fruit and seed set, number of days taken for fruit initiation, development and maturation were recorded.

3.3.16.4 Xenogamous Pollination

In the xenogamous pollination experiments, the flower buds (one day before anthesis) were emasculated (androecium removed) and bagged. During the peak period of stigma receptivity, the emasculated flowers were pollinated with pollen grains from flowers of different plants and pollen grains from different populations. Fruit development and maturation were noticed periodically.

3.3.17 Foraging behaviour of insects

As the flowers are showy and attractive the pollinators get attracted to the plants and observations were made on floral visitors. The numbers of floral visitors, percentage of floral visit were visually analysed and the data were recorded. Foraging period and nature of the individual visitors were observed. Percentage of visit of individual pollinators was calculated by the following formula.

$$\text{Percentage of visit} = \frac{\text{Number of individual pollinator visited the flower}}{\text{Total number of pollinator visited the flower}} \times 100$$

The insects were identified with the help of taxonomists at the Department of Agriculture Entomology, College of Agriculture, Vellayani, Kerala Agricultural University.

3.3.18 Time taken for fruit maturity

The number of days taken from flowering to fruit maturity was recorded by color change in fruit from green to brown and finally drying. The data was measured in number of days. Fruit development was studied from the day of pollination until maturation and dehiscence. Matured fruits from each accession were harvested and seeds were collected. The average number of fruits developed in each population and average number of seeds developed per fruit were also calculated.

3.3.19 Seed size, shape and weight

Seed size was recorded in centimetre using scale, visual observation was made to record the seed shape. Individual seed weight and hundred seed weight was recorded using weighing balance.

The proportion of the seed set was also calculated based on the number of seeds produced per flower by natural pollination in relation to the number of ovules per flower.

3.3.20 Seed moisture content

To analyze the moisture content of the seeds, the mature capsules were collected randomly before dehiscence. The seeds were carefully removed from the capsules and weighed in an electronic balance to record fresh weight of the seed. The weighed seeds were transferred to hot air oven and kept overnight at 80 ^{0}C and the dry weight was measured. Seed moisture content was calculated by the formula developed by the International Seed Testing Association (Bewley and Black, 1994).

$$\% \text{ of seed moisture content} = \frac{(\text{Fresh weight of seeds} - \text{Dry weight of seeds})}{\text{Fresh weight of seeds}} \times 100$$

3.3.21 Seed viability

The viability of the seeds was analyzed as per the method suggested by Enescu (1991). The seeds (0-4 weeks old) were collected and soaked in distilled water for 24 hours for the absorption of water. The seed coats were pierced without damaging the embryo. Few drops of tetrazolium-phosphate buffer solution (pH 7.5) was added and incubated at 30^0C for 24 hours. After the incubation period, the seeds were washed thoroughly with water and observed under microscope. The completely stained seeds were counted as viable and readings were recorded.

3.3.22 Seed germination

Matured fruits were covered with paper bags before dehiscence. The seeds were collected from individual plants separately during the fruiting seasons. The seeds were collected and stored in the laboratory conditions. Seeds were allowed to germinate in the plastic trays filled with garden soil. Three replicates of 50 seeds were germinated every week to determine the optimal week for seed germination and seedling establishment. Quantitative features such as the number of days taken for seed germination and percentage of seed germination in the field as well as in the laboratory conditions were also analyzed periodically. The percentage of seedling establishment was also calculated.

3.4 ROOT CHARACTERS

3.4.1 Storage root formation

Arrangement of the storage roots on the underground stem was visually assessed at harvest and recorded as closed cluster, open cluster, dispersed and

very dispersed by comparing the observed storage roots arrangement as per IPGRI (1991) descriptor.

3.4.2 Storage root stalk (cm)

This was recorded as the average of five storage roots stalk length (one storage root from each plant in net plot) which was measured as the length of stalk joining storage roots to the stems.

3.4.3 Number of storage roots per plant

The number of roots per stock of the tuber was counted and the mean of five plants were recorded.

3.4.4 Variability of storage root shape

This was recorded by visual observation and comparing with the descriptor data.

3.4.5 Variability of storage root size

These were recorded by visual observation and comparing with the descriptor data

3.4.6 Storage root cracking

These were recorded by visual observation and comparing with the descriptor data.

3.4.7 Predominant skin color

The predominant color of freshly harvested storage roots of each accession were recorded as white/ cream/ yellow/ orange/ brown/ pink/ red/ purple-red/ dark purple as per descriptor data (IPGRI, 1991).

3.4.8 Predominant flesh color

The predominant flesh color of the freshly harvested storage root were recorded following descriptor data (IPGRI, 1991).

3.5 CONSERVATION STRATEGIES

3.5.1 Vegetative propagation

From selected healthy plants hard wood, single node stem cuttings of 10-12 cm length were prepared and the oblique cut ends were dipped in 0.1% Bavistin (fungicide) for 5 minutes to avoid fungal contamination and then washed to remove excess amount of fungicide. After the treatments, the stem cuttings were planted in 15x25 cm polythene bags containing coir pith, sand and farmyard manure (3:2:1). They were kept under shade net for root initiation. The replicates of 50 cuttings were planted for each treatment.

3.5.2 Seed collection and propagation

Seeds were collected from each accessions during 2015-2016 and 100 seeds were sown in trays with mixture of coco peat. These trays were observed daily, recorded germination and development of rooted cuttings for months. Surviving individuals were transplanted to polythene bags.

3.6 HARVESTING

The crop was harvested at 365 DAP. While harvesting the whole plants were uprooted manually and the roots were separated by cutting. The roots were cleaned, washed and the observations were recorded on tuber characters. Along with this crop the mother plot was also harvested and two year old tubers were also collected.

3.7 YIELD CHARACTERS

The plants were harvested by pulling up individual plants at 365 DAP.

3.7.1 Number of tuber

The cluster tuber from the stalk region was counted and recorded. The mean of five tubers were computed and recorded.

3.7.2 Length of tuber (cm)

The length of the tuber in all five plants was measured using scale and expressed in centimetre.

3.7.3 Girth of tuber (cm)

Girth of the thickest portion of the tuber was measured and the mean diameter of all the five tubers per plant was computed and expressed in centimetre.

3.7.4 Fresh tuber yield per plant (g)

The harvested tubers of five plants were cleaned and weighed individually by using the electronic balance and the mean was worked out and recorded.

3.7.5 Dry tuber yield per plant (g)

The freshly harvested tubers from five plants were sliced and dried separately in oven at 50°C for seventy two hours till constant weight was obtained. The individual dried root was weighed in an electronic balance and mean weight was recorded.

3.8 PHYTOCHEMICAL SCREENING

Collected tubers of each accession were powdered and samples were subjected to systematic phytochemical screening by successive extraction (1:1 ratio) of samples in different solvents (methanol, ethanol, chloroform and hydro-ethanolic extract). Both qualitative and quantitative analyses of phytoconstituents were done following different procedures (Plate 7).

3.8.1 Preparation of extracts

20 g each of powdered sample (tuber powder) was soaked separately in 100 ml of methanol, ethanol, chloroform and hydro-ethanol for 24 h at 40^0C in water bath. The obtained extract was filtered using Whatman No. 1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till golden viscous mass was obtained. The prepared extracts were stored for further analysis.

3.8.2 Qualitative screening of phytoconstituents

3.8.2.1 Test for Alkaloids (Wagner's Reagent Test)

Fifty mg of the extract was stirred with few ml of dilute hydrochloric acid and filtered. Then three to five drops of Wagner"s reagent [1.27 g of iodine and 2 g of potassium iodide in 100 ml of water] were added through the side of the test tube and observed for the formation of reddish brown precipitate. The formation of reddish brown precipitate indicates the presence of alkaloids.

3.8.2.2 Test for Carbohydrates (Molisch's Test)

Few drops of Molisch"s reagent were added to two ml portions of the extracts followed by addition of two ml of concentrated H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet ring colour at the inter phase of the two layers confirmed the presence of carbohydrates.

3.8.2.3 Test for Glycosides (Keller Kelliani's Test)

Five ml each of extracts was treated with two ml of glacial acetic acid in a test tube containing one drop of five per cent ferric chloride solution followed by addition of one ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of glycosides. Appearance of violet ring below the brown

ring and greenish ring in the acetic acid layer indicated the presence of cardiac glycosides.

3.8.2.4 Test for Saponins (Foam Test/ Froth Test)

Fifty mg each of the extracts was diluted with distilled water and the volume made up to 20 ml. The suspension was shaken vigorously in a graduated cylinder for 15 minutes. Development of two cm layer of foam indicated the presence of saponins.

3.8.2.5 Test for Phytosterols

One ml each of the extracts was dissolved in 10 ml of chloroform and equal amount of concentrated sulphuric acid was added from the sides of the test tubes. The upper layer turned red and the lower layer showed yellow with green fluorescence. This indicated the presence of steroids.

3.8.2.6 Fats and Oils

To five drops of the sample were added one ml of one per cent copper sulphate solution and a few drops of 10 per cent sodium hydroxide. The formation of a clear blue solution confirmed the test.

3.8.2.7 Resins

To the extracts three to four ml of $CuSO_4$ / copper acetate was added separately and shaken vigorously for one to two minutes. The resulting solution was allowed to separate. Formation of green coloured precipitate indicated the presence of resins.

3.8.2.8 Test for Flavonoids (Alkaline Reagent Test)

Two ml of extracts was treated with few drops of 20 per cent sodium hydroxide solution. Formation of intense yellow colour, which becomes

colourless on addition of dilute hydrochloric acid, indicated the presence of flavanoids.

3.8.2.9 Proteins (Ninhydrin Test)

Two ml of filtrate was treated with two to five drops of ninhydrin solution placed in a boiling water bath for one to two minutes and observed for the formation of purple colour which indicated the presence of proteins.

3.8.3 Quantitative estimation of phytoconstituents

3.8.3.1 Alkaloids (%)

The total alkaloid content (%) in tubers harvested was estimated following the Gravimetric method (Harborne, 1973; Soni and Sosa, 2013). The flow chart for detailed estimation procedure is as follows:

0.5g of the sample was weighed and dispensed in 10 ml of 10 per cent acetic acid solution in ethanol. The mixture was shaken well and allowed to stand for four hours and then filtered. Later the quarter of the original filtrate volume was evaporated on a hot plate and 30 per cent ammonium hydroxide was added to precipitate the alkaloids. A pre-weighed Whatman No. 1 filter paper was used to filter off the precipitate, after the filtration the filter paper with the precipitate was dried in oven at 70 °C for 30 minutes, and then it was transferred to desiccators to cool and reweighed until the constant weight was obtained. Percentage alkaloids were worked out following the formula;

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{\text{Weight of the sample (g)}} \times 100$$

Where, W_1 is the pre-weighed filter paper; W_2 is the weight of the filter paper with the residue.

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3.8.3.2 Carbohydrates (%)

Quantitative estimation of carbohydrates in the samples (tuber powder) was estimated by Anthrone Method. Percentage carbohydrates was calculated as per the following formula,

$$\frac{\text{Absorbance of unknown}}{\text{Concentration of unknown}} = \frac{\text{Absorbance of standard}}{\text{Concentration of standard}}$$

3.8.3.3 Glycosides (%)

Total glycoside (%) was estimated by gravimetric method (Ibraheem and Maimako, 2014). Percentage glycoside was calculated as follows:

$$\% \text{ Glycosides} = \frac{W_2 - W_1}{\text{Weight of the sample (g)}} \times 100$$

Where, W_1, is the weight of the pre-weighed dish; W_2, is the weight of the dish with the precipitate.

3.8.3.4 Saponins (%)

Percentage saponin content in the tuber was estimated by gravimetric method (Rai *et al.*, 2013). Percentage saponin content was calculated as follows:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Weight of the sample (g)}} \times 100$$

Where, W_1, is the weight of the pre-weighed dish; W_2, is the weight of the dish with the precipitate.

3.8.3.5 Oil (Cold Percolation Method)

Estimation of oils was carried out by the method suggested by Sadasivam and Manickam (1991) and percentage oil is calculated as follows:

$$\text{Oil in ground sample (\%)} = \frac{\text{Weight of oil in sample (g)}}{\text{Weight of sample (g)}} \times 100$$

3.8.3.6 Fatty acid (mg KOH g^{-1})

One gram of oil was dissolved of in 50 ml of the neutral solvent in a 250 ml conical flask and a few drops of phenolphthalein was added. The contents was Titrated against 0.1N potassium hydroxide and shaken constantly until pink color was formed which persists for 15 s.

$$\text{Acid value (mg KOH } g^{-1}) = \frac{\text{Titer value x normality of KOH x 56.1}}{\text{Weight of the sample (g)}} \text{ X } 100$$

3.8.3.7 Flavonoids (mg^{-1})

Total flavonoid content (mg g^{-1} of sample) is determined by Aluminium chloride method using Quercetin or Rutin as standards (Vijay and Rajendra, 2014).

3.8.3.8 Proteins ($mg\ g^{-1}$)

The total protein content (mg gm^{-1} of sample) in tubers was estimated following the Lowry"s Method.

3.9 CHROMATOGRAPHY

Thin layer chromatography (TLC) was attempted in the present study, in which the sample was applied as a small spot on to the origin of at thin sorbent

layer (silica gel) supported on a glass or metal plate. The mobile phase moved through stationary phase by capillary action, sometimes assisted by gravity or pressure. Mobile phase consisted of a single solvent or mixture of solvents. The separated spots are visualized using reagents and identified by calculating the R_f value.

R_f values were determined using the formula;

$$\text{Retardation factor } (R_f) = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

3.9.1 Optimization of TLC solvent system

Different solvent system were tried for developing a TLC system for identification of constituents present in the extract, based on the literature survey and keeping in mind the chemical nature of the constituents.

One showing maximum separation was selected as mobile phase for the study. The different solvents were used for the development and detecting agent were tried to standardize the TLC procedure as follows,

- TLC Plate- Silica gel GF_{254} plates
- Sample- Crude hydroalcoholic extract of *I. digitata* L.
- Solvent system-
- Pet. Ether: Benzene
- Benzene: Chloroform
- Chloroform: Ethyl acetate
- Chloroform: Methanol
- Toulene: Ethyl acetate

HPLC analysis was also carried out to detect the flavonoids and alkaloids present in the sample. It was out sourced by following the procedure detailed below.

Powdered tubers of *I. digitata* L. were extracted by refluxing with water. 2.5 g each of both mature and immature tubers were powdered and refluxed using water as solvent for 45 minutes. The extracts were filtered and concentrated using Rotary evaporator. The sample (2.5 g aqueous extract in 10 ml water) was subjected to HPLC and the profile for both mature and immature tubers were the same. An HPLC instrument of Shimadzu made with injection volume of 20µl at flow rate of 1.0ml/minute run for 30minutes. The HPLC system consisting of LC-10ATVP pump, a rheodyne injector, SPDAVP UV-Visible detector and CLASS-VP6 software was used for the analysis. Merck C-18 (250 x 4.6mm) column with stationary phase of 5µ particle size was used and equilibrated with the initial solvent ratio for an hour. The mobile phase, consisting a gradient system of methanol and water where pump A: Water and Pump B: Methanol. Concentration of methanol increased from 10-50 percent within 0.01-20 min and rose to 100% at 30.00 minutes. The detection was done at 254 nm.

3.10 STATISTICAL ANALYSIS AND INTERPRETATION

3.10.1 Analysis of Variance (ANOVA)

The biometric observations recorded from the field evaluation were subjected to analysis of variance (Panse and Sukhatme, 1957) for the comparison among various accessions and to estimate variance components. The significance of mean sum of squares for each character was tested against the corresponding error degrees of freedom using F test (Fisher and Yates, 1967).

3.10.2 Estimation of genetic parameters

3.10.2.1 Genetic components of variance

For each character, the phenotypic and genotypic components of variance were estimated by equating the expected value of mean squares (MS) to the respective variance components (Jain, 1982). The variance components were estimated based on this. It is as follows:

i. Genotypic variance (GV)

$$GV = \frac{MST - MSE}{r}$$

ii. Environmental variance (EV)

$$EV = MSE$$

iii. Phenotypic variance (PV)

$$PV = GV + EV$$

Where, MSE=Mean sum of squares for error,

MST= Mean sum of squares for treatments.

3.10.2.2 Coefficients of Variation

The components namely, phenotypic, genotypic and environmental variances were used for estimation of coefficient of variation at both phenotypic and genotypic levels for all the traits were computed by following the formula as suggested by Singh and Chaudhary (1985).

i. Phenotypic coefficient of variation (PCV)

$$PCV = \frac{\sqrt{PV}}{\bar{x}} \times 100$$

ii. Genotypic coefficient of variation (GCV)

$$GCV = \frac{\sqrt{GV}}{\bar{x}} \times 100$$

PV- Phenotypic variance

GV- Genotypic variance

$\bar{x}$ - The mean of each character estimated over all the treatments.

The PCV and GCV was classified by Subramanian and Menon (1973) as,

Low - (<10%)

Moderate - (10-20%)

High - (>20%)

3.10.2.3 Heritability

For each trait, broad sense heritability (H^2) was calculated as the ratio of genotypic variance to phenotypic variance and expressed as percentage (Allard, 1999).

$$\text{Heritability } (H^2) = \frac{GV}{PV} \times 100$$

Heritability was categorized by Robinson *et al.* (1949) as

Low - (<30%),

Moderate - (31-60%)

High - (>60%)

3.10.2.4 Genetic Advance

Genetic advance, which is the measure of genetic gain under selection, depends upon standardized selection differential, heritability and phenotypic standard deviation. The genetic advance was calculated by the method proposed by (Fehr *et al.*, 1987)

$$\text{Genetic advance (GA)} = k.H^2 \sqrt{PV}$$

Where k is the standardized selection differential (2.06 at 5% selection)

Genetic advance as percentage mean was estimated using the formula given by Johnson *et al.* (1955).

$$\text{GA as percentage of mean} = k.H^2 \frac{\sqrt{PV}}{\bar{x}} \times 100$$

Genetic advance (% mean) was categorized as per the suggestion of Al-Jibouri *et al.* (1958).

Low - (0-10%)

Moderate - (10-20%)

High - (>20%)

3.10.3 Correlation analysis

Phenotypic, genotypic and environmental correlation coefficients were calculated using the respective variances and co-variances of the characters which showed significant variation in the ANOVA as suggested by Singh and Choudhary (1985).

Phenotypic correlation coefficients, $r_P = \frac{COV_P(X,Y)}{\sqrt{PV(X).PV(Y)}}$

Genotypic correlation coefficient, $r_G = \frac{COV_G(X,Y)}{\sqrt{GV(X).GV(Y)}}$

Where, COV_P (X,Y) and COV_G (X,Y) respectively denotes the phenotypic and genotypic co variances between the two traits X and Y. PV (X) and GV (X) denotes the phenotypic and genotypic variance for X and PV (Y) and GV (Y) indicate the phenotypic and genotypic variance for Y respectively.

3.10.4 Mahalanobis D^2 Analysis

The genetic divergence between genotypes was estimated using Mahalanobis"s D^2 statistic (1936). The distance D from the sample was computed using the formula.

$$D^2p = d^1 S^{-1} d$$

Where,

D^2p = Square of distance considering „p" variables

d =Vector observed differences of the mean values of all the characters $(x_{i1} x_{i2})$

S^{-1}= inverse of variance and covariance matrix

3.10.5 Clustering of D^2 values

All the genotypes used were clustered into different groups following Tocher"s method (Rao, 1926). The intra and inter distance were also computed the criterion used in clustering to the same cluster should atleast on the average, show a smaller D^2 values than those belonging to different clusters.

The device suggested by Tocher (Rao, 1952) was started with two closely associated populations and find a third population which had the smallest average of D^2 from the first two. Similarly, the fourth was chosen to have a smallest average D^2 value from the first three and so on. The permissible average D^2 value is fixed as the maximum D^2 values among all lowest D^2 values among the genotypes. If at any stage increase in average D^2 value exceeded the average of already fixed, because of the addition of a new genotypes, then that genotypes was deleted. The genotypes that are included already in that group were considered as the first cluster. This procedure was repeated till D^2 values of the other genotypes were exhausted omitting those that were already included in the former cluster and grouping them into different cluster.

3.10.5.1 Intracluster Distances

The intracluster distances were calculated by the formula given by Singh and Choudhary (1977).

$$\text{Intracluster distance square} = \frac{\Sigma Di^2}{n}$$

where,

ΣDi^2 is the sum of distance between all possible pairs of combinations of the entries included in a cluster.

n = Number of all possible pairs of combinations

3.10.5.2 Intercluster Distances

The intercluster distances were calculated by the formula described by Singh and Choudhary (1977).

$$\text{Square of the intercluster distance} = \frac{\Sigma Di^2}{n_i x n_j}$$

Where,

ΣDi^2 is the sum of distance between all possible pairs of combinations ($n_i n_j$) of the entries included in the clusters i and j.

n_i = Number of entries in cluster i

n_j = Number of entries in cluster j

Results

4. RESULTS

The study entitled, "Diversity analysis and reproductive biology of milk yam (*Ipomoea digitata* L.)" was carried out in the department of Plantation Crops and Spices, College of Agriculture, Vellayani, Thiruvananthapuram during 2014-2016. The data collected from the field experiment were statistically analyzed and the results are presented in this chapter.

4.1. SURVEY AND COLLECTION

4.1.1 Survey

During the present investigation the field survey was conducted during the month of January 2014, in the natural growing tracts of milk yam in Kerala, to collect accessions. During the survey apart from relevant details on soil and climate from the place of collection, ethnobotanic information regarding milk yam were also collected. The details of twenty accessions collected from eight different districts of Kerala are presented in the Table 1.

The collected accessions were named after the place of collection which included two accessions from Wayanad district [Meppadi (T1) and Panamaram (T3)], ten accessions from Thiruvananthapuram district [Vellayani (T2), Kalliyoor (T7), Punnamoodu (T9), Peringamala (T10), Pravachambalam (T12), Neyyattinkara (T13), Aruvikkara (T15), Pallichal (T16), Kunnathukal (T17), Amboori (T18)], two accessions from Thrissur district [Pattikadu (T4) and Peechi (T5)], one accession from Alappuzha district [Kayamkulam (T6)], one accession from Pathanamthitta district [Chittar (T8)], one accession from Eranakulam district [Kumbalamngagy (T11)], one accession from Kollam district [Sasthamkotta (T14)] and finally two accessions from Idukki district [Thopramkudy (T19) and Udumbanchola (T20)].

Table 1: Details of *I. digitata* L. accessions collected in the study.

Sl.no.	Accession	Place of collection	Form
1	T1	Meppadi, Wayanad District	Tuber and cutting
2	T2	Vellayani, Thiruvananthapuram District	Tuber
3	T3	Panamaram, Wayanad District	Tuber and cutting
4	T4	Pattikadu, Thrissur District	Tuber
5	T5	Peechi, Thrissur District	Tuber
6	T6	Kayamkulam, Alappuzha District	Tuber
7	T7	Kalliyoor, Thiruvananthapuram District	Tuber
8	T8	Chittar, Pathanamthitta District	Tuber
9	T9	Punnamoodu, Thiruvananthapuram District	Tuber
10	T10	Peringamala, Thiruvananthapuram District	Tuber
11	T11	Kumbalangy, Eranakulam District	Tuber
12	T12	Pravachambalam, Thiruvananthapuram District	Tuber
13	T13	Neyyattinkara, Thiruvananthapuram District	Tuber
14	T14	Sasthamkotta, Kollam District	Tuber
15	T15	Aruvikkara, Thiruvananthapuram District	Tuber
16	T16	Pallichal, Thiruvananthapuram District	Tuber
17	T17	Kunnathukal, Thiruvananthapuram District	Tuber
18	T18	Amboori, Thiruvananthapuram District	Tuber
19	T19	Thopramkudy, Idukki District	Tuber
20	T20	Udumbanchola, Idukki District	Tuber

4.1.2 Climatic data

Climate data for all the accessions were collected from the meteorological departments of the respective districts and are presented in Table 2. The climate data collected during the survey includes geographical location, altitude, temperature both maximum and minimum, soil type, mean relative humidity and rainfall of the particular places.

4.1.2 Ethno-botanical information

In addition to the climate data the ethno-botanical information of the accessions were also collected from the place of collection, which are presented in Table 3. From the collected data it was observed that in Thiruvananthapuram District the tuber powder along with cow milk was mainly used as galactagogue and with honey as a tonic whereas, in Kollam District the root powder was used as galactagogue and tonic for children. Pathanamthitta District people used this Vidari for hypertension, general debility and constipation. The people from Alappuzha, Eranakulam and Thrissur District use this powdered tuber as galactagogue, general tonic, aphrodisiac and for sexual debility. In case of Idukki and Wayanad Districts the tuber powder was used as galactagogue, for gastric ulcer and ulcerative colitis, aphrodisiac, and as tonic for children.

4.1.3 Soil type

Soil types of the places of the collected accessions were recorded and the data pertaining to soil type are presented in Table 2. Soil types of the particular places were assessed with the help of reference of Soil fertility assessment and information for enhancing crop productivity in Kerala by Rajesh kumar *et al.*, 2013. The soil types included non-gravelly laterite (Thiruvananthapuram), Laterite (Kollam), Red loam (Pathanamthitta), Sandy grey onttukara (Alappuzha), Sandy to clayey loam

Table 2: Climate data and soil type details of *I. digitata* L. accessions.

Sl.No	Acc.	Place of collection		Geographical location		Altitude (m above MSL)	Soil type	Temperature (°C)		RF (mm)	RH (%)
		District	Place	North latitude	East longitude			Max	Min		
1	T2	Thiruvananthapuram	Vellayani	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	30	Non gravelly laterite soil	30.8	21.7	2035	90
2	T7		Kalliyoor	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
3	T9		Punnamoodu	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	25	Non gravelly laterite soil	30.8	21.7	2035	90
4	T10		Peringamala	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
5	T12		Pravachambalam	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
6	T13		Neyyattinkara	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
7	T15		Aruvikkara	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
8	T16		Pallichal	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
9	T17		Kunnathukal	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	50-75	Non gravelly laterite soil	30.8	21.7	2035	90
10	T18		Amboori	8° 16' 59" and 8° 49' 59"	76° 28' 59" and 77° 16'59",	170	Non gravelly laterite soil	30.8	21.7	2035	90

Ref.- Soil fertility assessment and information for enhancing crop productivity in Kerala. (Rajesh kumar *et al.*, 2013)

Table 2 continued: Climate data and soil type details of *I. digitata* L. accessions.

Sl.no	Acc.	Place of collection		Geographical location		Altitude (m above MSL)	Soil type	Temperature (°C)		RF (mm)	RH (%)
		District	Place	North latitude	East longitude			Max	Min		
11	T14	Kollam	Sasthamkotta	8 0 45' and 9 0 07'	76 0 29' and 77 0 17'.	3	Laterite soils	32.8	25.7	2555	93
12	T8	Pathanamthitta	Chittar	90 5' and 9028'	76030' and 770	800	Red loam soils	39.0	20.7	3133	93.2
13	T6	Alappuzha	Kayamkulam	9o 05' and 9o 54'	76o 17' and 76o 3	>6	Sandy grey onttukara soil	30.7	23.9	2965	84
14	T11	Eranakulam	Kumbalangy	09 047' 13" and 10 0 10'44"	760	>8.0	sandy to clayey loam	31.4	23.2	3432	89
15	T4	Thrissur	Pattikadu	100 10' 22'' and 100 46' 54''	750 57' 20'' and 760 54' 23''	20-25	Midland laterite soil	32.3	23.3	3198	93
16	T5		Peechi	100 10' 22'' and 100 46' 54''	750 57' 20'' and 760 54' 23''	20-25	Midland laterite soil	32.3	23.3	3198	93
17	T19	Idukki	Thopramkudy	09 0 16' 30" and 10 0 21'00"	760 38' 00" and 770 24'30".	> 1500	Brown hydromorphic soils	31.5	14.0	3677	98.45
18	T20		Udumbanchola	09 0 16' 30" and 10 0 21'00"	760 38' 00" and 770 24'30".	>1500	Alluvial soil	31.5	14.0	3677	98.45
19	T1	Wayanad	Meppadi	110 26' to 120 00'	750 75' to 760 56'.	1400-2100	Laterite soil	29.0	18.0	2608	92.9
20	T3		Panamaram	110 26' to 120 00'	750 75' to 760 56'.	1400-2100	Laterite soil	29.0	18.0	2608	92.9

Ref.- Soil fertility assessment and information for enhancing crop productivity in Kerala. (Rajesh kumar *et al.*, 2013)

Table 3: Details of ethnobotanicaal information of *I. digitata* L. accessions collected.

Sl.No	Acc No	Place of collection		Ethnobotanical Information
		District	Place	
1	T2	Thiruvananthapuram	Vellayani	Root powder used as galactagogue
2	T7		Kalliyoor	Root powder used as galactagogue
3	T9		Punnamoodu	Root powder with cow's milk used as galactagogue
4	T10		Peringamala	Root powder with milk used as galactagogue and tonic
5	T12		Pravachambalam	Root powder used as galactagogue
6	T13		Neyyattinkara	Root powder used as tonic and galactagogue
7	T15		Aruvikkara	Root powder with milk used as galactagogue and aphrodisiac
8	T16		Pallichal	Root powder used as galactagogue
9	T17		Kunnathukal	Root powder with honey used as tonic
10	T18		Amboori	Root powder used for sexual debility, infertility, lactation, gastric ulcer and ulcerative colitis.

Table 3 continued: Details of ethnobotanical information of *I. digitata* L. accessions collected.

Sl.No	Acc. No	Place of collection		Ethnobotanical Information
11	T14	Kollam	Sasthamkotta	Root powder used as galactagogue and tonic for children
12	T8	Pathanamthitta	Chittar	Root powder used for hypertension, general debility, constipation
13	T6	Alappuzha	Kayamkulam	Root powder used as galactagogue and general tonic
14	T11	Eranakulam	Kumbalangy	Root powder used as galactagogue, aphrodisiac and for sexual debility
15	T4	Thrissur	Pattikadu	Root powder along with milk used as galactagogue
16	T5		Peechi	Root powder with milk used as galactagogue
17	T19	Idukki	Thopramkudy	Root powder along with honey used as galactagogue and tonic for sexual debility, infertility, lactation, hepato-splenomegaly
18	T20		Udumbanchola	Root powder along with milk used as galactagogue, for gastric ulcer and ulcerative colitis.
19	T1	Wayanad	Meppadi	Root powder used as galactagogue and general tonic
20	T3		Panamaram	Root powder used as aphrodisiac, galactagogue and tonic for children

(Ernakulam), Midland laterite (Thrissur), Brown hydromorphic (Thopramkudy), Alluvial (Udumbanchola) and Laterite (Wayanad).

4.2. MORPHOLOGICAL CHARACTERISATION OF ACCESSIONS

4.2.1 Vine characters

The collected accessions of *I. digitata* were raised in the same environment, at the same plant density, and observations on morphological characters of all accessions were recorded at 3rd, 6th, 9th, and 12th month of planting. Vine characters were recorded as the average expression of characters observed in the section of the main stem located in the middle portion of several main stems.

4.2.1.1 Twining

Ability of the vines to climb the adjacent stakes placed in those accessions showing twining characters were recorded following sweet potato descriptor (1991) at three months interval for a period of one year. Twining plants achieve vertical growth by revolving around supports of different sizes on which they exert a pressure. The twining showed variation at all the stages of plant growth.

At 3 MAP, all the accessions showed a slightly twining nature and later on the accessions showed moderately twining habit at 6 MAP. At 9 MAP, the plant showed twining habit and a similar trend was observed during the harvest *i.e.* at 12 MAP (Fig. 1.).

4.2.1.2 Plant Height (cm)

Plant height is a reliable index of growth. The data pertaining to plant height at different stages of crop growth (3 MAP, 6 MAP, 9 MAP and 12 MAP) are presented in Table 4. The data revealed significant variations in plant height at all

Table 4. Plant height (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	195.80	239.26	277.33	289.13
T2	190.93	232.13	272.00	283.87
T3	192.86	238.93	279.06	291.47
T4	214.13	269.26	310.46	321.93
T5	198.13	247.86	283.53	295.33
T6	96.80	133.53	150.66	167.27
T7	205.13	250.40	280.86	292.67
T8	192.06	238.40	273.66	286.67
T9	209.13	257.86	282.80	295.93
T10	176.33	226.60	262.13	275.13
T11	169.86	205.73	222.00	237.27
T12	198.66	249.46	293.40	312.00
T13	223.80	279.53	316.20	329.07
T14	195.26	241.20	291.13	303.67
T15	209.46	258.26	298.00	310.87
T16	211.73	253.66	292.46	304.87
T17	205.46	254.00	292.00	304.67
T18	204.80	256.86	295.73	308.53
T19	225.66	275.46	298.13	310.27
T20	243.73	299.73	308.00	322.00
S.Em	9.56	12.38	12.92	12.67
C.D	27.36	35.44	37.01	35.11
CV (%)	8.36	8.73	8.02	7.51

stages of plant growth. The gradual increase in plant height was observed starting from initial stage of growth till harvest.

Significant differences in plant height were noticed among accessions at 3 MAP, and the highest plant height (243.73cm) was recorded in accession T20. The accessions T13 and T19 were on par. The lowest plant height was observed in the accession T6 (96.8cm) which showed on par result with the accession T11.

At 6 MAP, the similar trend was followed and, the accession T20 recorded highest plant height (299.73cm) which showed on par values with the accessions T13 and T19 and the lowest plant height was recorded in the accession T6 (133.53cm).

At 9 MAP, plant height was significantly higher (316.2cm) in the accession T13 which was on par with accessions T4, T5, T9, T12, T14, T15, T16, T17, T18 and T19. The lowest (150.66cm) plant height was observed in accession T6.

At harvest the plant height varied significantly which ranged from 167.27cm to 329.07cm. The maximum plant height (329.07cm) was recorded in accession T13 which was on par with T4, T5, T9, T12, T14, T15, T16, T17, T18 and T19. The lowest plant height was recorded for the accession T6.

4.2.1.2.1 Plant Type (Descriptor Data)

Length of main vine scored using 0-9 scale descriptor data are presented in Fig.ure 2. The accessions showed variations at all the stages of plant growth. At 3 MAP, the accessions recorded spreading habit in which the plants height ranged from 151cm to 250cm. Later at 6 MAP, some of the accessions showed spreading and some extremely spread habit. From 9^{th} month onwards the vine exhibited extremely spreading habit in which the plant height was above 250cm and the similar spread was observed at the time of harvest (12 MAP).

4.2.1.3 Internode Diameter (cm)

Internode diameter measured with the help of a thread and scale, and the average expression of at least three internodes located in the middle section of the vine recorded is presented in Table 5. The results showed a significant variation in internode diameter at all stages of crop growth.

At 3 MAP, the internode diameter varied from 0.88cm to 2.82cm among the accessions. The highest internode diameter is reported from accession T6 and it was on par with T4 and T10. The lowest internode diameter was observed in accessions T19 and T20 which was on par with T14.

Similar trend was observed at 6 MAP, where the value ranged from 1.29cm to 3.08cm. A significant higher internode diameter was recorded in accession T6 which was on par with the accession T10 and the lowest internode diameter was recorded for the accession T14 which was on par with the accessions T1, T2, T5, T9, T11, T12, T13, T15, T16, T17, T18, T19 and T20.

At 9 MAP, the internode diameter varied from 1.72cm to 3.26cm. A significantly superior internode diameter was observed in the accession T6 (3.26 cm) which was on par with accessions T4, T5, and T10 and the lowest internode diameter was recorded from the accession T1which showed on par with the accessions T11, T12, T14, T16, T17, T18, T19 and T20.

At harvest, the internode diameter ranged between 1.98cm to 3.44cm. Maximum internode diameter was observed in accession T6 which was on par with the accessions T4, T5 and T10 and the lowest was recorded in the accessions T14 and T17.

4.2.1.3.1 Internode Diameter (Descriptor Data)

As per the descriptor values all the accessions showed very thick internode diameter at all the stages of plant growth (3 MAP, 6 MAP, 9 MAP and 12 MAP). All accessions had more than 12 mm of internode diameter (Fig. 3.).

4.2.1.4 Internode Length (cm)

The internode length of the vine measured from middle of the vine from one node to other node with the help of scale and expressed in centimetre is presented in Table 6. Internode length showed significant variation at all the stages of crop growth and there was a gradual increase of internode length at all the stages of crop growth.

At 3 MAP, the internode length ranged between 4.76cm to 12.65cm. The accession T8 recorded maximum length of internode (12.65cm), which was on par with the accessions T1, T5, T12 and T15. The lowest internode length was observed in accession T16 which showed on par result with the accession T1, T2, T3, T4, T6, T7, T9, T10, T11, T13, T14, T17, T18, T19 and T20.

Similar trend was noticed in all accessions in the later growth stages also. At 6 MAP, the accession T8 recorded the highest (14.56cm) internode length which was on par with accessions T1, T5, T12 and T15. The shortest internode length was observed in accession T16 (7.38cm) which was on par with the accessions T4, T13, T14, T17 and T20.

At 9 MAP, the internode length ranged from 9.76cm to 16.25cm. and the maximum internode length was recorded in accession T8 which was on par with accessions T5, T12 and T15. The minimum internode length was recorded in accession T16.

Table 5. Internode diameter (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	1.28	2.12	1.72	2.40
T2	1.46	1.76	2.16	2.32
T3	1.86	2.28	2.52	2.68
T4	2.12	2.69	3.24	3.33
T5	1.82	2.13	2.83	3.13
T6	2.81	3.08	3.26	3.44
T7	2.11	2.30	2.62	2.54
T8	1.88	2.21	2.72	2.88
T9	1.63	1.94	2.29	2.48
T10	2.62	2.82	3.06	3.15
T11	1.07	1.46	1.90	2.09
T12	1.22	1.54	1.98	2.15
T13	1.40	1.72	2.19	2.39
T14	0.98	1.29	1.80	1.98
T15	1.38	1.73	2.14	2.31
T16	1.38	1.58	1.96	2.15
T17	1.19	1.38	1.80	1.98
T18	1.19	1.51	1.93	2.12
T19	0.88	1.49	1.94	2.16
T20	0.88	1.39	1.84	2.02
S.Em	0.14	0.12	0.17	0.18
C.D	0.40	0.36	0.51	0.53
CV (%)	15.57	11.34	13.51	12.91

At harvest (12 MAP) the internode length among accessions ranged between 11.76cm to 18.13cm. Significantly higher internode length (18.13cm) was recorded in accession T8 which was on par with accessions T5, T12 and T15. Shorter internode length was recorded in the accession T14.

