MORPHOLOGICAL AND MOLECULAR ANALYSIS FOR ASSESSING INTRASPECIFIC VARIATION IN SWEET POTATO (*Ipomoea batatas* (L.) Lam.) AND INTERSPECIFIC DIVERGENCE IN *Ipomoea* spp.

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B. Sc. - M. Sc. (INTEGRATED) BIOTECHNOLOGY

DEPARTMENT OF PLANT BIOTECHNOLOGY

COLLEGE OF AGRICULTURE

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2020

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by

SASILEKHA S.

(2015-09-003)

THESIS

Submitted in partial

fulfillment of the

requirement for the degree

of

B. Sc. - M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of
Agriculture

Kerala Agricultural University, Thrissur

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KERALA, INDIA 2020

DECLARATION

I hereby declare that the thesis entitled "Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp." 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.

Place: Vellayani **SASILEKHA S.**

Date: 17/12/2020 (2015-09-003)

CERTIFICATE

Certified that this thesis entitled "**Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp.** "is a record of research work done independently by **SASILEKHA S.** (2015-09- 003) under my guidance and supervision and this has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place: Sreekariyam

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We, the undersigned members of the advisory committee of Ms. **SASILEKHA S.** (**2015-09-003**) a candidate for the degree of B. Sc. -M. Sc. (Integrated) Biotechnology, agree that the thesis entitled **"Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea spp*."** may be submitted by Ms. SASILEKHA S. in partial fulfillment of the requirement for the degree.

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ACKNOWLEDGEMENT

At the outset I express my gratitude to ALMIGHTY GOD for being with us throughout each and every step that I have taken towards the completion of dissertation. Gratitude comes from within and with joy and gratitude acknowledges the help of those who were involved in the successful completion of the endeavor.

First and foremost, I express my deep sense of gratitude and sincere thanks to my major advisor ***Dr. (Mrs.) Shirly Raichal Anil****, Principal Scientist, Division of Crop Improvement, ICAR-CTCRI, for her excellent guidance, sustained interest, mentoring, encouragement, motivation, love, help and valuable support throughout the period of my research work. I really can't express my gratitude in actual sense. She gave me the confidence to describe things in the best and easiest way. Her constructive criticisms and suggestions were invaluable in the successful completion of this work.*

My special thanks to the Director of ICAR-CTCRI ***Dr. (Mrs.) Archana Mukherjee*** *for permitting me to do my project work and for her support.*

I cordially offer my unboundful gratitude to revered member of the advisory committee ***Dr. (Ms.) N. Krishna Radhika,*** *Scientist, Division of Crop Improvement, ICAR-CTCRI, Thiruvananthapuram, for her constant help, encouragement and valuable suggestions during the research work and preparation of the thesis.*

I express my profound gratitude to ***Dr. (Mrs.) Swapna Alex, and Dr. (Mrs.) K. B. Soni*** *not only for their insightful comments and encouragement, but also for their valuable discussions, throughout support, intellectual advice, valuable guidance and critical suggestions related to the project.*

I would like to express my sincere thanks and gratitude to ***Dr. (Mrs.) Sheela M.N.,*** *Head, Division of Crop Improvement, ICAR-CTCRI for permitting me and extending all the facilities to complete my work.*

My wholehearted thanks to ***Dr. Asha Devi A, Dr. C. Mohan*** *and all other scientists at ICAR-CTCRI, for their for their valuable advices and constant motivation , help and support in the most difficult times.*

I express my heartfelt gratitude and sincere thanks to ***Mr. Prakash Krishnan****, who was always there for me and for his valuable suggestions, technical support and affectionate encouragement throughout my research work.*

My sincere gratitude is towards ***Mrs. Jyothy, Mrs. Athulya, Mr.Shersha, Mr. Abhilash Mr. Athul and Mr. Abin.*** *who helped me a lot to carry out this piece of work with all hearts.*

My acknowledgement would be lacking if I don't mention my gratitude to my beloved ones, ***Krishnaveni Vijayakumar, Akhila j., Preeja P. S., Aswani S. A., Sailakshmi and Ajeesh B. R.*** *for their selfless help and support throughout my work.*

I would like to thank my senior ***Mr. Sabarinath V. B.*** *for his help and support.*

I deeply indebted to all the scientists and staff members of ICAR- CTCRI, teachers in college, my seniors and juniors for their timely support. I acknowledge the favour of numerous persons who, though not been individually mentioned here, who have all directly or indirectly contributed to this work.

Finally I can't forget the support, prayer and encouragement of ***my parents and sister*** *which inspires me all the way throughout my studies. I thank my parents for all the support and strength they gave me throughout my life.*

Place: Thiruvananthapuram

Date: 17-12-2020 ***Sasilekha S.***

DEDICATED TO MY DEAR PARENTS

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LIST OF ABBREVATIONS

%	Percentage
^{o}C	Degree Celsius
µg	Microgram
µl	Microlitre
µM	Micromolar
A	Adenine
A230	Absorbance at 230 nm wavelength
A260	Absorbance at 260 nm wavelength
A280	Absorbance at 280 nm wavelength
AFLP	Amplified Fragment Length Polymorphism
bp	Base pair
C	Cytosine
CIP	International Potato Center
cm	centimetre
DNA	Deoxyribonucleic acid
dNTPs	Deoxy nucleotide tri phosphates
EDTA	Ethylene diamine tetra acetic acid
et al.	et alia
EtBr	Ethidium bromide
FAOSTAT	Food and Agriculture Organization Statistical Database
G	Guanine
g	gram
g	standard acceleration due to gravity at the earth's surface
h	Hour
ha	Hectare

ICAR-CTCRI-	Central Tuber Crops Research Institute
IPGRI	International Plant Genetic Resources Institute
IBPGR	International Board for Plant Genetic Resources
ISSR	Inter Simple Sequence Repeat
KAU	Kerala Agricultural University
Kb	Kilo bases
kg	Kilogram
L	Litre
m	Meter
M	Molar
mg	milligram
$MgCl_2$	Magnesium chloride
min	Minute
ml	Millilitre
mm	Millimeter
mM	Millimolar
MVSP	Multi Variate Statistical Package
NaCl	Sodium chloride
NAGS	National Active Germplasm Site
Ng	Nanogram
Nm	Nanometer
OD	Optical density
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
ppm	Parts per million
PVP	Polyvinylpyrrolidine

RAPD	Random amplified polymeric DNA
RDA	Recommended dialy allowance
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute
s	second
sp.	Species
spp.	Species (plural)
SSR	Simple sequence repeat
Taq	*Thermus aquaticus*
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UV	Ultra violet
V	Volt
v/v	volume/volume
w/v	weight/volume

INTRODUCTION

1. INTRODUCTION

Roots and tuber crops are the most important cultivated staple energy sources, after cereals, generally in tropical regions in the world. They include sweet potatoes, potatoes, cassava, yams, and aroids belonging to different botanical families but are grouped together as all types produced underground food. They are consumed as staple food and used as raw material for small-scale industries. They also used as an animal feed. Nutritionally, roots and tubers have high amount of carbohydrates that make them as a staple energy source (Chandrasekara and Kumar, 2016).

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a root crop of Convolvulaceae, the morning-glory family, grown during the winter season (October-December) in India (Solankey *et al*., 2014). It belongs to the genus *Ipomoea*, includes approximately 500–600 species, and sweet potato is the only cultivated species in the genus *Ipomoea* series Batatas (Austin *et al.*, 1988). The genus has four ploidy levels: hexaploid (2n=6x=90) *Ipomoea batatas*, tetraploid (2n=4x=60) *Ipomoea tiliaceae* and diploid (2n=30) *I. cordatotriloba*, *I. lacunosa* and *I.triloba*. (Austin, 1988). Sweet potato having thirteen related wild species (Austin 1988; Austin and Huáman 1996). Of these, *I. trifida* has been considered the most likely progenitor (Nishiyama 1971; Shiotani and Kawase 1989).

The exact location of its botanical origin is unknown but it was found in the Americas approximately 8000–10,000years ago and domesticated at least 4000years ago (Woolfe *et al*., 1992). The primary center of diversity is Central America, and South America (Peru, Ecuador) is considered as the secondary center of diversity (Zhang *et al*., 2000). China account for 70% world's sweet potato production and they are the major producer after Sub-Saharan Africa (Padmaja *et al*., 2009). In recent years, the global production of sweetpotato was more than 100 million tons (FAOSTAT 2019). Considering worldwide food crops, It is the sixth most one, following rice, wheat, potatoes, maize, and cassava (International Potato Center, 2018).

The sweet potato plant is a herbaceous perennial vine with alternate heart-shaped or palmately-lobed leaves and the flowers are medium-sized sympetalous.

The sweet potato roots are edible with smooth skin covering. The growing period of crop ranges from 90 to 120 days (Mohanraj and Sivasankar, 2014). Reproduction through vegetative propagation using storage roots or stem cuttings. The varieties are classified according to its tuber flesh colour, which ranges from beige to white, violet, red, pink, orange, yellow, and purple. Compared to red, orange and pink fleshed sweet potato, the sweetness of white or pale yellow flesh are less (Loebenstein *et al*., 2009).

Ipomoea batatas (L.) Lam, is a delicious vegetable that possesses high nutritional value. Orange and purple- fleshed varieties are considered as the dietary antioxidants. Storage roots of purple-fleshed sweet potatoes contain large amounts of anthocyanins (802-1747 mg/kg fresh weight) (Steed and Truong, 2008). Orange fleshed sweet potato with rich Beta- carotene helps in alleviating vitamin A deficiency (Ravi and Indira, 1999). Roots are valuable source of carbohydrates. Besides vitamin A, sweet potatoes are rich in vitamin B1, B2 (riboflavin), B3 (niacin), B6, biotin, E, and pantothenic acid. Moreover, dietary fibers like, potassium, manganese, copper, and iron are present and they are low in fat and cholesterol (Wang *et al*., 2016). It is also a valuable medicinal plant having anti-cancer, anti-diabetic, and anti-inflammatory activities. They also possess analgesic, spasmogenic, antimicrobial, spasmolitic, anticoagulant, hypoglycemic, psychotomimetic and hypotensive activities (Meira *et al*., 2012).

Sweet potato is considered as an "insurance crop" because, under suitable climatic conditions it can be grown all around the year and under adverse climatic conditions the loss of complete crop is rare. The crop is particularly important in Southeast Asia, Oceania, and Latin America regions (Bovell-Benjamin, 2007). It has been regarded as the 'potato' of the warm tropics due to its ability to grow under high temperatures and low inputs of water and fertilizer (Bohac *et al*., 1995).

Sweet potato varieties and accessions are collected and maintained in germplasm worldwide. The International Potato Center (CIP) possess one of the largest collections of sweet potato germplasm (Huaman and Zhang, 1997).

The ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) a constituent Institute under the Indian Council of Agricultural Research (ICAR) is the only research organization in the World dedicated solely to the research on tropical tuber crops. The Headquarters of ICAR-CTCRI is located at Sreekariyam, Trivandrum. It is called the National Active germplasm site (NAGS) of tuber crops except potato. Around 6000 accessions of various tropical tuber crops are being maintained at the institute in which 1400 accessions of sweet potato accession are maintained in the NAGS at ICAR-CTCRI, Sreekariyam and its regional Centre at Bhubaneswar. One of the major mandates of the Institute includes the Collection, Conservation and Characterization of tuber crops germplasm. Germplasm of each crop possess a lot of variability in the leaves, stem and storage root characteristics and lot of morphologically similar accessions also exist.