4.2.1.4.1 Internode Length (Descriptor Data)

Vine Internode length ranged from intermediate (6-9 cm), long (10-12 cm) and very long (>12 cm) at various stages of crop growth period (Fig. 4). At the initial months (3 MAP) the internode length was intermediate with 6 cm to 9 cm of length. At 6 MAP and 9 MAP, The plants showed long internodes which ranged from 10 cm to 12 cm and finally at 12 MAP, the plants showed very long internodes for all the accessions according to the sweet potato descriptor data of IPGRI (1991).

4.2.1.5 Predominant Color of Vine

As per the descriptor data the predominant color of vine of all accessions appeared to be green at all stages of crop growth period (3 MAP, 6 MAP, 9 MAP and 12 MAP).

4.2.2 Leaf Characters

The parameters like type and number of leaf lobes, shape of central leaf lobe, matured leaf size, matured leaf color, immature leaf color, petiole pigmentation and petiole length data were recorded and presented (Plate 4).

4.2.2.1 Type of Leaf Lobes

The data pertaining to type of leaf lobe at different stages of crop growth period for different accessions are presented in Table 9 (Fig. 5).

Table 6. Internode length (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	10.28	11.84	13.62	15.84
T2	9.50	11.07	13.45	15.45
T3	9.68	11.34	13.71	15.64
T4	7.27	9.19	11.62	13.80
T5	10.84	12.51	14.67	16.41
T6	8.90	10.47	12.77	14.65
T7	7.94	9.82	12.24	14.58
T8	12.65	14.56	16.25	18.13
T9	9.48	11.12	13.12	15.11
T10	9.44	11.06	12.98	14.89
T11	8.16	9.83	11.88	14.42
T12	11.62	13.39	15.11	16.80
T13	5.98	8.02	10.70	12.82
T14	6.10	7.60	10.15	11.76
T15	11.81	13.46	15.34	17.23
T16	4.76	7.38	9.76	12.37
T17	7.77	9.28	11.66	13.82
T18	8.14	10.22	12.36	14.58
T19	7.54	9.38	11.86	14.26
T20	7.44	9.11	10.89	14.05
S.Em	0.84	0.76	0.77	0.70
C.D	2.43	2.18	2.20	2.00
CV (%)	16.78	12.56	10.50	8.18

The type of leaf lobes varied from slightly lobed, moderately lobed to deeply lobed. At 3 MAP, all the accessions showed slightly lobed leaves.

After 6 MAP, the accessions T1, T2, T6, T10, T14, T15, T16, T18, T19 and T20 showed slight lobing of leaves, whereas, the accessions T3, T5, T11 and T12 showed moderate lobing.

Accessions T7, T8, T9, T16 and T17 showed deeply lobed leaves at all the stages of crop growth period (9 MAP and 12 MAP).

4.2.2.2 Number of Leaf Lobes

The data recorded on the predominant number of lateral and central leaf lobes observed on the leaves located on the middle section of the leaves at different stages of crop growth for all the accessions showed 7 leaf lobes irrespective of the stages of crop growth.

4.2.2.3 Shape of Central Leaf Lobe

The shape of central leaf lobe remained constant for all the accessions. All the accessions showed lanceolate shape of central leaf lobe at all the stages of plant growth period.

4.2.2.4 Matured Leaf Size (cm)

The average expression of both leaf length and leaf breadth of at least three leaves located in the middle portion of the vines of all accessions at different crop growth stages are presented.

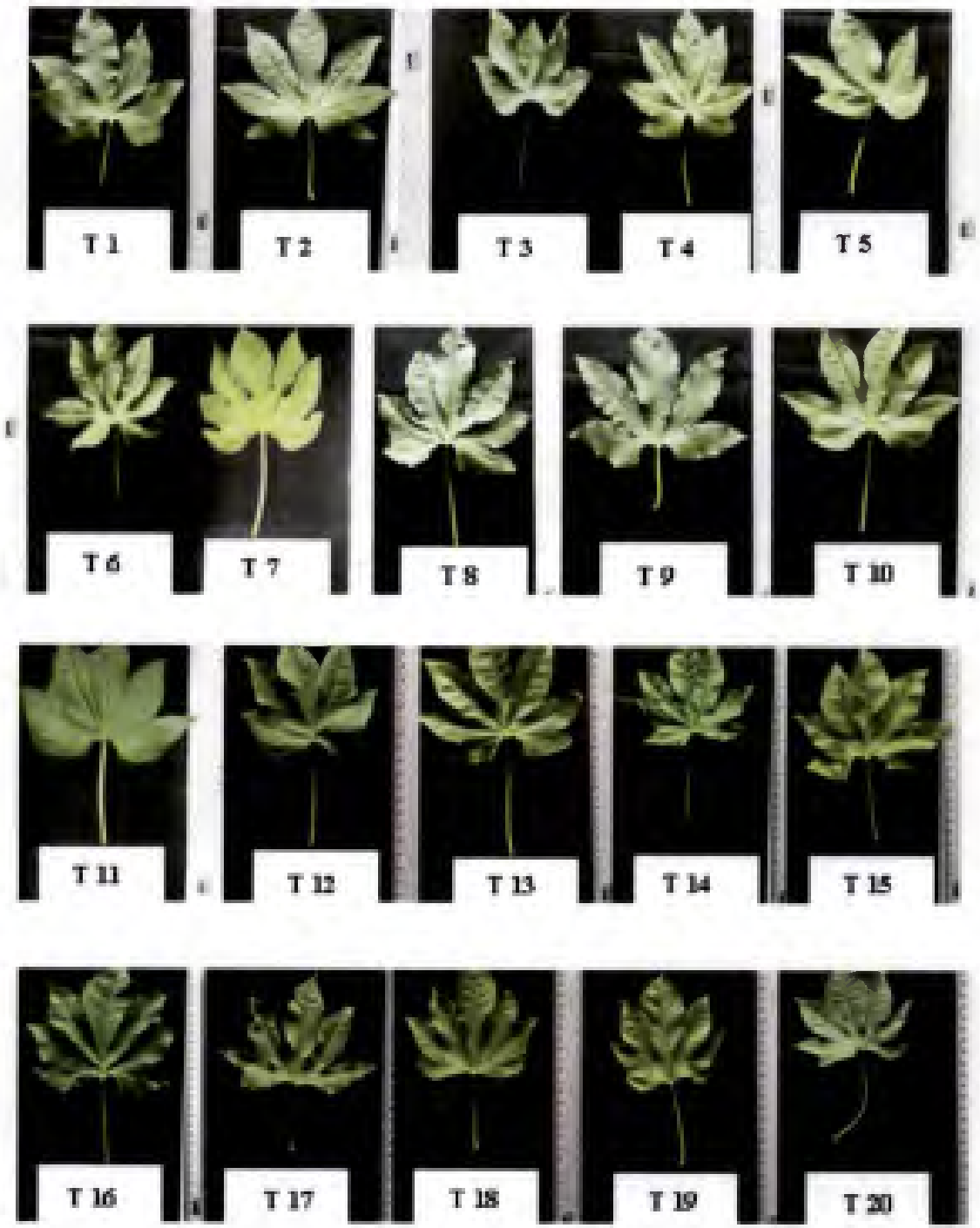


Plate 4. Variability in leaf characters of *I. digitata* L.

Table 7. Type of leaf lobe at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	Type of leaf lobe			
	3rd month	6th month	9th month	12th month
T1	3	3	3	3
T2	3	3	3	3
T3	3	5	5	5
T4	3	3	3	3
T5	3	5	5	5
T6	3	3	3	3
T7	3	7	7	7
T8	3	7	7	7
T9	3	7	7	7
T10	3	3	3	3
T11	3	5	5	5
T12	3	5	5	5
T13	3	3	3	3
T14	3	3	3	3
T15	3	3	3	3
T16	3	7	7	7
T17	3	7	7	7
T18	3	3	3	3
T19	3	3	3	3
T20	3	3	3	3

3- Slight

5- Moderate

7- Deep

4.2.2.4.1 Matured Leaf Length (cm)

Data recorded on leaf length at different stages of crop growth are presented in Table 8. and Fig. 6. Significant variation with a gradual increase in mature leaf length was noticed at all the stages of crop growth.

At 3 MAP, mature leaf length ranged between 7.16cm to 9.73cm. The accession T18 (9.73cm) recorded maximum leaf length which was on par with T1, T4, T5, T7, T12, T13, T17, T19 and T20. The minimum length was noticed in accession T10.

At 6 MAP, the leaf length varied from 7.66cm to 10.83cm. Significantly higher leaf length was noticed in accession T18 (10.83cm) which showed on par values with the accessions T1, T7 and T17. The lowest leaf length was recorded in the accession T10 (7.66cm).

At 9 MAP, the matured leaf lengths of the accessions varied between 8.00 cm to 11.24 cm. Accession T18 recorded significantly maximum matured leaf length (11.24 cm) which was on par with accession T7. The minimum leaf length (8.00 cm) was observed in the accession T10.

At harvest the leaf length ranged between 8.18 cm to 11.43 cm. the highest leaf length was observed for the accession T18 (11.43 cm) which was on par with the accessions T7, T17, T19 and T20. The lowest leaf length was observed for the accession T10.

4.2.2.4.2 Matured Leaf Breadth (cm)

The data recorded on leaf breadth at different stages of crop growth are presented in Table 9. and Fig. 7. There was significant variation in the leaf breadth at all stages of crop growth.

Table 8. Mature leaf length (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	9.00	9.96	10.38	10.90
T2	8.56	9.36	9.76	10.14
T3	8.73	9.56	9.85	10.23
T4	9.03	9.63	10.01	10.32
T5	8.96	9.66	10.10	10.42
T6	8.36	8.83	9.33	9.62
T7	9.63	10.34	10.72	10.96
T8	7.53	8.16	8.56	8.82
T9	7.66	8.30	8.79	8.98
T10	7.16	7.66	8.00	8.18
T11	8.33	9.00	9.32	9.43
T12	8.93	9.56	10.04	10.33
T13	9.03	9.86	10.30	10.50
T14	8.33	9.00	9.31	9.52
T15	8.00	8.70	9.08	9.55
T16	8.23	8.93	9.22	9.60
T17	9.00	10.23	10.79	11.10
T18	9.73	10.83	11.24	11.43
T19	9.03	9.86	10.29	10.59
T20	8.90	9.80	10.31	10.67
S.Em	0.30	0.30	0.29	0.29
C.D	0.87	0.88	0.85	0.85
CV (%)	6.16	5.73	5.28	5.13

Table 9. Mature leaf breadth (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	8.53	10.33	11.64	12.32
T2	8.40	10.10	11.50	11.26
T3	8.16	9.40	10.60	11.45
T4	8.30	9.83	11.20	11.54
T5	8.40	10.23	11.46	11.56
T6	8.56	10.26	11.46	11.38
T7	8.56	10.23	11.63	12.40
T8	8.76	10.50	12.13	11.51
T9	6.63	7.40	8.06	11.13
T10	8.80	10.73	12.20	11.46
T11	8.66	10.56	11.60	10.99
T12	8.50	10.63	11.93	11.64
T13	9.00	10.80	12.10	11.80
T14	8.70	10.53	11.90	11.01
T15	8.50	10.40	11.56	10.75
T16	7.93	9.33	10.40	10.15
T17	8.03	9.73	11.03	12.02
T18	8.46	10.50	11.86	11.62
T19	8.70	10.43	11.66	11.88
T20	8.80	10.33	11.83	11.70
S.Em	0.21	0.26	0.31	0.26
C.D	0.61	0.74	0.91	0.76
CV (%)	4.44	4.46	4.86	4.04

At 3 MAP, the leaf breadth varied from 6.63cm to 9.00cm. The maximum leaf breadth was recorded in the accession T13 (9.00cm) which was on par with the accessions T1, T2, T5, T6, T7, T8, T10, T11, T14, T15, T18, T19 and T20. The minimum breadth was recorded in accession T9 (6.63cm).

At 6 MAP, the leaf breadth ranged from 7.40cm to 10.80cm. the highest leaf breadth was observed for the accession T13 which was on par with the accessions T1, T2, T5, T6, T7, T8, T10, T11, T12, T14, T15, T18, T19 and T20. The lowest leaf breadth was observed for the accession T9 (7.40cm).

At 9 MAP, the leaf breadth varied from 8.06cm to 12.10cm. The accession T13 (12.10cm) recorded significantly highest leaf breadth which was on par to the accessions T1, T2, T4, T5, T6, T7, T8, T10, T11, T12, T14, T15, T18, T19 and T20. The lowest leaf breadth was noticed in accession T9 (8.06cm).

At harvest the leaf breadth varied from 10.15cm to 12.40cm among the accessions. The accession T7 showed the broadest leaves (12.40cm) and the value was on par with that of T1, T17, T19 and T20. However, the lowest leaf breadth was recorded in the accession T16 (10.15cm).

4.2.2.5 Matured Leaf Color

As per the descriptor data recorded, all the accessions showed green color for matured leaves at all the stages of crop growth period.

4.2.2.6 Immature Leaf Color

Immature leaves also showed green color at all the stages of crop growth stages as per the descriptor data.

4.2.2.7 Petiole Pigmentation

Petiole pigmentation among the accessions varied from green, green with purple near stem, green with purple near leaves, some petioles purple and others green (Table 10. and Fig. 8). The results recorded as per the descriptor data showed that the accessions T3, T5, T6, T7, T9, T12, T13, T18, T19, and T20 showed green petioles at all stages of crop growth period (3 MAP, 6 MAP, 9 MAP and 12 MAP). The accessions like T2, T4, T10, T11, T14, and T17 recorded green with purple near stem. Whereas, the accessions T8, T15 and T16 showed green with purple near leaf and the accession T2 recorded some petioles purple and others green type of petiole pigmentation.

4.2.2.8 Petiole Length (cm)

The data pertaining to petiole length (cm) during different stages of crop growth in different accessions is presented in Table 11. The accessions did not show significant difference in petiole length at all stages of crop growth. However a gradual increase in petiole length with increase in growth period was noticed in all accessions.

At 3 MAP, the petiole length ranged from 3.5 cm to 4.19 cm, at 6 MAP 5.47 cm to 7.57 cm, at 9 MAP 7.23 cm to 8.91 cm and at 12 MAP it ranged from 7.54 cm to 9.32 cm.

4.3 INFLORESENCE CHARACTERS AND REPRODUCTIVE BIOLOGY

4.3.1 Flowering Habit

All the accessions showed moderate flowering habit as per the descriptor data. In all accessions, flowering started from April and continued till October. The peak flowering period was noticed in the month of September.

Table 10. Petiole pigmentation at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	2	2	2	2
T2	8	8	8	8
T3	1	1	1	1
T4	2	2	2	2
T5	1	1	1	1
T6	1	1	1	1
T7	1	1	1	1
T8	3	3	3	3
T9	1	1	1	1
T10	2	2	2	2
T11	2	2	2	2
T12	1	1	1	1
T13	1	1	1	1
T14	2	2	2	2
T15	3	3	3	3
T16	3	3	3	3
T17	2	2	2	2
T18	1	1	1	1
T19	1	1	1	1
T20	1	1	1	1

1- Green

2- Green with purple near stem

3- Green with purple near leaf

8- Some petioles purple, others green

Table 11. Petiole length (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

Treatments	3rd month	6th month	9th month	12th month
T1	3.73	5.47	7.24	8.31
T2	4.04	6.99	8.37	8.59
T3	3.96	7.01	8.28	8.49
T4	3.73	6.70	7.93	8.17
T5	3.67	6.30	7.40	7.74
T6	3.77	6.76	8.09	8.43
T7	4.07	6.99	7.65	8.55
T8	3.78	6.67	7.93	8.24
T9	3.93	6.96	7.80	8.72
T10	4.13	7.57	8.00	9.32
T11	3.92	6.95	8.22	8.50
T12	3.85	6.64	8.91	9.19
T13	3.81	6.61	7.64	7.97
T14	4.19	6.98	8.37	8.63
T15	3.53	6.25	7.24	7.54
T16	3.57	6.43	7.32	8.34
T17	3.50	6.11	7.23	8.37
T18	3.59	6.40	7.43	8.49
T19	3.93	7.05	7.63	8.63
T20	3.89	6.76	7.76	8.10
S.Em	0.18	0.38	0.39	0.39
C.D	NS	NS	NS	NS
CV (%)	8.03	9.85	8.74	8.08

NS – Non significant

4.3.1.1 Inflorescence

No variation in inflorescence characters was noticed among accessions. In all accessions the inflorescence was axillary cymose which is aggregated in capitate clusters. Number of flowers in each inflorescence ranged from 1 to 12 the average being five. Number of inflorescence per plant varied from 6 to 23. At peak flowering season 2 flowers bloomed in each day per plant inflorescence^{-1}. The flowers had fused petals with a tubular corolla, five sepals (2 outer, 2 inner and one outer inner) with a long peduncle and a pedicel. Stamens were free and epipetalous with 5 anthers and a bifid stigma both white in color. Ovary is syncarpous superior and with axile placentation. The flowers were beautiful pinkish colored. The particulars of inflorescence characters are presented in Table 12 and Plate 5.

4.3.1.2 Flower Size (cm)

The data on flower size (cm) are given in Table 13. The length of flowers ranged from 5.5cm to 6.8cm with a mean value of 5.9cm. The breadth of the flowers ranged from 3.4cm to 4.9cm with the mean value being 4.2cm. The average flower size was considered as 5.9cm X 4.2cm with a peduncle length of 6.1cm.

The petal characters of flowers (Table 13) revealed that the corolla length ranged from 5.3cm to 6.8cm and breadth from 3.4cm to 4.9cm with an average size of 5.9cm x 4.2cm. Flowers had 3 large stamens of 2.1cm length and 2 small stamens of 1.9cm length. The average carpel length was 0.8cm with four numbers of carpel each with a locule.

4.3.1.3 Flower Color

(A) Flower bud

(B) Matured flower bud

(C) Flowering branch

(D) L.S. of flower

Plate 5. Inflorescence characters of *I. digitata* L.

Table 12. Inflorescence characters of milk yam accessions (*I. digitata* L.).

Sl.no	Infloresence characters	
1	Flowering Habit	Globrous twining shrub with moderate flowering
2	Flower Size	6.12 X 4.6 cm
3	Flower Color	Pink or reddish purple
4	Shape Of Limb	Companulate/ rounded
5	Sepal Shape	Ovate/ cup shaped/ whorled (2 outer, 2 inner and 1 outer inner
6	Sepal Apex	Whorled/ acute
7	Sepal Pubesence	Absent/ glabrous
8	Sepal Color	Light green
9	Color Of Stigma	White
10	Color Of Style	White
11	Stigma Exertion	Inserted
12	Seed Capsule Set	Profuse/ present
13	Flowering Habitat	Plain and deciduous forest
14	Corolla Arrangement	Convolulate
15	Fruit Type	Capsule

The flowers of all the accessions were bright showy with pink or reddish purple flowers.

4.3.1.4 Shape of Limb

The flowers had rounded limb which is considered as the companulate type as the petals did not show any cuts.

4.3.1.5 Sepal Shape

As per descriptor data the flowers of all accessions showed ovate sepals. There were five sepals in all the flowers among which two outer, two inner and one outer inner sepal. The sepals were cup shaped and had whorled arrangement. Morphological features of sepals revealed the presence of 3 large and 2 small sepals with an average length of 1.6cm x 1.8cm and breadth of 1.3cm x 0.5cm respectively (Table 14).

4.3.1.6 Sepal Apex

All flowers showed acute sepal apex as per the descriptor data.

4.3.1.7 Sepal Pubescence

The flowers had glabrous sepals and sepal pubescence was absent in all the flowers.

4.3.1.8 Sepal Color

The sepal color ranged from light green to green color in the accessions.

4.3.1.9 Color of Stigma

The color of stigma was white in all flowers irrespective of the accessions.

Table 13. Petal characters of milk yam accessions (*I. digitata* L.).

Flower number	Size of corolla (cm)		Androecium				Gynoecium		
			Stamen number		Stamen length (cm)		Carpel		Number of locule
	Length	Breadth	Large	Small	Large	Small	No.	Length (cm)	
1	6.1	4.9	3	2	2.1	1.9	4	0.9	4
2	5.9	3.7	3	2	2.3	1.8	4	0.9	4
3	5.6	4.4	3	2	1.9	1.8	4	0.8	4
4	6.0	4.6	3	2	2.2	2.1	4	0.6	4
5	6.8	4.5	3	2	2.1	1.9	4	0.9	4
6	6.1	4.9	3	2	2.4	2.2	4	0.9	4
7	6.3	4.5	3	2	2.5	1.9	4	0.8	4
8	5.7	3.8	3	2	2.1	1.8	4	0.9	4
9	5.9	3.7	3	2	2.2	1.9	4	0.7	4
10	5.5	3.4	3	2	2.1	2.0	4	0.9	4
Mean	5.9	4.2	3	2	2.1	1.9	4	0.8	4

Table 14. Morphological features of sepal characters of milk yam accessions (*I. digitata* L.).

Flower number	Number of sepals		Length (cm)		Breadth (cm)	
	Large	Small	Large	Small	Large	Small
1	3	2	1.5	1.2	0.9	0.7
2	3	2	1.5	1.1	0.9	0.7
3	3	2	1.7	1.5	0.8	0.4
4	3	2	1.6	1.3	0.8	0.5
5	3	2	1.5	1.2	0.8	0.6
6	3	2	1.8	1.4	0.8	0.6
7	3	2	1.6	1.3	0.7	0.4
8	3	2	1.6	1.3	0.9	0.6
9	3	2	1.5	1.3	0.9	0.6
10	3	2	1.7	1.4	0.9	0.7
Mean	3	2	1.6	1.3	0.8	0.5

4.3.1.10 Color of Style

Color of style also remained white for all the accessions.

4.3.1.11 Stigma Exertion

Style length varied from 22.1mm -25.5mm while the length of filament varied from 21.2mm - 25mm (Table 15). Hence the stigma was considered as inserted by assessing the relative position of stigma with that of the longest anther.

4.3.1.12 Seed Capsule Set

Seed capsule set was moderate to profuse among the accessions studied. With regard to fruit setting percentage (Table 16), on an average out of 7 fruits inflorescence^{-1} five fruits were found to be healthy and two fruits were aborted. Thus 71.42 per cent fruit set was estimated inflorescence^{-1}.

4.3.2 **Reproductive Biology**

4.3.2.1 Days Taken From Initiation till Opening of Flower Buds

The data recorded on bud initiation and flower opening of ten different inflorescence showed that, all the buds in the inflorescence took nearly 6-8 days from bud formation till opening of flower buds among all the accessions observed.

4.3.2.2 Size of Fully Matured Flower Bud (cm)

The average size of fully matured flower bud was 3.2cm x 3.5cm.

4.3.2.3 Frequency of Flower Opening

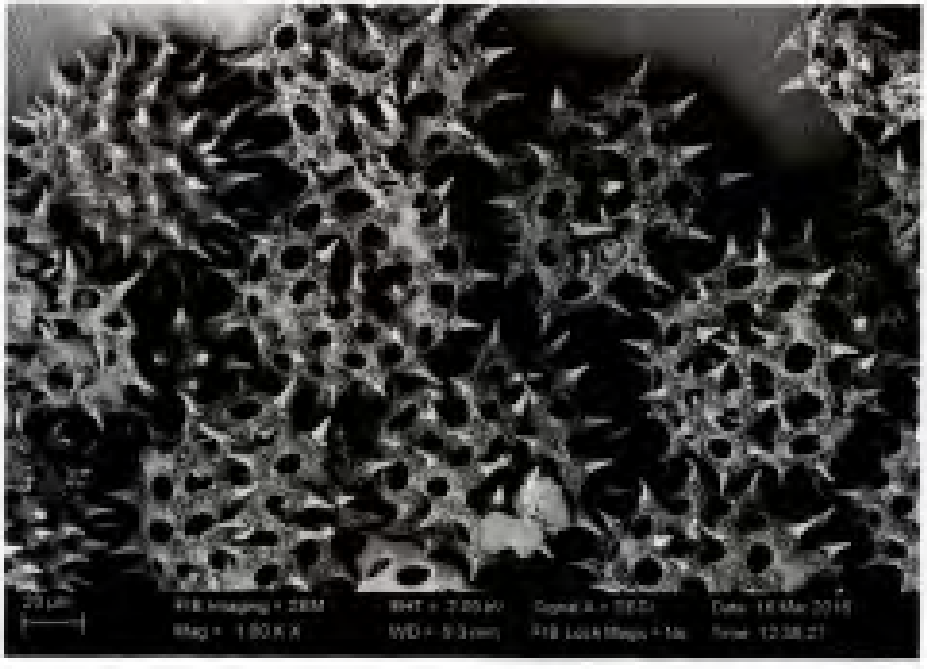
Each inflorescence on an average contained five flower and the matured flower buds of each inflorescence took one to three days for the opening of all flowers. Some inflorescences showed two flower blooms on the same day, and some

Table 15. Quantitative characters of the pollen and styles of *I. digitata* L.

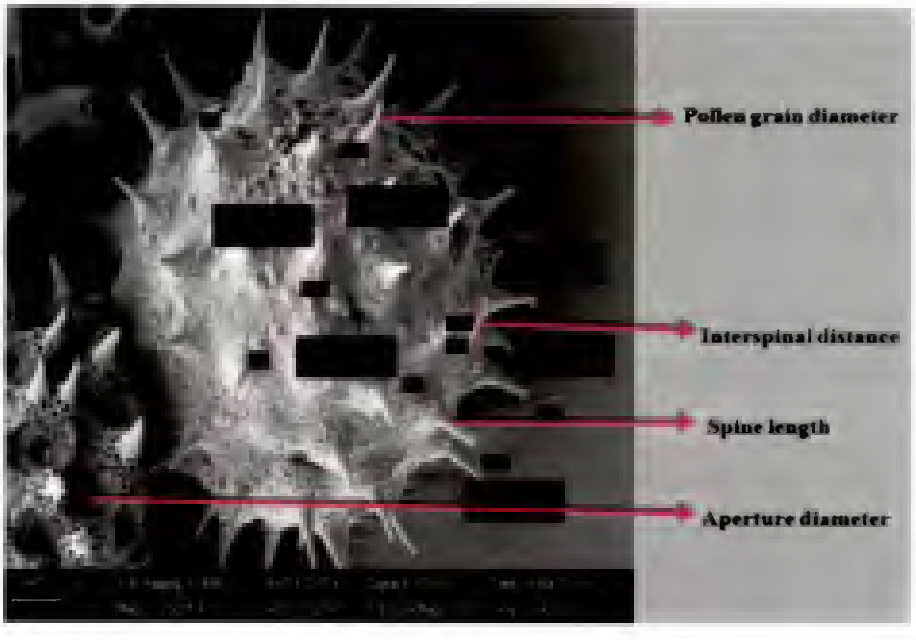
Pollen Number	Quantitative characters of I. digitata L.					
	Pollen characters (μm)				Reproductive characters (mm)	
	Pollen grain diameter	Spine length	Interspinal distance	Aperture diameter	Length of style	Length of filament
1	88.4	11.5	5.0	2.8	25.0	24.0
2	102.0	10.7	5.6	3.2	25.5	25.0
3	63.6	8.7	6.5	3.7	22.1	21.3
4	93.9	10.2	6.7	2.8	24.3	23.7
5	64.5	8.2	6.5	4.0	22.4	21.2
6	91.4	9.9	6.7	4.6	24.1	23.4
7	99.4	9.6	5.9	2.9	25.8	24.9
8	94.8	9.9	6.1	3.9	23.5	22.1

Table 16. Fruit setting in milk yam accessions (*I. digitata* L.).

Inflorescence	No. of fruits/inflorescence	Healthy fruit formed/ inflorescence	Aborted fruit/ inflorescence
1	7	7	0
2	4	3	1
3	9	7	2
4	6	5	1
5	8	6	2
6	1	1	0
7	11	6	5
8	7	4	3
9	2	1	1
10	10	6	4
Mean	7	5	2



(A) Group of pollen



(B) Pollen structure

Plate 6. Pollen morphology in *I. digitata* L.

flower buds of the inflorescence bloomed the next day after the first flower bloomed. Hence, the frequency of anthesis in the inflorescence varied from 1-3 days.

4.3.2.4 Peduncle length (cm)

The data pertaining to peduncle length are presented in Table 17. The peduncle length of the flowers varied from 5.1cm to 6.9cm the mean value being 6.1cm.

4.3.2.5 Diameter of Fully Opened Flower (cm)

The data recorded on diameter of ten fully opened flowers are presented in Table 17. The flower diameter of fully opened flower ranged from 5.5 cm to 6.5 cm, the average being 5.9cm.

4.3.2.6 Longevity of Flowers (hr)

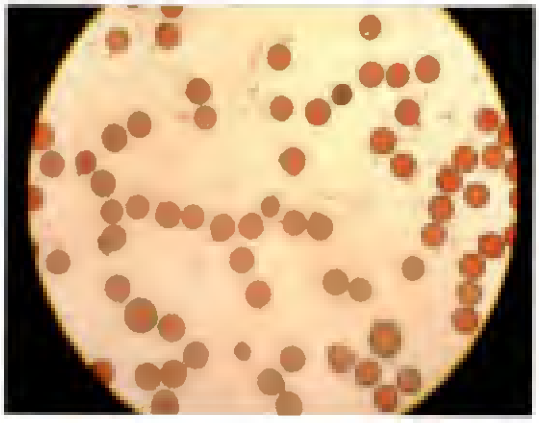
The flowers were observed to be short lived and their longevity varied from 10-12 hours *i.e.* the flower dehisced on the same day of anthesis.

4.3.2.7 Time of Anthesis and Anther Dehiscence

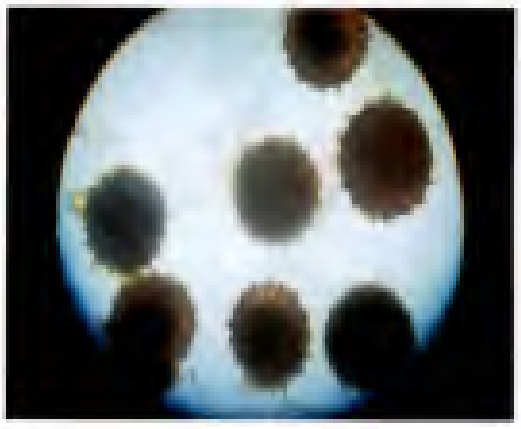
The anthesis was observed in the early morning hours between 4:30 am to 6:00 am. Anther dehiscence was also noticed on the same day of anthesis.

4.3.2.8 Pollen Morphology

The data recorded on pollen morphology are presented in Table 18. Pollen morphology studies of accessions revealed that they exhibited monard type of pollen grains with pantoporate aperture morphology and spinose exine ornamentation. The shape of the pollen was spheroidal with pointed spines. Microscopic observations on pollen morphology revealed that the pollen grain diameter ranged between 63.60 µm - 102 µm with an average pollen diameter of 87.25 µm . Spine length of the pollens

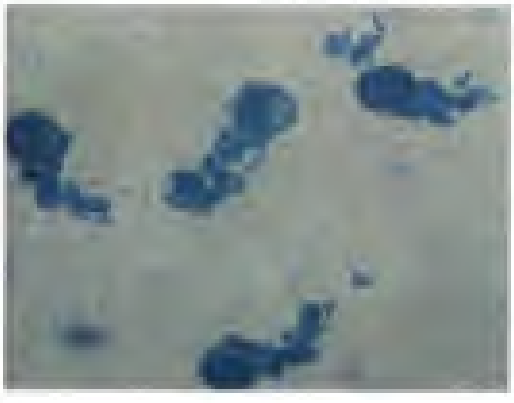


(A) Pollen fertility



(B) Pollen viability by IKI

(C) Pollen viability by TTC



(D) *In-vivo* pollen germination

Plate 7. Pollen biology of *I. digitata* L.

Table 17. Particulars of flower diameter and peduncle length (cm) of milk yam accessions (*I. digitata* L.).

Flower	Flower diameter (cm)	Peduncle length (cm)
1	6.0	5.5
2	6.0	5.9
3	5.5	9.2
4	6.1	6.5
5	5.9	6.9
6	6.2	6.6
7	5.8	5.1
8	6.0	5.3
9	6.5	5.4
10	5.6	5.3
Mean	5.9	6.1

Table 18. Particulars of pollen morphology of milk yam accessions (*I. digitata* L.).

Sl.no	Pollen morphology	Observation
1	Pollen type	Monad pollen grains
2	Aperture morphology	Pantoporate
3	Exine ornamentation	Spinose
4	Spine type	Pointed
5	Pollen grain shape	Spheroidal
6	Pollen grain diameter	63.6-102 µm
7	Spine length	8.28-11.5 µm
8	Interspinal distance	5-6.7 µm
9	Aperture diameter	2.8-4.6 µm
10	Length of style	23.5- 25.8 mm
11	Length of filament	21.2-25 mm
13	Pollen fertility	98 %

(A) Receptive stigma at field condition

(B) Non receptive stigma at field condition

(C) Microscopic view of receptive stigma

Plate 8. Stigma receptivity of *I. digitata* L.

ranged from 8.28 µm - 11.5 µm with the average being 9.83 µm. Interspinal distance ranged from 5.0 µm - 6.7 µm and the mean Interspinal distance was 6.12 µm. the aperture diameter of 2.8 µm to 4.6 µm. the length of style varied between 23.5mm to 25.8mm and filament ranged 21.2mm to 25mm (Plate 7).

4.3.2.9 Pollen fertility and viability

Pollen fertility was accessed by acetocarmine-glycerin staining technique and the data pertaining to pollen fertility are presented in Table 19. The pollen fertility ranged from 95.45 per cent to 100 per cent, and on an average 97.81 per cent of pollens was found to be fertile.

Pollen viability was calculated by iodine potassium iodide test and the results are presented in Table 20. The pollen viability ranged from 89.4 per cent to 100 per cent with an average value of 94.76. Pollen viability calculated by 2,3-5 Triphenyl tetrazolium chloride test also revealed that the viability of pollen grains ranged from 89.74 per cent to 100 per cent, the average value being 94.96 (Table 21 and Plate 7).

4.3.2.10 Stigma Receptivity and In-Vivo Pollen Germination

Continuous observation of the flowers from the time of anthesis revealed that the stigma of the flowers remained receptive for 8 hr from the time of anthesis for all the accessions. In the case of *in-vivo* pollen germination, maximum percentage of germination was observed immediately after anthesis and 2 hr after anthesis respectively.

4.3.2.11 Mode of Pollination

The data pertaining to mode of pollination are presented in Table 22 and Fig. 9. From the tagged plants, thirty flowers were selected for each experiment to know the induced fruit set under different breeding experiments.

Table 19. Pollen fertility (%) in milk yam accessions (*I. digitata* L.).

Field (40x)	Total no. of pollen	No. of stained pollen	Fertility (%)
1	62	61	98.38
2	71	79	97.18
3	13	13	100.00
4	17	17	100.00
5	39	38	97.43
6	44	42	95.45
7	27	26	96.29
Mean			97.81

Table 20. Pollen viability in milk yam accessions (*I. digitata* L.) by IKI test.

Field (40x)	Total no. of pollen	Stained pollen	Viability (%)
1	20	18	90.00
2	24	23	95.83
3	9	9	100.00
4	13	13	100.00
5	19	17	89.41
6	15	14	93.33
Mean			94.76

Table 21. Pollen viability in milk yam accessions (*I. digitata* L.) by TTC test.

Field (40x)	Total no. of pollen	Stained pollen	Viability (%)
1	84	78	92.85
2	53	48	90.56
3	91	89	97.80
4	87	86	98.85
5	39	39	100.00
6	39	35	89.74
Mean			94.96

Table 22. Induced fruit set in different modes of pollination in milk yam accessions (*I. digitata* L.).

Sl.no	Breeding experiments	No. of flowers pollinated	No.of flowers set fruits	% fruit set
1	Open pollination	30	24	80
2	Self pollination	30	18	60
3	Geitonogamous pollination	30	12	40
4	Xenogamous pollination	30	15	50

4.3.2.11.1 Open Pollination

Under open pollination, out of 30 flowers tagged 24 flowers were successful in setting fruits and 6 flowers were aborted. Thus the results revealed that 80 per cent fruit set was observed for open pollination.

4.3.2.11.2 Self Pollination

In the case of self pollination out of the 30 bagged flowers, 18 flowers set fruits successfully and 12 flowers were aborted. Thus in the present study, the extend of self pollination in *I. digitata* was observed to be 60 per cent.

4.3.2.11.3 Geitonogamous Pollination

In geitonogamous pollination, out of the 30 flowers tagged fruit set was observed in only 12 flowers 18 flowers were aborted. Thus the results revealed that geitonogamous pollination was only to the extent of 40 per cent fruit set. .

4.3.2.11.4 Xenogamous Pollination

The result showed that 50 per cent fruit set was seen in xenogamous pollination in which out of 30 flowers tagged only 15 flowers set healthy seeds while remaining 15 flowers were aborted.

4.3.2.12 Foraging Behaviour of Insects

Many insects were observed to visit the flowers for both nectar and pollen. As the flowers are showy and bright colored the pollinators are seen attracted to the crop. The data pertaining to foraging behaviour of insects are presented in Table 23 and Plate 9. The major pollinators recorded includes the Large carpenter bee which forage for both nectar and pollen, Blue banded bee (nectar and pollen), Skipper butterfly

Table 23. Pollinators and their foraging behaviours in milk yam accessions (*I. digitata* L.).

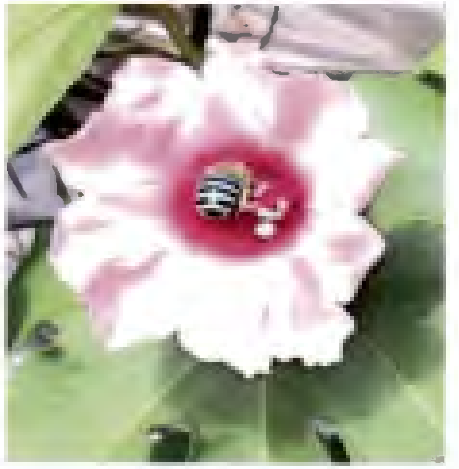
Sl. no	Common name	Scientific name	Family	Visiting time	Foraging nature
1	Large carpenter bee	*Xylocopa aestuans*	Apidae	Day	Nectar and Pollen
2	Blue banded bee	*Amegilla sp.*	Apidae	Day	Nectar and Pollen
3	Skipper butterfly	-	Hesperiidae	Day	Nectar
4	Scolid wasp	-	Scolidae	Day	Nectar and Pollen
5	Weaver ant	*Oecophylla smaragdina*	Formicidae	Day	Nectar
6	Butterfly	*Athyma sp.*	Nymphalidae	Day	Nectar

Table 24. Visitation rate of pollinators and insects on milk yam accessions (*I. digitata* L.).

visitors				
	5-8 am	8-11 am	11-2 pm	2-5 pm
Large carpenter bee	+	+	-	-
Xylocopa aestuans	+	-	-	-
Xylocopa sp. 2	+	+	-	-
Blue banded bee	+	+	+	+
Skipper butterfly	+	+	-	-
Scolid wasp	+	+	+	-
Weaver ant	+	+	+	+
Butterfly	+	+	-	+

+ - Present
- - Absent

(A) Large carpenter bee
(Xylocopa sp. 2)

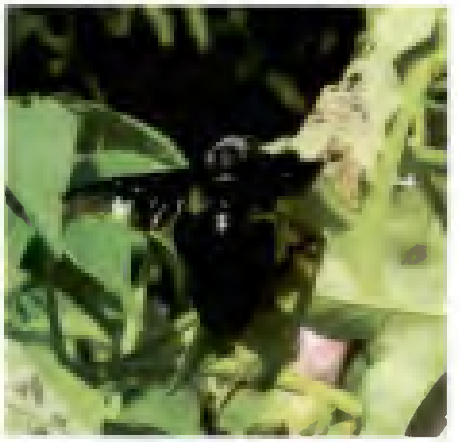


(B) Blue banded bee
(*Amegilla sp.*)

(C) Weaver ant
(*Oecophylla sp.*)

(D) Large carpenter bee
(Xylocopa aestuans)

(E) Skipper butterfly
(Hesperiidae sp.)



(F) Large carpenter bee
(*Xylocopa sp.* 1)

Plate 9. Pollinators of *I. digitata* L.

which visits the flower only for nectar, Scolid wasp (nectar and pollen), Weaver ant and Butterfly for nectar purpose. All pollinators were observed during the day time.

The data pertaining to visitation rate of insects are presented in Table 24. The flowers were visited by different insects. The pollinators are seen attracted by the showy bright colored flowers. Observations on the visitation time of different pollinators indicated that the large carpenter bee forage for both nector and pollen grains during the day time from 5-11am. Similar visits were observed for *Xylocopa aestuan, Xylocopa* sp. 2, Skipper butterfly and Butterfly which forage only for nectar purpose. Frequent visitation was observed in the case of blue banded bee and weaver ant. Scolid wasp was observed only till noon in a day. Maximum visitation was observed during morning hours and less during afternoon hours.

4.3.2.13 Time Taken For Fruit Maturity

The recorded fruit maturity time was 18 to 28 days from the day of flower opening.

4.3.2.14 Seed Size (cm), Shape and Weight (mm)

The data pertaining to seed characters are presented in Table 25. Fruit weight of the accessions ranged from 135.5mg to 216.8mg with a mean weight of 175.1mg. The number of seeds was 4 per capsule with an average size of 0.7cm x 0.8cm and with 143.4mg seed weight. The shape of the seed remained irregular (ovoid woolly black) with a thin seed wall.

4.3.2.15 Seed Germinability, Seed Viability and Moisture Content (%)

For estimating the moisture content, undehisced mature capsules were covered with paper bags and the seeds were collected. The moisture content of the seeds was calculated at weekly intervals from 0-4 week storage and the results are

Table 25. Particulars for the seed characters of *I. digitata* L.

Fruit number	Fruit weight (mg)	Seed			
		Number	Length (cm)	Breadth (cm)	Weight (mg)
1	216.8	4	0.3	0.8	140.4
2	154.4	3	0.7	1.1	107.1
3	199.9	4	1.1	0.9	138.4
4	157.7	4	0.6	0.9	136.0
5	135.5	4	0.9	0.9	157.6
6	182.5	4	0.3	0.3	165.6
7	163.7	4	0.9	0.7	181.6
8	181.1	3	1.1	0.9	117.9
9	154.4	4	0.7	1.0	153.2
10	205.9	4	0.8	0.9	136.4
Mean	175.1	3.8	0.7	0.8	143.4

Table 26. Seed biology of milk yam accessions (*I. digitata* L.).

S. no	Duration	Moisture content (%)	Viability (%)	Germinability (%)
1	At the time of dehiscence	12.3	82	25
2	0-1 week	13.6	60	15
3	1-2 week	12.1	56	15
4	2-3 week	10.3	53	15
5	3-4 week	10.2	42	15

(A) Matured fruits

(B) Matured fruits

(C) Germination paper

(D) Germinated seeds

Plate 10. Different stages of seed germination in *I. digitata* L.

presented in Table 26 and Plate 10. The results revealed that there was a considerable reduction in the seed moisture content from 12.3 per cent to 10.2 per cent within one month. The viability of the seeds at the time of dehiscence was 82 per cent and gradually decreased with the time of storage and reached 42 per cent at fourth week of storage. Seed germination depends on physical conditions around the seed and physiological status of the seed. The germinability of seeds varied from 25 per cent at the time of dehiscence to 15 per cent after a week (Fig. 10).

4.4 ROOT AND YIELD CHARACTERS

4.4.1 Root Characters

The parameters like storage root formation, storage root stock, number of storage roots per plant, variability of storage root shape, variability of storage root size, storage root cracking, predominant skin color and predominant flesh color are presented in Table 27 and Plate 11.

4.4.1.1 Storage Root Formation

The arrangement of storage root on underground stem of the accessions varied from open cluster (45%) to closed cluster (55%). The accessions T1, T2, T3, T6, T7, T11, T14, T15 and T19 showed closed clusters while the remaining accessions T4, T5, T8, T9, T10, T12, T13, T16, T17, T18 and T20 showed open cluster arrangement as per the descriptor data.

4.4.1.2 Storage Root Stalk

The storage root stalk of the accessions varied from very short (10%), short (85%) and intermediate types (5%). The accessions T2 and T16 showed very short storage stalk *i.e.* less than 2 cm length, whereas the accessions T1, T3, T4, T5, T6, T7, T8, T9, T10, T12, T13, T14, T15, T17, T18, T19 and T20 showed short storage

Table 27. Root characters of milk yam accessions (*I. digitata* L.).

Treatments	Storage root formation	Storage root stalk	Variability of storage root shape	Variability of storage root size	Predominant skin color	Predominant flesh color	Number of storage root per plant
T1	1	3	3	5	2	1	3
T2	1	1	3	3	2	1	2
T3	1	3	3	3	4	2	3
T4	3	3	7	3	2	2	2
T5	3	3	7	3	2	1	2
T6	1	3	3	3	2	1	3
T7	1	3	3	3	4	2	2
T8	3	3	7	3	5	2	1
T9	3	3	3	3	5	2	2
T10	3	3	3	3	2	2	2
T11	1	5	7	3	5	1	2
T12	3	3	3	3	5	1	2
T13	3	3	7	5	5	2	2
T14	1	3	7	3	3	2	2
T15	1	3	3	3	5	2	3
T16	3	1	3	5	5	2	2
T17	3	3	3	3	5	2	3
T18	3	3	7	3	3	1	2
T19	1	3	7	3	5	1	2
T20	3	3	3	5	5	2	3

Storage root formation: 1- closed cluster, 3- open cluster

Storage root stalk: 1- very short (<2), 3- short (2-5), 5- intermediate (6-8)

Variability of storage root shape: 3- uniform, 7- moderately variable

Variability of storage root size: 3- uniform, 5- slightly variable

Predominant skin color: 2- cream, 3- yellow, 4- orange, 5- brownish orange

Predominant flesh color: 1- white, 2- cream

Number of storage root per plant: average number of 5 plants

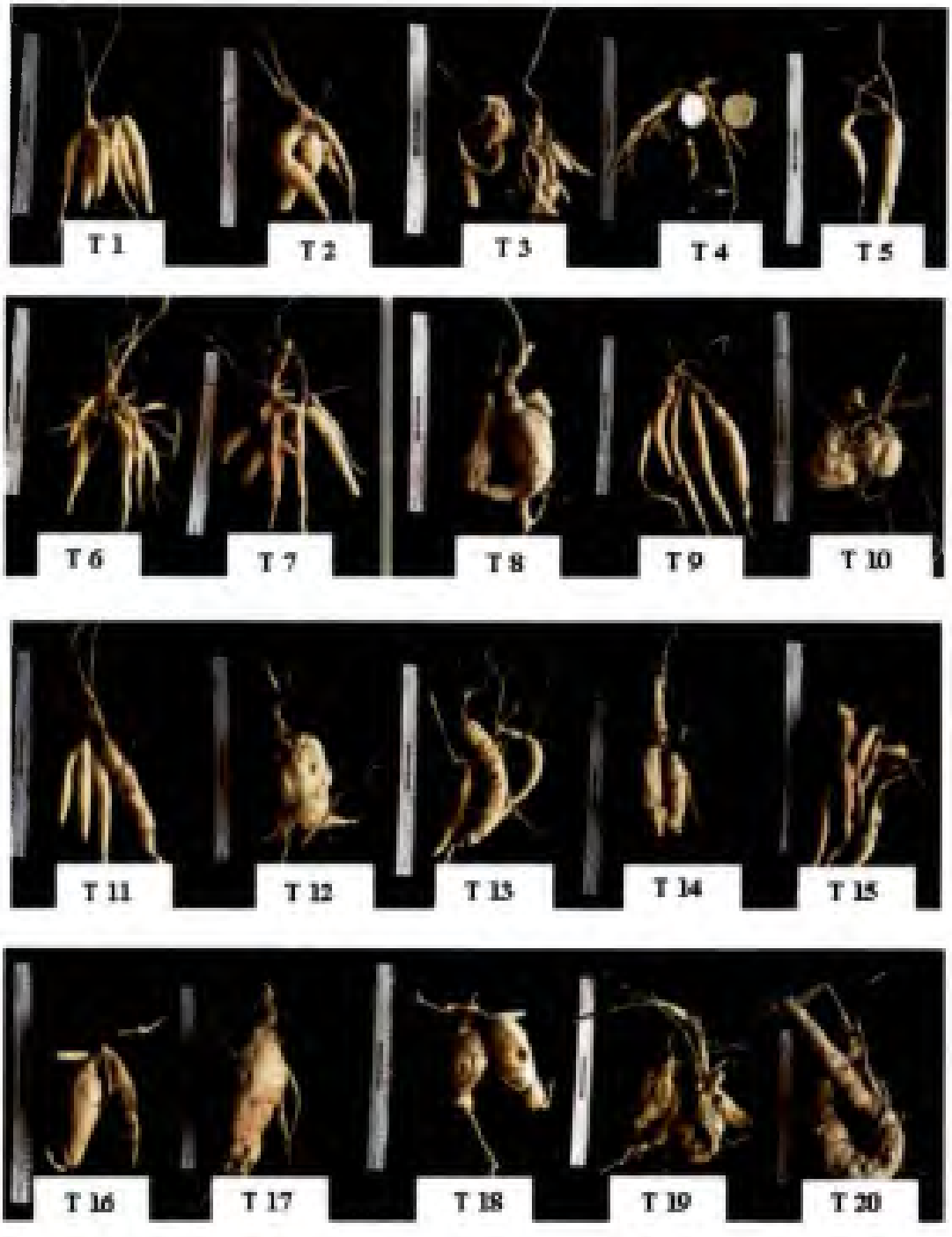


Plate 11. Variability in tubers of *I. digitata* L.

root stalks which measured 2cm - 5cm and accession T11 recorded intermediate type of storage root stalk which had 6cm to 8cm length.

4.4.1.3 Number of Storage Root Plant^{-1}

The number of storage roots per plant varied from 1.4 to 3.27. maximum number of storage root per plant was found in T20 (3.27) and it was on par with T1, T2, T3, T6, T15 and T17 and minimum number of storage root per plant was recorded in accession T8 (1.4).

4.4.1.4 Variability of Storage Root Shape

By visual observation and as per the descriptor, variability of storage root shape ranged from uniform (60%) to moderately variable (40%). The accessions T1, T2, T3, T6, T7, T9, T10, T12, T15, T16, T17 and T20 exhibited uniform variability of root shape whereas, the accessions T4, T5, T8, T11, T13, T14, T18 and T19 recorded moderately variable storage root shape (Table 27).

4.4.1.5 Variability of Storage Root Size

Variability of storage root size varied from uniform (80%) to slightly variable (20%) among the different accessions. The accessions T1, T16, and T20 exhibited slightly variable storage root size and the accessions T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12, T13, T14, T15, T17, T18 and T19 exhibited uniform variability of storage root size.

4.4.1.6 Storage Root Cracking

Storage root cracking was absent in all the accessions evaluated.

4.4.1.7 Predominant Skin Color

The predominant color of freshly harvested storage roots of each genotype was determined as per the descriptor. The predominant skin color of all the accessions varied from cream (30%), yellow (10%), brownish orange (50%) and orange (10%). The accessions T1, T2, T4, T5, T6, T7 and T10 reported cream skin color. The accessions T3 and T7 were orange skin colored types. Whereas the accessions T8, T9, T11,T12, T13, T15, T16, T17, T19 and T20 revealed brownish orange skin color for the tuber.

4.4.1.8 Predominant Flesh Color

Storage root flesh color of freshly harvested storage root cross section of all accessions was recorded based on the descriptor data. Accessions showed two distinct flesh colors of which the white color was predominant (60%) and cream color consisted of 40 per cent. The accessions T1, T2, T5, T6, T11, T12, T18 and T19 had white flesh color whereas, the accessions T3, T4,T7, T8, T9, T10, T13, T14, T15, T16, T17 and T20 exhibited cream flesh color.

4.4.2 Yield Characters

Yield is a complex character and is a function of several component characters and their interaction with environment. The parameters like number of tuber, length of tuber, girth of tuber, fresh tuber yield per plant and dry tuber yield per plant are presented in Table 28.

4.4.2.1 Number of Tuber

As observed in Table 28. significant differences were found among different accessions on number of tuber. The mean number of tuber among the accessions ranged from 2.07 to 3.47. The highest number of tuber (3.47) was recorded in

Table 28. Yield characters of milk yam accessions (*I. digitata* L.).

Treatments	Number of tuber	Length of tuber (cm)	Girth of tuber (cm)	Fresh tuber yield per plant (g)	Dry tuber yield per plant (g)
T1	3.13	35.29	13.43	503.00	250.67
T2	2.67	24.65	11.44	467.00	232.00
T3	2.87	24.43	11.93	620.27	390.73
T4	2.33	25.73	8.90	557.13	233.80
T5	2.67	34.04	9.93	447.67	289.20
T6	2.80	26.19	11.88	698.53	454.33
T7	2.33	31.70	9.23	299.25	142.33
T8	2.20	26.77	10.95	504.00	309.93
T9	2.67	23.25	12.71	262.00	151.53
T10	2.47	31.53	13.59	431.67	298.73
T11	2.07	27.74	10.77	752.13	432.53
T12	2.07	36.75	12.15	744.13	187.13
T13	2.27	29.73	14.71	564.80	233.67
T14	2.47	28.83	14.02	367.40	215.00
T15	3.00	26.67	13.77	870.47	681.73
T16	2.20	35.53	13.89	466.20	379.67
T17	2.67	31.63	10.11	821.93	458.40
T18	2.47	31.19	13.09	573.00	342.93
T19	2.07	33.91	10.63	539.07	281.47
T20	3.47	31.99	9.14	483.67	247.00
S.Em	0.23	1.95	1.03	92.79	72.74
C.D	0.66	5.58	2.95	265.59	208.22
CV (%)	15.81	11.32	15.15	29.29	40.56

accession T20 which was on par with T1, T3 and T15. The lowest number of tuber was found in the accessions T11 along with T12 and T19.

4.4.2.2 Length of Tuber (cm)

There was significant variation in length of tuber among the accessions. The tuber length ranged from 23.25cm to 36.75cm. Significantly longest root was found in the accession T12 (36.75cm) which showed on par values with the accessions T1, T5, T7, T10, T16, T17, T18, T19 and T20. Lowest tuber length was observed in the accession T9 (23.50cm).

4.4.2.3 Girth of Tuber (cm)

The girth of tuber also showed significant variations among the accessions and values ranged from 8.90cm to 14.71cm. The thickest roots (14.71cm) were recorded in accession T13 which was on par with T1, T3, T6, T9, T10, T12, T14, T15 and T16. The girth of tuber was observed minimum in the accession T4 (8.90cm).

4.4.2.4 Fresh Tuber Yield Plant^{-1} (g)

There was significant variation among the accessions for fresh tuber yield per plant, which varied widely from 299.25g to 870.47g. The accession T15 recorded the highest fresh tuber yield per plant (870.47g) which was on par with the accessions T3, T6, T11, T12 and T17. The lowest fresh tuber yield per plant was observed in accession T7 (299.25g) which was on par with remaining accessions except T18.

4.4.2.5 Dry Tuber Yield Plant^{-1} (g)

The dry tuber yield per plant showed significant differences. It ranged from 142.33 g to 681.73 g plant^{-1}. Highest dry weight of tuber per plant was recorded in accession T15 (681.73g) and the lowest value was observed in accession T7 (142.33g).

Yield analysis after the first year of planting revealed T15 ranked first with regard to number and girth of tuber as well as dry and fresh tuber yield per plant. Other promising accessions with regard to fresh tuber yield per plant included T17, T11, T12, T6 and T3.

4.5 PHYTOCHEMICAL SCREENING

Both qualitative and quantitative estimations of phytoconstituents were carried out following the different extraction methods for all the accessions and the results are presented in Table 29 and Table 30.

4.5.1 Qualitative screening of phytoconstituents

The data pertaining to qualitative estimation of phytoconstituents are presented in Table 29 and Plate 12. The data confirmed the presence of alkaloids, carbohydrates, glycosides, saponins, fats and oils, phytosterols, resins, flavonoids and proteins, in all accessions under different extraction methods. Hydro-ethanolic extract showed the presence of most of these compounds hence, it was considered best in the present study.

4.5.1.1 Alkaloids (Wagner's Reagent)

The qualitative analysis of alkaloids was done following wagener's reagent test and the results revealed the presence of alkaloids in methanol, ethanol and hydro ethanolic extract. The chloroform extract did not show the presence of alkaloids in Wagner's test.

4.5.1.2 Carbohydrates (Molisch's Test)

Test for carbohydrates was done following molisch's test which recorded positive response for ethanolic and hydro-ethanolic extracts. Whereas, it showed negative response for the methanol and chloroform extracts respectively.

Table 29. Qualitative phytochemical investigation of different extracts of milk yam accessions (*I. digitata* L.).

Name of Extract	Methanol Extract	Chloroform Extract	Ethanol Extract	Hydro-ethanolic Extract
Alkaloid's (Wagner's reagent)	+	-	+	+
Carbohydrate's (Molisch's test)	-	-	+	+
Glycoside's (Keller Kelliani's test)	-	-	-	+
Saponins (Foam test)	+	-	-	+
Fat and Oil's (copper sulphate test)	-	-	-	+
Phytosterols (Liebermann-Burchard test)	-	+	+	+
Resin's ($CuSO_4$)	+	-	+	+
Flavonoids (Alkaline reagent test)	-	-	+	+
Protein's (ninhydrin test)	+	-	+	+

+ : present,

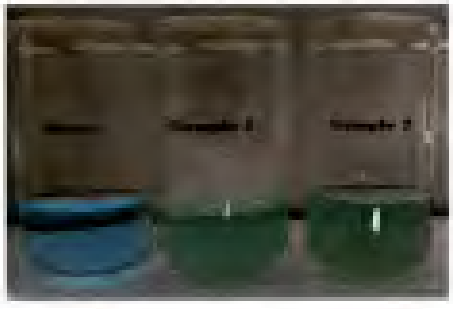
- : absent

(A) Protein

(B) Carbohydrate

(C) Flavonoids

(D) Fatty acids



(E) Resins

(F) Phytosterols

(G) Cardiac glycosides

(H) Saponin

Plate 12.Qualitative phytochemical investigation of *I. digitata* L.

4.5.1.3 Glycosides (Keller Kelliani's Test)

The presence of glycosides was detected only in hydro-ethanolic extract whereas, in all the other extracts *viz.,* methanol extract, ethanol extract and chloroform extract glycosides were not detected in all the accessions tested.

4.5.1.4 Saponins (Foam Test)

The presence of saponins was found to be positive for methanol and hydro-ethanolic extract. But chloroform and ethanol extracts showed negative results for the accessions tested.

4.5.1.5 Fats and oils (Copper Sulphate Test)

Fats and oils showed positive result only in hydro-ethanolic extract when tested with copper sulphate test. The extracts methanol, ethanol and chloroform showed the absence of fats and oils in the samples tested.

4.5.1.6 Phytosterols (Liebermann-Burchard Test)

Liebermann-bruchard test showed the presence of phytosterols in chloroform extract, ethanol extract and hydro-ethanol extracts but it was found absent in methanol extract.

4.5.1.7 Resins ($CuSO_4$)

The presence of resins was found in all the three extracts (ethanol extract, methanol extract, and hydro-ethanol extract) but it was absent in chloroform extract.

4.5.1.8 Flavonoids (Alkaline Reagent Test)

Ethanol extract and hydro ethanol extract showed the presence of flavonoids in alkaline reagent test. Whereas, the methanol extract and chloroform extract showed negative report for the accessions tested.

4.5.1.9 Protein's (Ninhydrin Test)

Ninhydrin test showed presence of proteins in methanol extract, ethanol extract and hydro-ethanol extract. But chloroform extract showed absence of protein in ninhydrin test.

4.5.2 Initial Quantitative Estimation of Phytoconstituents

The quantitative estimation of alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, fatty acids and oils was done following appropriate procedures (Sadasivam and Manickam, 1996) and the data are presented in Table 30.

4.5.2.1 Alkaloids (%)

Alkaloid content of all accessions ranged from 18.13 per cent to 34.69 per cent. Significantly highest alkaloid content was recorded in accession T20 (34.69%) which was on par with the accessions T1, T3, T7, T9 and T16. Lowest alkaloid content was observed in the accession T19 (18.13%) which was on par with T12 and T18.

4.5.2.2 Carbohydrates (%)

Carbohydrate content in the accessions was estimated by anthrone method. Among the accessions it ranged from 32.33 per cent to 62.31 per cent. Significantly highest carbohydrate content was seen in accession T7 (62.31%) which was on par with the accessions T1, T3 and T16. Significantly lowest carbohydrates content was recorded in the accession T12 (32.33%) which was on par with T14.

Table 30. Phytochemical constituents of milk yam accessions (*I. digitata* L.) at the time of collection.

Treatments	Alkaloids (%)	Carbohydrates (%)	Glycosides (%)	Saponins (%)	Proteins (mg g^{-1})	Flavonoids (mg g^{-1})	Fatty acid (mg KOH g^{-1})	Oils (%)
T1	31.12	61.21	0.62	9.23	5.12	0.61	0.19	1.09
T2	27.61	52.24	0.24	5.32	3.22	0.24	0.12	0.43
T3	30.99	59.99	0.55	9.21	4.11	0.31	0.16	0.94
T4	24.96	43.21	0.17	4.32	5.27	0.39	0.20	1.11
T5	24.20	48.92	0.21	4.76	3.14	0.27	0.17	0.72
T6	28.40	51.24	0.28	5.42	3.18	0.35	0.11	0.30
T7	32.33	62.31	0.61	9.11	5.32	0.67	0.22	1.08
T8	28.70	54.12	0.32	5.73	4.10	0.42	0.18	0.99
T9	30.33	56.21	0.34	8.00	5.02	0.60	0.24	1.13
T10	24.11	49.81	0.11	3.21	3.31	0.41	0.14	0.56
T11	28.12	46.14	0.31	5.24	3.93	0.39	0.13	0.45
T12	21.14	32.33	0.10	2.39	2.73	0.28	0.11	0.32
T13	28.63	49.11	0.23	5.37	4.41	0.43	0.16	0.89
T14	22.86	41.23	0.21	3.42	3.24	0.27	0.11	0.83
T15	27.65	43.21	0.24	5.32	4.09	0.47	0.17	0.86
T16	34.14	59.21	0.59	9.34	5.24	0.57	0.24	0.73
T17	25.14	50.00	0.32	5.53	5.21	0.56	0.19	0.14
T18	20.24	54.80	0.31	5.48	3.73	0.48	0.17	0.88
T19	18.13	47.71	0.24	3.77	3.91	0.36	0.16	0.43
T20	34.69	55.19	0.63	9.76	5.66	0.63	0.23	1.18
S.Em	1.67	3.49	0.02	0.29	0.26	0.02	0.01	0.05
C.D	4.63	9.67	0.06	0.82	0.74	0.08	0.03	0.14
CV (%)	10.65	11.87	12.47	8.58	11.07	11.70	11.41	11.82

4.5.2.3 Glycosides (%)

Glycoside content of collected accessions was estimated using the procedure given by Ibraheem and Maimako (2014). The percentage of glycoside varied from 0.10 per cent to 0.63 per cent among the accessions. The results revealed that significantly highest glycosides content was recorded in the accession T20 (0.63%) which was on par with the accessions T1, T7 and T16. Significantly lowest glycosides content was observed in the accession T12 (0.10%) which was on par with T4 and T10.

4.5.2.4 Saponins (%)

Saponin content was estimated through gravimetric method as suggested by Sutharsingh *et al.* (2011). The saponin content among the accessions ranged from 2.39 per cent to 9.76 per cent. Significantly highest saponin content was observed in accession T20 (9.76%) which was on par with T1, T3, T7 and T16. However, significantly lowest saponin content was observed in the accession T12 (2.39%) which was on par with T10.

4.5.2.5 Fatty acid (mg KOH g^{-1})

Fatty acid content was estimated using the protocol referred from Sadasivam and Manickam (1996). The fatty acid content varied between 0.11 mg KOH g^{-1} to 0.24 mg KOH g^{-1} .The results showed significantly highest fatty acid content in the accessions T9 and T18 (0.24 mg KOH g^{-1}) which was on par with the accessions T4, T13, T16 and T20. Significantly lowest fatty acid content was observed in the accession T15 (0.11 mg KOH g^{-1}) which was on par with T6, T2 and T11.

4.5.2.6 Oils (%)

Oil content in collected accessions was estimated using protocol referred from Sadasivam and Manickam (1996). Oil content in the samples ranged from 0.14 per cent to 1.18 per cent. Significantly highest oil content was recorded in the accession T20 (1.18%) which was on par with the accessions T1, T4, T7 and T9. Lowest oil content was observed in the accession T17 (0.14%).

4.5.2.7 Proteins (mg g^{-1})

Protein content of accessions was estimated by Lowry's method which ranged from 2.73 mg g^{-1} to 5.66 mg g^{-1}. The results showed that the highest protein content was observed in accession T20 (5.66 mg g^{-1}) which was on par with the accessions T1, T4, T7, T9, T16 and T17. However, significantly lowest protein content was observed in the accession T12 (2.73 mg g^{-1}) which was on par with T2, T5, T6, T14 and T10. .

4.5.2.8 Flavonoids (mg g^{-1})

Flavonoid content for the accessions varied from 0.24 mg g^{-1} to 0.67 mg g^{-1} of the sample which was estimated by Aluminium chloride method. The results reveal that significantly highest flavanoid content was recorded in T7 (0.67 mg g^{-1}) which was on par with the accessions T20 T1, and T9. However, significantly lowest flavonoid content was observed in the accession T2 (0.24 mg g^{-1}) which was on par with T3, T5, T12 and T14.

In the initial phytochemical screening T20 (Udumbanchola, Idukki) and T7(Kalliyoor, Thiruvananthapuram) were found to have highest phytochemical content followed by T11(Kumbalamgy, Eranakulam) and T16 (Pallichal, Thiruvananthapuram). All other accessions were found to have lower content of phytoconstituents in the tuber.

Table. 31: Qualitative phytochemical investigation of *I. digitata* L.

Sl. no	Phytochemical	Result	Qualitative method	Quantitative method	Reference
1	Alkaloids	+	Wagner's reagent	Gravimetric method	Harborne, 1973; Soni and Sosa, 2013; Rai *et al.*, 2013.
2	Carbohydrates	+	Molisch's test	Anthrone Method	Hedge and Hofreiter, 1962.
3	Glycosides	+	Keller Kelliani's test	Gravimetric method	Ibraheem and Maimako, 2014.
4	Saponins	+	Foam test	Gravimetric method	Obadoni and Ochuko, 2001; Sutharsingh *et al.*, 2011; Anjali and Sosa, 2013; Rai *et al.*, 2013.
5	Phytosterols	+	Liebermann-Burchard test	-	Hossain *et al.*, 2013; Oluwasesan *et al.*, 2013.
6	Fats	+	Gravimetric method	Gravimetric method	Sosa, 2013; Rai *et al.*, 2013.
7	Oils	+	Gravimetric method	Gravimetric method	Sosa, 2013; Rai *et al.*, 2013.
8	Resins	+	$CuSO_4$ test	-	Archana *et al.*, 2012; Oluwasesan *et al.*, 2013.
9	Flavanoids	+	Alkaline reagent test	Aluminium chloride method	Zhishen *et al.*, 1998; Vijay and Rajendra, 2014.
10	Proteins	+	Ninhydrin test	Lowry's Method	Lowry *et al.*, 1951.

+ - Present

4.5.3 Qualitative Estimation of Phytoconstituents harvested 365 DAP

The data pertaining to qualitative estimation of phytoconstituents showed positive results for the presence of alkaloids under Wagner's reagent test , carbohydrates (Molisch's test), glycosides (Keller Kelliani's test), saponins (Foam test), phytosterols (Liebermann-Burchard test), fats and oils (Gravimetric method), resins ($CuSO_4$ test), flavanoids (Alkaline reagent test) and proteins (Ninhydrin test), for all the accessions under different extraction methods. The data pertaining to quantitative estimation of phytoconstituents harvested 365 DAP are presented in Table 31.

4.5.4 Quantitative Estimation of Phytoconstituents harvested 365 DAP

The quantitative estimation of alkaloids, carbohydrates, glycosides, saponins , fats and oils, resins, flavanoids and proteins was done for the accessions harvested 365 days after planting, following different procedures (Sadasivam and Manickam, 1996) and the data are presented in Table 32. along with graphical representation in Fig. 11 and 11a. The data indicated significant variation in all phytoconstituents among the accessions.

4.5.4.1 Saponins (%)

Saponin content in accessions was estimated through Gravimetric method as suggested by Sutharsingh *et al.,* (2011). The saponin content of the accessions ranged from 5.46 to 9.33 per cent. Significantly highest saponin content was observed in accession T9 (9.33%) and it was on par with T3, T4, T7, T8, T11, T12, T13, T14 and T16. significantly lowest saponin content was observed in accession T19 (5.46%) which was on par with T15, T17, T2, T20, T5, T18, and T10.

Table 32. Phytochemical constituents of milk yam accessions (*I. digitata* L.) harvested after 365 days of planting.

Treatments	Saponins (%)	Glycosides (%)	CHO (%)	Alkaloids (%)	Proteins (mg/g)	flavanoids (mg/g)	Fatty acid (mg KOH/g)	Oils (%)
T1	7.33	0.33	56.80	28.03	3.43	0.45	0.13	0.40
T2	6.26	0.39	58.10	28.36	3.41	0.18	0.15	0.76
T3	8.00	0.39	59.16	30.70	3.93	0.20	0.12	0.83
T4	9.20	0.36	57.53	28.00	4.29	0.32	0.12	0.90
T5	6.73	0.43	56.83	30.30	4.22	0.29	0.13	0.73
T6	7.46	0.33	55.16	27.63	2.95	0.23	0.11	0.93
T7	7.60	0.34	54.23	29.23	4.05	0.20	0.12	0.30
T8	7.73	0.38	55.50	27.96	4.10	0.18	0.16	0.53
T9	9.33	0.52	57.83	30.33	4.30	0.19	0.16	0.36
T10	7.06	0.40	55.16	27.80	4.00	0.47	0.15	0.43
T11	8.93	0.46	56.53	30.20	3.60	0.55	0.15	0.53
T12	8.53	0.39	56.03	28.33	3.71	0.35	0.13	0.96
T13	7.86	0.44	53.76	28.60	4.05	0.35	0.15	1.06
T14	8.60	0.36	55.90	27.26	4.22	0.44	0.12	0.73
T15	6.00	0.42	54.96	28.40	4.37	0.24	0.11	1.06
T16	9.13	0.43	56.26	27.16	4.30	0.22	0.12	0.83
T17	6.20	0.36	56.10	29.23	3.22	0.41	0.12	0.83
T18	7.06	0.42	53.43	30.46	3.41	0.46	0.12	0.83
T19	5.46	0.35	57.10	28.16	4.20	0.49	0.12	0.53
T20	6.46	0.45	55.43	29.60	4.16	0.19	0.12	0.36
S.Em	0.64	0.01	0.65	0.50	0.30	0.02	0.00	0.07
C.D	1.84	0.03	1.86	1.43	0.85	0.06	0.02	0.20
CV (%)	14.80	5.82	2.01	3.01	13.32	11.44	9.89	18.19

4.5.4.2 Glycosides (%)

Glycoside content of collected accessions was estimated by the procedure given by Ibraheem and Maimako (2014). The percentage of glycoside varied between 0.33 per cent to 0.52 per cent among the accessions. The results revealed that significantly highest glycoside content was recorded in the accession T9 (0.52%) which was on par with T11, T20 and T13. However, significantly lowest glycosides content was observed in T6 (0.33%).

4.5.4.3 Carbohydrates (%)

The presence of carbohydrate in all the accessions was estimated by Anthrone Method. The carbohydrate content in the accessions ranged from 53.43 per cent to 59.16 per cent. Significantly highest carbohydrates content was seen in accession T3 (59.16%) which was on par with the accessions T2, T4 and T9. Whereas, significantly lowest carbohydrate content was recorded by the accession T18 (53.43%).

4.5.4.4 Alkaloids (%)

Alkaloid content of all the accessions ranged from 27.16 per cent to 30.7 per cent. Significantly highest alkaloid content was recorded in accession T3 (30.7%) which was on par with the accessions T5, T9, T11, T18 and T20. However, lowest alkaloid content was observed in the accession T16 (27.16 %).

4.5.4.5 Proteins (mg g^{-1})

Protein content of accessions was estimated by lowry's method which ranged from 2.95 mg g^{-1} to 4.37 mg g^{-1} among the accessions. The results showed that highest protein content was observed in accession T15 (4.37 mg g^{-1}) which was on par with the accessions T3,T4, T5, T7, T8, T9, T10, T11, T12, T13, T14, T16 T19

and T20. However, significantly lowest protein content was observed in accession T6 (2.95 mg g^{-1}).

4.5.4.6 Flavonoids (mg g^{-1})

Flavonoid content for the accessions varied from 0.18 mg g^{-1} to 0.55 mg g^{-1} of the sample which was estimated by aluminium chloride test. The results revealed that significantly highest flavonoid content was recorded in accession T11 (0.55 mg g^{-1}) which was on par with the accessions T11 and T19. However, significantly lowest flavanoid content was observed in accession T8 (0.18 mg g^{-1}).

4.5.4.7 Fatty acid (mg KOH g^{-1})

Fatty acid content was estimated using protocol referred from Sadasivam and Manickam (1996). The content fatty acid among the accessions varied between 0.11 mg KOH g^{-1} to 0.16 mg KOH g^{-1} .The results showed significantly highest fatty acid content in the accessions T8 and T9 (0.16 mg KOH g^{-1}) which was on par with the accessions T2, T10, T11 and T13. Significantly lowest fatty acid content was observed in the accession T6 (0.11 mg KOH g^{-1}).

4.5.4.7 Oils (%)

Oil content in collected accessions was estimated using protocol referred from Sadasivam and Manickam (1996). The oil content percentage in the samples of all the accessions ranged from 0.30 per cent to 1.06 per cent. The results reveal that significantly highest oil content was recorded in the accessions T13 and T15 (1.06 %) which was on par with the accessions T4, T6 and T12. significantly lowest oil content was observed in the accession T7 (0.3%).

4.5.5 Quantitative Estimation of Phytoconstituents harvested 2 years after planting

The quantitative estimation of saponins, glycosides, carbohydrates, alkaloids, proteins, flavanoids, fatty acid and oils, and was done for the accessions harvested two years after planting, following different procedures from Sadasivam and Manickam (1996) and the data are presented in Table 33.

4.5.5.1 Saponins (%)

Saponin content in accessions was estimated through gravimetric method as suggested Sutharsingh *et al.,* 2011. The saponin content ranged from 7 per cent to 9.51 per cent. Significantly highest saponin content was observed in accession T9 (9.51%) and it was on par with the accessions T4, T8, T11, T12, T13, T16 and T18. However, significantly lowest saponin content was observed in the accession T14 (7%).

4.5.5.2 Glycosides (%)

Glycoside content of collected accessions was estimated by using the procedure given by Ibraheem and Maimako (2014). The percentage of glycoside varied between 0.39 per cent to 0.59 per cent among the accessions. The results revealed that significantly highest glycosides content was recorded in the accession T9 (0.59 per cent) which was on par with the accessions T1, T2, T3, T4, T10, T11, T12, T13, T14, T15, T16, T17, T18, T19 and T20. However, significantly lowest glycosides content was observed in accession T6 (0.39%).

4.5.5.3 Carbohydrates (%)

The presence of carbohydrate in all the accessions was estimated by anthrone method. The carbohydrate content in the accessions ranged from 53.84 per cent to

Table 33. Phytochemical constituents of milk yam accessions (*I. digitata* L.) harvested 2 years after planting

Treatments	Saponins (%)	Glycosides (%)	CHO (%)	Alkaloids (%)	Proteins (mg/g)	Flavanoids (mg/g)	Fatty acid (mgKOH/g)	Oils (%)
T1	7.82	0.51	57.26	30.33	4.16	0.49	0.14	0.96
T2	7.23	0.54	54.43	29.16	4.14	0.23	0.18	0.89
T3	7.99	0.53	59.32	32.24	4.27	0.39	0.16	0.91
T4	9.34	0.53	58.56	30.07	4.39	0.38	0.17	0.93
T5	7.37	0.49	59.98	30.97	5.01	0.47	0.14	1.02
T6	7.93	0.39	56.63	31.36	3.90	0.33	0.13	0.98
T7	7.77	0.50	56.10	30.00	4.30	0.36	0.12	1.10
T8	8.13	0.42	57.80	27.47	4.67	0.32	0.18	1.03
T9	9.51	0.56	59.03	31.54	4.86	0.37	0.18	0.85
T10	7.63	0.51	56.00	29.99	3.99	0.51	0.16	0.92
T11	9.31	0.53	58.11	30.09	3.98	0.53	0.16	0.77
T12	9.23	0.52	59.23	30.53	4.41	0.48	0.18	0.87
T13	8.71	0.51	55.61	29.84	4.73	0.43	0.18	1.04
T14	7.00	0.56	56.01	29.61	4.42	0.51	0.16	0.82
T15	7.03	0.54	55.19	30.06	5.00	0.47	0.13	0.91
T16	9.19	0.53	58.33	30.04	5.42	0.43	0.15	1.02
T17	8.00	0.59	57.01	30.43	5.34	0.49	0.15	1.04
T18	8.23	0.57	54.99	33.10	4.33	0.48	0.17	0.98
T19	7.92	0.52	59.10	32.21	5.01	0.51	0.16	0.71
T20	7.40	0.54	56.97	34.69	5.09	0.34	0.16	0.65
S.Em	0.52	0.03	2.59	1.85	0.28	0.03	0.01	0.06
C.D	1.45	0.08	7.16	5.11	0.78	0.07	0.02	0.15
CV (%)	11.15	10.73	7.52	10.14	10.74	10.59	10.93	10.58

66.57 per cent. Significantly highest carbohydrates content was seen in accession T9 (66.57%) which was on par with the accessions T5, T8, T13, T15, T16, T17 and T18. Whereas, significantly lowest carbohydrates content was recorded in accession T4 (53.84%).