Morphological characterization of sweet potato accessions is done using the International Plant Genetic Resources Institute (IPGRI) descriptors. The variability and divergence between sweet potato species can be analyzed with the help of different molecular markers like Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Inter-Simple Sequence Repeats (ISSR) in which ISSR markers has been reported to differentiate closely related cultivars and clones (Zietkiewicz, *et al.*, 1994). Hence the objectives of this study were framed to identify diversity in sweet potato at intraspecific levels and to obtain interspecific divergence in *Ipomoea* spp using both morphological and molecular markers.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 ROOT AND TUBER CROPS

Tropical root and tuber crops play vital role in nutrition, food security and climate change adaptation. The diet plan of many countries includes root and tuber crops as an essential component. Tropical tuber crops comprise of major and minor (underexploited) plant species like, cassava, sweet potatoes, yams and aroids (Giri and Sakhale, 2019). They represent the second most important set of food crops in developing countries as global sources of carbohydrates, closely following the cereals. A considerable part in the supply of world's food is contributed by root and tuber crops. They can be favourably adapted to diverse soil and environmental conditions and due to this agronomic advantage they perform as a staple food source (Chandrasekara and Kumar, 2016). All these crops have two type of lifespan. Wild species are perennial but cultivars are annual and need to be replanted to produce desirable underground storage organs. They have variable ploidy levels but are predominantly allogamous and highly heterozygous. As a natural defence mechanism against predators, wild forms accumulate secondary metabolites and domestication entails reducing these anti-nutritional compounds while increasing those of value in human consumption (Lebot, 2009). Among different root and tuber crops, sweet potato is one of the nutritious, healthy and underutilized tropical tuber crops. As a food crop the significance of sweet potato was neglected but now gaining importance because of its nutritional composition. The low glycemic index has raised the value of sweet potato tubers as a health food.

2.2 SWEET POTATO

Ipomoea batatas (L.) Lam, commonly known as sweet potato belonging to the family Convolvulaceae (morning glory family), is an important root vegetable which is large, starchy, and sweet tasting dicotyledonous crop with a vital role in food production (Woolfe, 1992). Systematic classification of sweet potato includes the family, Convolvulaceae of the tribe, *Ipomoeae* and the genus *Ipomoea*. The genus comprises of around 500–600 species, mostly found in the tropics. They are annual or perennial herbaceous vines with a few erect shrubs. The crop belongs to the same sub-genus and section Eriospermum and Batatas is the series under which the species

Ipomoea batatas (L.) Lam. comes (Huaman, 1999). The botanical characteristics of the plant shows high variation, so they are distinguished on the basis of morphological traits, colour of root skin, yield potential, as well as size, colour and shape of branches and leaves (Zhang *et al*., 2000).

2.2.1 Wild species

The existence of interrelationships between *I. triloba, I. trifida, I. tiliacea, I. gracilis* and *I. batatas* was recognized by Van Ooststroom in 1953 and these five species were included in the Batatas section. The sweet potato and their relatives were then grouped in a new Batatas section, which included 11 species and two natural hybrids but McDonald and Austin (1990) revised the taxonomy of the Batatas section, which now includes 16 taxa. The chromosome number, cross-compatibility and the formation of edible roots make the sweet potato unique from other related species. Even though the ploidy level is different, it is assumed that there should be a common genome for the species which provides the ability to intercross with sweet potato.

Some wild species are closely related to sweet potato. Austin (1988) found that similarity of thirteen wild species with sweet potato of which the most likely progenitor was *I. trifida* (H.B.K.) Don. (Shiotani and Kawase, 1989). The sweet potato was considered to be originated from an hexaploid species (*I. trifida*), which is a hybridization product of diploid *I. leucantha* Jacq. and tetraploid *I. littoralis* Blume (Nishiyama, 1971) . Shiotani and Kawase (1989) also believed the origin of sweet potato from hexaploid *I. trifida*, based on the results obtained from artificial hybridization of the diploid and tetraploid species available. The supposition was later recognized and upheld by Munoz-Rodrıguez et al. (2018) based on the gene sequences comparison data in sweet potato and later Wu et al. (2018) confirmed it with the information obtained through whole-genome sequencing in *Ipomea* genus.

2.3 ORIGIN AND DISTRIBUTION

Sweet potatoes were domesticated in Central America, 5000 years ago (Bovell-Benjamin, 2014). The oldest prehistoric remnants of sweet potatoes were found in 8000 BC in the caves of the Chilca Canyon in Peru through radiocarbon

dating (Lebot, 2010; Woolfe, 1992). The proper location of origin of the crop is unknown however, on the basis of related species number and morphological variation, Austin (1988) postulated that sweet potato and its wild relatives originated in the region between the Yucatán Peninsula of Mexico and the Orinoco River in Venezuela. Due to the high diversity of both sweet potato and their related species found in the Americas, this area found to be the possible primary center of origin (Zhang *et al*., 2000 and Mwanga *et al*., 2017).

The plant was introduced in Western Europe in 1492 by Columbus on his return from his first voyage to the New World (Roullier *et al*., 2013). Then sweet potatoes spread rapidly to all the other parts of the world by Polynesian and European voyagers. It was first diffused to islands of Polynesia such as Easter Island, Hawaii, and eastern Polynesia, and then into New Zealand. On the beginning of 16th century, Spanish and Portuguese travelers and traders distributed it into the Indonesia and Philippines and rest of the Asia. Sweet potato was introduced to China during the end period of Ming dynasty or the great Ming in the year, 1594 (Goodrich, 1937). As a part of the introduction of sweet potato in China, the year 1594 was recognized and celebrated by the Chinese scholarship. An alternate suggestion was also made by Goodrich on the introduction of sweet potato that it may likely to enter China from the island of Luzon, Malaysia or the South Seas. As a result, areas like Fujian and Guangdong provinces have been widely admitted as the main areas through which sweet potato which is a known crop of America, entered China (Perez Garcia, 2018). It was during the 17th and 18th centuries, the crop was well established and started spreading throughout Asia, Latin America, and Africa.

2.4 PRODUCTION

Now the sweet potatoes are cultivated all over the world in a wide range. It can be survive in any condition so that it has become one of the major food crops in human consumption. There is no requirement of specified condition for the growth of the plant it can grow even in poor water and soil condition as a high-yield tuberous crop, such as in Pacific Islands, sub-Saharan Africa and different parts of Asia (Mu and Li, 2019).

According to FAOSTAT (2016), 105 million tons of sweet potatoes were produced worldwide in the year 2016. They were the sixth largest staple food crop in comparison to other crops, followed by corn (1060 million tons), wheat (749 million tons), rice (741 million tons), potato (376 million tons), and cassava (277 million tons). Production of sweet potato is not evenly distributed around the world but the cultivation is widespread around the world. China is the largest producer among other countries. As per the report of FAOSTAT (2016), 71 million tons production account by China itself that means 68% of the world's total production. A large number of the varieties of the plant have been cultivated in China.

In each year global production of sweet potato is more than 100 million tons, from which 95% production account by developing countries. Asia is the biggest sweet potato-producing region today and they produce 78 million tons of sweet potatoes. Africa produced 21 million tons and they were the second largest producer. Less than 5% production was contributed by the Americas, the native place of sweet potatoes. Europe produces only in small which is mainly in Portugal (Mu and Li, 2019).

2.5 AGRO CLIMATIC CONDITIONS

Certain peculiarities of sweet potato like it can be grown all-round the year under suitable climate conditions, harvesting can be employed multiple times with little quantity each time and rare case of complete crop failures under adverse weather conditions etc. suggests this crop as a typical crop for food security especially among marginal and poor populations. (Bovell-Benjamin, 2007).

Sweet potatoes are grown between latitudes 42° N and 35° S on the equator and 3000 m above the sea level. It is mainly grown in tropics and temperate regions either through vine cuttings or rooted sprouts and these are planted on mounds or ridges in the field (Woolfe, 1992).

The growth of sweet potatoes as any other crop, is influenced by the environment and the ability of plants to adapt. The crop can grow without fertilized soil, but the tuber production is affected by temperature significantly. At the temperature of 24˚C the tuber formation is optimized. The tuber is not well formed in higher locations with low temperature (Aldow, 2017). The ability of the plant to

adapt different environmental conditions makes the plant as a security in human food even in vulnerable regions (Glato *et al*., 2017).

Acidic soil is most preferred by the plant to grow with an optimum pH of 5.5-6.5. Though only some extent of drought can be tolerated and water logging is an issue while considering the growth of this crop. Sweet potato cultivation requires sufficient rain fall of around 75-150cm and plenty of sunshine but shade causes reduction in the yield (Nedunchezhiyan *et al*., 2007).

2.6 GERMPLASM

For the maintenance and conservation of cultivated plants and wild crops, germplasm collections play an important role. This can be useful for the evolution of potential new crops. Germplasm can be characterized on the basis of morphological characters of plants and such characters can be used for the establishment of a gene-bank collection. The main advantages of these morphological characters include reliability, easiness of visual observation, easy to study and relatively cheap to evaluate (Lin *et al*., 2007).

Sweet potato shows great diversity and genetic polymorphism (Andrade *et al*., 2017; Su *et al*., 2016; Wadl *et al*., 2018). Sweet potato germplasm classified on the basis of phenotypic descriptors (Huaman, 1991). Such descriptors are potentially helpful in sweet potato collection for analysing and studying genetic relationships between accessions and clonal identification as well as genetic distance estimation. For distinguishing sweet potato accessions phenotype characters have been used widely (Baker *et al*., 1998; Huaman, 1992; Huaman *et al*., 1999; Tsegaye *et al*., 2007; Veasey *et al*., 2007). For breeding purpose, sweet potato and *Ipomoea* germplasm with wide genetic and phenotypic diversity were used (Gichuki *et al*., 2003; Jarret *et al*., 2019; Khoury *et al*., 2015; Su *et al*., 2017).

Genetic diversity of sweet potato is conserved at several germplasm collections throughout the world. Sweet potato cultivars and other *Ipomoea* species (more than 8000 and 26,000 accessions respectively) were maintained at 83 genebanks in the world (Kuo, 1991; Rao *et al*., 1994). Kim *et al*. (2018) reported more than 20,000 *I. batatas* accessions in 10 collections from just nine countries, and Roca *et al*. (2007)

reported more than 27,000 sweetpotato accessions from 36 collections in 32 countries. According to FAO (2010), there are 35,478 sweet potato accessions held in ex situ gene banks globally. The most important and one of the largest sweet potato collection present in CIP, Lima, Peru (Huaman and de la Puente, 1988) with ten wild species (532 samples) and cultivated accessions (5526) from the entire world (CIP, 2019).

Security and the availability of crop germplasm ensured by, a two-tier system of management that is crop "base" and "active" collection. Long-term conservation is provided by base collection, there is no distribution among these accessions. Active collections are the vehicles for germplasm evaluation and distribution and serve as the links between conservation efforts and the user community. Management of active collections emphasizes availability and security. Base collections serve as back-ups for active collections (Jarret and Florkowski, 1990).

2.7 GENERAL PLANT MORPHOLOGY

Sweet potato is a clonally propagated, perennial herb, treated as an annual when cultivated. It has trailing or twining stems with glabrous or pubescent and green to purple in colour. The thin, long, stems that spread all over the surface and produce roots where nodes have soil contact. Depending on genotype the length of stem or vine varies from 1 to 5 m. The epidermis of the stem have hairs and is composed of a thin layer of cells and stomata. Cortex is present under the epidermis and composed of several layers of cells containing chlorophyll (Lebot, 2009).

Depending on the age of plant, high variability can be seen on the leaves, sometimes on the same plant and have a sprall leaf arrangement with a 2/5 phyllotaxy. They are exstipulate and simple, with 5 and 30 cm long petiole. The general outline of leaves can be categorized into seven types, rounded, reniform, triangular, hastate, lobed, cordate and almost divided. Varying colours are possessed by the leaves, from light green to deep purple.