4.5.5.4 Alkaloids (%)

Alkaloid content of all the accessions from different agro climatic regions were estimated by gravimetric method (Harborne, 1973). Alkaloid content of all the accessions ranged from 21.72 per cent to 37.23 per cent. Significantly highest alkaloid content was recorded in accession T19 (37.23%) which was on par with the accessions T2, T3, T5, T9, T13, T18 and T20. However, lowest alkaloid content was observed in the accession T16 (21.72%).

4.5.5.5 Proteins (mg g^{-1})

Protein content of accessions was estimated by lowry's method which ranged from 3.98 mg g^{-1} to 5.42 mg g^{-1}. The results showed that highest protein content was observed in accession T16 (5.42 mg g^{-1}) which was on par with the accessions T5, T8, T9, T13, T15, T17, T19 and T20. However, significantly lowest protein content was observed in the accession T11 (3.98 mg g^{-1}).

4.5.5.6 Flavonoids (mg g^{-1})

Flavonoid content for the accessions varied from 0.23 mg g^{-1} to 0.53 mg g^{-1} of the sample which was estimated by aluminium chloride method. The results revealed that significantly highest flavonoid content was recorded in accession T11 (0.53 mg g^{-1}) which was on par with the accessions T1, T9, T10, T12, T14, T15, T17 and T18. However, significantly lowest flavonoid content was observed in accession T2 (0.23 mg g^{-1}).

4.5.5.7 Fatty acid (mg KOH g^{-1})

Fatty acid content was estimated using protocol referred from Sadasivam and Manickam(1996). The content fatty acid varied between 0.12 mg KOH g^{-1} to 0.18 mg KOH g^{-1} .The results showed significantly highest fatty acid content in the accessions T2, T8, T9, T12 and T13 (0.18 mg KOH g^{-1}) which was on par with the accessions T3, T4, T10, T11, T14, T18, T19 and T20. However, significantly lowest fatty acid content was observed in the accession T7 (0.12 mg KOH g^{-1}).

4.5.5.8 Oils (%)

Oil content in collected accessions was estimated using protocol referred from Sadasivam and Manickam (1996). The oil content percentage in the samples ranged from 0.65 per cent to 1.04 per cent. The results reveal that significantly highest oil content was recorded in the accessions T9 and T13 (1.04%) which was on par with the accessions T1, T2, T3, T4, T5, T6, T7, T8, T10, T15 and T18. Whereas, significantly lowest oil content was observed in the accession T20 (0.65%).

4.6 CHROMATOGRAPHY

The HPLC and TLC methods developed for quantitation of alkaloids and flavanoids from tuber of *I. digitata* were found to be simple, sensitive and accurate and were validated in terms of linearity, precision, accuracy, system suitability, sample stability.

4.6.1 Thin Layer Chromatography (TLC)

Chromatographic fingerprinting of alcoholic extract of *I. digitata* was performed by TLC. The phyto documentation of the plates showed numerous bands under UV 366 nm and visible light after derivatization. The Rf at 0.2 value of *I. digitata* is presented in Table 34. The developed plates were photographed under UV

Table. 34. TLC profile of *I. digitata* L.

Phytoconstituents	Mobile phase	Spray reagent	Rf
Alkaloid	Toluene (65): Ethyl acetate (25): Diethylamine (10)	Dragandrsff's Reagent	0.2
Flavonoids	Ethylaceate (100): Glacial acetic acid (27): Formic acid (27): Water (11)	1% Aluminium chloride solution	0.6 0.8
Saponins	Chloroform (60): Glacial acetic acid (32): Methanol (12): Water (8)	Liebermann-Burchard Reagent	0.2 0.35

Table. 35. HPLC fingerprint of rutin and nicotinic acid fraction present in sample of *I. digitata* L.

I. digitata sample	Retention Time (min)	Area (mV.s)	Area (%)	Amount (mg g^{-1})
Rutin	2.050	15345.917	100	4.16
Nicotinic acid	1.843	56.466	100	17.20

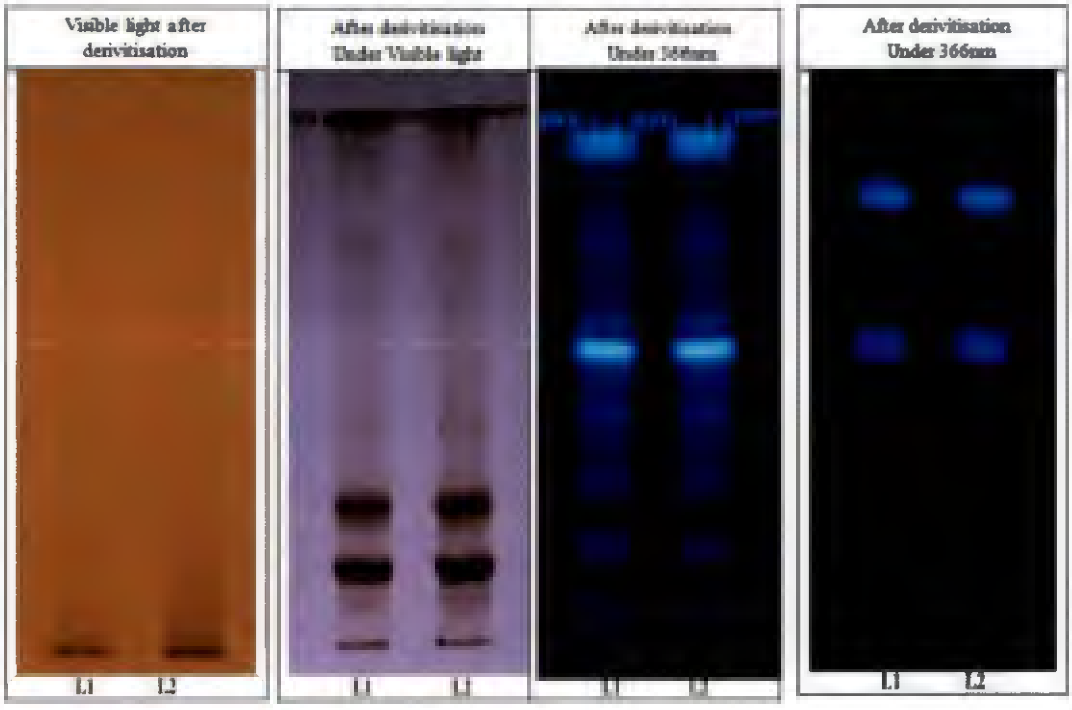


Plate 13. TLC profile of *I. digitata* L.

366 nm and under visible light which confirmed the presence of alkaloids, saponins and flavanoids respectively.

The results of the study showed the presence of Red spots at Rf 0.2, which showed presence of alkaloids when sprayed with drasgandraff's reagent, brown spots at Rf 0.2 and 0.35 confirmed the presence of saponins when sprayed with libermann-burchard reagent and blue spots at Rf .06 and 0.8 showed the presence of flavanoids when sprayed with 1per cent aluminum chloride solution as shown in Plate 13.

4.6.2 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography was performed with the aqueous extracts of different samples of tuber of *I. digitata*. Samples collected from best performing accessions were used for analysis. The HPLC profile of accessions in the aqueous extracts consistently exhibited a quantitative difference in the phytoconstituents particularly those eluting at 1.843 minute and 2.050 minute retention time (Rt) in the HPLC chromatograms. The data are presented in Table 35.

In the present research work, the identity of peak of rutin and nicotinic acid in sample solution, was confirmed by comparing the retention time (Rt) or retardation factor (Rf) with that of standard rutin and nicotinic acid (Fig. 12). The retention time (Rt) of rutin was found to be 2.050 min whereas, the retention time of nicotinic acid was found to be 1.843. The results confirmed the presence of flavanoids and alkaloids in the sample and two new compounds were identified in the present study which was not identified so far in *I. digitata i.e,* Rutin- A flavanoids and nicotinic acid- A alkaloid. The assay results indicate that amount of rutin (flavanoid) and nicotinic acid (alkaloid) estimated by HPLC method was 4.16 $\mu g\ g^{-1}$ and 17.20 $\mu g\ g^{-1}$ respectively.

4.7 STATISTICAL ANALYSIS

4.7.1 Phenotypic and Genotypic Correlations

Phenotypic and genotypic correlations were worked out among 19 characters to know the nature of associations existing among the characters. Significant correlation at 5 per cent and 1 per cent probability levels have been reported below and data is presented in the Table 36 and Table 37.

4.7.1.1 Number of Tuber

At both genotypic and phenotypic levels number of tuber per plant had positive and significant correlation with number of storage root per plant (0.9292; 0.6895). However, significant negative correlation at genotypic level was observed for the character saponins (-0.5951).

4.7.1.2 Length of Tuber (cm)

Length of tuber had positive and significant correlation with matured leaf length (0.4478) at genotypic level. Whereas, at phenotypic level the characters had no significant correlation with any of the traits.

4.7.1.3 Girth of Tuber (cm)

At both genotypic and phenotypic levels the character did not reveal positive and significant correlation with any of the traits. Whereas, at genotypic level girth of tuber showed negative significant correlation with matured leaf length (-0.5421) and matured leaf breadth (-0.5014). Whereas, no other character had significant association with any traits at phenotypic level.

Table. 36. Genotypic correlation coefficient among 19 characters in milk yam accessions (*I. digitata* L.)

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}
X_1	1	-0.2035	-1067	-0.0878	0.2524	0.9292**	0.0123	0.1052	0.2772	0.272	0.3225	-0.5951**	0.0891	-0.4148	-0.3219	-0.1256	-0.2257	0.3024	0.1668
X_2		1	-0.0192	-0.031	-0.2464	0.065	0.3357	-0.3647	-0.134	0.4478	0.3968	-0.2543	-0.2796	0.4094	-0.4214	-0.1516	-0.0727	-0.2575	-0.301
X_3			1	-0.1792	0.0204	0.0824	0.0243	-0.2624	-0.1509	-0.5421*	-0.5014*	0.3186	0.1823	0.2393	0.2695	0.4114	0.1241	-0.4068	-0.3877
X_4				1	0.8922**	0.4018	-0.2103	-0.166	0.3215	0.1889	-0.0699	-0.4799*	-0.1869	0.2662	-0.4754*	0.7992**	-0.5215*	0.0623	-0.1204
X_5					1	0.4664*	-0.3648	0.006	0.3236	-0.2215	-0.5618*	-0.3955	-0.0523	0.066	-0.4916*	0.5237*	-0.2221	-0.0684	-0.1156
X_6						1	-0.0851	-0.1118	-0.2037	0.4609*	0.2654	-0.5813**	-0.1982	-0.0335	-0.7352**	0.2561	-0.6616**	0.0478	0.1238
X_7							1	-0.494*	-0.1254	0.3943	0.139	-0.068	0.2224	-0.0062	-0.0581	0.0318	0.9043**	0.0807	-0.0756
X_8								1	0.319	-0.4262	0.1423	0.1431	-0.2524	-0.3012	0.107	0.0574	0.098	-0.155	0.1379
X_9									1	-0.17	0.2162	-0.3733	-0.0272	-0.3456	0.2737	-0.0787	-0.0729	0.2946	0.1217
X_{10}										1	0.7455**	-0.4177	-0.3569	0.1182	-0.6333**	0.1501	-0.3717	0.3753	-0.1685
X_{11}											1	-0.5119*	-0.5592*	0.2241	-0.0516	-0.4585*	-0.6201**	0.1676	-0.2424
X_{12}												1	0.3579	-0.0805	0.3459	0.0135	0.5444*	0.0113	0.1962
X_{13}													1	-0.1333	0.5447*	-0.0986	0.6238**	0.5688**	0.0693
X_{14}														1	-0.0338	-0.0611	-0.3418	-0.0481	-0.1851
X_{15}															1	-0.3866	0.1818	0.1823	0.0964
X_{16}																1	-0.152	-0.2053	-0.09
X_{17}																	1	-0.3003	0.0283
X_{18}																		1	0.1617
X_{19}																			1

**significant at 1% *significant at 5%

Table. 37. Phenotypic correlation coefficient among 19 characters in milk yam accessions (*I. digitata* L.)

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}
X_1	1	-0.1064	0.0118	0.1015	0.1773	0.6895**	-0.0626	-0.057	0.084	0.0761	-0.0267	-0.2015	-0.0175	-0.269	-0.3345	-0.1462	-0.0664	0.1223	0.055
X_2		1	0.081	0.0445	-0.0962	-0.0182	0.1906	0.2319	-0.072	0.3011	0.1386	-0.1321	-0.1176	0.3341	-0.1596	-0.0468	0.0462	-0.1283	-0.3103
X_3			1	0.1677	0.2908	0.0081	0.0205	-0.1253	-0.1479	-0.087	-0.3143	0.0498	0.1897	0.1437	0.0274	0.2304	-0.055	-0.2155	-0.0977
X_4				1	0.6501**	0.2412	-0.1742	-0.0947	0.1546	0.123	-0.1031	-0.0466	-0.0782	0.1821	-0.1645	0.4361	-0.3402	-0.0245	-0.0606
X_5					1	0.1692	-0.2167	-0.0238	0.0698	-0.0302	-0.269	-0.2484	0.0366	0.0098	-0.2739	-0.4031	-0.1613	0.0724	-0.1019
X_6						1	-0.1338	-0.0165	-0.0637	0.2352	0.0673	-0.3196	-0.1324	-0.0419	-0.6513**	0.1102	-0.2331	0.0633	0.0438
X_7							1	-0.3426	-0.0983	0.2772	0.0997	-0.0922	0.1806	-0.004	-0.0061	0.0582	0.3889	0.0905	0.0873
X_8								1	0.2531	-0.24	0.0161	0.0927	-0.1686	-0.1956	0.1431	0.0335	-0.0798	-0.0953	0.0488
X_9									1	-0.144	0.853**	-0.1547	-0.0125	-0.2055	0.2754	-0.0257	-0.0381	0.0953	0.1369
X_{10}										1	0.399	-0.2401	-0.2028	0.0953	-0.3549	0.1289	-0.2515	0.2454	-0.077
X_{11}											1	-0.2368	-0.3299	0.1357	-0.0072	-0.1796	-0.0972	0.227	-0.0876
X_{12}												1	0.22	-0.0214	0.2347	0.0234	-0.0187	-0.0843	0.1407
X_{13}													1	-0.1061	0.3829	-0.0458	0.1848	0.4622*	0.0263
X_{14}														1	-0.0078	-0.0375	-0.1477	-0.0498	-0.146
X_{15}															1	-0.1514	-0.0501	0.1259	0.1086
X_{16}																1	-0.1433	-0.1402	-0.1215
X_{17}																	1	0.1409	0.1455
X_{18}																		1	0.1373
X_{19}																			1

**significant at 1% *significant at 5%

4.7.1.4 Fresh Tuber Yield per Plant (g)

Fresh tuber yield per plant showed positive and significant correlation with dry tuber yield per plant (0.8922; 0.6501) at both the genotypic and phenotypic levels, respectively. At genotypic level oils (0.7992) also reported positive and significant association. Whereas, saponins (-0.4799), fatty acids (-0.4754) and proteins (-0.5215) showed significant negative correlation with the character.

4.7.1.5 Dry Tuber Yield per Plant (g)

Dry tuber yield per plant at genotypic level reported significant positive correlation with number of storage root per plant (0.4664) and oil content (0.5237). Whereas, the same character had negative significant correlation with matured leaf breadth (-0.5618) and fatty acids (-0.4916) content of the tuber. The character had no phenotypic correlation with any of the traits.

4.7.1.6 Number of Storage Root per Plant

At genotypic level number of storage root per plant is found to have significant and positive correlation with matured leaf length (0.4609). However, significant but negative correlation of this trait was observed for characters like saponins (-0.5813), fatty acid (-0.7352) and proteins (-0.6616) content of tuber. At phenotypic level, the character reported significant and negative correlation with fatty acids content (-0.6513).

4.7.1.7 Plant Height (cm)

At genotypic level plant height showed positive and significant correlation with protein (0.9043) content of tuber. The character has no significant correlation at phenotypic level. However, at both genotypic and phenotypic levels the character is found to have non-significant and positive correlation with matured leaf length,

matured leaf breadth, glycosides, fats and oils. The character also had non-significant and negative association with internode length, saponins, flavanoids and fatty acids at both levels.

4.7.1.8 Internode Diameter (cm)

Internode diameter does not exhibit any significant association with any of the traits at both genotypic and phenotypic levels. However, at both levels the character reported positively non-significant correlation with internode length, matured leaf breadth, saponins, fatty acids, oils and carbohydrates. The character also reported negative and non-significant association with matured leaf length, glycosides, flavanoids and alkaloids.

4.7.1.9 Internode Length (cm)

Internode length did not show any significant correlation with any of the trait at genotypic level. However, at phenotypic level the character has positive significant association with matured leaf breadth (0.853). At both genotypic and phenotypic level, the character had positive and non-significant correlation with fatty acids and carbohydrates. Also the character has negative and non-significant association with matured leaf length, saponins, glycosides and flavanoids.

4.7.1.10 Matured Leaf Length (cm)

At genotypic level, matured leaf length has positive significant correlation with matured leaf breadth (0.7455) and negative significant correlation association with fatty acids (-0.6333). Whereas, at phenotypic level the character has no significant relationship with any of the traits.

4.7.1.11 Mature Leaf Breadth (cm)

At genotypic level, mature leaf breadth has significant negative association with saponins (-0.5119), glycosides (-0.5592), oils (-0.4585) and proteins (-0.6201) content of the tuber. At phenotypic level the character has no significant association with any traits.

4.7.1.12 Saponins(%)

Saponins had significant positive genotypic correlation with protein (0.5444) content of the tuber. Whereas, it had no significant association with any of the characters at genotypic level. However, at both the levels the character has positive and non-significant association with glycosides, fatty acids, oils and carbohydrates. Also the character has negative non-significant association with flavanoids.

4.7.1.13 Glycosides (%)

Glycosides are found to have positive significant genotypic correlation with fatty acids (0.5447), proteins (0.6238) and alkaloids (0.5688) content of the tuber. The character also had positive significant phenotypic correlation with alkaloids (0.4622).

4.7.1.14 Flavonoids (mg g^{-1})

None of the characters at both genotypic and phenotypic levels exhibited positively significant association with flavonoids. However, at both levels flavonoids showed positive non-significant correlation with fatty acids, oils, proteins, alkaloids and carbohydrates.

4.7.1.15 Fatty Acids (mg KOH g^{-1})

Fatty acids do not exhibit any significant correlation with any of the characters at both genotypic and phenotypic levels. However, fatty acids at both levels showed positively non-significant correlation with alkaloids and carbohydrates. The character also exhibited negatively non-significant association with oils.

4.7.1.16 Oils (%)

None of the characters at both genotypic and phenotypic levels exhibited positively significant association with oils. However, at both levels oils showed positive and non-significant correlation with proteins, alkaloids and carbohydrates.

4.7.1.17 Proteins (mg g^{-1})

Proteins do not exhibit any significant association with any of the characters at both genotypic and phenotypic levels. However, at both levels proteins recorded positively non-significant association with carbohydrates.

4.7.1.18 Alkaloids (%)

At both genotypic and phenotypic levels, alkaloids do not exhibit any significant association with any of the traits. However, at genotypic level the character has positive and non-significant correlation with carbohydrates.

4.7.1.19 Carbohydrates (%)

Carbohydrates do not exhibit any significant association with any of the characters at both genotypic and phenotypic levels.

4.7.2 Estimation of Variability Components

The genotypic and environmental components of phenotypic variance are presented in Table 38. (Fig. 13.), along with the genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), which is the relative measure of variation used for comparison among characters measured in different units.

Maximum phenotypic (52.92%) and genotypic coefficient (33.99%) of variation were observed for dry tuber yield per plant followed by flavanoids (PCV 39.29 per cent and GCV 37.58%), oils (PCV 38.07% and GCV 33.44%) and fresh tuber yield per plant (PCV 38.24% and GCV 24.58%) respectively.

The minimum PCV and GCV were recorded by the characters such as glycosides as 3.08 per cent for PCV and 2.33 per cent for GCV. Matured leaf breadth (PCV 5.61% and GCV 3.88%) as well as matured leaf length (PCV 9.28% and GCV 7.73%), Protein (PCV 15.28% and GCV 7.47%), internode length (PCV 12.63% and GCV 9.61%) and fatty acid (PCV 13.86% and GCV 9.71%) showed low value for genotypic and phenotypic levels.

The characters, dry tuber yield per plant, fresh tuber yield per plant, number of tuber, girth of tuber and proteins showed maximum difference between PCV and GCV which indicates the influence of the environment on these characters is considerable. But the low difference between GCV and PCV for the characters glycosides, saponins, carbohydrates, matured leaf length, flavanoids and matured leaf breadth pointed out that the variation observed in these characters are mainly due to genetic reasons and environmental influence on these characters was less.

4.7.3 Heritability and Genetic Advance

According to Allard (1960) classification of heritability, heritability percentage of less than 30 per cent indicates low heritability, 30-60 per cent medium

Table. 38. Components for total variance along with heritability and genetic advance of 19 characters of milk yam accessions (*I. digitata* L.)

Sl. no	Characters	GCV	PCV	Heritability (%)	Genetic Advance (5%)	Genetic % as of Mean
1	Number of tubers	11.81	19.73	35.84	0.37	14.57
2	Length of tubers	11.95	16.46	52.73	5.34	17.88
3	Girth of tubers	12.52	19.65	40.59	1.94	16.43
4	Fresh tuber yield plant^{-1}	24.58	38.24	41.33	178.65	32.56
5	Dry tuber yield plant^{-1}	33.99	52.92	41.26	139.73	44.98
6	Number of storage root plant^{-1}	16.56	22.22	55.57	0.63	25.43
7	Plant height	12.16	14.57	69.66	58.33	20.91
8	Internode diameter	17.13	21.45	63.77	0.70	28.18
9	Internode length	9.61	12.63	57.96	2.23	15.08
10	Matured leaf length	7.73	9.28	69.41	1.33	13.27
11	Matured leaf breadth	3.88	5.61	47.98	0.63	5.54
12	Alkaloids	12.67	19.48	42.28	1.28	16.97
13	Carbohydrates	11.98	13.33	80.80	0.08	22.19
14	Flavonoids	37.58	39.29	91.50	0.24	74.05
15	Fatty acids	9.71	13.8	49.08	0.01	14.02
16	Oils	33.44	38.07	77.18	0.42	60.53
17	Proteins	7.47	15.28	23.94	0.29	7.53
18	Saponins	3.58	4.53	62.55	1.68	5.83
19	Glycosides	2.33	3.08	57.38	2.02	3.64

heritability and more than 60 per cent high heritability. Based on this classification in the present study high heritability was noticed in characters such as flavanoids (91.50 per cent) followed by carbohydrates (80.80%), oils (77.18%), plant height (69.66%), matured leaf length (69.41%) and saponins (62.55%).

Medium heritability was noticed in characters internode length (57.96%), glycosides (57.38 per cent), number of storage root per plant (55.57), length of tuber (52.73%), fatty acids (49.08%), matured leaf breadth (47.98 %), alkaloids (42.28 %), fresh tuber yield per plant (41.33%), dry tuber yield per plant (41.26 %), girth of tuber (40.59 %) and number of tuber (35.84 %). Whereas, the lowest heritability was expressed by the character proteins as 23.94 per cent.

Genetic advance as percentage of mean is independent of the unit of measurement and hence is used for the comparison of characters. Maximum genetic advance was obtained for the character flavanoids (74.05%) followed by oils (60.53%), dry tuber yield per plant (44.98%) and fresh tuber yield per plant (32.56%). The least genetic advance was obtained glycosides (3.64%).

4.7.4 Mahalnobis distance [D^2]

To study genetic diversity among the genotypes, Mahalanobis D^2 statistics was used. The D^2 statistics enables to discriminate between different accessions according to the diversity pattern (Mahalanobis, 1963). It gives clear idea about the diverse nature of the populations. The results could give an insight about diverse nature of the accessions in a cluster means which also gives the difference between accessions which belong to different clusters.

By employing D^2 statistics, the genetic diversity among 20 accessions was measured. For all the accessions, the correlated unstandardized mean values (x) for 11 characters under consideration were transformed to the correlated standardized value (y). The D^2 value which being the sum of squares of differences for each Y

value was calculated for all the combinations. Based on the D^2 values the accessions were grouped into 6 clusters using Ward's method. Of the 6 clusters, cluster 1 was the largest comprising of 8 accessions [*viz.,* Vellayani, Panamaram, Pattikadu, Kayamkulam, Chittar, Pravachambalam, Neyyttinkara and Pallichal] followed by cluster 2 with 6 accessions [Meppadi, Punnamoodu, Peringamala, Sasthamkotta, Kunnathukal and Amboori]. Whereas, cluster 3 had 3 accessions [Peechi, Kalliyoor and Udumbanchola]. Cluster 4 [Punnamoodu], cluster 5 (Kumbalamngagy) and cluster 6 (Aruvikkara) had least number of accessions. Grouping of *I. digitata* germplasm based on D^2 values are represented in Table 39.

4.7.4.1 Intercluster Distance

The intercluster D^2 and D values are given in Table 40. The intercluster D^2 values were maximum (126.95) between cluster 5 and cluster 6. The minimum distance (43.32) was observed between cluster 2 and cluster 5. If the D^2 value is more it indicates the relationship is highly diverse. Whereas, if the D^2 value is less it indicates they are more similar genetically.

4.7.4.2 Intracluster Distance

The intracluster cluster distances observed among the accessions varied from 0 to 29.24. The intracluster cluster distances was highest in cluster 2 (29.24), followed by cluster 1 (29.02) and cluster 3 (17.86).

4.7.4.3 Cluster Mean Analysis

The cluster means for 11 different characters (Table 41) were compared and indicated the considerable difference between clusters for all the characters.

Cluster means where high in cluster 3 for characters *viz.,* number of tuber, girth of tuber, fresh tuber yield plant^{-1}, dry tuber yield per plant, oils and proteins.

Table 39: Clustering pattern of accessions of *I. digitata* L.

Sl. No	Clusters	No. of Accession	Name of the Accessions
1	1	8	Vellayani (T2), Panamaram (T3), Pattikadu (T4), Kayamkulam (T6), Chittar (T8), Pravachambalam (T12), Neyyttinkara (T13) and Pallichal (T16).
2	2	6	Meppadi (T1), Peringamala (T10), Sasthamkotta (T14), Kunnathukal (T17), Amboori (T18) and Thopramkudy (T19).
3	3	3	Peechi (T5), Kalliyoor (T7) and Udumbanchola(T20).
4	4	1	Punnamoodu (T9), Thiruvananthapuram District.
5	5	1	Kumbalangy (T11), Ernakulam District.
6	6	1	Aruvikkara (T15), Thiruvananthapuram District.

Table. 40. Average inter and intra cluster distances (D^2 values) in milk yam accessions.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	29.02	70.10	52.56	48.97	110.87	52.98
Cluster 2		29.24	71.78	90.91	43.32	97.33
Cluster 3			17.86	90.95	121.64	70.75
Cluster 4				0	126.46	101.49
Cluster 5					0	126.95
Cluster 6						0

Table. 41. Cluster means of various characters of milk yam accessions (*I. digitata* L.).

Cluster	x_1	x_2	x_3	x_4	x_5	x_6	x_7	x_8	x_9	x_{10}
1	2.42	28.72	11.98	577.75	302.65	28.34	0.25	0.85	3.82	8.02
2	2.58	30.68	12.33	511.06	283.33	28.91	0.41	0.61	3.79	7.33
3	2.82	32.57	9.43	410.19	226.17	29.71	0.23	0.46	4.14	6.93
4	2.47	31.53	13.59	431.67	298.73	27.80	0.47	0.43	4.01	7.06
5	2.07	27.74	10.77	752.13	432.53	30.20	0.55	0.53	3.60	8.93
6	3.00	26.67	13.77	870.47	681.73	28.40	0.24	1.06	4.37	6.01

x_1- Number of tubers, x_2- Length of tubers, x_3-Girth of tubers, x_4- Fresh tuber yield/plant, x_5- Dry tuber yield/plant, x_6- Alkaloids, x_7- Flavonoids, x_8- Oils, x_9- Proteins and x_{10}- Saponin.

Whereas, the cluster 5 had high cluster mean for alkaloids, flavanoids, saponins and glycosides. Cluster mean was high for length of tuber in cluster 3. This shows that the accessions kumbalangya and aruvikar are wild accessions which showed high variability.

4.7.5 Divergence Analysis

4.7.5.1 Yield Characters

With regard to divergence of yield characters the character *viz.*, fresh tuber yield plant^{-1} recorded highest percentage of divergence being (37%) followed by dry tuber yield plant^{-1} (24%), length of tuber (18%), girth of tuber (12%) and number of tuber per stock (9 %) respectively. (Fig. 14)

4.7.5.2 Phytoconstituents

Contribution of phytoconstituents towards divergence reported that, highest divergence was observed for flavanoids (48%), followed by oils (16%), glycosides (14%), alkaloids (8%), carbohydrates (6%), fatty acids (3%), proteins (3%) and saponins (2%) respectively. (Fig. 14).

4.8 Climatic and Soil factor Influence

The data pertaining to influences of climate and soil factors on the content of milk yam are presented in the Table 42.

4.8.1 Altitude (m AMSL)

The accessions which were collected from higher altitude region (above 1000 m AMSL) showed higher active ingredient content (alkaloids, carbohydrates, glycosides, saponins, phytosterols, fats and oils, resins, flavanoids and proteins) followed by the accessions collected from 100-1000 m AMSL. Comparatively lesser

Table 42. Variability in phytoconstituents content in milk yam (*I. digitata* L.) under different climate and soil.

Climate		Saponins (%)	Glycosides (%)	CHO (%)	Alkaloids (%)	Proteins (mg g^{-1})	Flavanoids (mg g^{-1})	Fatty acid (mg KOH g^{-1})	Oils (%)
Altitude (meters AMSL)	0-100	5.48	0.28	48.94	27.11	4.09	0.42	0.17	0.68
	<1000	5.60	0.31	54.46	24.47	3.91	0.45	0.21	0.93
	>1000	7.99	0.51	56.02	28.73	4.70	0.47	0.18	0.91
Temperature (^{0}C)	20-25	9.22	0.58	60.60	31.05	4.61	0.46	0.18	1.01
	25-30	5.86	0.30	50.95	27.24	4.13	0.46	0.17	0.66
	> 30	5.28	0.29	48.07	25.95	4.17	0.39	0.17	0.81
Rain fall (mm)	1500-2000	5.90	0.30	50.92	27.13	4.22	0.47	0.15	0.70
	2000-3000	6.82	0.41	53.41	28.34	4.33	0.41	0.18	0.81
	3000-3500	5.59	0.31	49.21	26.46	3.91	0.38	0.17	0.79
Soil type	Laterite	6.18	0.33	51.61	27.32	4.28	0.44	0.18	0.77
	Red loam	4.57	0.26	47.67	25.78	3.67	0.34	0.17	0.91
	Sandy to clay loam	5.33	0.29	48.69	28.26	3.55	0.37	0.12	0.37
	Alluvial	6.76	0.43	51.45	26.41	4.78	0.49	0.19	0.80

active ingredients were observed in the accessions which were collected from the regions with 1-100m AMSL.

4.8.2 Temperature (^{0}C)

The accessions which were collected from different zones which fall under different temperatures showed that, the accessions collected from the temperature range of 20-25^{0}C were preferably high in phytoconstituents content than compared to the accessions collected from higher temperature areas above 25 ^{0}C.

4.8.3 Rain fall (mm)

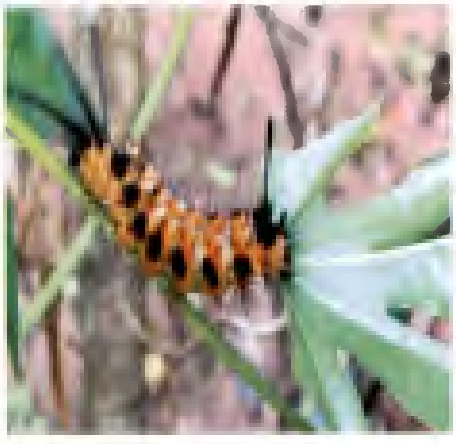
Highest phytoconstituents content was seen in the accessions collected from medium rain fall areas of 1000-2000mm. Decreased rainfall or increased rainfall above 2000mm showed the lower active ingredient contents.

4.8.4 Soil type

The accessions were collected from different Districts varied differently among the phytoconstituent content. The accessions which were collected from the areas of laterite soils and alluvial soils showed increased active ingredients comparably then the other accessions which were collected from areas of red loam and sandy to clayey loam soils.

(A) Egg

(B) Early instar larvae



(C) Late instar larvae

(D) Adult

Plate 15. Pest of *I. digitata* L. (*Echiomyia polymina* caterpillar)

Discussion

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V. DISCUSSION

The presence of phytoconstituents in milk yam (*I. digitata* L.) and the underutilized recognition of it as a medicinal plant of high health benefits, renders, a high profile for this crop. A systematic study on the extent of variation under uniform cultural conditions has not been reported so far. The present investigation is the bold attempt by integrating diversity analysis and reproductive biology to bring out a report on the reproductive biology of *I. digitata* L. from different zones of Kerala. Their existence was not known for several decades after their discovery and hence such studies assume great significance not only for conservation but also have direct relevance for genetic improvement and popularization of these pharmaceutically and nutraceutically potential species. Therefore, in the present investigation, a study on diversity analysis and reproductive biology of milk yam was carried out and results of the experiment are discussed in this chapter under the following heads;

1. Survey and collection
2. Morphological characterisation
3. Inflorescence characters and Reproductive biology
4. Root and yield characters
5. Phytochemical screening
6. Chromatography
7. Selection of promising genotypes
8. Variability components
9. Cluster analysis
10. Climate and soil factor influence

5.1. SURVEY AND COLLECTION

The fundamental objective of collecting plant genetic resources is to capture the maximum amount of variation in the smallest number of samples (Marshall and Brown, 1975). During the present investigation a roving survey was carried out at

different districts of Kerala, twenty diverse accessions were collected from eight different districts of Kerala which included two accessions from Wayanad district, ten accessions from Thiruvananthapuram district, two accessions from Thrissur district, one accession each from Alappuzha district, Pathanamthitta district, Eranakulam district, Kollam district and two accessions from Idukki district respectively.

5.1.1. Ethnobotanic Information on Milkyam

The ethnobotanical information gathered from different districts revealed that in Kerala milk yam powder is generally used as a galactagogue/general tonic/aphrodisiac along with milk/honey This obsevation is in consonance with the reports by Sarkar, (1991); Singh *et al.* (2004); Singh and Panda (2005); Behera *et al.* (2006); Jain (2011); Asha *et al.* (2013) regarding the folkloric uses of milk yam from different states of India.

5.1.2. Soil Type

Wide variation in the soil types of the places from where the accessions were collected was noticed. Soil texture in most of the soil series ranged from sandy loam to sandy clay loam. In Thiruvananthapuram milk yam was found to grow luxuriantly in non gravelly laterite type, whereas, in Kollam they were found in laterite type soil. In Pathanamthitta District they were found to occur in red loam soils whereas, in Alappuzha and Eranakulam districts milk yam flourishes naturally in sandy to clayey loam soils. In Thrissur they were found to grow in midland laterite soil whereas, in Idukki hydromorphic and alluvial soils supported their growth. From Wayanad the accessions were found in laterite soil type. The occurrence of *I. digitata* L. in these widely varying soil conditions shows that it is has wide adaptability to varying soil conditions.

5.2. MORPHOLOGICAL CHARACTERISATION

Adequate characterization for agronomic and morphological traits is necessary to facilitate the utilization of germplasm by breeders (Upadhyaya *et al.*, 2008). To achieve this, the collected accessions were grown in the field under uniform conditions. Since the milk yam vines resembles sweet potato in morphological and storage root characters, for characterizing the morphological and storage root traits the descriptor developed by international Potato Centre for sweet potato was used with certain modifications. Morphological diversity is assessed by measuring variation in phenotypic traits such as flower color, shape of leaves and growth habit (Rao, 2004). Phenotypic traits have long been used in selecting crops that best suits needs of farmers (Gepts, 2004).

5.2.1. Vine characters

Vines depend on external support to prevent shading by neighbouring plants. In milk yam twining of the vine increased with the increase in days after planting. At 3 MAP, all the accessions showed slightly twining and it increased gradually from 3 MAP till maturity in all accessions (Fig. 1). Profuse twining was observed at 9 MAP and at 12 MAP which coincides with the active growth stages of the plants. High twining provides better anchorage for the plant and also helps to withstand weather aberrations. Not much variation in twining was noticed among the accessions which indicate that it is a genetic character not controlled by environment.

Plant height varied significantly among the accessions and at 12 MAP it ranged from 167.27cm to 329.07cm. The highest plant height was observed in Accession T20 (Udumbanchola, Idukki District). The higher length might be the indication of higher vigour and faster growth rate and it may also be due to productivity of soil and higher rainfall. Benefits obtained from long vines are that it can be used as a source of planting materials and can be used as livestock feed. The lowest plant height was recorded from accession T6 (Kayamkulam, Alappuzha

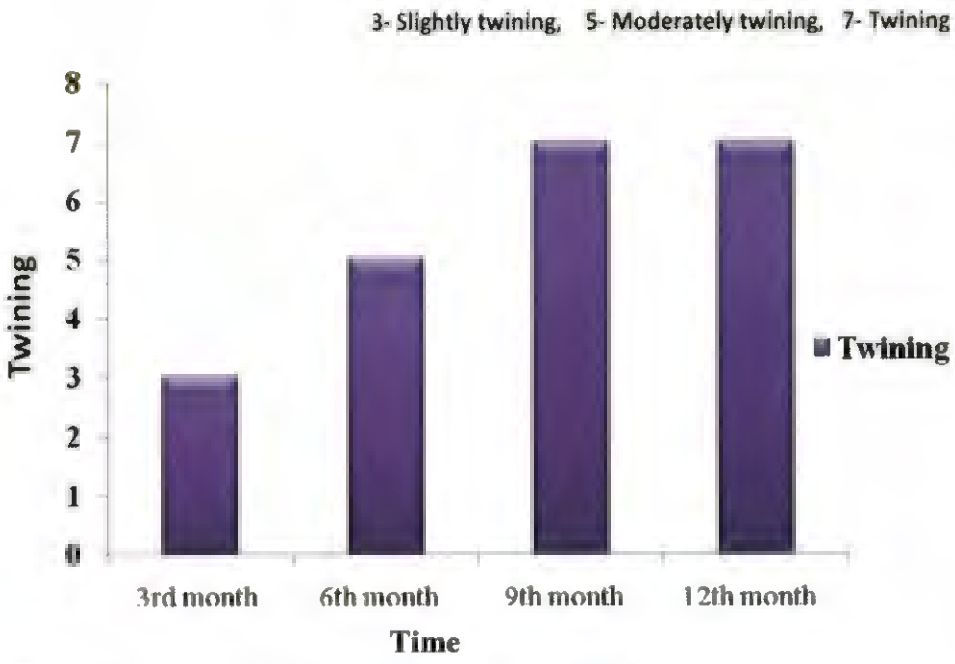


Fig 1. Twining at different growth stages of milk yam accessions (*I. digitata* L.).

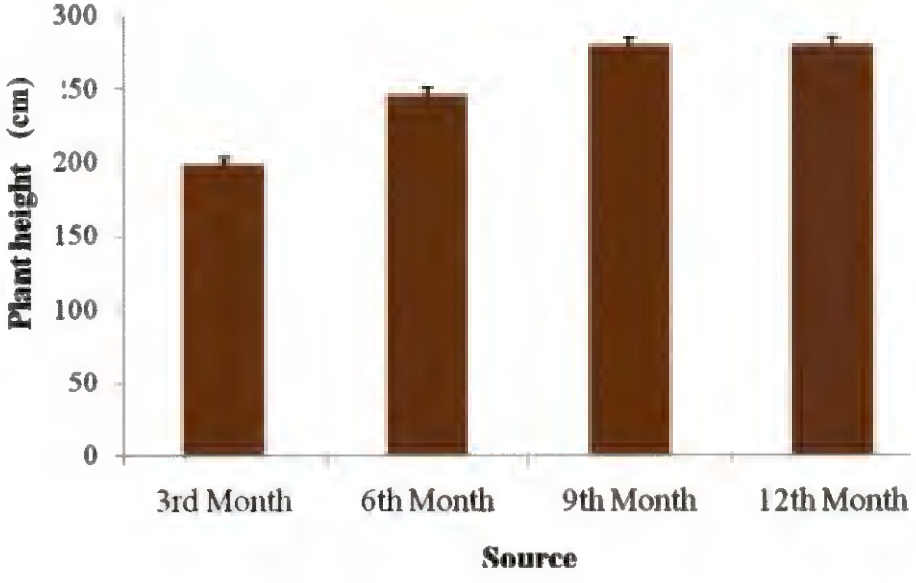


Fig 2. Plant height (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

District) and the shorter lengths may be due to bushy habit of the cultivars. Cultivars with less bushy habitat are more likely to grow tall and vice-versa. Plant height differs due to the genetic makeup of the accessions.

At 3 MAP to 6 MAP, the plants exhibited spreading habit (97.5%) in which plant height ranged from 151-250 cm and later on after 6 MAP, they exhibited extremely spreading habit (90%) as represented in Fig. 2. similar findings were recorded by Ahmed *et al.*, (1996) in their study on sweet potato. They reported that, about 48.36 per cent of the accessions were found to be semi-compact types followed by compact types (29.20%) and only 1.88 percent of extremely spreading types.

Regarding the internode diameter of the main vine, it was observed that diameter increased gradually from 3 MAP till maturity (Fig. 3) and it varied significantly among the accessions ranging from 1.98 cm to 3.44 cm. Such variation might be due to their different genetic makeup and response to soil and climatic conditions. Rashid *et al.* (2002), Onunka *et al.* (2012) and Yooyongwech *et al.* (2014) stated that vine diameter is a genetic character and may differ from genotype to genotype under similar soil and environmental conditions.

Internodal length of the vine at 12 MAP ranged from 11.76 cm to 18.13 cm. The result revealed that the internode length increased rapidly until 6 MAP after that increased slowly till 12 MAP. The longer internodes as seen in accession T8 (Chittar, Pathanamthitta District) might be the indication of higher vigour and faster growth rate. In the study it was observed that accessions which produced higher tuber yields were having longer internodes (T12,T15) and those with shorter internode length(T14, T16) were found to be poor yielders (Fig. 4). Kareem (2013) reported that medium sized nodal length (14-15 cm) gave the best yield in sweet potato.

Predominant color of vine remained green for all the accession at all stages of crop growth *viz.*, 3 MAP, 6 MAP, 9 MAP and 12 MAP. This may be due to more sunshine hours prevailing in the region, high chlorophyll content and nutritive soil.

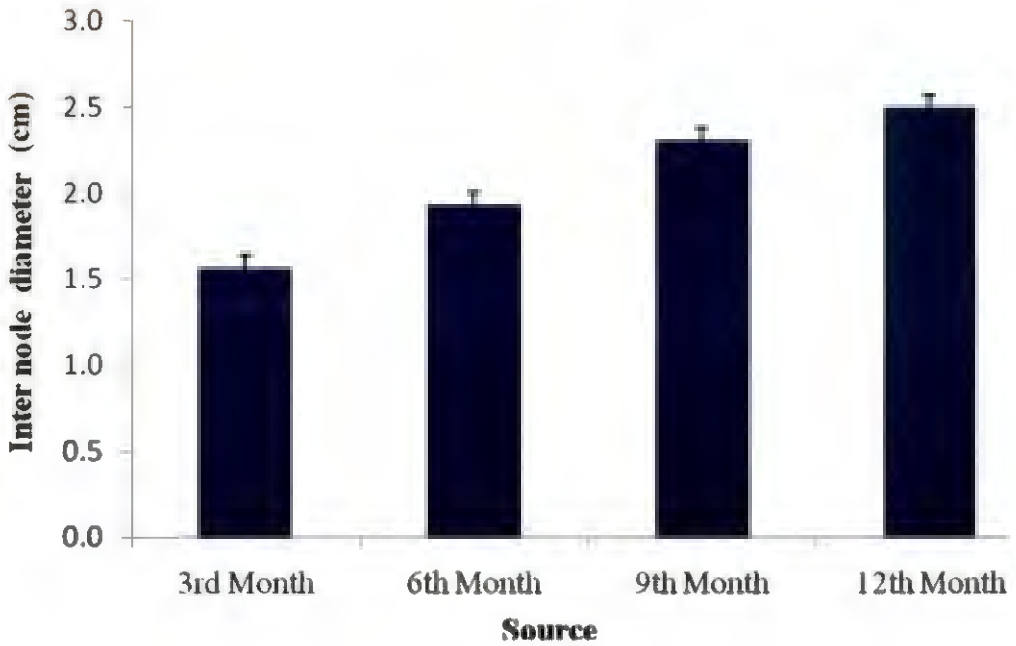


Fig 3. Internode diameter (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

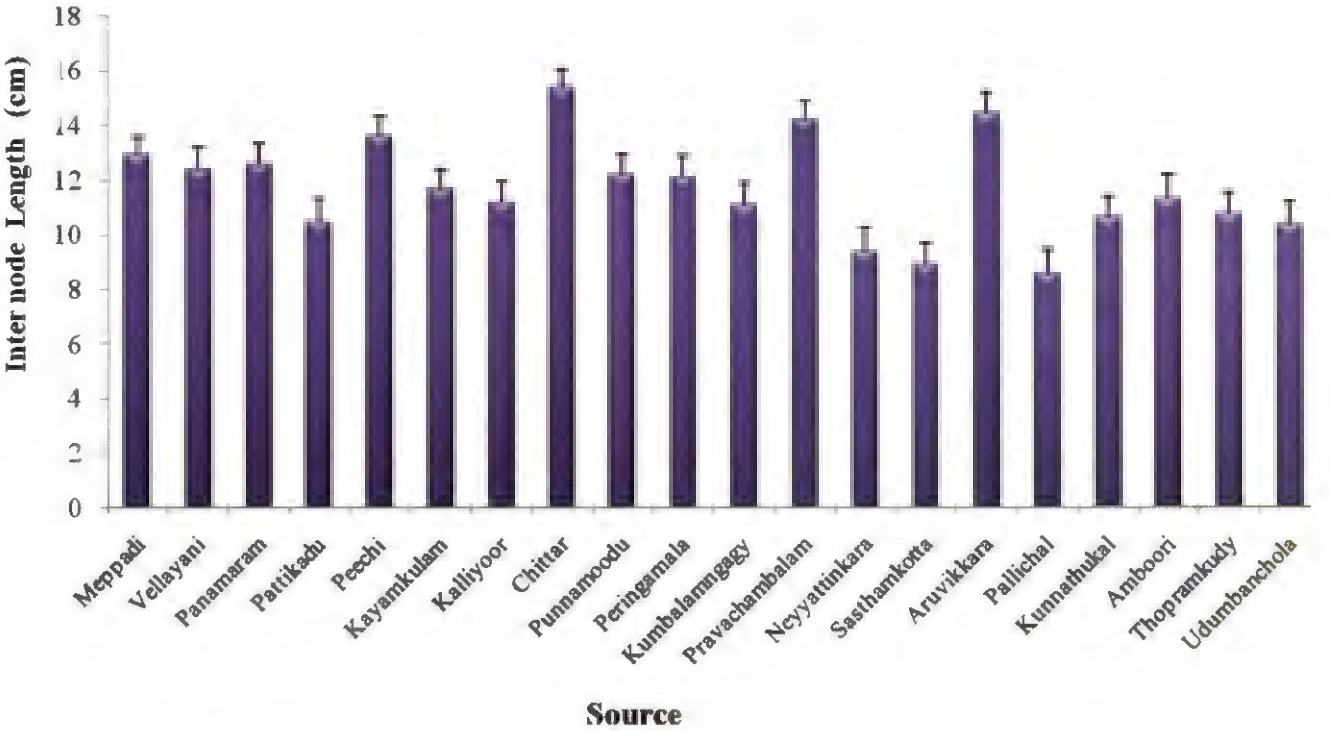


Fig 4. Internode length (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

These findings were also corroborated with the findings of Saraswati and Prabawardani (2007) who reported that the predominant green color of vine was due to the influence of nutrient supply (N and K), exposure to sunlight and availability of water at critical stages of crop growth.

5.2.2. Leaf Characters

Leaf characterization is basic for any crop improvement programme. Such characterization has been used for various purposes *viz.,* identification of duplicates, variability patterns and correlation of characteristics. In the present study, wide range of variation was observed for type of leaf lobe, number of leaf lobes, shape of central leaf lobe, matured leaf size, mature leaf color, immature leaf color, petiole pigmentation and petiole length among all the accessions.

The leaves of milk yam accessions at 3 MAP exhibited slight lobbing pattern, at 6 MAP some accessions showed slightly lobed pattern, some moderately lobed and some deeply lobed patterns (Fig. 5). Lobed leaves differ in the degree of the cut, ranging from superficial to deeply lobed. Martin *et al*., (1974) described *I. digitata* L. as a wild species closely resembling sweet potato. The observations on leaf characters are in conformity with the results of Fongod *et al.,* (2012) who reported that 55 per cent of sweet potato leaves were slightly lobed, followed by deep lobed (30%) and very deep lobed (20%).

The number of leaf lobes of all accessions was 7 at all stages of crop growth which agrees with the general leaf morphology already described in literature.

All the accessions possessed lanceolate leaf shape. Variation in leaf shape are often more inheritable and independent of the environment (Dickinson *et al.,* 1987). Another study had mentioned that the shape of the leaves is a response to the plant long term ecological and evolutionary history. The limiting factors from the

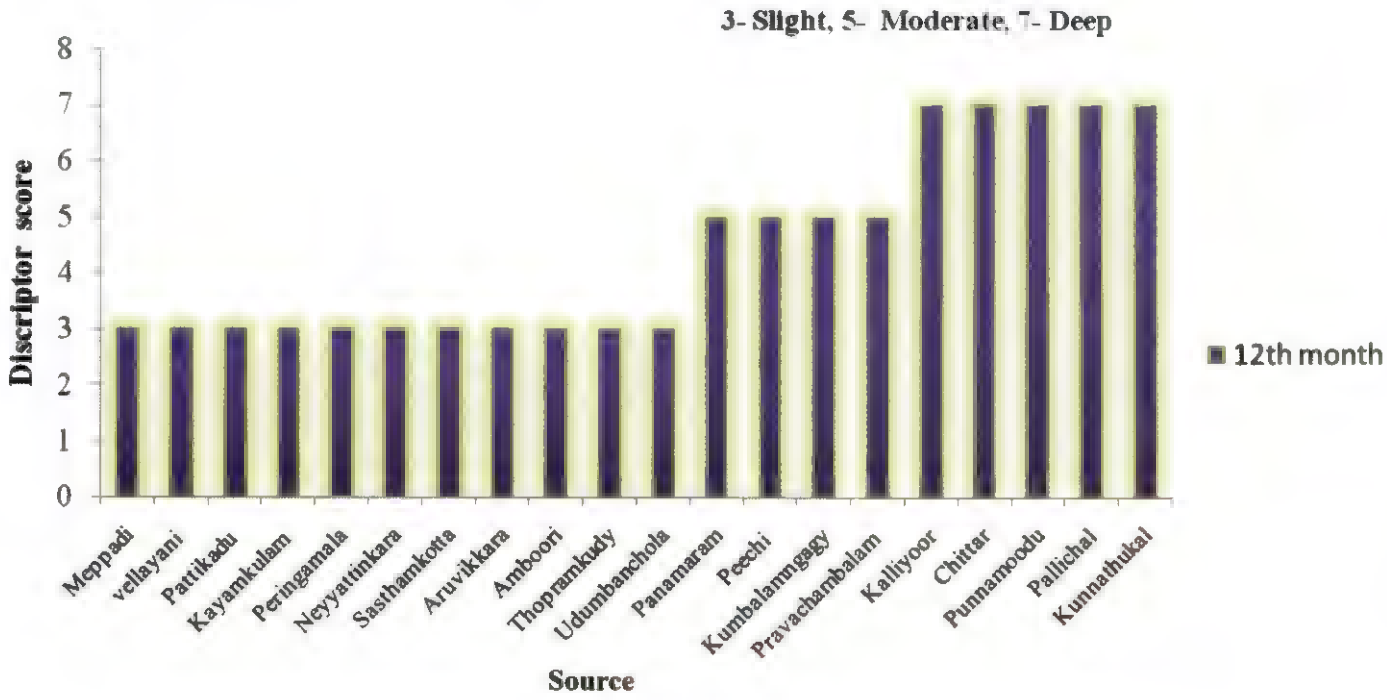


Fig 5. Type of leaf lobe at different growth stages of milk yam accessions (*I. digitata* L.).

environment may also modify the finished form and shape of a tree's leaves (Magdolna, 2005).

The matured leaf length (Fig. 6) of *I. digitata* L. at harvest ranged from 8.18cm to 11.43cm and the leaf breadth (Fig. 7) varied between 10.15cm and 12.10cm among the accessions. Higher the leaf length and breadth, higher will be the leaf area which increases the photosynthetic activity of the plant and also increases the tuber yield. Larger surface area in leaves has been associated with increased light absorption. Kareem (2013) reported that accessions with large leaf size can easily trap sunlight and hence carry out better photosynthesis required for carbohydrates synthesis than those with small leaf size. In the present study, accessions with larger leaf size were found to have higher tuber yield which is in agreement with the above statement.

The matured and immature leaves of all milk yam accessions remained green colored at all the crop growth stages indicating that green color may be an incompletely dominant trait. Hernandez *et al.* (1965; 1967) observed leaf color as a quantitative character which was controlled by several genes in complimentary action.

The petiole pigmentation among different accessions varied from green, green with purple near stem, green with purple near leaves, some petioles purple and others green (Fig. 8). Similar observations in sweet potato accessions has been reported by Fongod *et al.* (2012) and Veasey *et al.* (2008).

5.3. INFLORESCENCE CHARACTERS AND REPRODUCTIVE BIOLOGY

5.3.1 Inflorescence Characters

The genus *Ipomoea* Linn., include most fascinated species with peculiar flower structures and spectacular colors. Floral structural characters such as color, shape and size assume an important role in predicting the pollinator type. No variation in inflorescence or floral characters was noticed among the accessions. In all

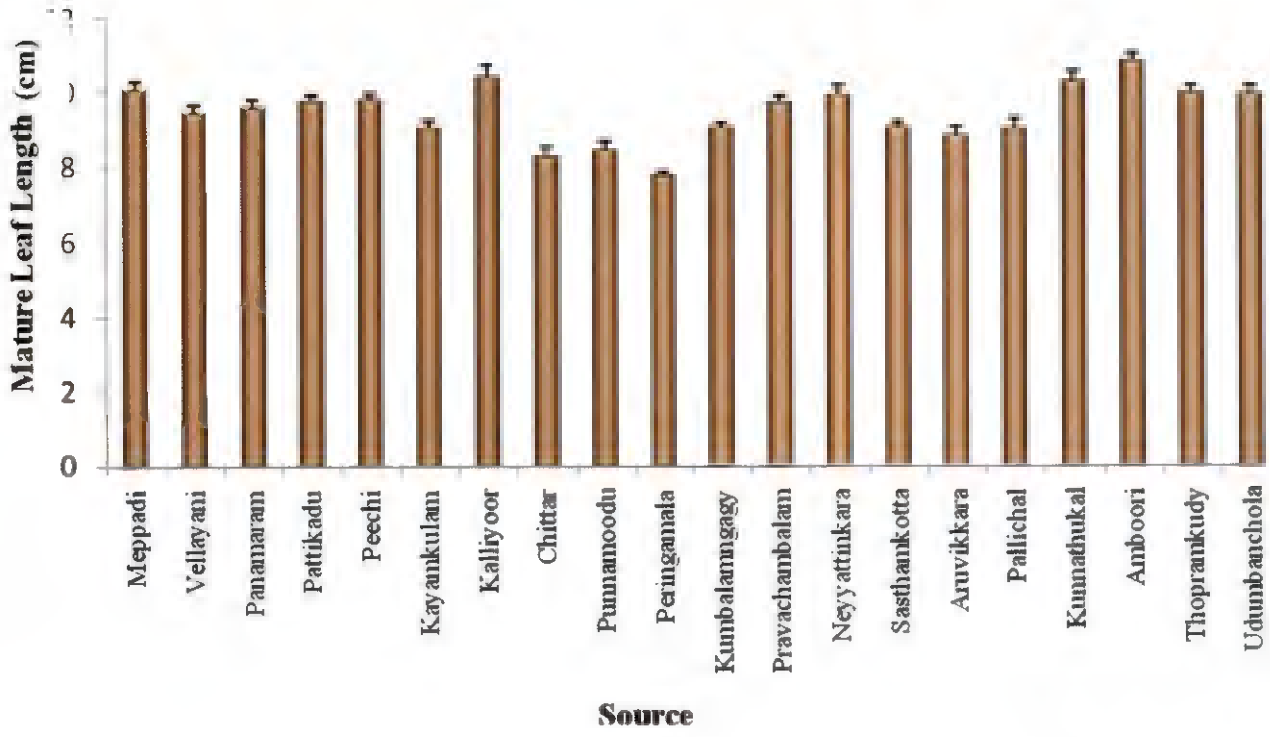


Fig 6. Mature leaf length (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

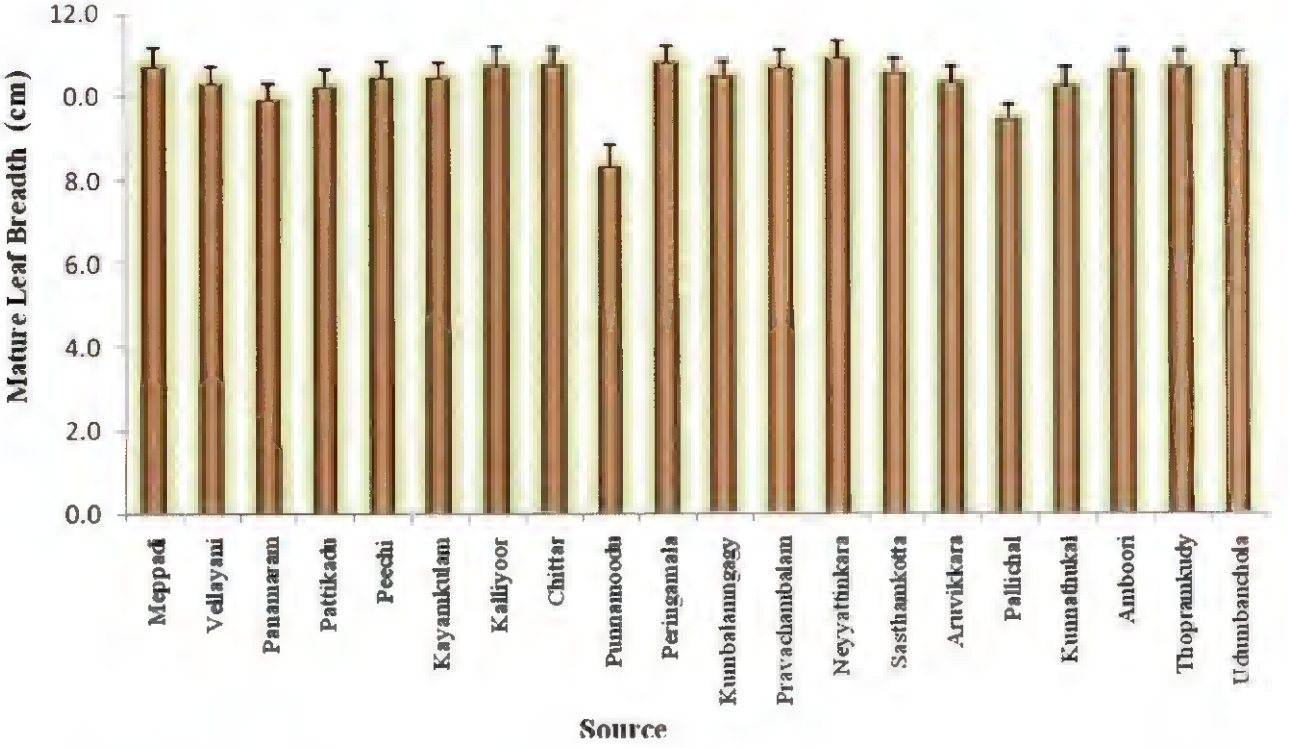


Fig 7. Mature leaf breadth (cm) at different growth stages of milk yam accessions (*I. digitata* L.).

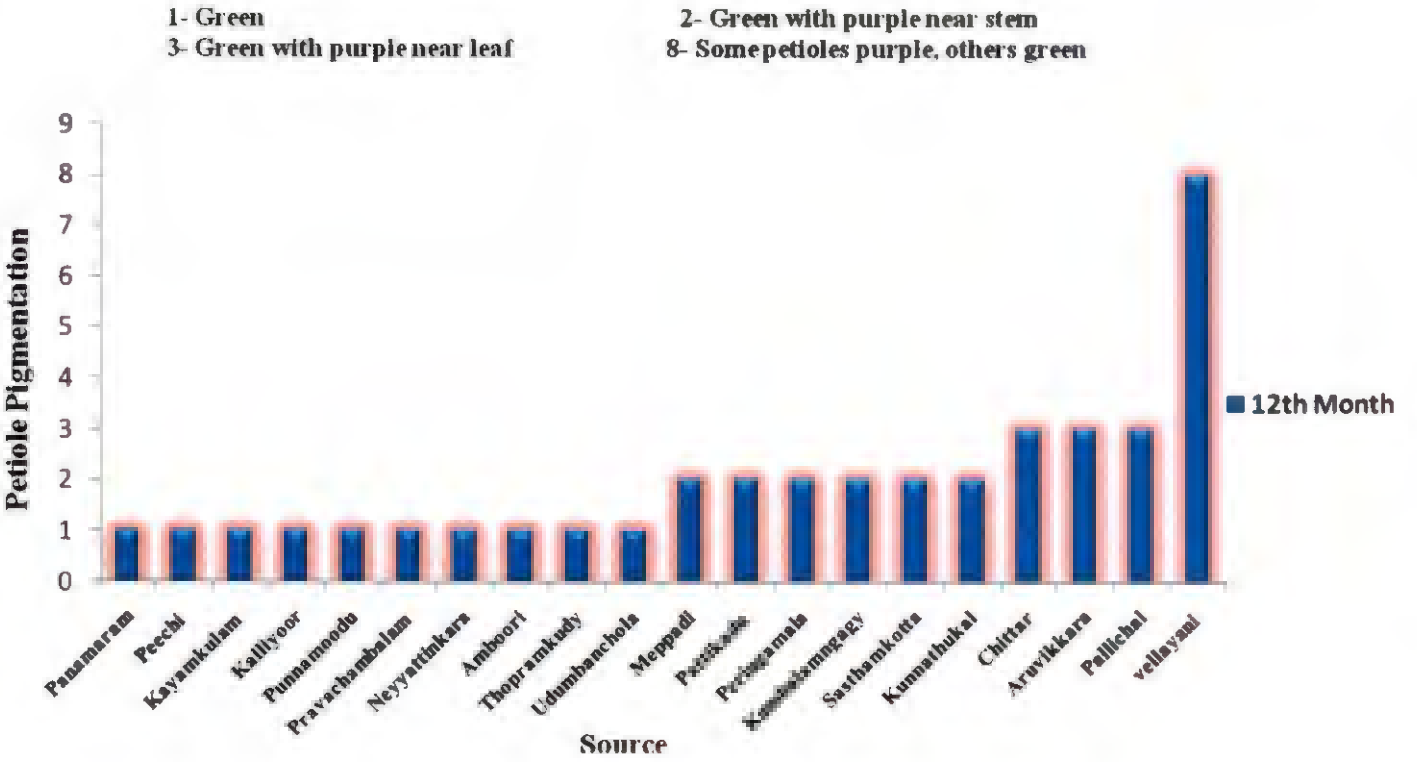


Fig 8. Petiole pigmentation at different growth stages of milk yam accessions (*I. digitata* L.).

accessions the inflorescence was axillary cymose which is aggregated in capitate clusters. Number of flowers in each inflorescence ranged from 1 to 12 the average being five. Number of inflorescence per plant varied from 6 to 23. At peak flowering season 2 flowers bloomed in each day per plant inflorescence^{-1}. Mean flower size was observed to be 6.12 X 4.6cm. The flowers were beautiful pinkish colored. The flowers had fused petals with a tubular corolla, five sepals (2 outer, 2 inner and one outer inner) with a long peduncle and a pedicel. Stamens were free and epipetalous with 5 anthers and a bifid stigma both white in color. Length of the style showed 22.1 -25.5mm variation with length of filament being 21.2 - 25mm in milk yam. Similar observations have been reported from other members of the convolvulaceae family. In *I. acuminata* the medium height of the style was 27.245 ± 2.431 mm and the medium height of the filament was 26.439 ± 3.147mm. In *I. batatas* (19.77 ± 1.956mm that is higher than the medium height of the filament), *I. cairica* (19.827mm; 17.952mm) and *I. quamoclit* the medium height of the style and the medium height of the filament had both bigger and smaller height variations of the style as reported by (Terada, *et al.,* 2005).Ovary is syncarpous superior and with axile placentation. The observed floral characters closely agreed with that already described in available literature.

In the present study, flower opening was seen during the early morning hours between 4:30am to 6:00am. Maimoni-Rodella and Rodella (1992), studying the floral biology of *Ipomoea acuminata* also reported that the flowers open between 4:30am. and 5:30am., presenting the exposed pollen grains and the receptive stigma at 10:00am. and 12:00am. *I. quamoclit* had their flowers opening at 6:00am. and closing at 2:00pm. (Terada *et al.,* 2005).

5.3.2 Reproductive Biology

The studies on reproductive biology of angiosperms have received much attention in recent years (Bawa *et al.*, 1985; Shukla and Pandey, 1991; Sreekala *et al.,*

2007, 2008). Detailed information on the reproductive biology is essential for developing effective strategies for their conservation and sustainable utilization. The results emerging from the detailed analysis of reproductive biology of *I. digitata* L. are discussed in the following sub headings in the light of available literature. The floral structure and floral parts of *I. digitata* L. are presented in Plate 10 and Plate 11.

5.3.2.1 Phenology

Phenology is the study of phenol-events which are critical for the survival and reproduction of plant species (Rathcke and Lacey, 1985). Therefore, the detailed information regarding the phenology is a prerequisite for the studies on the breeding systems and silvicultural practices. The most important and intensely studied pheno-events in majority of the cases are on flowering only (Widen, 1990). The timing of flowering influence the competition for pollinations and thus availability of pollinators (Bawa, 1983). Phenological pattern of flowering can be analyzed at various levels such as individuals, populations and communities (Bawa *et al.*, 1990). Community based phenological patterns have been already explored by several workers whereas population based phenological studies are rather limited in spite of the fact that such events have direct bearing on the survival and establishment (Primack, 1980; Bawa *et al.*, 1990).

Population based analysis of phenological events in *I. digitata* L. was carried out in which all plants in the population started flowering during the months of June-July, extended up to October and reached a peak during September. The flowers of *I. digitata* lasted only for a day showing that the flowers are ephemeral. Rodella and Yanagizawa (2007) also reported that in three *Ipomoea spp.* the flowers are ephemeral, lasting only one day.

5.3.2.2. Pollen Morphology

Morphological analysis of pollen grains is made based mainly on the aperture characters. The other palynological characters such as exine ornamentation, pollen size and shape are also taken into consideration as supplementary factors. The aperture characters are considered to be of primary importance, exine surface pattern secondary and the others as tertiary. Pollen size and shape are relatively less diagnostic as they are not stable characters. A wide range of grain size and shape may occur in the same taxon. Moreover the method of pollen preparation also affects considerably the pollen size and shape (Walker and Doyle, 1975).

The recent introduction at Scanning Electron Microscopy (SEM) has proved to be a useful tool for palynological studies with increased accuracy and precision. This has opened up possibilities for better understanding of the exine ornamentation pattern and enabled application of exine features in studies involved systematic relationships of microtaxa particularly sub species, varieties, cultivar, cytotypes, bio forms etc. (Ravikumar and Nair, 1985).

Pollen morphology studies of *I. digitata* L. (Plate 12) using SEM revealed that they exhibited monard type of pollen grains with pantoporate aperture morphology and spinose exine ornamentation. Similar findings have been reported on the pollen grains of the species of *Ipomoea* and other genera of Convolvulaceae by Ayyangar, (1980). They reported polyforate (pantoporate) and echinate pollen in the species of *Ipomoea.* The pollen of *I. digitata* L. belonged to the basic types. The basic type in the family perhaps is the pantoporate and spinose type from which other morphotypes have evolved by the process of zonation, reduction and fusion as outlined by Erdtman in his scandinavian pollen flora (Nair and Rehman, 1963).

The pollen shape of *I. digitata* L. are spheroidal with pointed spines (Plate 13) which are known as echinoconiae, as evidenced by the present investigation; This confirms the work of Hallier, (1893) and Erdtman, (1952). A detailed study on the

pollen morphology of *Ipomoea* species have been carried out by Nair (1965), In which he reported that the pollen of *I. purpurea*, had monard pollen grains and they differ from the rest of the species of *Ipomoea,* in having large sized pollen.

In the present investigation larger pollens were noticed in *I. digitata* L. *i.e.* the pollen diameter ranged from 63.6 µm to 102 µm, spine length varied from 8.28 µm to 11.5 µm with inter spinal distance of 5 µm to 6.7 µm and aperture diameter of 2.8 µm to 4.6 µm. Walker and Doyle (1975), assigned standards to pollen grain sizes *viz.,* minute grain <10µm; small grain 10-24µm; medium grain 25-49µm; large grain 50-99 µm; very large grain 100-199µm and gigantic grain < or=200µm. All pollen grain sizes of *I. digitata* lie in the very large size. According to Muller, (1979) increase in pollen size indicates an increased adaptation to different systems of animal pollination. Rajurkar *et al.* (2011) reported the pollen diameter of *I. trilobata* to be 68.93-78.9 µm. The pantoporate aperture pollen grains are considered to be an advanced character (Woodehouse, 1935). This indicates that both primitive and evolved type of pollen is found in the same genus and based on the present palynological data, *I. digitata* can be considered as an advanced species in the genus of Ipomoea. The major groups of pollen grains in Convolvulaceae found were monard pollens as reported by Nair and Rehman, (1963). Hsiao and Kuoh (1995) had divided eighteen *Ipomoea* spp. into two groups based on spines and the ridges of bacula around the extrapolar region.

5.3.2.3. Pollen Viability and Fertility

Pollen viability is critical for any studies on pollen biology. Successful seed set and establishing newer population generally depend upon viable pollen grains. Treating the pollen grains with non-vital stains such as acetocarmine, aniline blue in lacto phenol essentially imparts colors to the contents of the pollen as well as fixed/dead pollen. It may be useful to determine the degree of pollen sterility in plants of hybrid origin or those grown under unfavourable conditions (Alexander, 1969,

174032

1980). From time to time, pollen viability tests have been fine-tuned and constantly upgraded. In the present investigation, 2, 3, 5- triphenyl tetrazolium chloride (TTC) test and iodine potassium iodide (IKI) test were used for assessing pollen viability and maximum pollen viability (94.76%) was noticed at the time of anthesis but it gradually decreased towards dehiscence (Plate 16 and Plate 17). This is in agreement with the reports of Terada *et al.* (2005) who found that pollen-grain viability of *Ipomoea* species decreases only when the time for flower closure approaches. They reported that there were highest frequencies of viable pollen grains in *I. batatas* between 6:35am. and 7:00am. and the variations in the frequency of viability of the pollen grains keep along the day until the flower closing was seen. Pollen fertility of *I. digitata* L. was assessed by acetocarmine-glycerin staining technique which ranged from 95.45 per cent to 100 per cent, and on an average 97.81 per cent of pollens were found to be fertile (Plate 15).

5.3.2.4. Pollen Germination

An understanding of the factors controlling pollen germination and tube elongation is essential to facilitate interspecific and intergeneric hybridization (Vasil, 1974). The success of hybridization largely depend upon the chemical composition and physiological state of pollen. The basic needs for improvement of plants while undertaking breeding programmes are pollen fertility, viability and its longevity. The present investigation suggests that in *I. digitata* L., different concentration (0.5-1%) of sucrose enhanced the *in-vivo* pollen germination and the maximum percentage of germination was observed immediately after anthesis and 2h after anthesis respectively. Sucrose (5%) is the best carbohydrate source for pollen germination and tube elongation in many plants (Shivanna and Johri, 1985). In some plants, temperature plays a crucial role in both pollen germination and tube growth apart from the basic nutrients (Kuruvilla *et al.*, 1989). In *I. digitata* L. Brewbakers medium in room temperature was found to be the most suitable for pollen germination and subsequent tube elongation (Plate 19).

5.3.2.5. Stigma Receptivity

The stigma is the recipient of pollen grains. There is a great variation in the morphology of stigma (Heslop-Harrison and Shivanna, 1977; Heslop-Harrison, 1990). Traditionally, the stigmas are classified into dry and wet types based on the presence or absence of stigmatic exudates at the time of pollination. *I. digitata* L. was characterized by wet and bifid stigmas with solid style. In species having a wet stigma and solid style, the secretion is predominantly produced from the stigmatic tissues (Heslop-Harrison and Heslop-Harrison, 1992). The stigma of milk yam remained receptive for 8 h from the time of anthesis and highest receptivity was observed immediately after anthesis and 2h after anthesis respectively. The above results are in confirmation with the findings of Terada, *et al.* (2005), who reported that in *I. quamoclit* stigma was receptive between 6:00 a.m. and 9:00 a.m. and in *I. batatas* between 6:35 am. and 1:00 pm.

5.3.2.6. Pollinators and Foraging Behavior

The flower colors of *Ipomoea species* are attractive to bees, according to Proctor and Yeo (1973) and Kevan (1979). In the present study many pollinators are observed visiting the flowers for both nectar and pollen. The pollinators are seen attracted by the showy and bright colored flowers. Observed pollinators visited the plants include *Xylocopa aestuan, Xylocopa* sp. 2, skipper butterfly and butterfly which forage only for nectar purpose. Highest visitation was observed with blue banded bee and weaver ant. Scolid wasp was seen till the noon time in a day. Maximum visitation was observed during morning hours and less during afternoon hours. Similar observations have been made by Rodella and Yanagizawa (2007) in *Ipomoea cairica*.

Insects belonging to Andrenidae, Anthophoridae, Apidae, Halictidae, and Megachilidae are frequent visitors of most species (Austin 1997, Piedade 1998, Kiill and Ranga 2003, Terada *et al.,* 2005). The family Convolvulaceae to which milk yam

belongs is characterised by melittophily, which involves many bee groups (Piedade 1998, Kiill *et al,.* 2000, Kiill and Ranga 2000, 2003, 2004, Terada *et al.,* 2005, Maimoni-Rodella and Yanagizawa 2007), including some of those observed in the present study. *Apis mellifera* and some species of the genera *Trigona* and *Bombus* have been identified as pollinators of Merremia species which also belongs to the family Convolvulaceae (Piedade 1998, Kiill and Ranga 2000, Kiill *et al.,* 2000).

5.3.2.7. Pollination and Breeding Experiments

Breeding system includes all aspects of sex expression that effect the relative genetic contribution to the next generation of individuals within the species. Flowering plants display a wide variety of breeding systems from strict outcrossing to strict autogamy (Bawa *et al.,* 1985). The breeding systems influence the genetic structure of the populations. In the present study the milk yam plants showed 80 per cent fruit set in open pollination, 60 per cent in self-pollination, 40 per cent in getinogamous pollination and 50 per cent in xenogamous pollination respectively (Fig. 9). However, because flowering on a single plant may last for more than two months, both self- and sib-fertilization may occur, with the proportion of each dependent on the genotype, the environment, and the presence of pollinating insects. Observed bee visitation patterns and the opening of several flowers on the same plant allowed geitonogamy because insects sequentially visited multiple flowers from a single individual. Geitonogamy is another form of autogamy in self-compatible plants, and is brought about by the same features that promote out-crossing, but may reduce xenogamy (Faegri and van der Pijl 1980).