The flowers are solitary or in clusters of up to 22 buds, growing out of the leaf axils. Their peduncles vary from 3 to 15 cm long. A flower is composed of five sepals, five petals, stamens (5) and a compound pistil. There are small and lanceolate bracteoles and with a five-lobed calyx and a corolla with funnel shape. Corolla

contains five stamens with variable length and they are attached at the base. Colours vary from pink to white or purple. The filaments and the anthers are also white.

Glabrous or hirsute fruits with diameter of 5–10 mm dehiscent capsules are present. Usually a total of four seeds are present in which only one or two will develop fully. The seeds have brown or black colour with 2-3 mm length with varying shape that ranges from rounded, angular or irregular. The seeds are bi-lobed cotyledons and convex on one side and flat on the other with a hard testa.

From the nodes of the cutting of the plant an extensive, fibrous root system is developed. When the nodes are contact with the soil surface root can be developed. The adventitious roots serve as a storage root for plants, around five to ten storage roots are produced per plant. The skin colour and the flesh colour vary in different cultivars. The root tuber color ranges from white to yellow, red, orange, beige, brown, and purple. The flesh colour ranges between beige, red, white, violet, pink, yellow, purple and orange (Loebenstein, 2009).

2.8 NUTRITIONAL AND HEALTH IMPORTANCE

Sweet potato is a large, starchy, and sweet tasting vegetable possessing lots of health benefits and medicinal properties. The nutritional composition of sweet potato helps in meeting the nutritional requirements of human beings. The carbohydrate rich roots and protein rich leaves helps in elevating the human health (Preedy *et al*., 2011). Sweet potato is not only a source of carbohydrates, but also a major source of vitamins A, pantothenic acid, C, B1, B2, B3, B6 and vitamin E (Hill *et al*., 1992;Wang *et al*., 2016). The functional value of sweet potato in the food market is due to the presence of natural health promoting components like β-carotene, phenolic acids, anthocyanins, carbohydrates, vitamins and high quality proteins (Grace *et al*., 2014).

Sweet potato leaves have good nutritious value which is high when compared to tuber. The leaves are rich in fat, fiber, protein, vitamins A and C, carbohydrates, moisture contents, and mineral elements like zinc, manganese, iron, sodium and calcium moreover toxicants like tannins, cyanide, oxalate and phytic acids are very low in level (Antia *et al*., 2006).

The anti-inflammatory, anti-cancerous and antidiabetic property make them as a valuable medicinal plant. Even though it is a rich in carbohydrate, it also reported to have low glycaemic index (<55), suggesting its suitability as a food for diabetic people (Bjorck *et al*., 2000). The leaves are used to treat type 2 diabetes and inflammation (Pochapski *et al*., 2011). A study of Ludvik *et al* in 2004 proved that sweet potato roots could be eaten as such to treat anemia, hypertension, and diabetes particularly in Kagawa and Japan. Purple-fleshed sweet potato variety is having anthocyanin and phenolics with antioxidant and anti-inflammatory activities (Grace *et al*., 2014, Vishnu *et al.*, 2019). A study of Van Jaarsveld in 2006 proved that carotenoid rich orange fleshed sweet potato (125g) consumption in children result in a gradual increase in the vitamin A production in body, mainly in developing countries. In the year of 2012, Panda and Sonkamble studied the sweet potato root anti-ulcerative property in a rat model.

The fresh roots and leaves are processed into animal feed, starch, flour, noodles, natural colorants, candy and alcohol (Lebot, 2009). The high concentration of stable anthocyanins present in the purple-fleshed sweet potato tubers and leaves could be an excellent alternative to synthetic colour in food products (Truong and Avula, 2010). The leaves are used as fish feed in India particularly the Monpa ethnic groups of Arunachal Pradesh (Namsa *et al*., 2011). The leaves provide protection from cancer, allergies, aging, HIV and cardiovascular problems (Islam, 2006). Purple sweet potato plays a chief role in the prevention of obesity (Hwang *et al*., 2011).

2.9 MORPHOLOGICAL CHARACTERIZATION

Morphological characterization has play major role in plant genetic diversity assessments for the use and conservation of plant genetic resources (Mwanga *et al*., 2017). Many studies have been done in sweet potato by using this approach to remove duplicated accessions in germplasm, to analyse genetic diversity within a specific geographical area and produce knowledge on breeding among sweet potatoes (Veasey *et al*., 2007; Koussao *et al*., 2014). Plant morphological characterization can be done by measuring vegetative and reproductive structures (Huaman, 1999). As compared to other tuber crops morphological characterization in sweet potato is quite difficult, because it is asexually propagated, so the crop is susceptible to mutation as

well as it shows high variability in phenotypical and morphological traits (Huaman *et al*., 1999; Woolfe, 1992).

Morphological descriptors (CIP, 2010 and Huaman, 1991, 1992) are often used to characterize phenotypes and for making selections in breeding programs (Wadl *et al*., 2018). A total of 104 potential sweet potato descriptors were identified (Huaman,1991); however, several researchers used morphoagronomic characters other than the International Plant Genetic Resources Institute sweetpotato descriptors (Galal and El Gendy, 2017; Reddy *et al*., 2018).

Environmental conditions have a major influence on morphological traits (Prakash *et al*., 1996); moreover the morphological descriptors often provide less genetic information (Rao, 2004). So morphological characterization is integrated with molecular characterization as the molecular markers are less affected by environment (Westman and Kresovich, 1997).

2.10 MOLECULAR MARKERS

The molecular studies need a good tool for assessing genetic diversity in plants so the development of molecular markers provides a new field to this study. It is widely adopted as a powerful tool for cultivar identification germplasm characterization, diversity analysis and phylogenetic studies in different crop plants (Yang *et al*., 2015).

It works by finding the nucleic acid sequence differences between person to person. These differences include translocations, duplications, point mutations, deletions and insertions. A large variety of DNA molecular markers can be employed for analysis of variation (Mondini *et al*., 2009) and it is an efficient tool to link these phenotypic and genotypic variation (Varshney *et al*., 2005).

There are mainly three method for the development of molecular markers, the first one is the genomic DNA amplification through random PCR and the second one is directly from the DNA library and the last one is the combined form of these two. These are grouped on the basis of heterozygosity (co-dominant marker) or homozygosity (dominant marker) (Hartl, 1988). The most commonly used dominant DNA markers include, Random Amplified Polymorphic DNA (RAPD), DNA

amplification fingerprinting (DAF), Inter simple sequence repeat (ISSR) and Amplified Fragment Length Polymorphisms (AFLP), whereas co-dominant markers include, Restriction Fragment Length Polymorphisms (RFLP), Microsatellites (SSR), Expressed sequence tag (EST) and Single Nucleotide Polymorphism (SNP). Both dominant and co-dominant markers can be used to detect polymorphism in DNA (Hartl and Jones, 2005).

The genetic variation analyses use RAPDs as the first PCR-based molecular markers (Welsh *et al*., 1990 and Williams *et al*., 1991). These markers are generated using short primers (decamers), through the random amplification of genomic DNA. These short primers help to raise the probability of finding the appropriate homologous sequences for annealing. DNA polymorphisms are then constructed by "deletions or rearrangements at or between the primer binding sites in the genome". The prior target sequence knowledge is not needed for this marker (Williams *et al*., 1991).

The main drawback of RAPDs is their low reproducibility, to overcome this limitation a Dutch company named Keygene developed AFLP technology (Vos *et al*., 1995). This technique is based on the fusion of techniques like DNA restriction digestion and PCR technology. It combines the power of RFLP with the flexibility of PCR-based technology so it considered as an intermediate between RAPDs and RFLPs technologies (Lynch and Walsh, 1998). "Genome representation" is a key feature of AFLP and is capable of screening representative DNA regions distributed randomly throughout the genome simultaneously. Usually around 50-100 bands were produced by using AFLP. This generates fingerprints without any prior DNA sequence knowledge. The technique can be used to map genes and distinguish closely related individuals at the sub-species level (Althoff *et al*., 2007).

A tract of tandemly repeated DNA motifs found within eukaryotic genomes are called Microsatellites or Simple Sequence Repeats (SSR) (Dietrich *et al*., 1992; Bell and Ecker, 1994; Morgante and Olivieri, 1993). These consist of sequences of repeated short motifs generally between 2-6 bp long and are typically repeated 5–50 times. Variations in length of the microsatellite generate polymorphism and these variations are caused generally due to slippage of strand during the replication

process of DNA, where the matching process done via either addition or excision of repeated sequences (Schlotterer and Tautz, 1992).

ISSR-PCR technique involves the amplification of DNA segments between two identical microsatellite repeat regions oriented in opposite directions. ISSRs use longer primers (15-30 mers) as compared to RAPD primers (10 mers) and no prior sequence knowledge is required (Zietkiewicz *et al*., 1994).

2.11 Inter Simple Sequence Repeats (ISSR)

ISSRs are fragments of DNA with a size ranging from 100 to 300bp located between adjacent, oppositely aligned microsatellite regions. A single primer composed of microsatellite sequences are used for the polymerase chain reaction (PCR) amplification of inter sequence repeat DNA. Primers are designed with repeat sequence of di-, tri-, tetra and penta nucleotides with an anchor made up of few bases and it is being successfully used among a wide variety of plants (Zietkiewicz *et al*., 1994).

The technique ISSR PCR, which defeat the limitations of other molecular markers such as expensiveness of AFLP, development of primers with flanking sequences for SSR and low reproducibility of RAPD. ISSR follows simple Mendelian inheritance so it segregates mostly as dominant markers. However, they also segregate as co-dominant markers in some cases, thus it can distinguish both homozygote and heterozygote (Sankar and Moore, 2001). ISSR analysis can be applied in gene mapping studies, taxonomic studies of closely related species as well as in studies involving genetic identity, parentage, clone and strain identification (Gupta *et al*., 1994; Zietkiewicz *et al*., 1994; Reddy *et al*., 2002), DNA fingerprinting (Kojomo *et al*., 2002).

Advantages of ISSR are simple, quick, and highly reproducible; no need of radioactivity labelling and it usually shows high degree of polymorphism (Kojima et al., 1998). No prior sequence knowledge is required for ISSR fingerprinting (Bornet and Branchard, 2001).

2.12 MOLECULAR MARKERS IN SWEET POTATO

Molecular markers have become a reference for information on genetic relationships more accurately and quickly. Sweet potatoes generally have a fairly high diversity but are often not known because it looks similar to each other.

High degree of polymorphism is exhibited by microsatellite or simple sequence repeat (SSR) markers. Hence many such markers have been emerged for sweet potato (Jarret and Bowen, 1994; Buteler et al., 1999; Hu et al., 2004). Lee *et al.* (2019) determined genetic variation in sweet potato by assessing genetic diversity among 558 sweet potato germplasms conserved in the Gene bank using nine cpSSRs. They found various genetic variations in the germplasms depending on the country from which they are collected.

SSR analysis of 40 orange fleshed sweet potato (OFSP) hybrid clones from International Centre for Potato and released varieties of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI) was done for assessing the level of genetic variability among them. Using 10 SSR primer pairs a total of 39 bands were obtained among which 29 bands were polymorphic (71.81%), with a mean of 4 bands per primer and a range of 2-6 bands per primer. It was found that a wide range of diversity existed among the tested accessions at the molecular level, using the SSR primers (Nair *et al*., 2017).

Genetic variability among clones of sweet potato in America (Villordon *et al*., 1995), Chile (Sagredo *et al*., 1998), Malaysia (Ramisah *et al*., 2000) have been analyzed with the help of RAPD markers and detect linkage between RAPD markers with sweet potato resistance genes against nematodes (Ukoskit *et al*., 1997).

Random amplified polymorphic DNA (RAPD) technique employed in 30 sweet potato accessions collected from Agricultural Research Council-Vegetable and Ornamental Plants (ARC-VOP) germplasm in South Africa. The variation in leaf proteins and RAPD markers of South African orange- and cream-fleshed sweet potatoes were assessed. The protein assays have 55.6% polymorphism and RAPD assays gave 98% of polymorphism (Feng *et al*., 2018).