5.3.2.8. Seed Germinability, Seed Viability and Moisture Content

Seed, being a biological propagule serves as a connecting link between two generations of the plant and is responsible for maintaining the genetic continuity of the species (Noggle and Fritz, 1977). A seed thus contains the message to reconstitute an entire plant with similar shape structure and function (Van 1981). Studies on seed

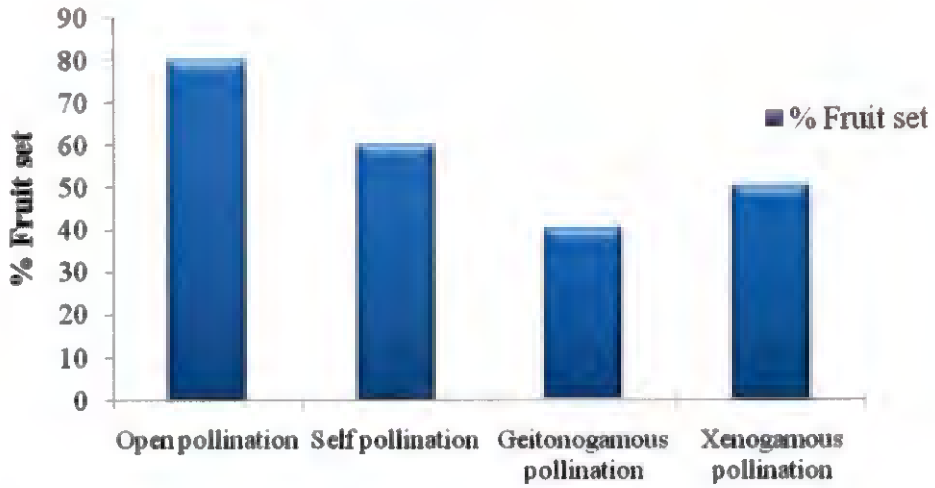


Fig 9. Induced fruit set in different modes of pollination in milk yam accessions (*I. digitata* L.).

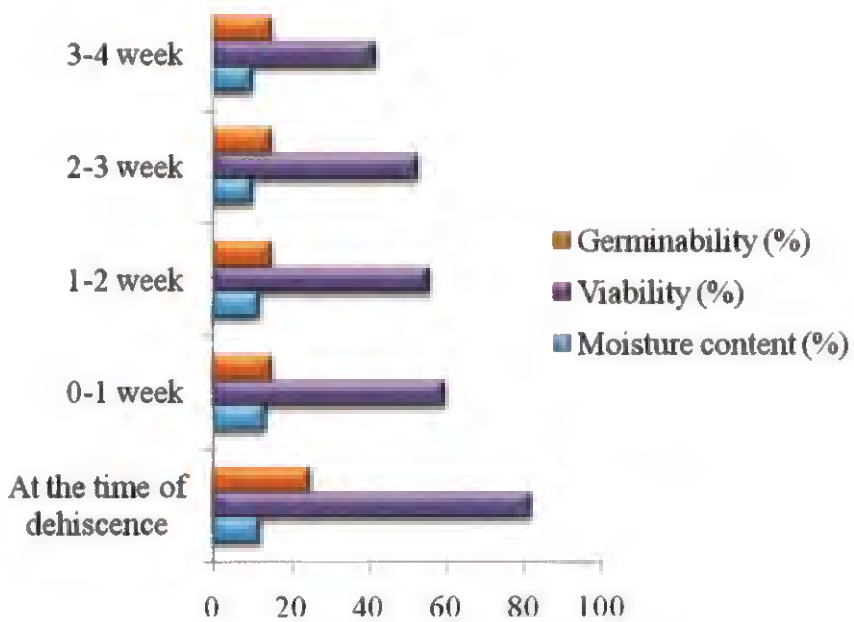


Fig 10. Seed biology of milk yam accessions (*I. digitata* L.)

germination strategies *viz.,* seed storage, viability and seed development have great significance in exploiting wider genetic and ecological characteristics particularly, the individual species recovery.

Milk yam plants took almost 18 to 28 days for fruit maturity from the day of flower opening. The appropriate period for collecting seeds is 27 to 28 days after blooming which is the field maturity period as noted by the increase in viable seeds to about 80 to 90 per cent and decrease in moisture content to approximately 14 per cent (Suwanketnikom and Julakasewee 2005).

A considerable reduction in the seed moisture content from 12.3 per cent to 10.2 per cent within one month of storage in milk yam has been noticed. The observed viability of the seeds at the time of dehiscence was 82 per cent and it gradually decreased and reached 42 per cent at fourth week (Fig. 10). The reduction in viability may be attributed to the loss of moisture during storage. Similar results were found by Jayasuriya *et al.* (2007) in sweet potato.

Seed germination depends on physical conditions around the seed and physiological status of the seed. In milk yam, the germinability of seeds varied from 25 per cent at the time of dehiscence and decreased to 15 per cent after a week (Fig. 14). Decrease in germinability may be due to result of the impermeability of the seed coat which was observed in many *Ipomoea* species (Elmore *et al.*, 1990). However, this type of dormancy might be referred to as coat- imposed dormancy while the other type of dormancy is influenced by the embryo (Bradbeer, 1988; Kelly *et al.*, 1992).

5.4. ROOT AND YIELD CHARACTERS

5.4.1. Root Characters

Mesenbet (2015) reported that the arrangement of storage root on underground stem of the genotypes varied from open cluster (40%), disperse (12%) to very disperse (44%) root formations. In the present investigation, the arrangement

of storage root on underground stem of the milk yam accessions varied from open cluster (45%) to closed cluster (55%). Poole (1952) reported that the characters of sweet potato such as tuber formation, skin color, flesh color and nature of leaf margins are controlled by two pair of genes in complementary action. Similar results were reported by Jones *et al.* (1966, 1988), Rashid *et al.* (2002).

The average root stalk length of milk yam accessions varied from very short (10%), short (85%) and intermediate types (5%) (Fig. 16). Although the length of the root stalk is generally considered cultivar specific and is used as a formal morphological characterization feature, this study demonstrates that root stalk length may be profoundly influenced by the interaction of the accessions with its environment. The numbers of storage root per plant varied significantly among the accessions and ranged from 1.4 to 3.27. Higher number of storage roots per plant enhances the total yield of the tuber.

Variability of storage root shape ranged from uniform (60%) to moderately variable (40%). The uniformity of root shape was controlled by a few genes with dominance inherited from their accessions and the presence of intermediate characters demonstrates the incomplete dominance as well as the occurrence of multiple alleles for a particular character as reported by Hammett (1966) in sweet potato.

The matured storage root size varied from uniform (80%) to slightly variable (20%) among the accessions. Root size is said to be a variable character and studies have indicated that heritability estimates for root size was low indicating non additive genetic variance (Jones *et al.*, 1966, 1988).

Root cracking was not found among any of the accessions in the present study. Root cracking (caused by *Meloidogyne incognita*) is the severe damage caused by nematodes to the tuber crops. The accessions locally available among various tracts of Kerala may be resistant to the disease. The disease invades roots when,

secondary roots are deformed and tubers may crack due to nematode infestation which was absent in case of milk yam.

Of the 20 accessions, a wide variety of skin colors of the storage root ranging from brown, cream, orange and yellow were observed in the present study. Maximum percentage (50%) of accessions possessed brownish orange skin followed by cream (30%), yellow (10%) and orange color (10%). The color of the skin as well as flesh is determined by pigments such as carotenoids and anthocyanin. The combinations of which may vary to produce skin and flesh of white, cream, orange, yellow (Hernandez *et al.,* 1965; 1967). They also indicated that colored skin is incompletely dominant over white or cream skin color in sweet potato.

Flesh color of storage root is controlled by the presence or absence, type and amount of pigments present in the internal tissue. Generally observed flesh colors of *Ipomoea spp.* are white, cream, yellow, orange (carotenoids) and brown (anthocyanin), purple with light, intermediate and dark shades of each. In the study, two distinct flesh colors have been observed in milk yam *viz.,* white and cream. Among them white color was predominant (60%) and cream color (40%) which depends on the presence of pigment. The appearance of white color in the progeny may be due to transgressive segregation which might have occurred in the accession. As the study was conducted using tubers and vine cuttings from seedlings, numerous combination of alleles from the parents would have occurred, resulting in the expression of character. Veasey *et al.* (2008) also observed 73 per cent of cream predominant flesh color among sweet potato accessions. The uniformity of tuber color was controlled by a few genes with partial dominance Hammett (1966). According to Hernandez *et al.* (1965; 1967) white and cream-orange color behaved as a typical character and several additive genes were involved in controlling the carotenoid pigment in case of sweet potato.

5.4.2. Yield Characters

5.4.2.1 Number, Length and Girth of Tubers

In milk yam the mean number of tubers per plant ranged from 2.07 to 3.47 among all the accessions. The highest number of tubers was obtained in accession T1 (3.47 plant^{-1}) while the lowest number of tubers was found in the accession T11, T12 and T19 (2.07 plant^{-1}). Production of lowest number of small sized roots can be mainly due to cultivar difference or accession difference. Similar findings were also reported by Kapinga *et al.* (2010) and Onunka *et al.* (2012). Generally the production of more number of tubers per plant results in higher yield. The length of tubers varied from 23.25 cm to 36.75 cm. It was found that the accession T12 (Pravachambalam, Thiruvananthapuram District) recorded the highest length of tubers (36.75 cm) and the lowest (23.25 cm) was found in accession T9 (Punnamoodu, Thiruvananthapuram District). The variation was found among the accessions both in the genetical and growth characteristics. Variability in tuber length in sweet potato has been reported by Rashid *et al.* (2002). he also reported variability in tuber length of sweet potato and reported that the length of the tubers is inversely correlated to number of tubers and vice-versa. However, in present study no such correlation has been observed.

The girth of tubers among accessions varied from 8.9 to 14.71 cm. It was found that the Accession T13 (Neyyatinkkara, Thiruvananthapuram District) produced the highest girth (14.71cm) and the lowest (8.9cm) was found in Accession T4 (Pattikadu, Thrissur District). The girth of the tuber varied due to the growth pattern of the plant which was influenced by the genotypic characteristics as well as adaptation capacity to the soil type and climatic conditions. Rashid *et al.* (2002) reported that diameter of storage root varied from accession to accession due to varied genetic makeup of the accessions. Tairo *et al.* (2008) reported that in sweet potato accessions which had tubers with more girth produced less number of tubers

per plant and leads to lesser yield. As the tuber girth increases the tuber length decreased, the tuber girth to length ratio increases and vice versa. The lower the ratios better the tuber shape and higher weights of tubers contributing to higher yields. But in milk yam no such relationship was noticed. Accessions with higher tuber girth recorded more number of tubers (T3, T15, T1, T20 etc). Similarly, accessions with more tuber length (T12, T16, T10 etc.) recorded higher tuber girth also.

5.4.2.2 Fresh Tuber Yield

Fresh tuber yield per plant at harvest exhibited a wide variation among all the accessions ranging from 262 g to 870 g plant^{-1}. The highest fresh tuber weight (870g) per plant was found in the accession T15 (Aruvikkara, Thiruvananthapuram District) and the lowest (262g) was found in the accession T9 (Punnamoodu, Thiruvananthapuram District). The highest yield might be due to high number as well as girth of tubers. It may also be attributed to the inherent genetic makeup as well as better adaptability.

5.4.2.3 Dry Tuber Yield

Dry tuber yield per plant varied significantly among the accessions. The mean dry weight of the tuber ranged from 142 to 681g. The highest dry tuber yield per plant (681 g) was found in the accession T15 (Aruvikkara, Thiruvananthapuram District) while the lowest tuber yield was found in accession T7 (Kalliyoor, Thiruvananthapuram District). This could be attributed mainly to the highest fresh tuber yield in T15. However, the accession which produced the lowest fresh tuber yield (T9) was not the lowest (T7) in dry tuber yield. This may be due to the higher moisture content in T7 compared to T9 tubers. Tuber yield is said to be a variable quantitative character and studies had indicated that heritability estimates for tuber yield was low indicating non additive genetic variance (Jones, 1988). Jones *et al.*, (1969) reported that storage root weight is an important component of yield in sweet

potato and it could be expressed as differential plant vigour. The photograph showing tubers of twenty different accession of *I. digitata* L. are presented in Plate 24.

5.5. PHYTOCHEMICAL SCREENING

5.5.1. Qualitative Phytochemical screening

Collected tubers of each accession were powdered and samples were subjected to systematic phytochemical screening by successive extraction (1:1 ratio) of samples in different solvents (methanol, ethanol, chloroform and hydro-ethanolic extract). Both qualitative and quantitative analyses of phytoconstituents were done following appropriate procedures. The data pertaining to qualitative estimation of phytoconstituents confirmed the presence of alkaloids, carbohydrates, glycosides, saponins, fats and oils, phytosterols, resins, flavonoids and proteins, in all accessions under different extraction methods.

The presence of alkaloid was reported from methanol, ethanol, hydro ethanolic extracts. The results are in conformity with the findings of Essiett *et al.*, (2014), Hemalatha *et al.* (2014), Devakumar *et al.* (2014), Khan *et al.* (2009) who reported the presence of alkaloid in methanol and ethanol extracts of *I. digitata* L.

Through Molisch's test, the presence of carbohydrates was detected in ethanolic and hydro-ethanolic extracts. Das and Himaja (2004) reported the presence of carbohydrates in ethanolic extract,Arockiamary *et al.* (2014) in aqueous extract and Khan *et al.* (2009) in hydro- ethanolic extracts of milk yam.

The presence of glycosides was tested through Keller Kelliani's Test and the results indicated its presence in hydro-ethanolic extract. This result corroborates with the findings of Essiett *et al.* (2014) and Hemalatha *et al.* (2014). Presence of saponin in methanol and hydro-ethanolic extract was confirmed through Foam test which was supported by the reports of Ralte (2014), Arockiamary *et al.* (2014) and Khan *et al.* (2009).

In the present study, the presence of phytosterols in chloroform extract, ethanol extract and hydro-ethanol extracts has been detected through Liebermann-Bruchard test. Similar results were reported by Pradeepa *et al.* (2016) for steroids in aqueous and acetone extracts, Das and Himaja (2004) for ethanolic extract, Hemalatha *et al.* (2014) for chloroform and methanol extracts. Phytosterols are known to be important for their cardiotonic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics (Callow, 1996).

Presence of fats and oils in hydro-ethanolic extract was detected through Copper Sulphate test.. Hemalatha *et al.* (2014) reported the presence of fats and oils in chloroform and methanol extract while Namrata *et al.* (2014) reported the presence in methanol extract.

In the study, presences of resins are reported from ethanol, methanol and hydro-ethanol extracts. The results are in conformity with the findings of Khan *et al.* (2009).

The presence of flavanoids were reported from methanol extract and chloroform extract in the present study which corroborated with the findings of Pradeepa *et al.* (2016) who reported flavanoids from ethanol, methanol, acetone and aqueous extracts. Essiett *et al.* (2014) reported the presence of flavanoid in ethanolic extract, Hemalatha *et al.* (2014) observed flavanoids in chloroform, methanol and ethyl acetate extract.

Ninhydrin test showed presence of proteins in methanol extract, ethanol extract and hydro-ethanol extract. Similar observations were made by Khan *et al.*, (2009) in water extract, Hemalatha *et al.* (2014) reported proteins from methanol, ethyl acetate and water extracts.

In the present study, among the different extracts used for qualitative estimation of phytoconstituents in milk yam, hydro-ethanolic extract showed the presence of most of these compounds hence, it was considered as the best.

5.5.2. Quantitative Phytochemical screening

Collection of herbs at right maturity is one of the parameters which affect the efficacy of medicinal plants. Only mature (bigger size) tubers of *I. digitata* L. have been used for preparing galactagogues and immuno modulatory herbal medicines by the traditional medical practitioners. Hence phytoconstituents of mature and immature tubers were analysed for detecting any variability between them, through phytochemical screening of the accessions at different harvest periods. In addition, mature tubers of all accessions harvested during germplasm collection were also analysed for finding out variation in phytoconstituents among ecotypes and also regional variation.

5.5.2.1 Regional Variation in Phytoconstituents

In the initial screening significant quantitative variation in primary metabolites like carbohydrates, proteins, fats and oils and secondary metabolites like alkaloid, glycoside, saponins, and flavonoids was noticed among the accessions collected from different agroclimatic areas. Carbohydrate content of the accessions varied from 32.33% to 62.31% whereas tuber protein content ranged from 2.73-5.66 mg g^{-1}, fatty acid 0.11 to 0.24 mg KOH g^{-1} and oil content 0.14 to1.18%. In the case of secondary metabolites, alkaloid content ranged from 18.13-34.69 per cent, glycoside varied between 0.10 to 0.63 per cent , Saponin content ranged from 2.39 to 9.76 per cent and flavonoids content from 0.24 mg g^{-1} to 0.67 mg g^{-1}. Investigation on phytochemical constituents present in *Ipomoea digitata* L. tubers by Monjur-Al-Hossain *et al.* (2013) also ensured the presence of constituents like alkaloids, tannins, steroids, gums, glycosides, carbohydrates and saponins. The phytochemicals present in the plant make it a phytoestrogen source since its activity is similar to estrogen present in human body which justifies its use in curing ailments related to female reproductive system (Ashajyothi *et al.*, 2003).

In the initial screening two accessions from Wynad (T1 and T3),one from Idukki (T20) and two accessions from Thiruvananthapuram (T7 and T16) were found to have higher content of primary and secondary metabolites. This phytochemical

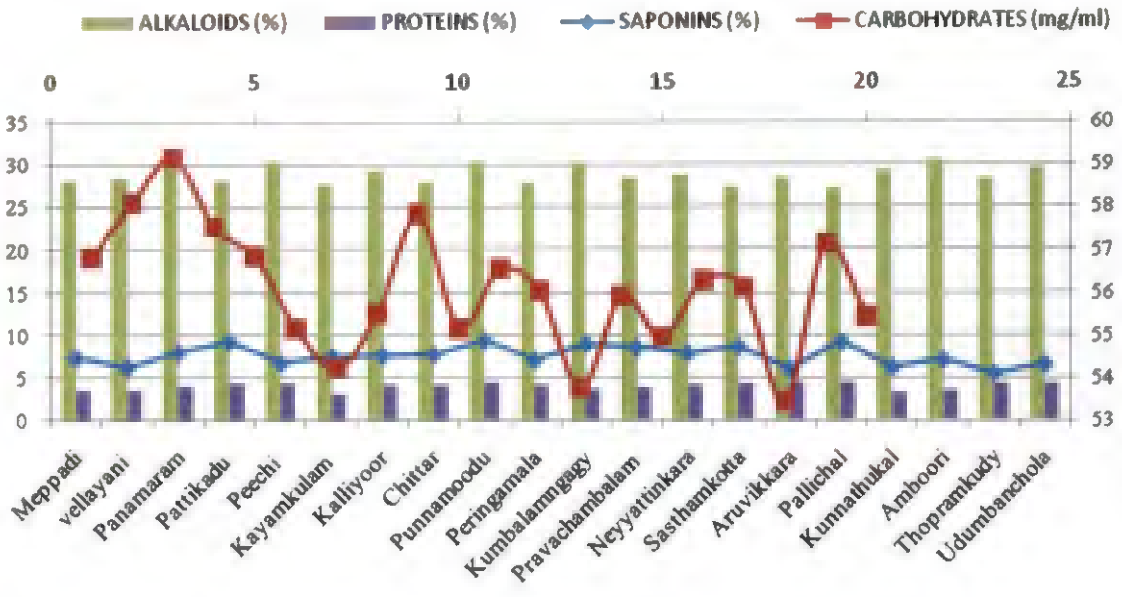


Fig 11. Quantitative phytochemical investigation of *I. digitata* L. harvested at 365 DAP.

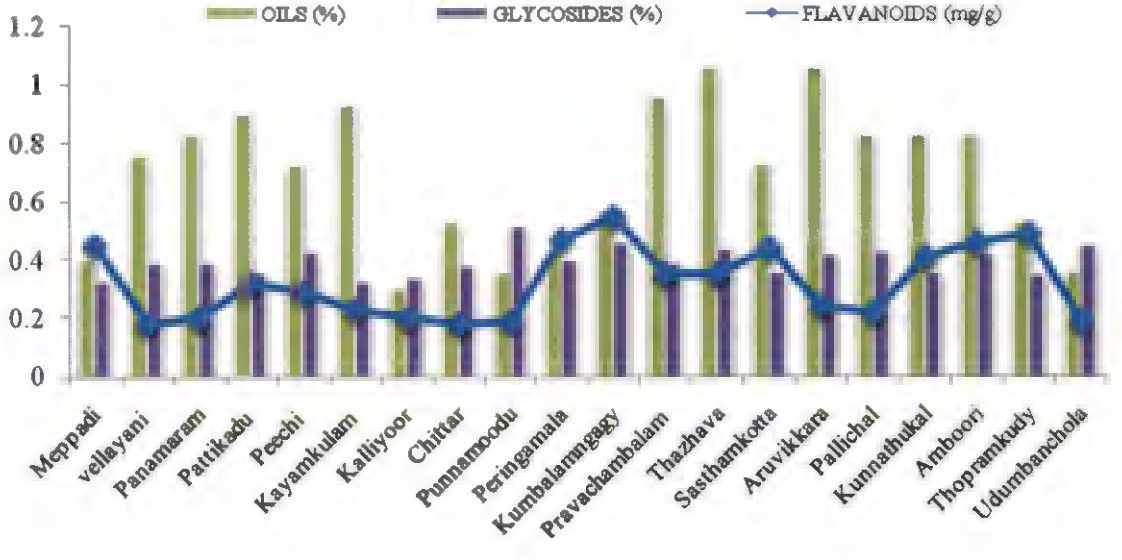


Fig 11a. Quantitative phytochemical investigation of *I. digitata* L. harvested at 365 DAP.

variability among accessions is justifiable based on the report of Foster, (2005) that the types and concentrations of secondary metabolites vary with plant species, tissue type, physiological development, and conditions to which the plant is exposed. Another corroborating report is that of Ramakrishna and Ravishankar, (2011) who reported that environmental factors viz. temperature, humidity, light intensity, the supply of water, minerals, and CO_2 influence the growth of a plant and secondary metabolite production. In the light of the above observations it may be concluded that the quantitative variation in phytoconstituents among accessions can be due to agroclimatic variations, maturity of the tubers or due to genetic variation.

5.5.2.2 Maturity based Phytochemical Screening

When the accessions were raised under uniform climatic and cultural conditions and the tubers were harvested and analysed one and two years after planting, tremendous variation in phytoconstituent content was observed from initial screening and also among the accessions. Under the new environment majority of accessions recorded an increase in both primary and secondary metabolite content (Fig. 11) whereas accessions T1, T3 (Wyanad), T7 (Thiruvananthapuram) and T20 (Idukki) had either equal or lower values. In a significant study on analysis of major active compounds of *Atractylodes lancea* from China, Bodekar, (1970) observed that not only plants growing in different geographical areas with different morphological characters could have different chemical constituents but also plants with similar morphological features growing on the same site may have different contents of chemical constituents, which justifies the above observation.

Another significant observation was the increase in both primary and secondary metabolite content of the tubers with increasing maturity. This was true with all accessions and in agreement with the findings of Khan *et al.* (2009) who reported that mature tubers of *I. digitata* L. possess about twice the quantity of phytoconstituents than immature tubers. The observation that mature tubers harvested two years after planting contained better concentration of phytoconstituents

than the immature source harvested at 365 DAP, proves beyond doubt the authenticity of traditional recommendation.

5.5.2.3 Variability in Phytoconstituent Content in Tubers

5.5.2.3.1 Primary metabolites

In a study carried out by Das *et al.* (2015) revealed the presence of carbohydrates, alkaloids, glycosides, saponins, phytosterols, flavonoids, fats and fixed oils, resins, proteins, gums and mucilagenous in *I.digitata* tubers. They also reported the absence of phenolic acids and tannins in it. Nair (2000) reported that *I. digitata* tubers are rich in carbohydrate, starch, protein, vitamins etc., In the present study, when the tubers were harvested two years after planting the carbohydrate content ranged from 53.84 to 66.57 per cent. Pradeepa *et al.* (2016) reported carbohydrate content of 57.75- 74 per cent; Essiett *et al.* (2014) reported a carbohydrate content of 40-60 per cent. A herbo - mineral drug containing different medicinal plants along with *Ipomoea digitata*, Zinc – ash complex and high energy carbohydrate molecules were evaluated for its clinical efficacy by Rani *et al.* (1997). An overall improvement ranged between 69 – 77 per cent was reported in all the patients having symptomatology of general weakness, appetite, sleeplessness etc. which could prove its potential revitalizing effect in humans.

The protein content of *I. digitata* L. accessions harvested one year after planting, ranged from 2.95 mg g^{-1} to 4.37 mg g^{-1} and at 2 year after planting from 3.98 mg g^{-1} to 5.42 mg g^{-1}. This observation is in slight contradiction to the report of Khan *et al.* (2009) that immature tubers *I. digitata* L. contain more proteins (6.60%) than the mature tubers (4.4%).

The fatty acid content varied from 0.11 mg KOH g^{-1} to 0.16 mg KOH g^{-1} at 365 DAP; and 0.12 mg KOH g^{-1} to 0.18 mg KOH g^{-1} at 2 YAP while the oil content in the tubers of milk yam ranged from 0.30 per cent to 1.18 per cent. Mishra *et al.* (1964) also have reported that *I. digitata* L. tubers contain 1.3% fixed oil and the components include oleic acid (60.1%), linoleic acid (19.38%), palmitic acid (8.15%), and linolenic acid (1.11%) in mixed acid fraction. The compounds identified

in the tuber of *I. mauritiana* possessed various biological activities like, hexadecanoic acid – Palmitic acid(14.22%) has antioxidant and anti-inflammatory activity (Aparna *et.al.,* 2012)

5.5.2.3.2 Secondary metabolites

At the initial phytochemical screening the alkaloid content of the tuber ranged between 18.13-34.69 per cent, at 365 DAP the accessions showed 27.16-30.7 per cent and at 2 YAP 21.72-37.23 per cent which showed an increasing trend towards maturity. The results are in conformity with Devakumar *et al.* (2014) reported alkaloid content of 0.57–0.90 mg g^{-1}. Patel *et al.* (2001) reported alkaloid isolated from seed and tubers have certain pharmacological properties such as cycotoxic, antispasmodial and hallucinogenic.

The percentage of glycoside varied between 0.10 to 0.63 per cent among the accessions during initial screening and later it showed increased glycoside content from 0.33 to 0.52 per cent (365 DAP) and 0.39 to 0.59 per cent (2 YAP) respectively. Reports of Masateru *et al.* (2009) revealed the presence of resin glycosides in the leaves and stems of *I. digitata* L. The resin glycosides of *I. digitata* L. are known as purgative ingredients and hence have medicinal value (Ashajyothi *et al.,* 2013). Ono *et al.* (2009) reported the presence of isobutyric, (S)-2-Methylbutyric, Tiglic, n-decanoic, n-dodecanoic cinnamic acids and two glycosidic acids - Quamoclic acid A and Operculinic acid - A. A resin glycoside 'digitajalapin' was also extracted from *I. digitata* by the same group of scientists. Ancient literatures of Ayurveda says resin glycosides in milk yam are responsible for the anti-inflammatory activity. According to Viji and Paulsamy (2016), Hexadecanoic acid and Hexadecan-1-ol trans -9 are the compounds with anti-inflammatory activities in milk yam tubers. Paniculatin is a glycoside isolated from *Ipomoea digitata* L. Administration of paniculatin resulted in elevated blood pressure, showed stimulant effect on myocardium and respiration, a vasoconstrictor and bronchoconstrictor effect with spasmogenic effect on smooth muscles of gut and also an oxytocic activity (Matin *et al.,*1969).

In *I. digitata* L. Khan *et al.* (2009) reported a saponin content of 7.28 per cent in immature tubers and 1.24 per cent in mature tubers. However, in the present study saponin content of *I. digitata* L. accessions ranged from 2.39 to 9.76 per cent at initial screening; 5.46 to 9.33 per cent at 365 DAP; 7 to 9.51 per cent at 2 YAP. The saponins have antifungal, antiviral and antitumour properties Wagner (2003). Saponins are known to have anti-inflammatory, anti-yeast, anti-fungal, anti-tumour and antiviral activities that supports its usefulness in traditional medicine (Sofowora, 2008). According to folklore and Indian systems of medicine, milk yam is used against skin infections like acne, dandruff, body malodor etc. (Jain *et al.,* 2011). A supporting study was done by Mahendra *et al.* (2015) to find out the antimicrobial activity of milk yam extracts (petroleum ether, chloroform, ethyl acetate and methanol) against skin pathogens like *Malassezia furfur*, *Propionibacterium acnes* and *Corynebacterium diphtheria* (@ 1, 20 and 50 mg ml^{-1}). A mild antibacterial activity was exhibited by chloroform and ethyl acetate extracts against *C. diphtheria* (@ 20 and 50 mg ml^{-1}) both with an inhibition zone of 13-14 mm. Petroleum ether extract showed inhibition against *C. diphtheria* at 50 mg ml^{-1}. Viji and Paulsamy (2016) found out a compound named 'tetradecanal' having antimicrobial activity from acetone extract of milk yam. E-15-Heptadecenal and Octadecanoic acid are the other specific compounds in the extract capable of killing bacteria. 1- Docosanol methyl ether identified from the acetone extract of milk yam tubers is supposed to have the ability to inhibit *in vitro* replication of many lipid enveloped viruses, including HSV (Herpes Simplex Virus).

Flavonoids are group of natural phenolics which generate H_2O_2, that can scavenge free radicals, posses the capability of chelating metal ions and inhibition of enzymes like NADPH (Benavente *et al.,* 1997). Flavonoids content for the milk yam accessions varied from 0.24 mg g^{-1} to 0.67 mg g^{-1} initially; at 365 DAP 0.18 mg g^{-1} to 0.55 mg g^{-1} and at 2YAP 0.23 mg g^{-1} to 0.53 mg $g^{-}1$. Viji and Paulsamy (2016) observed that flavonoids contents in tubers of *I. digitata* L. varied much across the

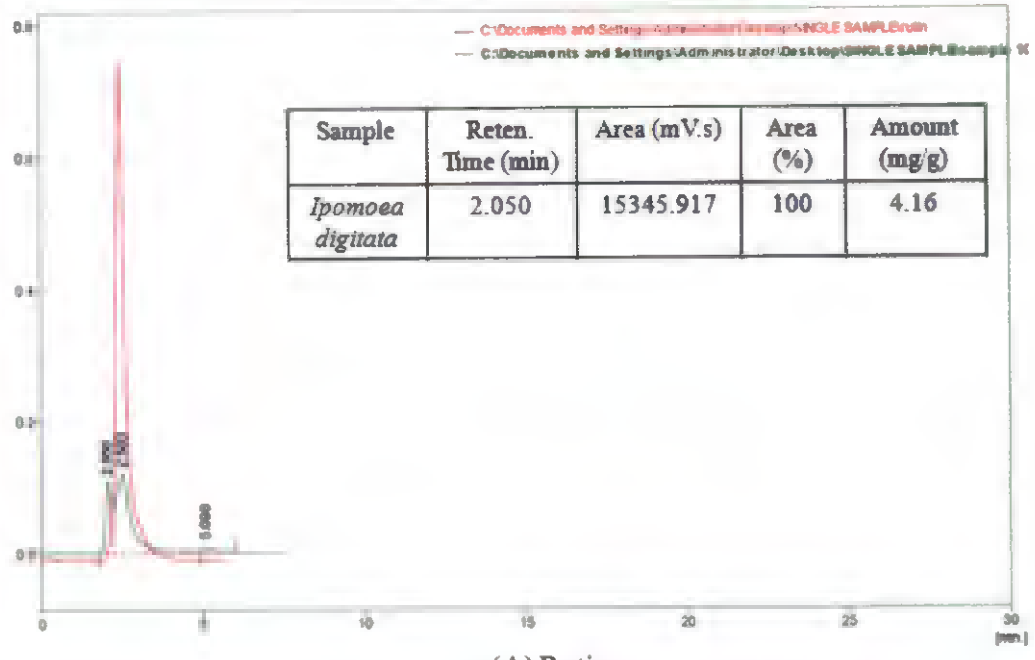
solvents used. The acetone extract of tubers possesed high flavonoids (174.44 mg RE g^{-1} extract) followed by methanol (97.50 mg RE g^{-1} extract). Administration of hydroalcoholic extract of milk yam tubers at a dose of 100 – 200 mg kg^{-1} body weight for 28 days showed significant antidiabetic activity and it may be due to the presence of active principles like flavonoids and β – sitosterol (Pandey *et al.*, 2013). Essiett *et al.* (2014) reported flavonoids content in *Ipomoea alba, Ipomoea batatas* and *Ipomoea nil.* It varies from 0.11 mg g^{-1} to 0.48 mg g^{-1}. Pietta (2000) reported flavonoids are potent water-soluble antioxidant and free radical scavengers, which prevent oxidative cell damage and they have strong anticancer activity. Further it has been reported that flavonoids constituents of plant possess antioxidant activity properties (Hesham and Nishiyama, 2002) and it was found to be useful in treatment of liver damaged (Maurya, 2004).

5.5.2.3.3 Comparison among Accessions for Phytoconstituent Content

When the accessions were compared for phytoconstituent, T11 (Kumbalangy, Ernakulam District) and T3 (Panamaram, Wyanadu District) recorded superior values in both first and second year analysis. Accessions T13 and T9 (Neyyattinkara and Punnamoodu) recorded the highest values for all phytoconstituents during the second year analysis. Accession T3 from Wynad recorded significantly superior phytocontents in both initial and final analysis indicating its genetic superiority.

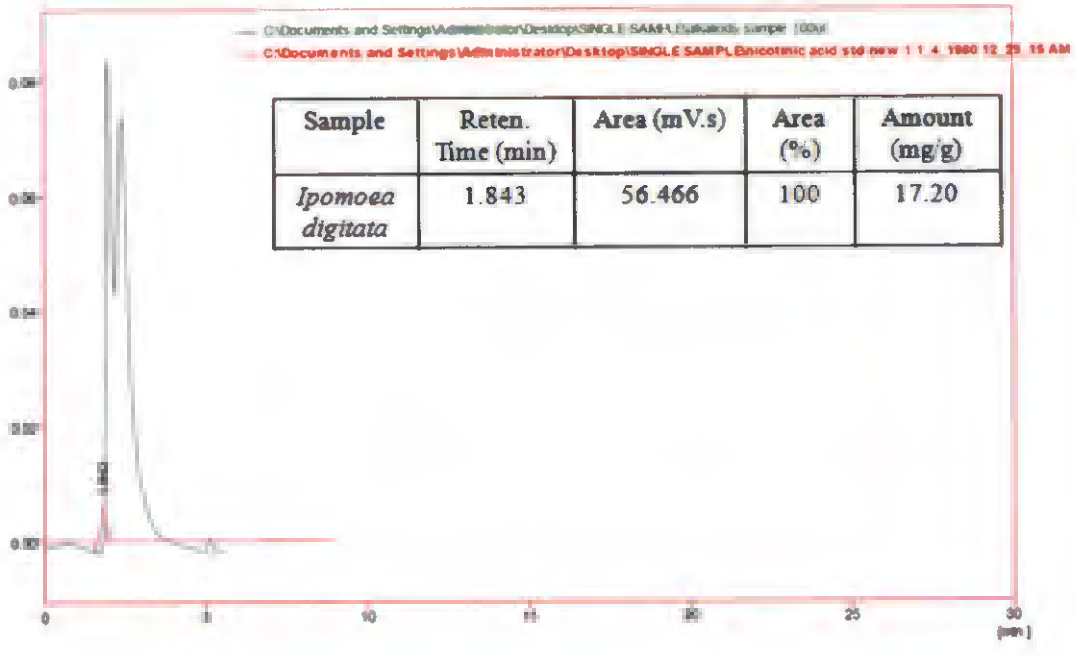
5.6. CHROMATOGRAPHIC ANALYSIS

Several species of *Ipomoea*, the largest genus of the family Convolvulaceae have been reported to have hallucinogenic indole alkaloids along with other phytoconstituents. In the present study the TLC fingerprint of the selected best accession showed the presence of alkaloids when sprayed with drasgandraff's reagent, saponins when sprayed with libermann-burchard reagent and flavanoids when sprayed with 1per cent aluminum chloride solution (Plate 13). In agreement with this result, the species like *I. aquatica*, *I. palmata*, *I. carnea* and *I. obscura* have been reported to be rich in indole alkaloids by Vijaykumar (1971). Detection of



Sample	Reten. Time (min)	Area (mV.s)	Area (%)	Amount (mg/g)
Ipomoea digitata	2.050	15345.917	100	4.16

(A) Rutin



Sample	Reten. Time (min)	Area (mV.s)	Area (%)	Amount (mg/g)
Ipomoea digitata	1.843	56.466	100	17.20

(B) Nicotinic acid

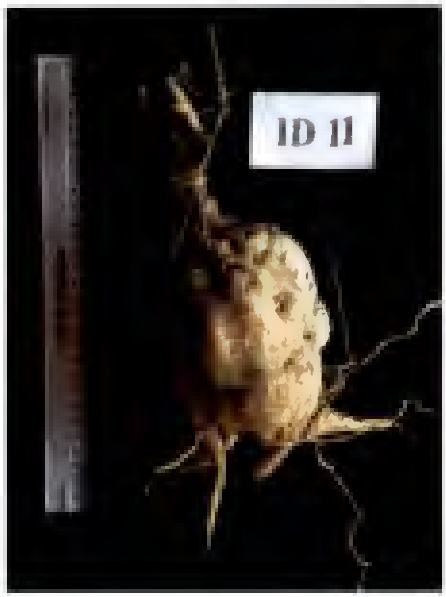
Fig 12. HPLC fingerprint of *I. digitata* L.

alkaloid umbelliferone from *I. digitata* by TLC profile was also reported by Dighe and Adhyapak (2011).

In the present study, TLC analysis confirmed the presence of flavanoids and alkaloids in the sample. In addition two new compounds were aso identified which was not reported so far in *I. digitata* L. *i.e,* Rutin- a flavonoid and nicotinic acid- an alkaloid (Fig. 12). The assay results indicate that amount of rutin (flavonoid) and nicotinic acid (alkaloid) estimated by HPLC method was 4.16 $\mu g\ g^{-1}$ and 17.20 $\mu g\ g^{-1}$ respectively. Earlier studies of Dighe and Adhyapak (2011), has recorded the mean amount of umbelliferone as 0.0719 mg g^{-1}. Rutin is a polyphenolic flavonoid. Supplementation of Rutin significantly reduced glucose levels, which could prompt the intact functional β cells to produce insulin and helps to fight against diabetes. (Kamalakkannan *et al.,* 2006; Chakravarthy *et al.,* 1980 ; Chakravarthy *et al.,* 1989; Hii and Howell, 1985; Coskun *et al.,* 2005). Nicotinic acid intake is an effective dyslipidemic agent with a broad spectrum of effects, including raising high-density lipoprotein (HDL) cholesterol, reducing lowdensity lipoprotein (LDL) cholesterol, reducing high lipoprotein (a), and reducing triglycerides (Witztum and Steinberg 1996; Carlson 2005).