AFLP analysis was used to characterize the genetic diversity of 97 accessions of sweet potato from Tanzania, using 10 primer combinations (Elameen *et al*., 2008) and to eliminate duplicates and identify diverse germplasm sources. AFLP analyses produced clear 202 polymorphic fragments. The results showed that the dice similarity coefficient ranged from 0.388 to 0.941.

Zhang et al. (2000) selected 69 accessions of sweet potatoes from 13 countries of tropical America to study the genetic diversity using AFLP markers and a variation of 90% shown within regions and a notable variation of 10% between regions (Caribbean, Peru-Ecuador, Central America and Colombia-Venezuela).

2.13 ISSR IN SWEET POTATO

Inter-Simple Sequence Repeat (ISSR) markers have high reliability and polymorphic ability. Therefore it is widely used in crop plants especially in sweet potato for genetic diversity analysis.

To assess the population structure and genetic diversity of cultivated sweet potato accessions in China, and to create the genetic relationships between their germplasm, Zhang *et al.* (2014) analysed 240 accessions with 17 screened ISSR markers. A total of 196 alleles were identified and the mean genetic similarity coefficient of 0.7302 were observed with Nei's gene diversity of 0.3167 and shared allele distance of 0.2698. The results revealed clear genetic structure among these sweet potato accessions (Zhang *et al*., 2014).

Liu *et al*. (2019) studied the genetic relationship among 129 sweet potato accessions amplified with 20 selected ISSR primers in China. 232 bands were amplified, of which 230 were polymorphic bands, with a polymorphism of 99.14%, and the average bands amplified by each primer were 11.60. The average genetic distance between wild varieties and local varieties, between wild varieties and introduced varieties and wild varieties and bred varieties were 0.4688, 0.4618 and 0.4643 respectively. The results of cluster analysis showed that wild species were completely distinguished from cultivars.

Genetic diversity analysis in one hundred sweet potato collection in China using 14 ISSR primers (He *et al*., 2007) generated a total of 239 polymorphic bands with about 17 polymorphic bands per primer. An average genetic distance of approximately 0.6428 revealed the high genetic diversity in Chinese sweet potato landraces.

2.14 ISSR IN WILD RELATIVES OF SWEET POTATO

ISSR markers have not been used extensively for the studies pertaining to relationship at the interspecific levels mainly because of the concerns of non-homology in co migration of DNA bands which are specific to fast evolving markers such as RAPD (Rieseberg, 1996). But in recent years, studies on genetic relationship shown that ISSR markers can impart the same level of accuracy like RFLP markers (Nagaoka and Ogihara, 1997). The advantage of ISSRs in sort outing relationships at both intra- and interspecific levels were done in the following years. Genetic relationships among cultivated sweet potato and other wild relatives was identified using ISSR and chloroplast DNA restriction analysis by Huang and Sun (2000) and Hu *et al*. (2003).

Genetic diversity and relationships of 10 different species of series Batatas (total 40 accessions), assessed using 15 ISSR markers and restriction-site variation in 4 non-coding regions of chloroplast DNA generated 2071 ISSR fragments and an average of 52 bands per accession were amplified. Of the examined species, *I. trifida* was found to be the close relative of cultivated sweetpotato ie., the hexaploid *I. batatas* (Huang and Sun, 2000).

A study of Hu *et al* (2003) found that ISSR markers is suitable for the finger printing of cultivated sweet potato and its wild species and it is useful for linkage map construction. Eight primers were selected to analyze genetic diversity and relationship among 34 accessions of sweet potato and related wild species.

MATERIALS AND METHOD

3. MATERIALS AND METHODS

The study entitled "Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp." was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020. This section describes the procedures and experimental materials used in the study.

3.1 PLANT MATERIALS

Ipomoea batatas accessions collected from various geographic locations are conserved in the sweet potato germplasm repository of ICAR-CTCRI (Plate 1), from which 41 accessions were selected and 6 wild *Ipomoea* species were collected from different geographic locations. The plants were raised in the field with two plants per accession spaced at 90 cm x 90 cm spacing.

The details of the accessions are given in Table 1.

Plate- 1 Sweet potato germplasm at ICAR-CTCRI (NAGS)

Table 1. Passport data of the sweet potato and wild accessions used for the study

Sl. No.	Sweet pota accessions	Accession No.	Place of Collection	Sl.No	Sweet pota accessions	Accession No.	Place of Collection
1.	Iba1	S 607	Kerala	25.	Iba 25	S 692	Kerala
2.	Iba2	S 681	Kerala	26.	Iba 26	S 703	Kerala
3.	Ib3	S 623	Kerala	27.	Iba 27	S 759	Hybrid
4.	Iba4	S 626	Kerala	28.	Iba 28	S 655	Kerala
5.	Iba5	S 632	Kerala	29.	Iba 29	S 641	Kerala
6.	Ib6	S 644	Kerala	30.	Iba 30	S 131	LR,U.P
7.	Iba7	S 668	Kerala	31.	Iba 31	SD-11	Hybrid
8.	Iba 8	S 682	Kerala	32.	Iba 32	AR-2017-4	LR,Kerala
9.	Iba 9	S 684	Kerala	33.	Iba 33	S 809	Orissa
10.	Iba 10	AR-2017-1	LR, Kerala	34.	Iba 34	S1801	Kerala
11.	Iba 11	S-1401	Kerala	35.	Iba 35	S1802	Kerala
12.	Iba 12	526/7	Hybrid	36.	Iba 36	S 621	Kerala
13.	Iba 13	SP-2	Hybrid	37.	Iba 37	S 646	Kerala
14.	Iba 14	SP-6	Hybrid	38.	Iba 38	S 823	Orissa
15.	Iba 15	SP-8	Hybrid	39.	Iba 39	S 965	Maharashtra
16.	Iba 16	SP-10	Hybrid	40.	Iba 40	S 618	Kerala
17.	Iba 17	SP-11	Hybrid	41.	Iba 41	S 680	Kerala
18.	Iba 18	AR-2017-3	*LR,Kerala	42.	*Ipomoea palmata*	*I.palmata*	Kerala
19.	Iba 19	AR-2017-2	LR,Kerala	43.	*Ipomoea obscura*	*I.obscura*	Kerala
20.	Iba 20	S 629	Kerala	44.	*Ipomoea triloba*	*I.triloba*	Kerala
21.	Iba 21	Jas-10-Pink	Kerala	45.	*Ipomoea aquatica*	*I.aquatica*	Kerala
22.	Iba 22	S 617	Kerala	46.	*Ipomoea vitifolia*	*I.vitifolia*	Kerala
23.	Iba 23	S 625	Kerala	47.	*Ipomoea nil*	*I.nil*	Kerala
24.	Iba 24	S 663	Kerala				

*LR-Land race

3.2 MORPHOLOGICAL OBSERVATION

Descriptors based on International Plant Genetic Resources Institute (IPGRI) (CIP *et al*., 1991) were used to take morphological observations. Observations on 18 vegetative morphological characters on vine and leaf were made after 45 days of planting.

3.2.1 Morphological Data Analysis

The morphological data were recorded by phenotypic observation of the selected accessions. Each character and traits were recorded by assigning certain numerical value to them as per the IPGRI descriptor states of sweet potato (CIP *et al.,* 1991) as shown in Table No. 2. All the recorded morphological data were tabulated in excel sheet for further statistical analysis.

Table 2. Descriptor states of sweet potato for morphological characterization (CIP *et al.*, 1991).

Sl. No.	Characters	Descriptor states
1	Twining	Non-twining-0,Slightly twining-3,Moderately twining-5,Twining-7,Very- twining-9
2	Plant type	Erect (<75 cm)-3, Semi-compact (75 – 150 cm)-5, Spreading (151 – 250 cm)-7, Extremely spreading (>250 cm)-9
3	Ground cover	Small-3, Medium-5, High-7
4	Internode diameter	Very thin (< 4mm) -1, Thin (4 - 6 mm) -3, Intermediate (– 9 mm) -5,Thick (10 – 12 mm) -7, Very thick (>12 mm) 9
5	Internode lengt	Very short (<3 cm)-1, Short (3 – 5 cm)-3, Intermediate (6 9 cm)-5, Long (10 – 12 cm)-7, Very long (> 12 cm)-9
6	Predominant vine colour	Green-1,Green with few purple spots-3, Green with many purple spots-4, Green with many dark purple spots-5, Mostly purple-6, Mostly dark purple-7, Totally purple-8, Totally dark purp

		9
7	Secondary vine colour	Absent-0, Green base-1, Green tip-2, Green nodes-3, Purp base-4, Purple tip-5, Purple nodes-6
8	Vine tip pubescence	None-0, Sparse-3, Moderate-5, Heavy-7, Very heavy-9
9	General leaf outline	Rounded-1, Reniform-2, Cordate -3, Triangular-4, Hastate 5, Lobed-6, Almost divided-7,
10	Type of leaf lobes	No lateral lobes-0, Very slight (teeth)-1, Slight-3, Moderate-5, Deep-7, Very deep-9
11	Number of leaf lobes	No lateral leaf lobes-0, Central tooth without lateral lobe- 3 Lateral lobes- 3, 5 Lateral lobes-5, 7 lateral lobes-7, 9 lateral lobes-9
12	Shape of centra leaf lobe	Absent-0,Teeth-1,Triangular-2, Semi-circular-3, Semi-elliptic-4, Elliptic-5, Lanceolate-6, Oblanceolate-7, Linear (broad)-8, Linear (narrow)-9
13	Mature leaf siz	Small (<8 cm)-3, Medium (8 – 15 cm)-5, Large (16 – 25 cm)-7, Very large (>25 cm)-9
14	Abaxial leaf ve pigmentation	Yellow-1, Green-2, Purple spot at base of main rib-3, Purple spots in several veins-4, Main rib partially purple-5 Main rib mostly or totally purple-6, All veins partially purple-7, All veins mostly or totally purple-8, Lower surface and veins totally purple-9
15	Mature leaf col	Yellow-green-1, Green-2, Green with purple edge-3, Greyish-4, Green with purple veins on upper surface-5, Slightly purple-6, Mostly purple-7, Green upper and purpl lower-8, Purple both surfaces-9
16	Immature leaf color	Yellow-green-1, Green-2, Green with purple edge-3, Greyish-4, Green with purple veins on upper surface-5, Slightly purple-6, Mostly purple-7, Green upper and purpl lower-8, Purple both surfaces-9
17	Petiole	Green-1, Green with purple near stem-2, Green with purp

	pigmentation	near leaf-3, Green with purple at both ends-4, Green with purple spots throughout petiole-5, Green with purple stripes-6, Purple with green near leaf-7, Some, petioles purple, others green-8,Totally or mostly purple-9
18	Petiole length	Very short (<10 cm)-1, Short (10 – 20 cm)-3, Intermediat (21 – 30 cm)-5, Long (31 – 40 cm)-7, Very long (>40 cm)-9

3.2.2 Diversity parameters

A percentage distribution of traits was calculated. Percentage distributions of traits across the 47 accessions were estimated by calculating the proportion of accessions expressing particular trait as percentage.

3.2.3 Cluster Analysis

To bring out the patterns of similarity and dissimilarity, data was subjected to UPGMA method of clustering based on similarity coefficients. Morphometric analysis was performed using Multivariate Statistical Package (MVSP 3.22) to group morphologically similar accessions under one group and dissimilar accessions in distant groups based on Euclidean distance. The clustering of accessions was depicted through a dendrogram after the analysis.

3.2.4 Principal Component Analysis

The tabulated data was subjected to multivariate analysis by numeric taxonomic techniques. PCA was performed to analyze the contribution of traits in separating accessions into different groups and to compare the clustering of accessions with respect to each cluster in a dendrogram. The PCA analysis reduces the dimensions of a multivariate data to a few principal axes, generates an Eigen vector for each axis and produces component scores for the characters (Ariyo and Odulaja; 1991 Sneath and Sokal, 1973).