5.7. SELECTION OF PROMISING ECOTYPES

Yield and secondary metabolite content are the important characteristics considered for commercial cultivation of medicinal plants apart from other characteristics such as ease of propagation, resistance to pest and disease, adaptability to different soil and climatic condition etc. In the present investigation analysis of variance was done to characterise milk yam (*I. digitata* Linn.) ecotype population variability. Biometric analysis of milk yam ecotypes demonstrated significant variability in vine, leaf (except petiole length), biochemical and yield characters. Tuber yield and phytoconstituents are of fundamental importance in the selection of milk yam ecotypes for commercial cultivation. The accessions which produced significantly superior fresh tuber yields were T15 (870.47g), T17 (821.93g), T11 (752.3g), T12 (744.13g), T6 (698.53g) and T3 (620.27g). Among these accessions,



T11 (Kumbalangy, Ernakulam District)

T3 (Panamaram, Wayanad District)

Plate 14.Two promising accessions of *I. digitata* L. identified.

T11 (Kumbalangy, Ernakulam District) and T3 (Panamaram, Wyanadu District) recorded significantly superior values for phytoconstituents in both first and second year analysis. Accessions T13 and T9 (Neyyattinkara and Punnamoodu) recorded the highest values for all phytoconstituents during the second year analysis; however their tuber yield was comparatively lesser. Two promising accessions with high tuber yield and significant phytochemical content identified in the study are T3 (Panamaram) and T11 (Kumbalangy) respectively which can be utilized in further breeding programmes (Plate 14). Both these accessions have predominantly green coloured vines with twining and extremely spreading habit, very thick (>12 mm) and long internodes (10-12 cm), moderately seven lobed leaves with axillary cymose inflorescence and moderate flowering habit. Accessions T3 have green coloured petioles while accession T11 have green petioles with purple colour near stem. T3 is characterised by uniform shaped tubers in closed cluster. Predominant skin colour of the tuber is orange and flesh colour being cream. It recorded a fresh tuber field of 620.27g plant^{-1}. T11 is characterised by slightly variable tubers in closed cluster. Predominant skin colour of the tuber is brownish orange and flesh colour being white. It recorded a fresh tuber field of 752.13 g plant^{-1}

5.8. VARIABILITY COMOPONENTS

5.8.1. Phenotypic and Genotypic Coefficient of Variation

Phenotypic variance measures the magnitude of variation arising out of difference in phenotypic values while the genotypic variance measures the magnitude of variation due to difference in genotypic value. High values of the coefficients indicate wider diversity. Similarly, narrow differences between GCV and PCV reveals low sensitivity to the environmental effects (Lush, 1945).

The phenotypic coefficient of variation (PCV) ranged from 3.08 (glycosides) to 52.92 (dry tuber yield per plant), while genotypic coefficient of variation (GCV) ranged from 2.33 (glycosides) to 37.58 (flavanoids). According to Sivasubramaniah

and Menon (1973), PCV and GCV values greater than 20 per cent are regarded as high, values between 10 per cent and 20 per cent to be medium whereas values less than 10 per cent are considered to be low. Based on these categories PCV and GCV values were high for fresh tuber yield per plant, dry tuber yield per plant, flavanoids and oils. In addition number of storage root per plant and internode diameter had moderate GCV but high PCV values.

High GCV and PCV could be an advantage as they can offer opportunity for selection of superior accessions with respect to the character of interest. Particularly high GCV is an indication of the less influence of environmental factors in the expression of such traits and the higher possibility to improvements through selection and hybridization (Tsegay *et al.,* 2007; Thiyagu *et al.,* 2013). In conformity with this results, Jones *et al.* (1969) reported considerable high variances for fresh tuber yield per plant and dry tuber per plant in sweet potato. Hossain *et al.* (2000) also reported high PCV and GCV values for vine length and number of storage roots per plant. Solankey *et al.* (2015) reported maximum PCV and moderate GCV values for number of storage root per plant and internode diameter indicating the presence of wide genetic variability for morphological traits in sweet potato.

Moderate PCV and GCV (>10% and <20%) values were computed for number of tubers, length of tubers, girth of tubers, plant height, alkaloids and carbohydrates. In addition, number of storage root per plant and internode diameter had moderate GCV but high PCV values. The characters *viz.,* internode length, fatty acids and proteins reported moderate PCV but low GCV values. Those traits having considerable genetic variability offer limited opportunity for crop improvement through selection. Consistent with these results, Tsegay *et al.* (2007) reported that, the GCV was relatively moderate for characters *viz.,* internode length, internodes diameter and number of storage root per plant. Vimala and Hariprakash (2011) reported moderate genotypic and phenotypic variations for number of tuber, length of tubers, girth of tubers and plant height. Both PCV and GCV values for matured leaf

length, matured leaf breadth, saponins and glycosides were low (<10%) and internode length, proteins and fatty acids recorded low GCV value which indicating the limited scope of improvement of these traits through selection. In agreement with this result, Wassu *et al.* (2015) reported that, the lowest PCV and GCV values for leaf length, leaf breadth and internode length. The traits with low PCV and GCV values suggested the higher influence of environment for their expression and thus the phenotypic basis selection would not be effective for the improvement of the trait (Das *et al.*, 2012).

Generally, if both PCV and GCV estimates were high it indicates high genetic variability for effective selection. The PCV values were greater than GCV but the differences between the two values were narrow which indicating the variability due to genetic constituent of the genotypes was less influenced by environmental factors (Tsegay *et al.*, 2007). Hence, selection for desirable traits would be effective for crop improvement.

5.8.2. Estimates of Heritability

Heritability estimates aid in determining the relative amount of heritable portion of variation. Heritability values itself does not provide any indications of the amount of genetic progress that would result from selecting the best individuals. Ramanujam and Tirumalachar (1967), they concluded that heritability estimates in broad sense accompanied with genetic advance would be more reliable.

In this study, broad sense heritability (H^2) ranged from 23.94% (proteins) to 91.50 per cent (flavanoids). As suggested by Robinson *et al.* (1955), heritability values are categorized as low (0-30%), moderate (31-60%) and high (> 61 %). According to this delineation high heritability values were calculated for plant height, internode diameter, matured leaf length, carbohydrates, flavanoids, oils and saponins which indicated that these traits were less influenced by environmental factors.

The present study was consistent with the finding of Lin *et al.* (2007) who reported high heritability for plant height, internode diameter and leaf length. Kareem *et al.* (2013) also reported high heritability for plant height. Kapinga *et al.* (2011) reported high heritability for carbohydrates, flavanoids and saponins. If the heritability of a character is very high around 80 per cent or more, selection for such character is fairly easy. This is because there would be a close correspondence between the genotypic and phenotypic variations due to relatively small contribution of the environment to the phenotype expression of the trait (Singh *et al.*, 1993).

Moderate heritability values (30-60%) estimates were observed for number of tubers, length of tubers, girth of tubers, fresh tuber yield per plant, dry tuber yield per plant, number of storage root per plant, internode length, matured leaf breadth, alkaloids, flavanoids and glycosides. In addition, low heritability estimates were computed for proteins. In contrast to this study result, Kapinga *et al.* (2011) reported high heritability for proteins, internode length and alkaloids. Traits with moderate heritability values suggested the limited scope of improvement and with low heritability the practically impossibility of improvement of these characters through selection. Singh (1993) suggested that, if heritability is less than 40 per cent selection may be considerably difficult or virtually impracticable to improve the characters due to the masking effect of the environment on the characteristics of genotype.

5.8.3. Genetic Advance

Heritability is a component of the computation of expected progress and is most meaningful when accompanied by genetic advance. Genetic advance would be less in case where the non-additive genetic variance is more than additive genetic variance (Lush, 1954).

The expected genetic advance expressed as a percentage of the mean by selecting the 5 per cent of the genotypes varied from 3.64 per cent (glycosides) to 74.05 per cent (flavanoids). This indicated that selection of the 5 per cent of high

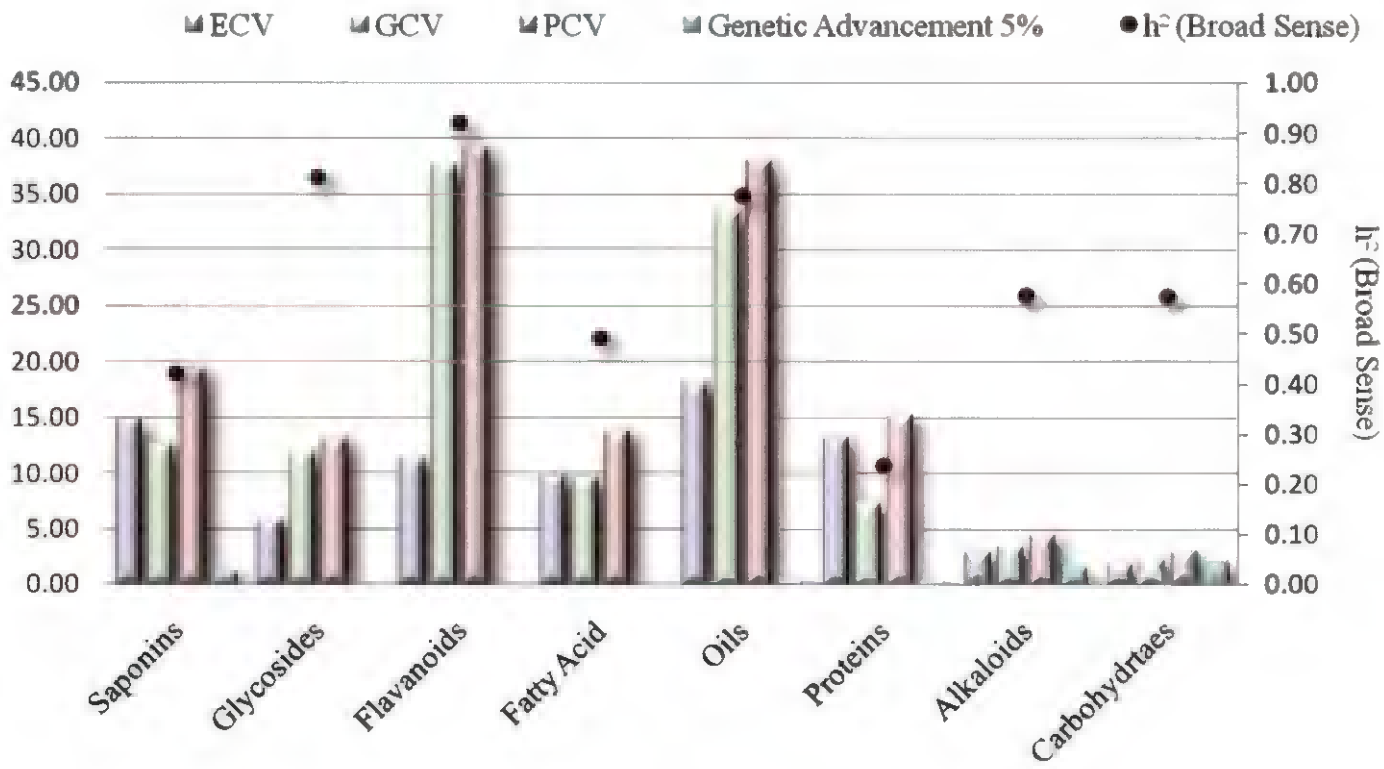


Fig 13. Genetic parameters of milk yam accessions (*I. digitata* L.)

performing genotypes from the base population could result in an advance of 3.64 per cent to 74.05 per cent over the population mean (Table 42).

As suggested by Johnson *et al.* (1955) the genetic advance as percent of mean was categorized as low (0-10%), moderate (10-20%) and high (> 20%). As per this categorization, high expected genetic advances as percent of mean were observed for fresh tuber yield per plant, dry tuber yield per plant, number of storage root per plant, plant height, internode diameter, carbohydrates, flavanoids and oils. Similar findings were reported by Tsegay *et al.* (2007) which revealed that the highest genetic advance as percent of mean for plant height, vines inter node length, vines inter node diameter, storage root number per plant and tuber yield. The high genetic advance indicated that the traits are controlled more of by additive genes. Hence, selection is likely to be more effective and make an advance of the mean value of each character (Panse, 1957). Whereas Moderate (10-20%) genetic advance as percent of mean was recorded for number of tubers, length of tubers, girth of tubers, internode length, matured leaf length, alkaloids and flavanoids. Low values of genetic advance were recorded for mature leaf breadth, proteins, saponins and glycosides (Das *et al.*, 2012).

Heritability estimates though provide basis for selection on the phenotypic performance, the estimates of heritability and genetic advance should always be considered simultaneously. This is because that high heritability is not always associated with high genetic gain (Johnson *et al.*, 1955). In this study, high heritability along with high genetic advance estimates were recorded for plant height, internode diameter, carbohydrates, flavonoids and oils.

High heritability associated with high genetic advance is due to additive gene effect but if heritability is due to dominance and epistasis, the genetic gain would be low. Hence selection for these characters would provide quite effective since the characters governed by additive genes (Panse, 1957). Lin *et al.* (2007) reported high heritability along with high genetic advance as percent of mean for plant height and

plant internode diameter. This finding is in agreement with the findings of Tsegay *et al.* (2007). Kareem *et al.* (2013) observed high heritability and genetic advance for Carbohydrates, flavonoids and oils. Similar results were expressed by Thiyagu *et al.* (2013) and Solanky *et al.*, 2015. Heritability and genetic advance are reported to be helpful in understanding the types of gene action involved in expression of polygenic characters (Johansson *et al.*, 1955).

Medium heritability along with moderate genetic advance was computed for number of tubers, length of tubers, girth of tubers, internode length, alkaloids and fatty acids. While moderate heritability coupled with low genetic advance was observed for matured leaf breadth and glycosides. Ali *et al.* (2008) suggested that selection is hardly possible to improve traits which exhibited low values both for heritability and genetic advance (or) moderate and low values combinations. This is due to the higher influence of environment on the expression of the characters and limits the scope of improvement by selection due to the presence of non additive (dominant and/ or epistasis) type of gene action.

5.8.4. Genotypic and Phenotypic Correlations

Genotypic and Phenotypic correlation reveals that the degree of association between different characters. Thus, it helps for plant improvement programme to a required balance, when two opposite characters are being selected. It also helps to improve different characters simultaneously (Falconer, 1981)

Phenotypic and genotypic correlations of all possible combinations of 19 traits of 20 *I. digitata* accessions are presented in Table. 36 and 37. The phenotypic and genotypic correlation coefficient values ranged between -0.6513 to 0.8530 and -0.7352 to 0.9292, respectively. Some of the quantitative traits had highly significant ($P < 0.01$) and significant ($P < 0.05$) positive phenotypic and genotypic correlations. Whereas, the other traits showed positive but non-significant negative correlations. The assessment of genetic potentiality of yield contributing traits and their

association with other traits is important to carry out the effective selection for isolating productive genotypes. In general, the genotypic correlation coefficients were observed to be higher than the corresponding phenotypic correlation coefficients in magnitude for most of the characters indicating inherent association among most characters. Thus, indicating the suppression of phenotypic expression under the influence of environmental factors and the presence of inherent association between various characters. Nedunzhiyan and Reddy (2000), Choudhary *et al.* (2000), Sahu *et al.* (2005) and Tirkey (2011) also found similar results in their studies on *I. batatas*.

5.8.5. Correlation of yield characters with other traits

Yield, in general, is a complex polygenic trait and is difficult to improve directly. Estimating its genotypic and phenotypic correlation coefficients with yield related traits and other components is important to utilize the available variability through selection (Jones, 1969). This study was undertaken to determine associations among yield and yield related traits and to identify the major traits of importance that could be used as a basis for clonal selection.

At phenotypic level, Number of tubers had positive and highly significant ($P<0.001$) phenotypic correlation with number of storage root per plant. From this, it is clear that with the increase in the number of tubers, the number of storage root per plant also increases. Thus the yield per plant also increases. These findings were corroborated with the findings of Teshome *et al.* (2004) and Gedamu *et al.* (2010). Fresh tuber yield per plant had positive and significant correlation with dry tuber yield per plant. Similar results were expressed by Teshome *et al.* (2004), Gunjanjaha (2008) who reported significant association between fresh and dry tuber yield. Number of storage root per plant had significant and negative correlation with fatty acid. The result implies that with the increase in number of storage root per plant, the fatty acid content of the tuber decreases. The results are in conformity with the results of Ahmed *et al.*, (1996). Sossah *et al.* (2014) expressed number of storage root per

plant and fatty acid content was highly significant but negatively correlated to each other. He also opined to get tuber with more fatty acid content; cultivar with less number of storage roots would be preferred.

At genotypic level, Number of tubers per plant had positive and highly significant correlation with number of storage root per plant. The character also had negative and significant correlation with the saponin content of the tuber. Gasura (2008) reported positive correlation between number of tuber and yield. Dash *et al.* (2015) reported non-significant positive association between these traits. Teshome *et al.* (2004) reported that the characters number of tuber and number of storage root per plant are economical characters directly correlating with yield. Length of tubers had positive and significant correlation with matured leaf length. The results indicated that as the length of matured leaf increases the length of tubers would increase. Madawal *et al.* (2015) reported significant and negative association of tuber length with vine length, internode length, tuber girth and leaf area index.

Girth of tubers showed significant and negative correlation with matured leaf length and matured leaf breadth. The result of the study indicates that with the increase in the length of matured leaf length and matured leaf breadth, the girth of tubers will be decreased and thus the tuber yield also reduces. Contrary to this finding, Dash *et al.* (2015) reported that tuber diameter showed highly significant positive correlation with the tuber yield at genotypic level. Fresh tuber yield per plant showed positive and highly significant correlation with dry tuber yield per plant and oil content of the tuber. In addition, fresh tuber yield per plant had significant but negative correlation with saponin, fatty acid and protein content of the tuber. Dry tuber yield per plant had significant and positive correlation with number of storage root per plant and oil content. These results showed that any positive increase in number of storage root per plant will suffice the boast in dry tuber yield. In addition, the character had negative and significant correlation with matured leaf breadth and

fatty acids. Dash *et al.* (2015) reported dry tuber yield showed highly significant correlation with dry matter of vine at genotypic level.

Number of storage root per plant had significant and positive correlation with matured leaf length. This indicates that as there is increase in length of matured leaf there is increase in the number of storage root per plant which will suffice to boast the tuber yield. The results are in conformity with results of Gunjanjaha (2008), Madawal *et al.* (2015). However, negative but highly significant correlation of this trait was observed with saponin, fatty acid and protein. This implies more the number of storage roots per plant less will be quantity of phytoconstituents. Similar findings were reported by Sossah *et al.* (2014) for proteins and saponins.

5.8.6. Correlation among other characters

Morphological characters and phytoconstituents which showed significant and positive or negative correlation with each other at phenotypic and genotypic levels (Table. 36 and Table. 37).

Plant height showed positive and significant correlation with protein at genotypic level. This indicates that protein content was more likely to be associated with plants with greater height. Barbara *et al.* (2015) reported higher protein content in spreading (151-125cm), extremely spreading (>250) plant type in Convolvulaceae family. Dash *et al.* (2015) reported negative and non-significant association between plant type and total soluble salts. Internode length has positive and significant association with matured leaf breadth at phenotypic level. The result of the study indicates that with the increase length of the internode, magnitude of the matured leaves also increases. Similar results were expressed by Madawal *et al.* (2015) reported internode length had positive and non-significant association with leaf area index. He also expressed positive and significant relation between internode length and tuber yield per vine of sweet potato.

Matured leaf length was positively significant with matured leaf breadth and negatively significant with fatty acids at genotypic level. The result of the study indicates that with the increase in the length of matured leaf, the magnitude of its breadth also increases. At genotypic level, mature leaf breadth has significant negative association with saponins, glycosides, oils and proteins content of the tubers. The result implies that with the increase in the breadth of leaf, phytoconstituents of the tubers are likely to reduce.

Saponins had significant positive genotypic correlation with protein. Glycosides are found to have positive significant genotypic correlation with fatty acids, proteins and alkaloids content of the tuber. The result of the study indicate that wherever the glycoside content of tuber is more, it is more likely that the presence of proteins, alkaloids and fatty acids also will be more. This result was in conformity with Barbara *et al.* (2015) who reported saponins, cardiac glycosides, starch, sugars, proteins, vitamin C, ascorbic acid as well as phosphorus, calcium and magnesium in *I. batatas.*

5.9. CLUSTER ANALYSIS

5.9.1. Genetic Diversity

Quantification of genetic diversity existing within and between groups of accessions is important and particularly useful in proper choice of parents for realizing higher heterosis and obtaining useful recombinants. Several methods have been advocated by various workers to estimate genetic divergence in crop plants, but Mahalanobis' generalized distance estimated by D^2 statistic is a unique tool for discriminating populations considering a set of parameters together rather than inferring from indices based upon morphological similarities, eco-geographical diversity and phylogenetic relationships.

Understanding the extent of variability prevailing for each character in the accession collected, would just imply the scope for improving the character studied through selection. In order to assess the diversity in 20 accessions of Milk yam, 11 quantitative characters were considered and their fitness was assessed using Mahalnobis' D^2 analysis. Based on D^2 analysis, 20 accessions were grouped into 6 clusters with high range of D^2 values ranging between 43.32 and 126.95. Cluster I is the biggest cluster consisting of 8 accessions, while II had 6 accessions followed by Cluster III with 3 accessions and remaining clusters (IV, V and VI) were solitary clusters. The formation of solitary clusters may be due to total isolation preventing the gene flow or intensive natural/human selection for diverse adaptive complexes. Similar findings were reported by Veasey *et al.* (2008); Koussao *et al.* (2014); Setiawati and Karuniawan (2013) and Khalik *et al.* (2012).

The considerable magnitude of intracluster distance was observed in cluster II (29.24), followed by cluster I (29.02) and cluster III (17.86). This reveals the presence of divergent genotypes within these three clusters. It indicated that there is good scope for selection within the cluster. While the other clusters showed no intracluster distance, indicating their independent identity and importance due to the unique characters possessed by the accessions. The inter cluster D^2 value was found to be highest between cluster V and VI (126.95) indicating high diversity among the accessions. Cluster V with accession from Kumbalamngagy and cluster VI with accession from Aruvikkara were the most divergent. These accessions can serve as potent accessions for breeding programme to obtain desired traits. The lowest inter cluster D^2 value was found between cluster II and cluster V (43.32), indicating that the cluster II which has 6 accessions were genetically very close to cluster V, which has one accession.

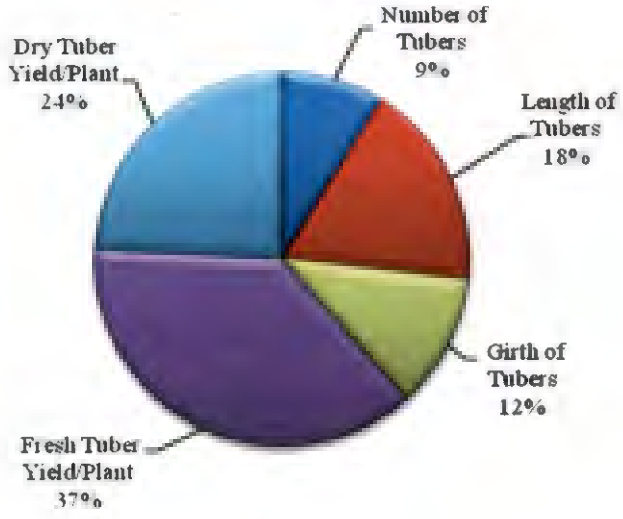
Accessions with different geographical origin may sometimes fall in different cluster, because of no definite relationship between genetic diversity and geographical origin. Though, geographical diversity is important, it may not be the

only factor determining genetic divergence and the factors other than genetic diversity such as genetic drift, selection pressure and environment may be responsible for differential grouping of the accessions. In some cases, accessions originated in the same place may also have different genetic architecture and likewise certain cultivars may possess similarity with respect to some traits though they had origin at different centers. This was confirmed by observing the accessions of Vellayani, Peringamala, Kalliyoor, Punnamoodu and Aruvikkara were the accessions of same district (Thiruvananthapuram), but was grouped under different clusters. This could be due to these accessions having distinct and contrasting characters. Similar findings are reported by Basavaprabhu (2010) and Shahaji (2011).

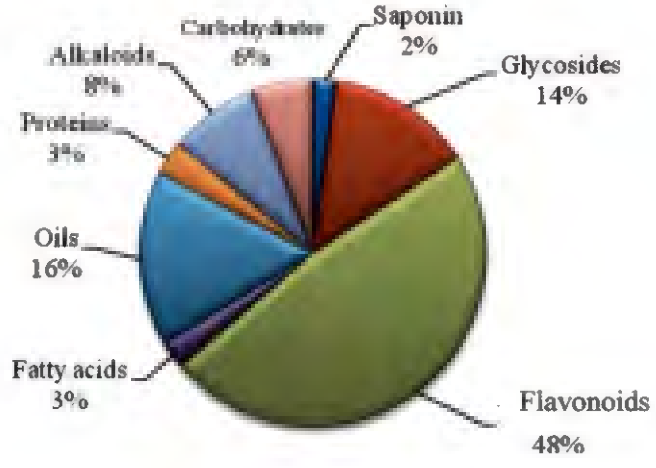
Tahasildar (2005) opined that the wide adaptability would be possible due to factors like heterogeneity, genetic architecture of populations, past selection history, developmental traits and degrees of general combing ability. It appears that varieties having same geographical origin might differ and may possess wide divergence factors. Since rapid ecotype differentiation will be taking place even in the absence of reproductive isolation (Kumar, 2009).

5.9.2. Contribution of Different Traits towards Divergence

Among the yield characters studies, the most important character contributing to the divergence was fresh tuber yield per plant (37%), this was followed by dry tuber yield per plant (24%), length of tubers (18%), girth of tubers (12%) and number of tubers per stock (9 %) respectively. Silva *et al.* (2013) reported that number of tubers per plant had the greatest contribution towards divergence. While Ardelean *et al.* (2004) observed the characters such as tuber girth, number of tubers and dry tuber yield per plant had the greatest contribution towards divergence (Fig. 14). Bonilla *et al.* (2014) indicated number of storage roots per plant followed by tuber length and tuber girth had greatest contribution towards divergence. Most interesting thing is all the yield characters studied exhibited their contribution towards divergence.



(A) Yield character



(B) Phytoconstituents

Fig 14. Contribution (%) of characters of milk yam accessions (*I. digitata* L.) towards divergence.

Among the phytoconstituents studies, the most important character contributing to the divergence was flavanoids (48%), followed by oils (16%), glycosides (14%), alkaloids (8%), carbohydrates (6%), fatty acids (3%), proteins (3%) and saponins (2%) respectively. Thus, the three constituents *viz.*, flavanoids, oils and glycosides constituted more than 75 per cent contribution towards divergence. The rest of phytoconstituents did not reported significant contribution towards divergence.

5.9.3. Analysis of Cluster Means

Analysis of cluster means indicates diversity demonstrated by different cluster for a character. Based on the range of means, it is possible to know the character influencing divergence. The variation observed in cluster means also points to the degree of variability. In the present investigation, cluster VI is found to have high cluster means for number of tubers, girth of tubers, fresh tuber yield per plant, dry tuber yield per plant, oils and proteins. Cluster V is found to have high cluster means for alkaloids, flavonoids and saponin. Whereas, cluster IV has high cluster mean for length of tubers. Similar results were obtained by Veasey *et al.* (2008) for number of tubers, fresh tuber yield per plant, dry tuber yield per plant and girth of tubers. Silva *et al.* (2013) for dry tuber yield per plant in sweet potato.

In the present study, it was observed that considerable amount of genetic diversity was present among the entries with respect to yield and other characters. The entries in cluster V and VI were genetically superior. The superior cluster with respect to characters such as number of tubers, girth of tubers, fresh tuber yield per plant, dry tuber yield per plant, oils and proteins was cluster VI. The superior cluster for alkaloids, flavonoids and saponin is cluster V. whereas, the superior cluster for length of tubers was cluster IV.

5.10. INFLUENCE OF CLIMATIC AND SOIL FACTORS ON PHYTOCONSTITUENTS

Climate change may directly alter plant fitness (Galen and Stanton, 1991, 1993), as well as alter the reproductive success of plants and their interactions through impacts on flowering phenology (Hughes, 2000; Beattie *et al.*, 1973; Schemske, 1977; Gross and Werner, 1983; Lacey and Pace, 1983; Schmitt, 1983; English-Loeb and Karban, 1992; Bishop and Schemske, 1998). The milk yam accessions were collected from places varying in their climatic conditions such as areas with altitude ranging from below 10m to 2000m above MSL, with monthly mean temperature varying from 23.5^{o}C to 30^{o}C, average monthly rainfall from 2035 mm 3677mm, and with relative humidity ranging from 84 per cent to 98 per cent. The soil conditions, temperature, rainfall, humidity, bioclimatic conditions, physio geographic conditions etc. are important determinants of the yield of secondary metabolites in medicinal plants apart from their intrinsic (genetic) factors. In the present investigation also showed variation in phytochemical content among accessions collected from different environmental conditions was observed during the initial phytochemical screening. These variations in active ingredient content of milk yam tubers with respect to different climatic parameters are discussed below. Wide variation in phytochemical content of the accessions were noticed and the existing variations in phytocontents among the populations were proved to be coupled with geographical altitude and local ecological conditions (temperature, rainfall, humidity, soil pH, etc.) but not with genetic basis in several studies.

5.10.1 Altitude

A perusal on the data on the phytoconstituents of milk yam accessions revealed that accessions collected from higher (>1500 meters AMSL) altitudes (T1,T3, and T20) had higher content of active ingredients (alkaloids, carbohydrates, glycosides, saponins, fats and oils, resins, flavonoids and proteins) compared to those

collected from 100-1000 m and places with 1-100 m altitude range. When the same accessions were raised in the lower altitude (Vellayani 30 m AMSL) and tubers were analysed for two consecutive years gradual reduction in phytochemical content was noticed. On the other hand in accessions collected from very low (<8m) altitudes (T4, T6 and T11) marked increase in phytoconstituents were noticed when grown under similar conditions. Increase in the quantity of phytochemicals viz., phenols, flavonoids, tannins, alkaloids and saponins with increase in altitude in popular medicinal plant *Primula denticulata* has been reported by Khaleefa Aslam *et al.* (2015). The authors are of the opinion that the high production of these phytochemicals may be attributed to fluctuation in temperature and non availability of nutrients. In *Desmodium gangeticum* , Jayanthy *et al.* (2013) also reported that the the quantity of lupeol was high in the roots collected from high altitude area and lowest in the ones collected from the plains. Researchers postulated that climate change could affect the chemical composition and, ultimately the survival of some medicinal plants in high altitude region (Vashistha *et al.,* 2009). According to this theory contents of secondary metabolites decreases from the equator to the poles (Bakus and Green, 1974; Siska *et al.,* 2002). The report of Osman *et al.* (1976) that alkaloid content increases with increase in altitude, as stress conditions induce polyamine formation which results in nitric oxide biosynthesis that moves freely through the cells acting as potential chemical elicitor of alkaloid production holds good for this study also. Copaja (2003) also reported that saponin production is higher at high altitudes where the prevailing environmental conditions are stressful.

5.10.2 Temperature

Among several environmental factor temperature stress is one of important factor known to stimulate the production of free radical scavenging enzymes as a result of these which secondary metabolites contents vary in the plants tissue. The accessions which were collected from different zones which fall under different temperatures showed that, the accessions collected from the temperature range of 20-

25^0C were preferably high in phytoconstituents content compared to the accessions collected from higher temperature areas above 25^0C. Temperature stress can affect secondary metabolites and other compounds that plants produce, which are usually the basis for their medicinal activity (Zobayed *et al.,* 2005). Generally when plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed not allocated to growth is instead allocated to secondary metabolites (Mooney *et al.,* 1991). Some report that secondary metabolites increase in response to elevated temperatures (Litvak *et al.,* 2002), while others report that they decrease (Snow *et al.,* 2003). Stress particularly the temperature stress can affect secondary metabolites and other compounds that plants produce, which usually are the basis of their medicinal activity (Salick *et al.,* 2009).

5.10.3 Rainfall

Highest phytoconstituents content was seen in the accessions collected from medium rain fall areas of 1000-2000 mm. decreased rainfall or increased rainfall above 2000 mm showed the lower active ingredient contents. Parker (1977); Sharkey and Yeh (2001) have described effect of elevated rainfall on contents of volatile compounds which were found to be increased. Mossi *et al.* (2009) suggested the existence of a correlation between environmental factors such as average annual temperature, climate, vegetation, geomorphology, latitude and altitude increased the tannin production.

5.10.4 Soil

Soil nutrients are directly linked with biosynthesis of secondary metabolites as well as their abundance or limited availability has effect on contents of secondary metabolites in plants. Coley *et al.* (1985) has reported variation in secondary metabolites contents occur with resource availability. The accession was collected from different Districts varied differently among the phytoconstituent content. The

accessions which were collected from the areas of Laterite soils and alluvial soils showed increased active ingredients comparably then the other accessions which were collected from areas of red loam and sandy to clayey loam soils. The active ingredient contents and the quality of medicinal plants are closely related to soil fertility (Lu *et al.,* 2006). Because the accumulation and synthesis of the active ingredients was an overall reflection of the interaction of multiple ecological factors, such as temperature, humidity, and solar radiation. The present study included the soil types like non gravelly laterite (Thiruananthapuram) (Laterite (Kollam), Red loam (Pathanamthitta), (Sandy grey onttukara (Alappuzha), sandy to clayey loam (Ernakulam), Midland laterite (Thrissur), Brown hydromorphic (Idukki (Thopramkudy) Alluvial (Udumbanchola) and Laterite (Wayanad).

Summary

6. SUMMARY

The current investigation on "Diversity analysis and reproductive biology of milk yam (*Ipomoea digitata* L.)" was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, during the year 2014-16, with the objectives of estimating the magnitude of genetic variation in morphological and biochemical traits in milk yam accessions and identifying superior accessions with high tuber yield and active ingredient content. Apart the study also envisaged a detailed understanding of the reproductive biology as well as regional variation, influence of climatic and soil factors on phytochemical content in milk yam.

The study was initiated with a survey conducted in natural growing tracts of Kerala and collection of twenty accessions of milk yam (both tubers and vines). Tubers of each accession were subjected to preliminary phytochemical screening and further the accessions were raised in the field during 2015-16 for analyzing phenotypic diversity and were evaluated for yield and active ingredient content in randomized block design replicated thrice.

Various morphological characters *viz,* vine, leaf, plant type, inflorescence characters etc. were studied in detail for arriving at the morphological descriptions. Plant is a perennial twining herb which showed gradual increase in trend of twining characters with slightly twining to profuse twining from 3 MAP to 9 MAP. Plant height ranged from 167.27cm to 329.07cm with accession T20 (Udumbanchola, Idukki District) scoring highest. The shorter accession T6 (Kayamkulam, Alappuzha District) displayed a bushy habitat of *I. digitata* L. The lanceolate green leaves of milk yam accessions which were simple, alternate, entire, glabrous, acute at apex with long petioles started exhibiting slightly lobbing pattern from 3 MAP to moderately to deeply lobbing pattern later at 6 MAP.

The lavish pink coloured flowers of *I. digitata* L. were axillary cymose, aggregated in capitate clusters, calyx fivelobed, bicolor-oblong, concave, glabrous,

subobtuse at apex, corolla five lobed, stamens five, included in corolla tube, filiform style ad capitate stigma. The flowers showed a moderate flowering habit with a flower size of 5.9 X 4.2cm. They opened in the early morning hours between 4:30am to 6:00am. Ovary is syncarpous superior and with axile placentation.

The ephemeral flowers of *I. digitata* L. lasted for a single day with anthesis started around 05:00h and lasted mostly until afternoon when the corollas wilted and fell off. At the opening time, for all species, the stigma was receptive and the anthers were dehisced.

Pollen morphology studies of *I. digitata* L. revealed that they exhibited monard type of pollen grains with Pantoporate aperture morphology and spinoseexine ornamentation and spheroidal in shape with pointed spines. While the pollen diameter ranged from 63.6 µm to 102 µm, spine length varied from 8.28 µm to 11.5 µm with inter spinal distance of 5 µm to 6.7 µm and aperture diameter of 2.8 µm to 4.6 µm.

The pollen viability showed a gradual decrease at closure time, but a viability per cent of 94.76% was noticed at the time of anthesis but it gradually decreased towards dehiscence. Also the maximum per cent of *in-vivo* pollen germination was observed after immediate anthesis and 2h after anthesis respectively.

In the present study the plants showed 80 per cent fruit set in open pollination, 60 per cent in self pollination, 40 per cent in getinogamous pollination and 50 per cent in xenogamous population respectively.

Various pollinators visited plants for nectar and pollen rewards. While the highest visitation was observed with blue banded bee and weaver ant.Scolid wasp was seen till the noon time in a day. Maximum visitation was observed during morning hours and less during afternoon hours.

The accessions in the present study took almost 18 to 28 days for fruit maturity from the day of flower opening. The appropriate period for collecting seeds should be during 27 to 28 days after blooming which is the field maturity period as

noted by the increase in viable seeds to about 80 to 90 per cent and decrease in moisture content to approximately 14 per cent.

The results revealed that there was a considerable reduction in the seed moisture content from 12.3 per cent to 10.2 per cent within one month and the germinability of seeds varied from 25 per cent at the time of dehiscence and decreased to 15 per cent after a week.

Root and yield characters of the plant followed arrangement of storage root on underground stem of the accessions varying from open cluster (45%) to closed cluster (55%). While, the number of storage root per plant varied significantly and ranged from 1.4 to 3.27 with storage root shape spanning from uniform (60%) to moderately variable (40%). Of the 20 accessions, a wide variety of skin colors of the storage root ranging from brown, cream, orange and yellow were observed in the present study. Maximum percentage (50%) of accessions possessed brownish orange skin followed by cream (30%), yellow (10%) and orange color (10%) with milky white latex exudation.

The mean number of tubers per plant ranged from 2.07 to 3.47 among all the accessions. The highest number of tubers was obtained in accession T1 (3.47 plant^{-1}) while the lowest number of tubers was found in the accession T11, T12 and T19 (2.07 plant^{-1}). It was found that the Accession T12 (Pravachambalam, Thiruvananthapuram District) produced highest length of tubers (36.75cm) and the lowest (23.25cm) was found in accession T9 (Punnamoodu, Thiruvananthapuram District). Fresh tuber yield per plant recorded at harvest exhibited a wide variation among all the accessions ranging from 262 g to 870 g plant^{-1}. The highest fresh tuber weight (870g) per plant was found in the Accession T15 (Aruvikkara, Thiruvananthapuram District) and the lowest (262g) was found in the Accession T9 (Punnamoodu, Thiruvananthapuram District).