3.3 MOLECULAR CHARACTERIZATION

3.3.1 DNA extraction

3.3.1.1 Sample collection

The samples used in this study were tender young leaves collected from different wild species of *Ipomoea* and of sweet potato accessions maintained in the germplasm of CTCRI, Thiruvananthapuram.

3.3.1.2 Dellaporta *et al*. (1983) method of DNA extraction

DNA was extracted from fresh tender and young leaf samples of sweet potato and wild species of *Ipomoea* using Dellaporta *et al*. (1983) method of DNA extraction .Leaves collected were weighed out; about 1 g was ground in a pre-cooled mortar and pestle to a fine powder using liquid nitrogen. 15 ml extraction buffer containing β-mercaptoethanol, EDTA, Tris HCl, sodium chloride and PVP (Polyvinylpyrrolidone) was added to the powdered mixture and ground once more. The homogenised lysate was transferred into 50 ml oakridge tubes and incubated at 4ºC. 20% SDS (1 ml) was added to the homogenised lysate and incubated at 65ºC for one hour in water bath. Added 5 ml of 5M potassium acetate and mixed by inversion followed by 20 minutes incubation at 4ºC. The mixture was then centrifuged at 12,000 rpm for 20 minutes at 4ºC. The resultant supernatant was transferred to another fresh sterile tube. Equal volume of ice cold isopropanol was added and mixed by gentle inversion and incubated at -20ºC for one hour or at 4ºC overnight. The mixture was then centrifuged at 12000 rpm for 10 minutes at 4ºC and the supernatant was discarded and pellet dried lightly by inverting the tubes on tissue paper. The pellet was dissolved in 1 ml of sterile distilled water and incubated at 65ºC for 15 minutes or at 4ºC overnight. In order to remove RNA contamination 10 µl of RNase was added and the mixture was incubated at 37º C for 1hr. The DNA solution was transferred to a microcentrifuge tube and this mixture was further extracted in an equal volume of chloroform: isoamyl alcohol (24:1) by centrifugation at 12000 rpm for 15 minutes at 4ºC. The aqueous phase was then transferred into a fresh 1.5 ml tube. Double the volume of ice cold absolute ethanol and 50 µl 3M sodium acetate was added and mixed by inversion then incubated for one hour at -20ºC or at 4ºC overnight in order to precipitate the DNA. The extract was then centrifuged at 12000 rpm for 10 minutes at 4º C. The supernatant was discarded without disturbing the pellet. The retained pellet was washed with 70% chilled ethanol by centrifugation at 12000 rpm for 8 minutes at 4º C. The pellet was air dried in RT after discarding the supernatant. Finally the air dried pellet was dissolved in 500µl of sterile distilled

water and stored at -20º C until use. The presence and quality of the extracted DNA was checked in 0.8 % agarose gel.

3.3.2 Analysis of the extracted DNA

3.3.2.1 *Checking the quality of DNA using Agarose Gel Electrophoresis*

The quality of the DNA was checked by agarose gel electrophoresis in 0.8 % agarose. Clean, dry gel casting tray was set up with combs to make a mould and placed on a horizontal flat surface. 0.8% agarose gel was prepared in 1X TBE buffer in a conical flask, and then heated in microwave oven until completely melted. After cooling the solution, about 1µl of ethidium bromide (EtBr) was added. It was then poured into casting tray containing sample comb and allowed to solidify at room temperature. After the solidification, then the combs were removed followed by placing gel into the electrophoresis chamber and covered with 1X TBE buffer. Extracted DNA sample (4µl) mixed with 1X loading dye (3µl) and was loaded into the corresponding wells of the gel. The gel was allowed to run in gel electrophoresis unit for 30 minutes at 100V. The gel was then visualized under UV light and the image was documented using the Gel Doc System (G: Box, M/s Syngene).

3.3.2.2 Quantification of DNA

The absorbance of DNA samples at 260, 280 and 230 nm were recorded to determine the quantity and quality of DNA isolated. The extracted DNA was quantified using Thermo Scientific NanoDrop™ 1000 Spectrophotometer. The spectrophotometer was calibrated to blank (zero absorbance) with 1µl of sterile distilled water. Then the concentration of each DNA samples (1µl) was recorded. The quality of DNA was determined by A260/A280 and A260/A230.

3.3.2.3 Dilution of DNA samples

Quantified DNA samples were diluted with nuclease free water to obtain a working stock of concentration of 10 ng/µl, based on the concentration obtained after DNA quantification.

3.4 PRIMER SCREENING

The diluted DNA samples of two sweet potato accessions were taken for primer screening. Initially primer screening was done with the following 17 ISSR primers:

UBC 807, UBC 808, UBC 809, UBC 818, UBC 825, UBC 827, UBC 847, UBC 860, $(ACC)_6Y$, $(GA)_9AT$, $(GA)_9AC$, UBC 811, UBC 849, UBC 851, UBC 810, UBC 848 and UBC 817.

3.4.1 Primer dilution

Generally primers were in lyophilized form. It was centrifuged and oligos were first dissolved in nuclease free water. Initially the main primer stock was made at 100 micro molar concentrations by adding a volume of nuclease free water as much as ten times the DNA in nanomoles present in the tube. Working stock was prepared in a concentration of 10 micro molar by diluting 10 µl of main stock with 90 µl of nuclease free water and stored in -20ºC refrigerator.

3.4.2 PCR amplification

The diluted samples were amplified for screening using the above mentioned 21 ISSR primers at standardized conditions of temperature and time. The components of PCR (Table 3) and PCR cycle used for amplification are given below.

Table 3. PCR MASTER MIX PREPARATION

Components	**Stock concentration**	**Required concentration**	**Volume for on reaction (15µl)**
Taq buffer (With 15 Mm $MgCl_2$)	10x	1x	1.5 µl
$MgCl_2$	25 mM	0.5 mM	0.3 µl
dNTP mix	2.5 mM each	0.3 mM each	1.8 µl
Primer (ISSR)	10 µM	0.33 µM	0.5 µl
Taq DNA polymerase	5U/µl	1U	0.2 µl
DNA template	10 ng/µl	40 ng	4 µl
Final volume made up to 15 µl with d H_20			

3.4.2.1 PCR conditions

PCR was carried out in Proflex Thermocycler. The PCR programme was as follows;

Lid -105ºC

Initial denaturation - 94ºC − 5 min

Denaturation - 94ºC − 30 sec

Annealing - 56.3ºC − 1 min } 35cycles

Extension - 72ºC − 1 min

Final extension - 72ºC − 8 min

Hold - 4 ºC

Amplification of PCR products was checked by agarose gel electrophoresis for selecting the suitable and efficient primers. The amplified products were resolved in a 2 % agarose gel along with 100 bp ladders and the bands on gel were visualized under UV light of G: Box gel documentation system using GeneSyS software (M/s. Syngene). The primers with scorable bands were selected for amplifying the 41 sweet potato accessions and 6 wild species of *Ipomoea*. The details of the selected primers are shown in Table 4.

Table 4. List of selected ISSR primers for final sweet potato characterization

Sl. No.	Primer Name	Sequence	Annealing temp.(ºC)
1	UBC 807	AGAGAGAGAGAGAGAGT	56.3ºC
2	UBC 808	AGAGAGAGAGAGAGAGC	56.3ºC
3	UBC 809	AGAGAGAGAGAGAGAGG	56.3 ºC
4	UBC 811	ATATATATATATATATT	56.3 ºC
5	UBC 818	CACACACACACACACAG	56.3 ºC
6	UBC 825	ACACACACACACACACT	56.3ºC

7	UBC 827	ACACACACACACACACG	56.3ºC
8	UBC 847	CACACACACACACACARC	56.3ºC
9	$(ACC)_6Y$	ACCACCACCACCACCACCY	56.3ºC
10	$(GA)_9AT$	GAGAGAGAGAGAGAGAGAT	56.3ºC
11	$(GA)_9AC$	GAGAGAGAGAGAGAGAGAC	56.3ºC

After doing the PCR with these selected primers, the amplicons were subjected to agarose gel electrophoresis.

3.5 ISSR ANALYSIS OF SWEET POTATO ACCESSIONS

The DNA of all the 47 accessions was subjected to molecular characterisation with the selected ISSR primers. To identify the molecular weight of obtained bands and for polymorphism studies, the PCR products were resolved in 2% agarose gel along with 100bp ladders.

3.5.1 Agarose Gel Electrophoresis

Agarose gel electrophoresis is an efficient and effective way of separating nucleic acids like DNA. Agarose gel can also use for isolating a particular band. The DNA molecules will separate based on their size in the gel. In general, the higher the concentration of agarose, the smaller the pore size so, sieving effect will increase and the separation will be easier.

3.5.1.1 Gel preparation

Agarose (2 g) was weighed out and transferred into a 250 ml conical flask. 100 ml 1X TBE buffer was added to it and the solution was boiled in microwave oven till the agarose completely dissolved in buffer (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel). The solution was allowed to cool to which 1µl/ml ethidium bromide was added. The casting tray was prepared and combs were kept in position. Pour the agarose slowly into a gel tray to avoid bubbles which will disrupt the gel. Any

bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip. Place newly poured gel at 4 °C for 10-15 minutes or let sit at room temperature for 20-30 minutes, until it has completely solidified.

3.5.1.2 Gel loading and running

The electrophoresis tank was filled with 1X TBE buffer. The solidified gel was transferred to electrophoresis unit and placed completely immersed in the buffer. The combs from the solidified gel were removed slowly without disturbing the wells. Sample (6 µl) was loaded into the wells, along with 3µl of 100 bp ladder into the first lane of the gel for reference. The gel was allowed to run at 120V for about 1.5 to 2 hr. The bands were visualized under UV transilluminator and documented using gel documentation system.

3.5.2 Gel profile visualization

The gel including the tray was taken out and was viewed under the ultraviolet light of G: Box gel documentation system (M/s. Syngene) using GeneSYS softwere. The image was taken under appropriate exposure and saved in JPEG format for scoring.

3.6 MOLECULAR DATA ANALYSIS

3.6.1 Scoring of bands

The gel images of resolved PCR products were taken. Clear and reproducible bands were taken for scoring. Scoring was carried out in the form of binary scoring format via assigning “1” for the presence of a specific band and “0” for the absence of band.

3.6.2 Polymorphic Marker Ratio

Based on the scoring data obtained polymorphic marker ratio was calculated. When a particular band is present in one accession and absent in any of the other accessions, the band is said to be polymorphic. The percentage of polymorphism was calculated. The equation for calculating percentage of polymorphism is given below.

$$\text{Percentage of polymorphism} = \frac{\text{No. of polymorphic bands}}{\text{Total number of bands}} \text{ X } 100$$

Thus the ratio of the polymorphic bands to the total number of bands was determined.

3.6.3 Cluster Analysis

The binary data prepared was used to generate a dendrogram which grouped the 47 accessions, on the basis of Jaccard's similarity coefficient using unweighted pair group method with arithmetic average (UPGMA) (Sneath and Sokal, 1973) using NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Biostatistic, New York, U. S. A., software version 2.02 package) (Rohlf, 1998). To determine the similarity and diversity between every two accessions, pair-wise distance (similarity) matrices was computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC. Morphological and molecular data was compared using Mantel's test (Mantel, 1967) by adopting random permutations.

RESULTS

4. RESULTS

The study entitled "Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp." was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020 are described in this chapter.

4.1 MORPHOLOGICAL CHARACTERIZATION

Forty seven accessions of sweet potato selected from the National Active sweet potato germplasm site at ICAR-CTCRI were used for the study. The selected accessions included 41 sweet potato accessions from the National Active sweet potato germplasm site at ICAR-CTCRI and 6 wild species (*Ipomoea aquatica, Ipomoea palmata, Ipomoea obscura, Ipomoea vitifolia, Ipomoea nil* and *Ipomoea triloba*) collected from ICAR-CTCRI campus and KAU, Vellayani. Habits of accessions selected for the study are shown in plate 2. Leaf lobe variability of accessions is depicted in plate 3.

Iba 3

Iba 4

Iba 5

Iba 8

Iba 10

Iba 18

Iba 25

Iba 38

Iba 36

Ipomoea palmata

Ipomoea aquatica

Ipomoea triloba

Plate 2: Habit of different accessions used in the study

Ipomoea palmata

Ipomoea nil

Ipomoea triloba

Ipomoea vitifolia

Ipomoea aquatica

Ipomoea obscura

Plate 3: Leaf lobe variability in the wild species

4.1.1 Percentage distribution

Percentage distribution of traits across the selected accessions was calculated and the table is shown below (Table 5, Plate 4).

Table 5. Frequency distribution of morphological traits

Sl. No:	Characters	Observed Traits	Frequency Distribution
1	Twining	Non- twining	89%
		Twining	7%
		Very twining	4%
2	Plant type	Erect	17%
		Semi - erect	49%
		Spreading	28%
		Extremely spreading	6%
3	Ground cover	Low	32%
		Medium	34%
		High	28%
		Total	6%
4	Vine internode length	Very short	36%
		Short	45%
		Intermediate	11%
		Long	4%
		Very long	4%
5	vine internode diameter	Very Thin	59%
		Thin	28%
		Intermediate	13%
6	Predominant vine colour	Green	49%
		Green with few purple spots	6%
		Green with many purple spots	6%
		Green with many dark- purple spots	15%
		Mostly purple	9%
		Mostly dark purple	9%
		Totally dark purple	6%
7	Secondary vine colour	Absent	51%
		Green base	13%
		Green tip	11%
		Purple base	8%
		Purple tip	2%
		Purple nodes	15%
8	Vine tip	None	9%

	pubescence	Sparse	87%
		Moderate	2%
		Heavy	2%
9	General leaf outline	Reniform	4%
		Cordate	7%
		Triangular	17%
		Hastate	6%
		Lobed	64%
		Almost divided	2%
10	Type of leaf lobes	No lateral lobes	11%
		Very slight (teeth)	17%
		Slight	15%
		Moderate	32%
		Deep	15%
		Very deep	10%
11	Number of leaf lobes	Central tooth without lateral lobe	21%
		3 Lateral lobes	26%
		5 Lateral lobes	38%
		7 lateral lobes	13%
		9 lateral lobes	2%
12	Shape of central leaf lobe	Absent	6%
		Toothed	13%
		Triangular	19%
		Semi-elliptic	25%
		Elliptic	11%
		Lanceolate	11%
		Oblanceolate	9%
		Linear (narrow)	6%
13	Mature leaf size	Small	87%
		Medium	11%
		Large	2%
14	Abaxial leaf vein pigmentation	Green	51%
		Purple spot at base of main rib	15%
		Purple spots in several veins	2%
		Main rib partially purple	4%
		Main rib mostly or totally purple	4%
		All veins partially purple	13%
		All veins mostly or totally purple	11%
15	Mature leaf colour	yellow-Green	2%
		Green	66%

		Green with purple edge	28%
		Greyish-green	2%
		Green with purple veins on upper surface	2%
16	Immature leaf color	Yellow Green	2%
		Green	21%
		Green with purple edge	30%
		Slightly purple	13%
		Mostly purple	13%
		Purple both surfaces	21%
17	Petiole length	Very short	2%
		Short	25%
		Intermediate	32%
		Long	20%
		Very long	21%
18	Petiole pigmentation	Green	36%
		Green with purple near stem	4%
		Green with purple near leaf	11%
		Green with purple at both ends	25%
		Green with purple spots throughout petiole	13%
		Green with purple stripes	2%
		Totally or mostly purple	9%

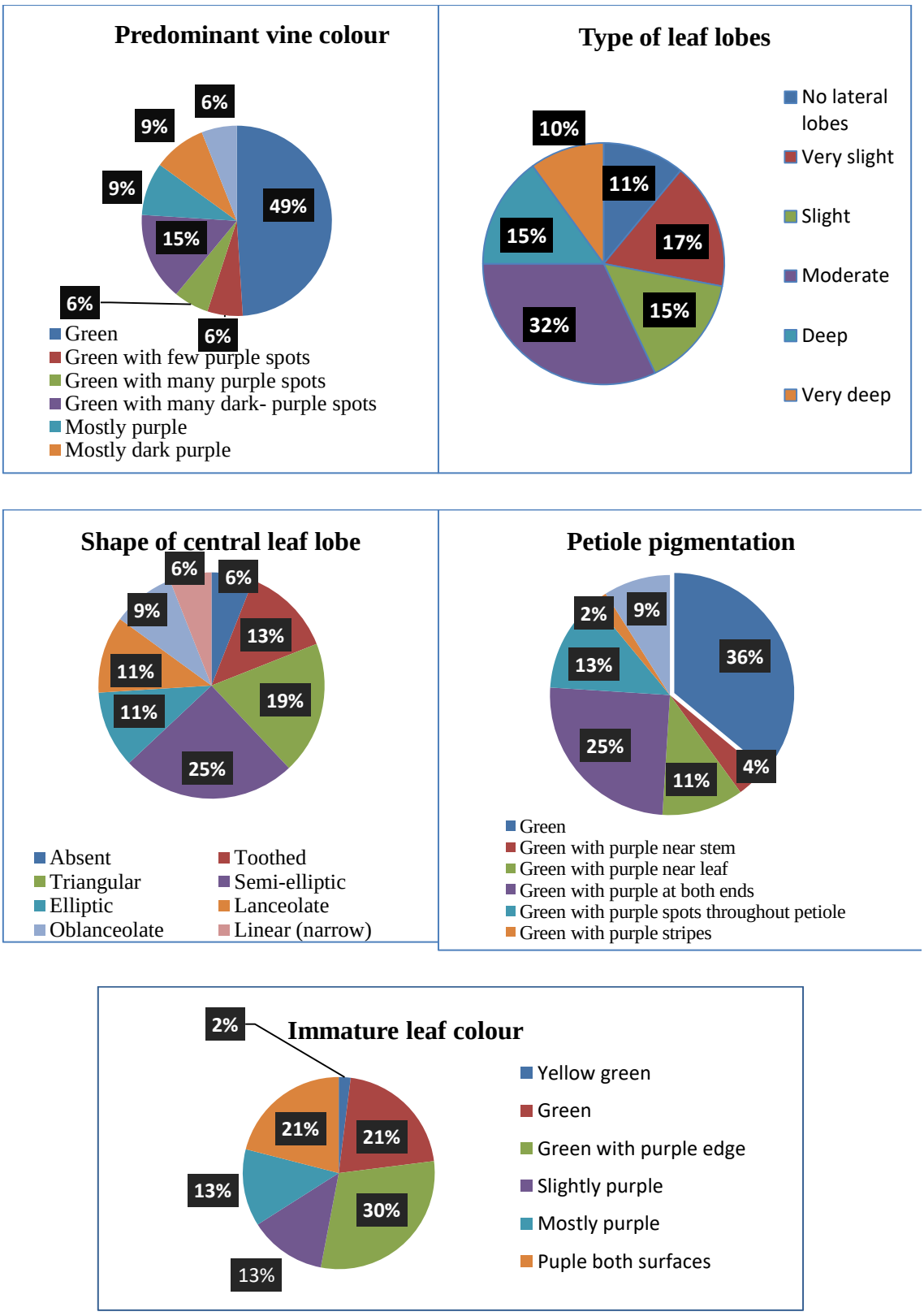


Plate 4: Frequency distribution of major variables

4.1.2 Cluster analysis

Cluster analysis based on morphological descriptors using Multivariate Statistical Package (MVSP 3.22) generated a dendrogram (Figure 1). UPGMA Cluster analysis based on 18 descriptors separated all the 47 accessions into two principal clusters at a Euclidean distance of 1.7. The pattern of clustering of accessions is described in Table 6. Accessions showing highest similarity are Iba 5 (S 632) & Iba 17 (SP-11) and Iba 20 (S 629) & Iba 24 (S 663) at a Euclidean distance of 0.4 (Log 10 transformed).

Table 6. The clustering pattern of different accessions

Clusters	**Sub-Clusters**	**Accessions**
Cluster 1	1A	*I. triloba, I.obscura*
	IB	*I.palmata, I.vitifolia, I.nil*
Cluster 2	2A	Iba40, Iba33, Iba35, Iba21, Iba16, Iba32, Iba31, Iba10
	2B (Outlier)	*I.aquatica*
	2C	Iba2, Iba4, Iba39 Iba36, Iba6, Iba34, Iba38, Iba30, Iba27, Iba28, Iba13, Iba7, Iba41, Iba26, Iba25, Iba18, Iba23, Iba24, Iba20, Iba8, Iba29, Iba22, Iba11, Iba9, Iba17, Iba5, Ib14, Iba3, Iba12, Iba19, Iba37, Iba15, Iba1

4.1.1 Principal component analysis (PCA)

Principal component analysis was done by considering all the 18 variables to find out the significant characters contributing to separation of accessions in the dendrogram using Multivariate statistical analysis package. The Eigen values were high for the first three principal components which accounted for the 60.9% of the variability among the characters studied (Table 7).

PC1 accounted for 29% of the variation. The first principal component had predominant vine colour, secondary vine colour, leaf lobes type, leaf lobe number, shape of central leaf lobe and petiole pigmentation with high values. The second principal component accounted for 19% of the variation which included twining, ground cover, vine internode length, petiole pigmentation, abaxial leaf vein pigmentation and immature leaf colour. The third principal component accounted for 12.6% of the variation which included predominant vine colour, leaf lobe type, shape of central leaf lobe, immature leaf colour, petiole length and petiole pigmentation. The trait included in PC1, PC2 and PC3 was petiole pigmentation. The trait included in PC1 and PC3 was predominant vine colour, shape of central leaf lobe and leaf lobe shape. Immature leaf colour was the trait included in both PC2, PC3 and PC4. Hence predominant vine colour, leaf lobes type, shape of central leaf lobe, immature leaf colour and petiole pigmentation was important in distinguishing the accessions.