Phytochemical screenings of the accessions were donequalitatively and quantitatively using different extraction methods for all the accessions. The data

pertaining to qualitative estimation of Phytoconstituents showed the presence of alkaloids, carbohydrates, glycosides, saponins, phytosterols, fats and oils, resins, flavanoids and proteins, for all the accessions under different extraction methods.

The accessions T20 (Udumbanchola, Idukki) along with T1 (Meppadi, Wayanad), T7, T16 and T9 (Kalliyoor, Pallichal and Punnamoodu, Thiruvananthapuram) were recorded significantly superior for phytochemical contents owing to their regional variations. Phytochemical screening of the accessions at different harvest periods, revealed the variation in phytoconstituents in mature and immature tubers. In the present study the mature tubers harvested at time of collection and at 2 YAP contained better concentration of phytoconstituents than the immature source harvested at 365 DAP, which enables us to conclude the authenticity of traditional recommendation. Also the present study confirmed the presence of two new compounds which was not identified so far in *I. digitata* L. *i.e,* Rutin- A flavanoids and nicotinic acid- An alkaloid. The assay results indicate that amount of rutin (flavanoid) and nicotinic acid (alkaloid) estimated by HPLC method as 4.16 µg g^{-1} and 17.20 µg g^{-1} respectively.

Significant regional variations in phytoconstituents exist in milk yam accessions and accessions from Wyanad, Idukki and Thiruvananthapuram are rich in phytoconstituents. The phytochemical content in milk yam tubers increases with maturity and accessions with higher tuber yields need not be rich in phytoconstituents and vice versa. Two promising accessions with high tuber yield and significant phytochemical content identified in the study are T3 (Panamaram) and T11 (Kumbalangy). Apart from collecting, detailed information of reproductive biology of milk yam, the study also revealed the influence of environment in yield and protein content of milk yam tubers. Glycoside, alkaloid, carbohydrates, and flavonoid content in tubers were found to be genetically controlled. The exclusive presence of phytoconstituents in milk yam (*I. digitata* L.) and the underutilized recognition of it as a medicinal plant of high health benefits renders, a high profile for this crop. A

systematic study on the extent of the variation under uniform cultural conditions has so for not been reported. The present investigation is the bold attempt by integrating diversity analysis and reproductive biology to bring out a report on the reproductive biology of *I. digitata* L. from different zones of Kerala.

The field experiment analysis of variance revealed significant variation in vine, leaf (except petiole length) and yield characters. The accessions which produced highest fresh tuber yield were T15 (870.47g), T17 (821.93g), T11 (752.3g), T12 (744.13g), T6 (698.53g) and T3 (620.27g). Among these accessions, T11 (Kumbalangy, Ernakulam District) and T3 (Panamaram, Wyanadu District) recorded superior values for phytoconstituents in both first and second year analysis. Accessions T13 and T9 (Neyyattinkara and Punnamoodu) recorded the highest values for all phytoconstituents during the second year analysis; however their tuber yield was comparatively lesser. Hence among all the other accessions this two accessions (T13 and T9) with a desirable traits can be considered for future crop improvement program.

Morphological characters and phytoconstituents showed significant and positive/ negative correlation with each other at phenotypic and genotypic levels.The characters, fresh and dry tuber yield per plant, number as well as girth of tubers, and protein content in the tuber showed the highest difference between PCV and GCV which indicates that the influence of environment on these characters is considerable. But lower difference between GCV and PCV for the biochemical characters *viz.,* glycoside, alkaloids, carbohydrates, and flavonoid content in tubers and morphological characters *viz.,* mature leaf length and breadth pointed out that the variation observed in these characters were mainly due to genetic reasons.

High heritability coupled with high genetic advance was observed for morphological characters *viz.,* plant height, internode diameter and biochemical parameters *viz.,* carbohydrates, flavanoids and oils. This indicates additive gene action for these characters and genetic improvement can be done by selection based on phenotypic performance.

The phenotypic coefficient of variation (PCV) ranged from 3.08 (glycosides) to 52.92 (dry tuber yield per plant), while genotypic coefficient of variation (GCV) ranged from 2.33 (glycosides) to 37.58 (flavanoids). PCV and GCV values were high for fresh tuber yield per plant, dry tuber yield per plant, flavanoids and oils. In addition number of storage root per plant and internode diameter had moderate GCV but high PCV values. The PCV and GCV (>10% and <20%) values were computed for number of tubers, length of tubers, girth of tubers, plant height, alkaloids and carbohydrates were moderate. In addition, number of storage root per plant and internode diameter had moderate GCV but high PCV values. The characters *viz.*, internode length, fatty acids and proteins reported moderate PCV but low GCV values. Thus, offering limited opportunity for crop improvement through selection for the characters with considerable genetic variability.

High heritability was observed for plant height, internode diameter, leaf length, carbohydrates, flavanoids and saponins making selection feasible and effective for crop improvement programmes. While , Moderate heritability values (30-60%) estimates were observed for number of tubers, length of tubers, girth of tubers, fresh tuber yield per plant, dry tuber yield per plant, number of storage root per plant, internode length, matured leaf breadth, alkaloids, flavanoids and glycosides suggesting the limited scope of improvement.

The expected genetic advance expressed as a percentage of the mean by selecting the 5 per cent of the genotypes varied from 3.64 per cent (glycosides) to 74.05 per cent (flavanoids). This indicated that selection of the 5 per cent of high performing genotypes from the base population could result in an advance of 3.64 per cent to 74.05 per cent over the population mean. High expected genetic advances as percent of mean were observed for fresh tuber yield per plant, dry tuber yield per plant, number of storage root per plant, plant height, internode diameter, carbohydrates, flavanoids and oils revealing the highest genetic advance as percent of mean for plant height, vines inter node length, vines inter node diameter, storage root number per plant and tuber yield. The high genetic advance indicated that the traits

are controlled more of by additive genes. Hence, selection is likely to be more effective and make an advance of the mean value of each character. Whereas Moderate (10-20%) genetic advance as percent of mean was recorded for number of tubers, length of tubers, girth of tubers, internode length, matured leaf length, alkaloids and flavanoids. Low values of genetic advance were recorded for mature leaf breadth, proteins, saponins and glycosides. Thus, in this study, high heritability along with high genetic advance estimates were recorded for plant height, internode diameter, carbohydrates, flavonoids and oils.

Phenotypic and genotypic correlations of all possible combinations of 19 traits of 20 *I. digitata* L. accessions were ranged between -0.6513 to 0.8530 and -0.7352 to 0.9292, respectively. Some of the quantitative traits had highly significant ($P < 0.01$) and significant ($P < 0.05$) positive phenotypic and genotypic correlations. Whereas, the other traits showed positive but non-significant negative correlations. The assessment of genetic potentiality of yield contributing traits and their association with other traits is important to carry out the effective selection for isolating productive genotypes. In general, the genotypic correlation coefficients were observed to be higher than the corresponding phenotypic correlation coefficients in magnitude for most of the characters indicating inherent association among most characters. Thus, indicating the suppression of phenotypic expression under the influence of environmental factors and the presence of inherent association between various characters.

The yield contributing characters at phenotypic level had number of tubers with positive and highly significant ($P<0.001$) phenotypic correlation with number of storage root per plant proving the increase in the number of tubers with, the increase in number of storage root per plant.

At genotypic level, Number of tubers per plant had positive and highly significant correlation with number of storage root per plant. The character also had negative and significant correlation with the saponin content of the tuber. Girth of tubers showed significant and negative correlation with matured leaf length and

matured leaf breadth indicating that with the increase in the length of matured leaf length and matured leaf breadth, the girth of tubers will be decreased and thus the tuber yield also reduces.

In addition, fresh tuber yield per plant had significant but negative correlation with saponin, fatty acid and protein content of the tuber. Dry tuber yield per plant had significant and positive correlation with number of storage root per plant and oil content. These results showed that any positive increase in number of storage root per plant will suffice the boost in dry tuber yield.

Matured leaf length was positively significant with matured leaf breadth and negatively significant with fatty acids at genotypic level. Indicating that with the increase in the length of matured leaf, the magnitude of its breadth also increases. At genotypic level, mature leaf breadth has significant negative association with saponins, glycosides, oils and proteins content of the tubers. Implying that with the increase in the breadth of leaf, phytoconstituents of the tubers is likely to reduce.

Saponins had significant positive genotypic correlation with protein. Glycosides are found to have positive significant genotypic correlation with fatty acids, proteins and alkaloids content of the tuber.

Mahalanobis' D^2 statistics was employed as a statistic tool for assessing the genetic divergence in the study. Based on D^2 analysis, 20 accessions were grouped into 6 clusters with high range of D^2 values ranging between 43.32 and 126.95. Cluster I is the biggest cluster consisting of 8 accessions, while II had 6 accessions followed by Cluster III with 3 accessions and remaining clusters (IV, V and VI) were solitary clusters. The considerable magnitude of intracluster distance was observed in cluster II (29.24), followed by cluster I (29.02) and cluster III (17.86) revealing the presence of divergent genotypes within these three clusters. This also indicates that there is good scope for selection within the cluster. While the other clusters showed no intracluster distance, indicating their independent identity.

Cluster V with accession from Kumbalamngagy and cluster VI with accession from Aruvikkara were the most divergent. These accessions can serve as potent

accessions for breeding programme to obtain desired traits. The lowest inter cluster D^2 value was found between cluster II and cluster V (43.32), indicating that the cluster II which has 6 accessions were genetically very close to cluster V, which has one accession.

Among the yield characters studies, the most important character contributing to the divergence was fresh tuber yield per plant (37%), this was followed by dry tuber yield per plant (24%), length of tubers (18%), girth of tubers (12%) and number of tubers per stock (9 %) respectively. Among the phytoconstituents studies, the most important character contributing to the divergence was flavanoids (48%), followed by oils (16%), glycosides (14%), alkaloids (8%), carbohydrates (6%), fatty acids (3%), proteins (3%) and saponins (2%) respectively. Thus, the three constituents*viz.*, flavanoids, oils and glycosides constituted more than 75 per cent contribution towards divergence. The rest of phytoconstituents did not reported significant contribution towards divergence.

An immense scope for research in *I. digitata* L. exists since its therapeutic and functional potential is not exploited deeply. Phytochemical profiling of the tubers is necessary to get a gross idea of the contents present in the drug which will not only help to have a chemical mapping of the drug but also will be helpful in assessing the probable mode of action of the drug. Its tuberisation morphology as well as anatomy, phytochemical characterization using sophisticated and accurate analytical tools like chromatography, spectroscopy etc. can be beneficial for authenticating the crude drug to prevent its adulteration with Vidari (*Peuraria tuberosa*).

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DIVERSITY ANALYSIS AND REPRODUCTIVE BIOLOGY OF MILK YAM (*Ipomoea digitata* L.)

by

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ABSTRACT

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ABSTRACT

The present study "Diversity analysis and reproductive biology of milk yam (*Ipomoea digitata* L.)" was carried out in the Department of Plantation Crops and Spices, College of Agriculture, Vellayani, during 2013-16, with the objectives of estimating the magnitude of genetic variation in morphological and biochemical traits in milk yam accessions and identifying superior accessions with high tuber yield and active ingredient content. A detailed study of reproductive biology and investigations on regional variation, influence of climatic and soil factors on phytochemical content in milk yam were also envisaged in the study.

A survey was conducted in natural growing tracts of Kerala and twenty accessions of milk yam (both tubers and vines) were collected. Tubers of each accession were subjected to preliminary phytochemical screening. The accessions were raised in the field during 2015-16 for analyzing phenotypic diversity and were evaluated for yield and active ingredient content in randomized block design with three replications. Ethnobotanical information collected in the study showed that in Thiruvananthapuram District, the tuber powder along with cow milk was mainly used as galactagogue and with honey as a tonic whereas, in Kollam and other District the root powder was mainly used as galactagogue and tonic for children. Traditional medical practitioners use tubers of *I. digitata* for preparing galactagogues and immuno modulatory herbal medicines. Data collected on climatic soil characters of natural growing tracts of milk yam revealed that it has wide adaptability to varying climatic and soil conditions.

In the initial phytochemical screening significant regional variation in phytoconstituents were noticed and accessions T20 (Udumbanchola, Idukki) along with T1 (Meppadi, Wayanad), T7, T16 and T9 (Kalliyoor, Pallichal and Punnamoodu, Thiruvananthapuram) recorded superior values for phytochemical content. High performance liquid chromatography analysis of tuber powder (T3 and T11) detected the presence of two compounds *viz.,* Rutin and Nicotinic acid in the tuber which has not been reported so far.

In the field experiment analysis of variance revealed significant variation in vine, leaf (except petiole length) and yield characters. The accessions which produced highest fresh tuber yield were T15 (870.47g), T17 (821.93g), T11 (752.3g), T12 (744.13g), T6 (698.53g) and T3 (620.27g). Among these accessions, T11 (Kumbalangy, Ernakulam District) and T3 (Panamaram, Wyanadu District) recorded superior values for phytoconstituents in both first and second year analysis. Accessions T13 and T9 (Neyyattinkara and Punnamoodu) recorded the highest values for all phytoconstituents during the second year analysis; however their tuber yield was comparatively lesser.

Detailed investigations on reproductive biology of milk yam revealed that the plants exhibit moderate flowering habit and produced cymose inflorescence with bright pinkish showy flowers (5.9 x 4.2 cm) with longevity of 8-10 hrs. Pollen grains of milk yam were identified to be of monard type with pantoporate aperture morphology and spinose exine ornamentation. Shape of pollen was spherioidal with pointed spines. Diameter of pollen ranged from 63.6-102µm, spine length (8.28-11.5µm), inter spinal distance (5-6.7µm), aperture diameter (2.8-4.6µm), style length (23.5-25.8mm) and length of filament ranged from 21.2-25mm. Stigma remained receptive for eight hours from the time of anthesis. 97.81 per cent of pollens were fertile and 94.96 per cent pollens were found to be viable. 80 per cent fruit set was observed in open pollination. Five different pollinators were identified visiting the flowers for both nectar and pollens. Fruit is a capsule containing four seeds with average size of 0.7 cm x 0.8 cm.

The characters, fresh and dry tuber yield per plant, number as well as girth of tubers, and protein content in the tuber showed the highest difference between PCV and GCV which indicates that the influence of environment on these characters is considerable. But lower difference between GCV and PCV for the biochemical characters *viz.*, glycoside, alkaloids, carbohydrates, and flavonoid content in tubers and morphological characters *viz.*, mature leaf length and breadth pointed out that the variation observed in these characters are mainly due to genetic reasons.

High heritability coupled with high genetic advance was observed for morphological characters *viz.*, plant height, internode diameter and biochemical parameters *viz.*, carbohydrates, flavonoids and oils. This indicates additive gene action for these characters and genetic improvement can be done by selection based on phenotypic performance.

Significant regional variations in phytoconstituents exist in milk yam accessions and accessions from Wyanad, Idukki and Thiruvananthapuram are rich in phytoconstituents. The phytochemical content in milk yam tubers increases with maturity and accessions with higher tuber yields need not be rich in phytoconstituents and vice versa. Two promising accession with high tuber yield and significant phytochemical content identified in the study are T3 (Panamaram) and T11 (Kumbalangy). Apart from collecting, detailed information of reproductive biology of milk yam, the study also revealed the influence of environment in yield and protein content of milk yam tubers. Glycoside, alkaloid, carbohydrates, and flavonoid content in tubers were found to be genetically controlled.

An immense scope for research in *I. digitata* L. exists since its therapeutic and functional potential is not exploited deeply. Phytochemical profiling of the tubers is necessary to get a gross idea of the contents present in the drug which will not only help to have a chemical mapping of the drug but also will be helpful in assessing the probable mode of action of the drug. Its tuberisation morphology as well as anatomy, phytochemical characterization using sophisticated and accurate analytical tools like chromatography, spectroscopy etc. can be beneficial for authenticating the crude drug to prevent its adulteration with Vidari (*Peuraria tuberosa*).

കേരള അഗ്രികൾച്ചറൽ യൂണിവേഴ്സിറ്റി
കോളേജ് ഓഫ് അഗ്രികൾച്ചർ, വെള്ളായണി
ഡിപ്പാർട്ട്മെന്റ് ഓഫ് പ്ലാന്റേഷൻ ക്രോപ്സ് ആന്റ് സ്പൈസസ്

സംക്ഷിപ്തം

പാൽമുതുക്കിന്റെ വൈജാത്യവിശകലനവും പ്രത്യുല്പാദന ബയോളജിയും എന്ന വിഷയത്തെക്കുറിച്ചുള്ള ഗവേഷണപഠനം 2013-16 കാലയളവിൽ വെള്ളായണി കാർഷികകോളേജിലെ തോട്ട സുഗന്ധവിള വിഭാഗത്തിൽ നടത്തിയ വിവിധ സ്ഥലങ്ങളിൽ നിന്നും ശേഖരിച്ച പാൽമുതുക് സസ്യങ്ങളുടെ രൂപ സാദൃശ്യത്തിലും ജനിതക ഘടനയിലും രാസഘടനകളിലുമുള്ള ജനിതക വ്യതിയാനത്തിന്റെ അളവ് നിർണയിക്കുക. ഉയർന്ന വിളവും രാസഘടകങ്ങളുമുള്ള ഇനങ്ങളെ കണ്ടെത്തുക. കാലാവസ്ഥയുടെയും മണ്ണിന്റെയും രാസഘടകങ്ങളുടെ അവയിലുണ്ടാകുന്ന സ്വാധീനം കണ്ടെത്തുക, തുടങ്ങിയവയായിരുന്നു ഈ ഗവേഷണത്തിന്റെ ലക്ഷ്യങ്ങൾ

കേരളത്തിലുടനീളം നടത്തിയ സർവ്വേയിൽ നിന്നും വിവിധ താലൂക്കുകളിൽ നിന്നായി ഇരുപതിനങ്ങളുടെ വള്ളികളും കിഴങ്ങുകളും ശേഖരിച്ച് ഡിപ്പാർട്ട്മെന്റിൽ നട്ട് വളർത്തി അവയുടെ കിഴങ്ങുകളുടെ രാസപരിശോധനയും നടത്തി. ഇതോടൊപ്പം തന്നെ പാൽമുതുകിനെപ്പറ്റിയുള്ള വിവിധ നാട്ടറിവുകളും ശേഖരിച്ചു. ശേഖരിക്കപ്പെട്ട ഇരുപതിനങ്ങളും 2015-16 കാലയളവിൽ മൂന്നിനെയും മൂന്ന് റപ്ലിക്കേഷനോടുകൂടിയ റാൻഡമൈസ്ഡ് ബ്ലോക്ക് ഡിസൈനിൽ നട്ട് വളർത്തി അവയുടെ രൂപത്തിലും രാസഘടനയിലുമുള്ള വ്യത്യാസങ്ങൾ രേഖപ്പെടുത്തി പാൽമുതുകിന്റെ പ്രത്യുല്പാദന ബയോളജിയെപ്പറ്റിയുള്ള വിശദമായ പഠനവും ഇതോടൊപ്പം നടത്തി.

കിഴങ്ങുകളുടെ പ്രാരംഭ രാസപരിശോധനയിൽ കാര്യമായ പ്രാദേശിക മാറ്റമുള്ളതായി ശ്രദ്ധിക്കപ്പെട്ടു. ഇടുക്കി, വയനാട്, തിരുവനന്തപുരം ഡിസ്ട്രിക്ടുകളിൽ നിന്നുള്ള ഇനങ്ങളാണ് രാസഘടനയിൽ മെച്ചപ്പെട്ടതായി കണ്ടെത്തിയത്. കിഴങ്ങുകളുടെ ഹൈ ഫെർഫോമൻസ് ലിക്വിഡ് ക്രോമാറ്റോഗ്രഫി വിശകലനത്തിൽ ഇതുവരെ റിപ്പോർട്ട് ചെയ്തിട്ടില്ലാത്ത റൂട്ടിൻ, നിക്കോട്ടിനിക് ആസിഡ് എന്നീ സംയുക്തങ്ങളുടെ സാന്നിധ്യവും കണ്ടെത്തി.

വൈവിധ്യത്തെക്കുറിച്ചുള്ള വിദഗ്ദ്ധപരിശോധനയിൽ വള്ളി, ഇല (പീറ്റിയോൾ നീളം ഒഴികെ), വിളവ്, പ്രതീകങ്ങളിൽ ഗണ്യമായ വ്യത്യാസങ്ങൾ പ്രകടമായിരുന്നു. T15 (870.47g), T17 (821.93g), T11 (752.3g), T12 (744.13g), T6 (698.53g), T3 (620.27g) ഇവ ഏറ്റവും മെച്ചപ്പെട്ട വിളവ് നൽകി. ഇവയിൽ T11 (കുമ്പളങ്ങ, എറണാകുളം ജില്ല) T3 (പനമരം, വയനാട് ജില്ല) ഇവ ഫൈറ്റോകോൺസ്റ്റിറ്റ്യൂമെന്റുകളുടെ അളവിൽ ഒന്നാം വർഷവും രണ്ടാം വർഷവും മികച്ച മൂല്യങ്ങൾ രേഖപ്പെടുത്തി. രണ്ടാം വർഷ വിശകലനത്തിൽ T13, T9 ആ സെക്ഷനുകൾ (നെയ്യാറ്റിൻകര, പുന്നമുട്) അവയുടെ ഫൈറ്റോകോൺസ്റ്റിറ്റുമെന്റുകളുടെ അളവിൽ ഏറ്റവും ഉയർന്ന മൂല്യം രേഖപ്പെടുത്തിയെങ്കിലും അവയിൽ കിഴങ്ങു വർഗങ്ങളുടെ അളവ് താരതമ്യേന കുറവായിരുന്നു.

പാൽ മുതുകിന്റെ റീപ്രൊഡക്ടീവ് ബയോളജി വിശകനം നടത്തിയതിൽ നിന്ന് ഈ ചെടികൾ മിതമായ പുഷ്ഠിക്കൽ സ്വഭാവം ഉള്ളവയായും തവിട്ടു നിറത്തിലുള്ള പൂക്കളോടുകൂടി 8-10 മണിക്കൂർ ദൈർഘ്യത്തിൽ സൈമോസ് ഇൻഫ്ളോറസെൻസ് പ്രകടിപ്പിക്കുന്നതായും മനസിലാക്കാൻ കഴിഞ്ഞു. പാൽ മുതുകിന്റെ പൂമ്പൊടി പാൻഡ്രോപേറ്റ് അപ്പാർച്ചർ മോർഫോളജിയും സ്പൈനോസ് എക്സൈൻ ഓർണമെന്റേഷനുമുള്ള മൊണാർഡ് ടൈപ്പ് ആണെന്നു തിരിച്ചറിഞ്ഞിട്ടുണ്ട്. പരാഗരേണുവിന്റെ വ്യാസം (63.6-102 μm), സ്പൈൻ നീളം (5-6.7 μm), അപ്പർച്ചർ നീളം (2.8-4.6 μm), ജനിദണ്ഡ് നീളം (23.5-25.8 μm), ഫിലമെന്റ് നീളം (21.2-25 μm) ആന്തസിസിനുശേഷം 8 മണിക്കൂറോളം സ്റ്റിഗ്മ റിസപ്റ്റീവായി കാണപ്പെട്ടു. 97.817 പരാഗരേണുക്കൾ ഫെർടൈലായും 94.96% വൈയബിളായും കാണപ്പെട്ടു. തുറന്ന പരാഗണത്തെ തുടർന്ന് 80% ഫലങ്ങൾ ഉണ്ടായി. തേനിനും പൂമ്പൊടിക്കുമായി പൂക്കളെത്തേടി അഞ്ച് വ്യത്യസ്ത പരാഗികൾ എത്തുന്നതായി കണ്ടെത്തി. ഫലം 0.7cm x 0.8cm വലിപ്പമുള്ള നാല് വിത്തുകൾ അടങ്ങുന്നതാണ്.

പുതിയ കിഴങ്ങിന്റെ വിളവ് ഉണങ്ങിയ കിഴങ്ങിന്റെ വിളവ്, കിഴങ്ങുകളുടെ എണ്ണവും ഗിർത്തും, പ്രോട്ടീൻ അളവ് എന്നീ മൂല്യങ്ങൾ പരിശോധിച്ചപ്പോൾ PCV, GCV എന്നിവയിൽ വലിയ വ്യത്യാസമുള്ളതായും ഈ പ്രതീകങ്ങളുടെ അളവിൽ പരിസ്ഥിതിയുടെ ഗണ്യമായ സ്വാധീനം ഉള്ളതായും കാണാൻ സാധിച്ചു. കിഴങ്ങിലുള്ള ഗ്ലൈക്കോസൈഡ്, ആൽക്കലോയ്ഡ്സ്, കാർബോഹൈഡ്രേറ്റ്സ്, ഫ്ലേവനോയിഡ്സ് എന്നീ ബയോകെമിക്കൽ പ്രതീകങ്ങളുടെ PCVയും GCVയും തമ്മിലുള്ള വ്യത്യാസം, പാകമായ ഇലയുടെ നീളം, വീതി എന്നീ മോർഫോളജിക്കൽ പ്രതീകങ്ങളിലുള്ള വ്യത്യാസം ഇവ നിരീക്ഷിച്ചതിൽ നിന്നും ഇവ പ്രധാനമായും ജനിതക കാരണങ്ങളാലാണെന്നു കണ്ടെത്തി.

മോർഫോളജിക്കൽ പ്രതീകങ്ങളായ ചെടിയുടെ ഉയരം, ഇന്റർനോഡ് ഡയമീറ്റർ, ബയോകെമിക്കൽ പ്രതീകങ്ങളായ കാർബോഹൈഡ്രേറ്റ്, ഫ്ളേവനോയ്ഡ്സ് ഓയിൽസ് ഇവയിൽ ഉയർന്ന ഹെറിബിലിറ്റിയും അഡ്വാൻസും നിരീക്ഷിക്കാൻ കഴിഞ്ഞു. ഈ പ്രതീകങ്ങളിലുള്ള അഡിറ്റീവ് ജീൻ ആക്ഷനാണ് ഇത് സൂചിപ്പിക്കുന്നത്. ഫിനോടിപ്പിക് ഫെർഫോമൻസ് അനുസരിച്ച് സെലക്ഷൻ നടത്തി ജനറ്റിക് ഇപ്രൂവ്മെൻർ സാധ്യമാക്കാം.

പാൽമുതുക്ക് ആസെക്ഷനുകളിൽ ഫൈറ്റോകെമിക്കൽ കോൺസ്റ്റിറ്റുവെന്റുകളുടെ അളവിൽ ഗണ്യമായ പ്രാദേശിക വ്യതിയാനം നിലനിൽക്കുന്നതായും വയനാട്, ഇടുക്കി, തിരുവനന്തപുരം എന്നിവടങ്ങിൽ നിന്നുള്ള ആസെക്ഷനുകളിൽ ഫൈറ്റോ കോൺസ്റ്റിറ്റുമെന്റുകൾ കൂടുതലായുള്ളതായും കണ്ടെത്തി. മൂപ്പ് കൂടുന്നതനുസരിച്ച് കിഴങ്ങിന്റെ രാസഘടകങ്ങളുടെ അളവ് കൂടുന്നതായി കണ്ടു. ഉയർന്ന കിഴങ്ങു വിളവുള്ള ആസെക്ഷനുകളിൽ രാസഘടകങ്ങളുടെ അളവ് കൂടിയിരിക്കണമെന്നില്ല. T3 (പനമരം), T11 (കുമ്പളങ്ങ) ഇവയിൽ ഉയർന്ന കിഴങ്ങുവിളവും ഗണ്യമായ അളവിൽ ഫൈറ്റോകെമിക്കൽ കണ്ടന്റുകളും ഉള്ളതായി കണ്ടെത്തി. പാൽമുതുക്കിന്റെ പ്രോട്ടീൻ കണ്ടെന്റിന്റെ അളവിൽ പരിസ്ഥിതിയുടെ സ്വാധീനവും പഠനവിധേയമാക്കി.

പാൽമുതുക്കിന്റെ വൈദ്യശാസ്ത്രപരമായ സാധ്യതകൾ ഇതുവരെ മുഴുവനായി പ്രയോജനപ്പെടുത്തിയിട്ടില്ല. അതിനാൽ ഇനിയും ഉയർന്ന ഗവേഷണ സാധ്യതകളാണുള്ളത്. ഫൈറ്റോ കെമിക്കൽ പ്രൊഫൈലിംഗ് വഴി കിഴങ്ങിന്റെ ഔഷധമൂല്യമുള്ള ഘടകങ്ങളെക്കുറിച്ചുള്ള അറിവ് ലഭിക്കുക വഴി ഔഷധത്തിന്റെ കെമിക്കൽ മാപ്പിംഗ് നടത്താനും പ്രവർത്തന രീതി മനസിലാക്കാനും കഴിയും. പാൽമുതുക്ക് കിഴങ്ങിന്റെ ട്യൂബറൈസേഷൻ മോർഫോളജി, അനാട്ടമി ഫൈറ്റോ കെമിക്കൽ പ്രതീകവൽക്കരണം ഇവ ക്രൊമറ്റോഗ്രഫി, സ്പെക്ട്രോമെട്രി ഇവയും പ്രയോജനപ്പെടുത്തി നടത്താൻ കഴിഞ്ഞാൽ വിദാരി പോലുള്ള സസ്യങ്ങളുമായി അഡൾട്ടറേഷൻ കണ്ടെത്താൻ സാധിക്കും.

Appendices

Appendix I.

Descriptor details of 33 morphological characters of *I. digitata* Linn.

Sl.no	Morphological characters	Descriptor scale
Vine Characters		
1.	Twining	0 Non twining 3 Slightly twining 5 Moderately twining 7 Twining 9 Very twining
2.	Plant type	3 Erect (< 75 cm) 5 Semi erect (75-150 cm) 7 Spreading (151-250 cm) 9 Extremely spreading (>250 cm)
3.	Internode diameter	1 Very thin (< 4 mm) 3 Thin (4-6 mm) 5 Intermediate (7-9 mm) 7 Thick (10-12 mm) 9 Very thick (> 12 mm)
4.	Internode length	1 Very short (<3 cm) 3 Short (3-5 cm) 5 Intermediate (6-9 cm) 7 Long (10-12 cm) 9 Very long (>12 cm)
5.	Predominant colour of vine	1 Green 3- Green with few purple spots 4- Green with many purple spots 5- Green with many dark purple spots 6- Mostly purple 7- 7- Mostly dark purple 8- Totally purple 9- Totally dark purple

Leaf Characters		
6.	Type of leaf lobes	0 No lateral lobes 1 Very slight 3 Slight 5 Moderate 7 Deep 9 Very deep
7.	Number of leaf lobes	
8.	Shape of central leaf lobe	0 Absent 1 Toothed 2 Triangular 3 Semi- circular 4 Semi-elliptic 5 Elliptic 6 Lanceolate 7 Oblanceolate 8 Linear (broad) 9 Linear (narrow)
9.	Matured leaf size	3 Small (<8 cm) 5 Medium (8-15 cm) 7 Large (16-25 cm) 9 Very large (>25 cm)
10.	Mature leaf colour	1 Yellow-green 2 Green 3 Green with purple edge 4 Greyish-green (due to heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Mostly purple 8 Green upper, purple lower 9 Puprle both surfaces

11.	Immature leaf colour	1 Yellow-green 2 Green 3 Green with purple edge 4 Greyish-green (due to heavy pubescence) 5 Green with purple veins on upper surface 6 Slightly purple 7 Mostly purple 8 Green upper, purple lower 9 Puprle both surfaces
12.	Petiole pigmentation	1 Green 2 Green with purple near stem 3 Green with purple near leaf 4 Green with purple at both ends 5 Green with purple spots throughout petiole 6 Green with purple stripes 7 Purple with green near leaf 8 Some petioles purple, other green 9 Totally or mostly purple
13.	Petiole length	1 Very short (<10 cm) 3 Short (10-20 cm) 5 Intermediate (21-30 cm) 7 Long (31-40 cm) 9 Very long (>40 cm)

Inflorescence Characters		
14.	Flowering habit	0 None 3 Sparse 5 Moderate 7 Profuse
15.	Flower size	Flower length (cm) X Flower width (cm)
16.	Flower color	1 White 2 White limb with purple throat 3 White limb with pale purple ring and purple throat 4 Pale purple limb with purple throat 5 Purple 6 Other
17.	Shape of limb	3 Semi- stellate 5 Pentagonal 7 Rounded
18.	Sepal shape	1 Ovate 3 Elliptic 5 Obovate 7 Obolong 9 Lanceolate
19.	Sepal apex	1 Acute 3 Obtuse 5 Acuminate 7 Caudate

20.	Sepal pubescence	0 Absent 3 Sparse 5 moderate 7 Heavy
21.	Sepal color	1 Green 2 Green with purple edge 3 Green with purple spot 5 Green with purple areas 6 Some sepals green, some purple 7 Totally pigmented – pale purple 9 Totally pigmented – dark purple
22.	Color of stigma	1 White 5 Pale purple 9 Purple
23.	Color of style	1 White 3 White with purple at the base 5 White with purple at the top 7 White with purple spots throughout 9 Purple
24.	Stigma exsertion	1 Inserted (shorter than longest anther) 3 Same height as highest anther 5 Slightly exerted 7 Exerted
25.	Seed capsule set	0 None 1 Scarce 3 Sparse 5 Moderate 7 Profuse

Root Characters		
26.	Storage root formation	1 Closed cluster 3 Open cluster 5 Dispersed 7 Very dispersed
27.	Storage root stalk	0 Absent 1 Very short (< 2 cm) 3 Short (2-5 cm) 5 Intermediate (6-8 cm) 7 Long (9-12 cm) 9 Very long (> 12 cm)
28.	No. Of storage root plant^{-1}	Average of ten plants
29.	Variability of storage root shape	3 Uniform 5 slightly variable 7 moderately variable
30.	Variability of storage root size	3 Uniform 5 slightly variable 7 moderately variable
31.	Storage root cracking	0 Absent 3 Few cracks 5 Medium nuber of cracks 7 Many cracks
32.	Predominant skin color	1 White 2 Cream 3 Yellow 4 Orange 5 Brownish orange 6 Pink

		7 Red 8 Purple-red 9 Dark purple
33.	Predominant flesh color	1 White 2 Cream 3 Dark cream 4 Pale yellow 5 Dark yellow 6 Pale orange 7 Intermediate orange 8 Dark orange 9 Strongly pigmented with anthocyanins

Appendix II

Weekly mean weather data at the time of collection of accessions: CRS pampadumpara Idukk (January 2014)

Week No.	Temperaure(°C)		Relative humidity (%)	Rainfall (mm)
	MAX	MIN		
1	22.1	16.0	98.6	107.5
2	23.2	15.4	69.6	117
3	25.3	14.6	92.2	123.5
4	24.9	14.1	95.8	109.5
5	25.2	16.8	87.7	0
Mean	24.2	15.3	88.8	91.5

Weekly mean weather data at the time of collection of accessions: Thrissur (January 2014)

Week	Temperature		Relative humidity		Sun shine (hrs/day)	Rainfal (mm)
	Maximum	Minimum	I	II		
1	32.6	22.4	74.0	34.0	9.2	0.0
2	32.8	23.1	68.0	37.0	8.1	0.0
3	33.2	23.7	63.0	38.0	8.8	0.0
4	33.7	22.3	60.0	33.0	9.9	0.0
5	33.4	22.5	62.1	34.1	9.2	0.0
Mean	33.0	22.9	66.2	35.5	9.0	0

Appendix II continued.

Weekly mean weather data at the time of collection of accessions: Wayanad (January 2014)

Week	Temperature		Relative humidity		Sun shine (hrs/day)	Rainfal (mm)
	Maximum	Minimum	I	II		
1	28.1	15.3	95.1	56.6	8.5	0
2	29.0	16.3	85.1	68.1	8.0	0
3	28.1	16.9	97.1	62.9	6.8	0
4	28.3	15.8	91.5	55.1	9.0	0
5	28.4	14.4	95.3	58.0	9.0	0
Mean	28.4	15.8	92.9	60.1	8.2	0

Weekly mean weather data at the time of collection of accessions: Vellayani (January 2014)

Week	Temperature		Relative humidity		Sun shine (hrs/day)	Rainfal (mm)
	Maximum	Minimum	I	II		
1	30.9	21.5	94.8	77.5	8.8	0
2	28.9	22.2	94.4	77.4	7.5	0
3	31.0	21.7	94.1	76.1	9.2	0
4	31.2	20.6	90.4	69.8	9.3	0.5
5	31.3	21.9	92.2	68.5	9.3	0
Mean	30.8	21.7	93.2	73.9	8.9	0.1

Appendix III

Weather data for the cropping period (Jan-2014 to Dec- 2014): Vellayani

Standard month	Temperature (0C)		Relative humidity (%)		Sun shine hours	Rain fall (mm)
	Maximum	Minimum	Maximum	Minimum		
January	30.7	21.6	93.2	73.9	8.8	2.9
February	31.3	22.3	92.4	69.7	9.4	8.1
March	32.4	22.8	91.7	66.4	9.8	3.1
April	32.3	24.5	91.3	73.6	9.1	10.7
May	31.8	24.8	90.4	78.2	8.6	15.4
June	30.8	25.1	92.5	78.5	9.0	3.5
July	30.0	24.3	91.4	77.4	9.1	4.3
August	29.5	23.7	91.0	81.5	8.4	29.4
September	30.2	24.2	90.9	78.4	8.8	9.0
October	30.4	23.7	83.6	86.0	7.9	9.0
November	30.1	23.3	91.8	79.2	7.6	5.5
December	30.2	23.2	91.8	75.0	8.4	10.0

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Appendix IV

Weather data for the cropping period (Jan-2015 to Jan- 2016): Vellayani

Standard month	Temperature (0C)		Relative humidity (%)		Sun shine hours	Rain fall (mm)
	Maximum	Minimum	Maximum	Minimum		
January	30.6	21.5	93.8	65.0	9.2	4
Feburary	31.5	22.3	91.7	64.7	9.4	0
March	32.4	23.6	89.9	67.1	9.5	11.7
April	32.7	24.4	90.7	73.2	8.8	15.2
May	32.1	25.3	90.7	83.2	7.3	27.1
June	31.4	24.4	90.8	82.8	8.8	13.8
July	31.3	24.6	89.1	79.8	5.4	9.6
August	31.6	24.5	89.5	76.4	9.5	6.7
September	31.4	24.4	91.4	83.7	8.1	16.1
October	31.3	24.0	92.4	80.5	8.1	15.3
November	31.5	23.7	92.7	79.5	7.7	12.1
December	31.6	23.8	94.5	83.8	6.2	18.5
January	32.3	22.6	91.9	72.4	7.8	0.4