Table 7. Principal component analysis in 47 accessions of sweet potato

(Highly loaded variables in combined analysis given in boldface)

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Twining	-0.010	**0.547**	0.098	**0.335**	-0.069
Plant type	-0.034	0.233	0.044	**0.283**	0.187
Ground cover	0.029	**0.315**	0.088	**0.254**	**0.206**
Vine internode length	0.028	**0.429**	0.038	0.176	0.084
Vine internode diameter	0.087	-0.039	-0.071	-0.178	**0.334**
predominant vine colour	**0.375**	-0.073	**0.561**	0.054	0.052
Secondary vine colour	**0.345**	-0.014	0.182	-0.082	**0.319**
Vine tip pubescence	-0.022	-0.050	-0.014	0.063	0.166
General outline of leaf	-0.205	-0.044	0.086	0.060	0.018
Leaf lobes type	**-0.519**	-0.119	**0.333**	0.008	0.196
Leaf lob number	**-0.342**	-0.009	0.183	0.085	**0.234**
Shape of central leaf lobe	**-0.430**	-0.099	**0.329**	-0.094	0.093
Mature leaf size	-0.025	0.113	0.009	0.070	0.029
Abaxial leaf vein pigmentation	0.159	**-0.321**	0.157	**0.409**	0.108
Mature leaf colour	0.001	-0.049	0.036	0.033	-0.026
Immature leaf colour	-0.074	**-0.399**	**-0.305**	**0.681**	-0.059
Petiole length	0.105	-0.080	**-0.293**	-0.048	**0.732**
Petiole pigmentation	**0.273**	-0.208	**0.401**	0.084	-0.109
Eigen values	22.152	14.475	9.617	7.744	6.179
Percent variation	29.170	19.062	12.664	10.197	8.137
Cumulative percentage	29.170	48.232	60.896	71.093	79.230

4.2. MOLECULAR DATA ANALYSIS

4.2.1 DNA isolation and quantification

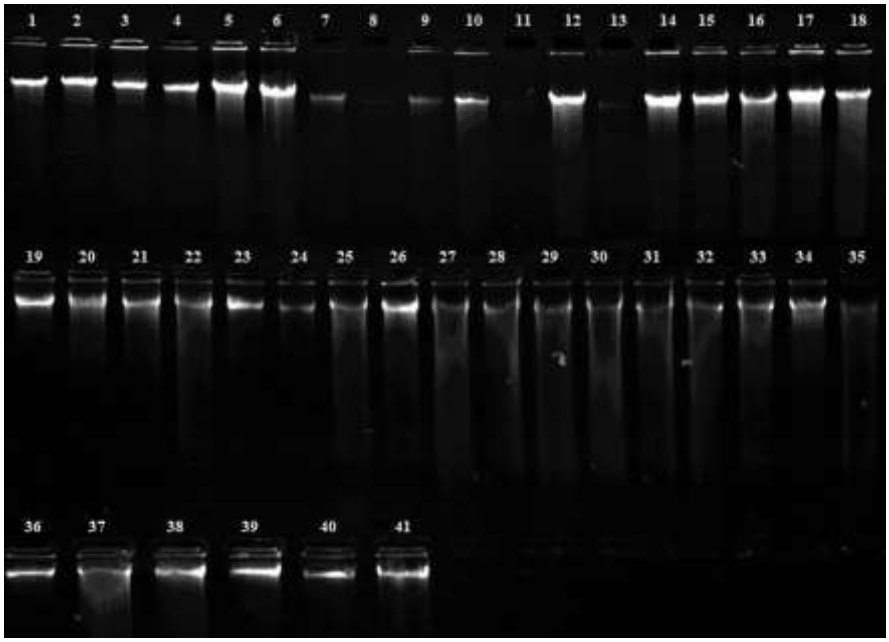
DNA of 47 accessions was isolated using the method described by Dellaporta *et al*. (1983). The quality of isolated DNA was checked in 1% agarose gel (Plate 5). The quantities as well as purity of the DNA obtained from spectrophotometric readings are included in Table 8. The concentration of the isolated DNA ranged from 297.8 ng/µl to 2942.9 ng/µl.

Table 8. Spectrophotometric readings of DNA isolated using Dellaporta method (Dellaporta *et al*., 1983).

Sl. No.	Sample Name	A260/A280	Concentration (ng/µl)
1	Iba1	1.91	2886.7
2	Iba2	2.12	645.3
3	Iba3	1.90	1784.4
4	Iba4	2.07	2143.6
5	Iba5	2.29	2176
6	Iba6	2.27	2248.8
7	Iba7	2.30	1488.3
8	Iba8	2.30	2847.3
9	Iba9	2.19	1235.2
10	Iba10	2.31	866
11	Iba11	2.02	2942.9
12	Iba12	2.21	1427.8
13	Iba13	1.93	587.5
14	Iba14	2.17	1808.1
15	Iba15	2.17	1357.8
16	Iba16	2.27	1190
17	Iba17	2.20	561.4
18	Iba18	2.24	1447.6
19	Iba19	1.91	2584.1
20	Iba20	2.30	1044.4
21	Iba21	2.24	2644.6
22	Iba22	2.23	695.9
23	Iba23	2.31	1287.5
24	Iba24	2.34	1108.8

25	Iba25	2.32	1635.7
26	Iba26	2.28	1232.8
27	Iba27	2.31	1567.2
28	Iba28	2.17	1854.6
29	Iba29	1.96	500.5
30	Iba30	2.13	424
31	Iba31	2.16	429.4
32	Iba32	2.09	1558.9
33	Iba33	2.06	1736.2
34	Iba34	2.34	1886.7
35	Iba35	2.34	1765.4
36	Iba36	2.18	1275.4
37	Iba37	2.32	1345.6
38	Iba38	2.18	1623.8
39	Iba39	2.12	2171.7
40	Iba40	2.26	1618.7
41	Iba41	2.18	1235.3
42	*I.palmata*	2.35	754.2
43	*I.obscura*	2.35	813.4
44	*I.triloba*	1.91	2791
45	*I.aquatica*	2.17	297.8
46	*I.vitifolia*	2.21	1435.1
47	*I.nil*	1.98	2150.8

A. Sweet potato accessions



B. Wild accessions

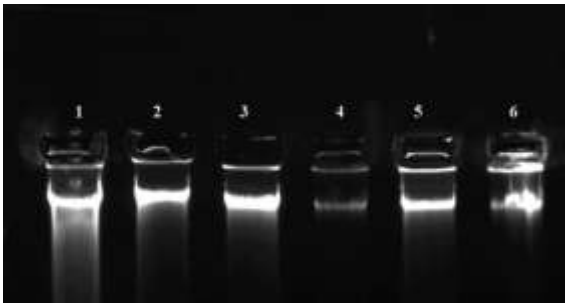


Plate 5: Checking the quality of DNA of 41 accessions (A) and 6 wild accessions (B) of Sweet potato using 0.8% Agarose gel electrophoresis

6 Wild accessions include, *Ipomoea aquatica*, *Ipomoea palmata*, *Ipomoea obscura*, *Ipomoea vitifolia*, *Ipomoea nil* and *Ipomoea triloba* from Lane 1 to 6 respectively.

4.2.2 ISSR analysis of sweet potato accessions

After preliminary primer screening using 17 ISSR primers, 11 primers gave clear and reproducible bands which were selected for PCR amplification. After the completion of PCR using selected markers, the amplicons were resolved in 2% agarose gel and the obtained amplicons are shown in Plate 6 to Plate 15.

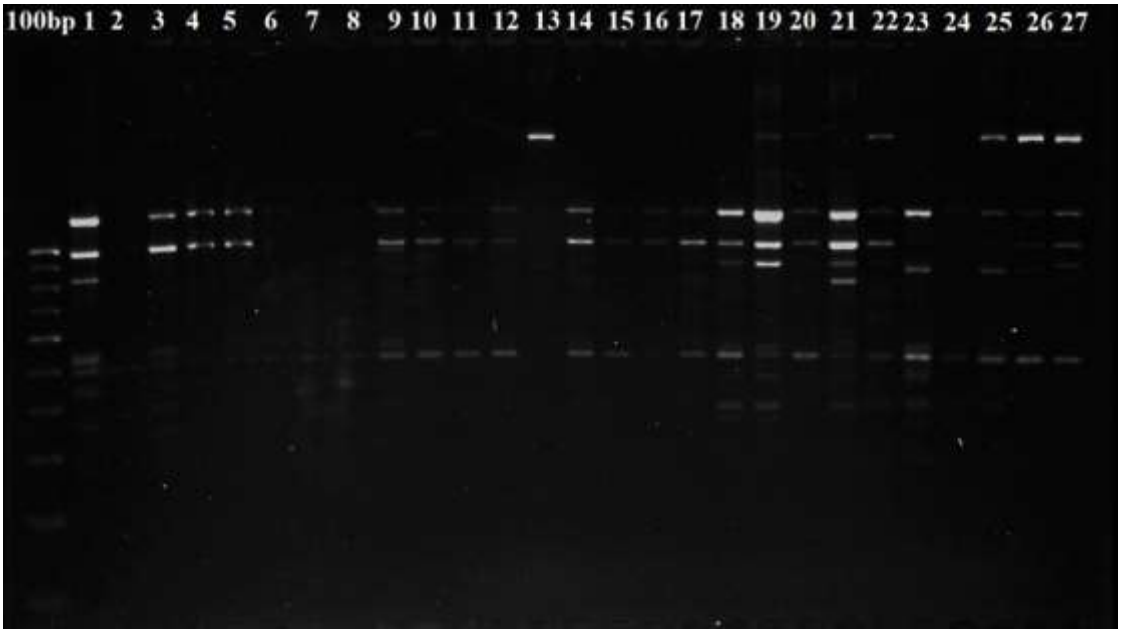
4.3 ANALYSIS OF MOLECULAR MARKER DATA

4.3.1 ISSR profile

Using 11 ISSR primers (Plates 11-17), a total of 211 bands were generated and all the bands were polymorphic. An average of 19.2 polymorphic bands per primer was obtained. Total number of bands per primer ranged from 12 (UBC 807) to 23 (UBC 825, $(GA)_9AC$ and UBC 827). A total of 100% polymorphism was obtained (Table 9).

Table 9 Polymorphism detected in 54 accessions of *Ipomoea* with 11 ISSR primers

Sl. No.	Primer	Total number of bands	Number of polymorphic bands	Percent Polymorphism (%)
1	UBC-807	12	12	100
2	UBC-808	17	17	100
3	UBC-809	21	21	100
4	UBC-811	14	14	100
5	UBC-818	18	18	100
6	UBC-825	23	23	100
7	UBC-827	23	23	100
8	UBC-847	19	19	100
9	$(ACC)_6Y$	20	20	100
10	$(GA)_9AT$	21	21	100
11	$(GA)_9AC$	23	23	100
Total		**211**	**211**	**100**
Mean		**19.2**	**19.2**	



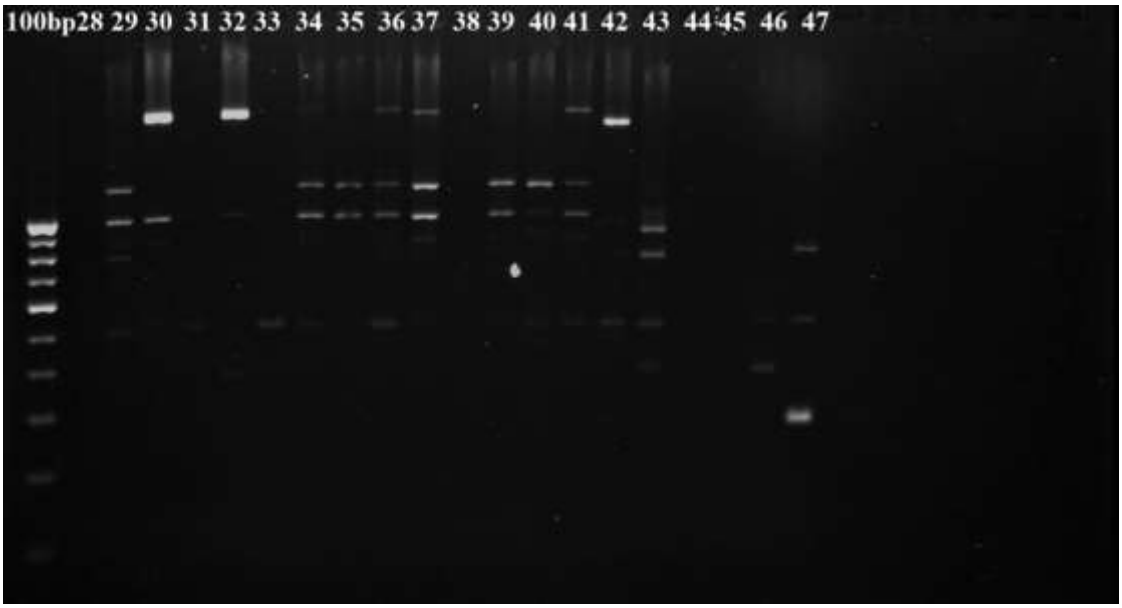
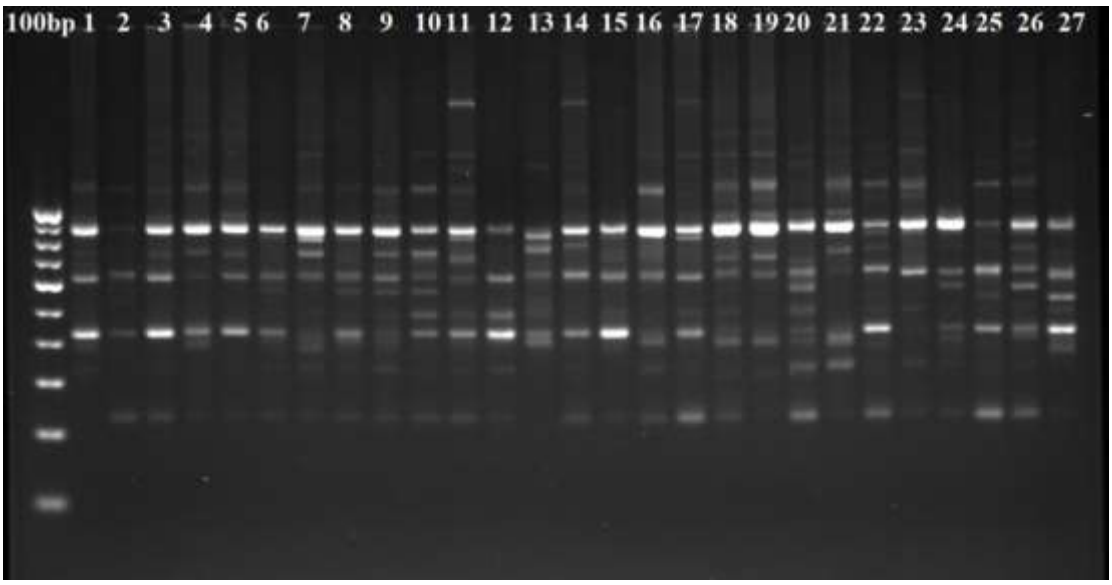


Plate 6: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC-811



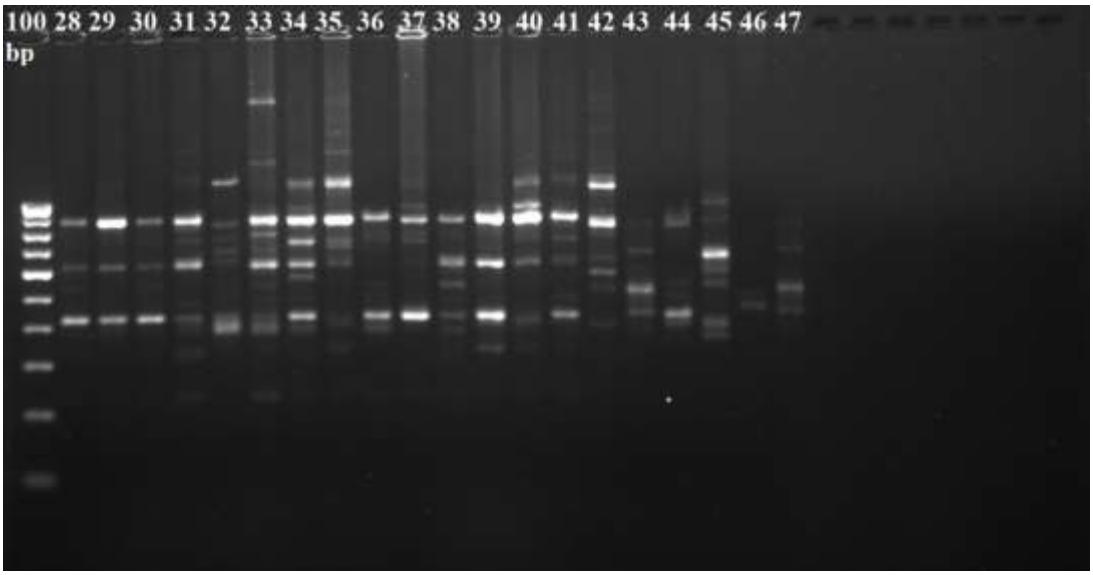


Plate 7: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC-818

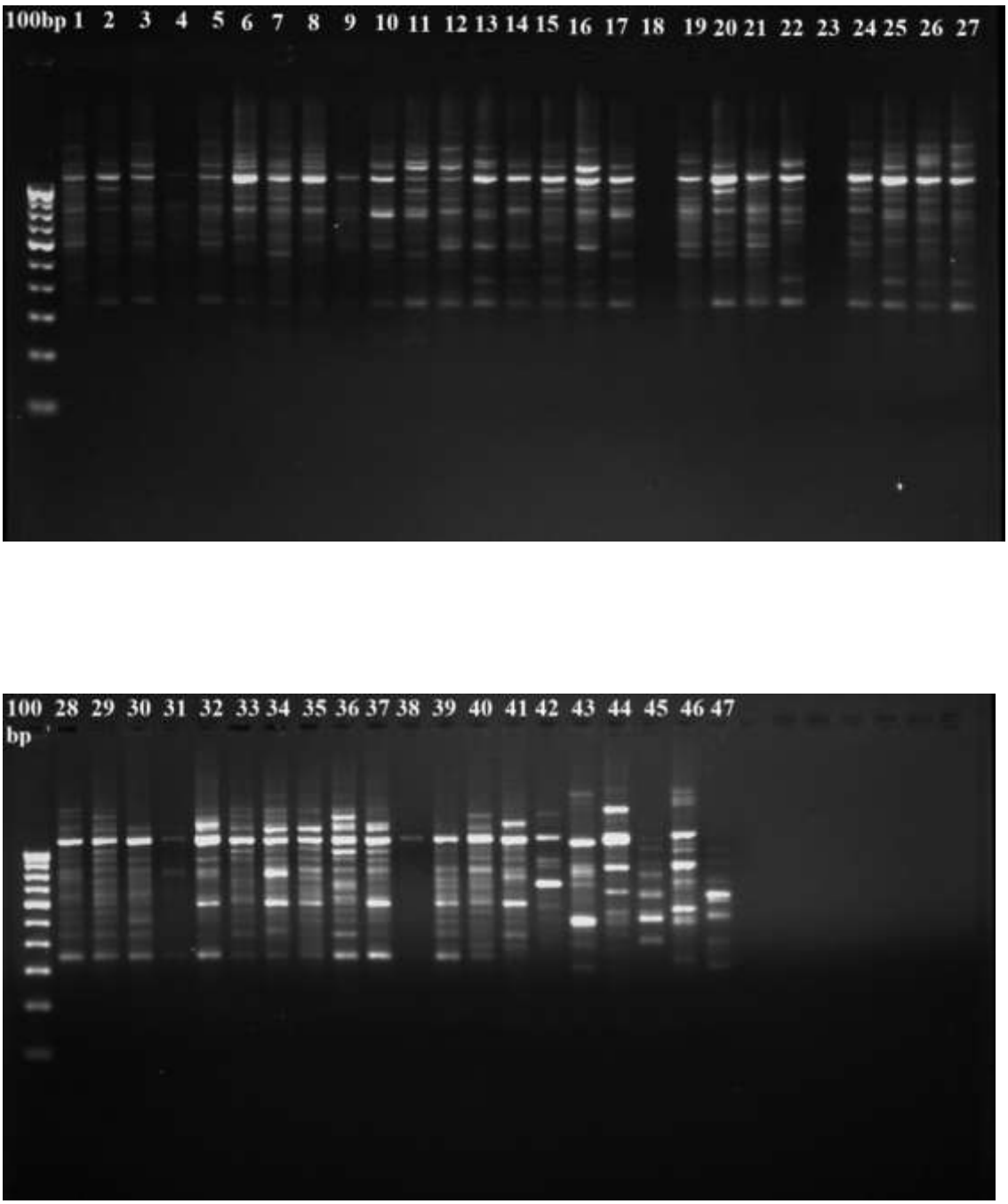


Plate 8: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer $(ACC)_6Y$

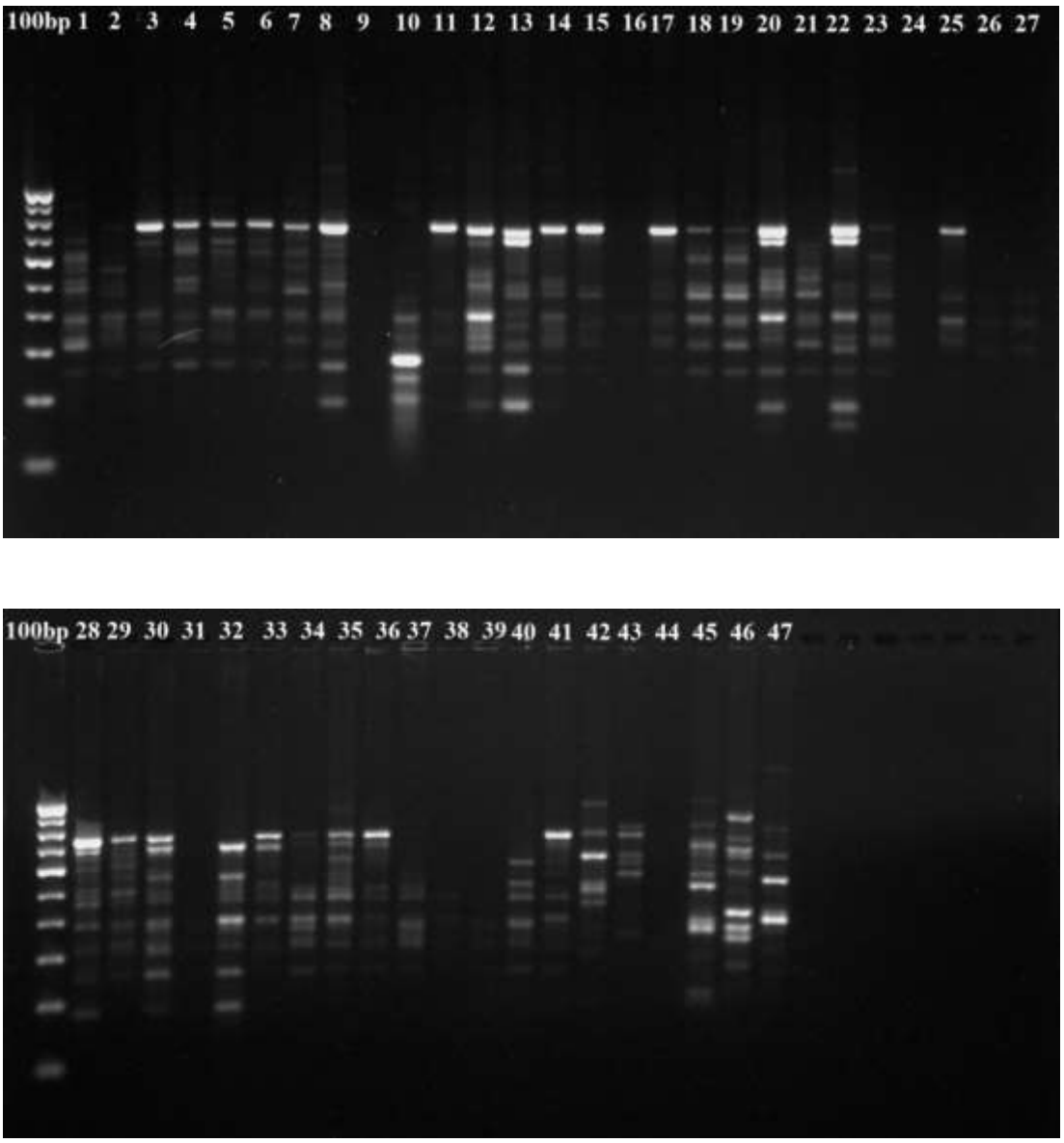


Plate 9: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer $(GA)_9AT$

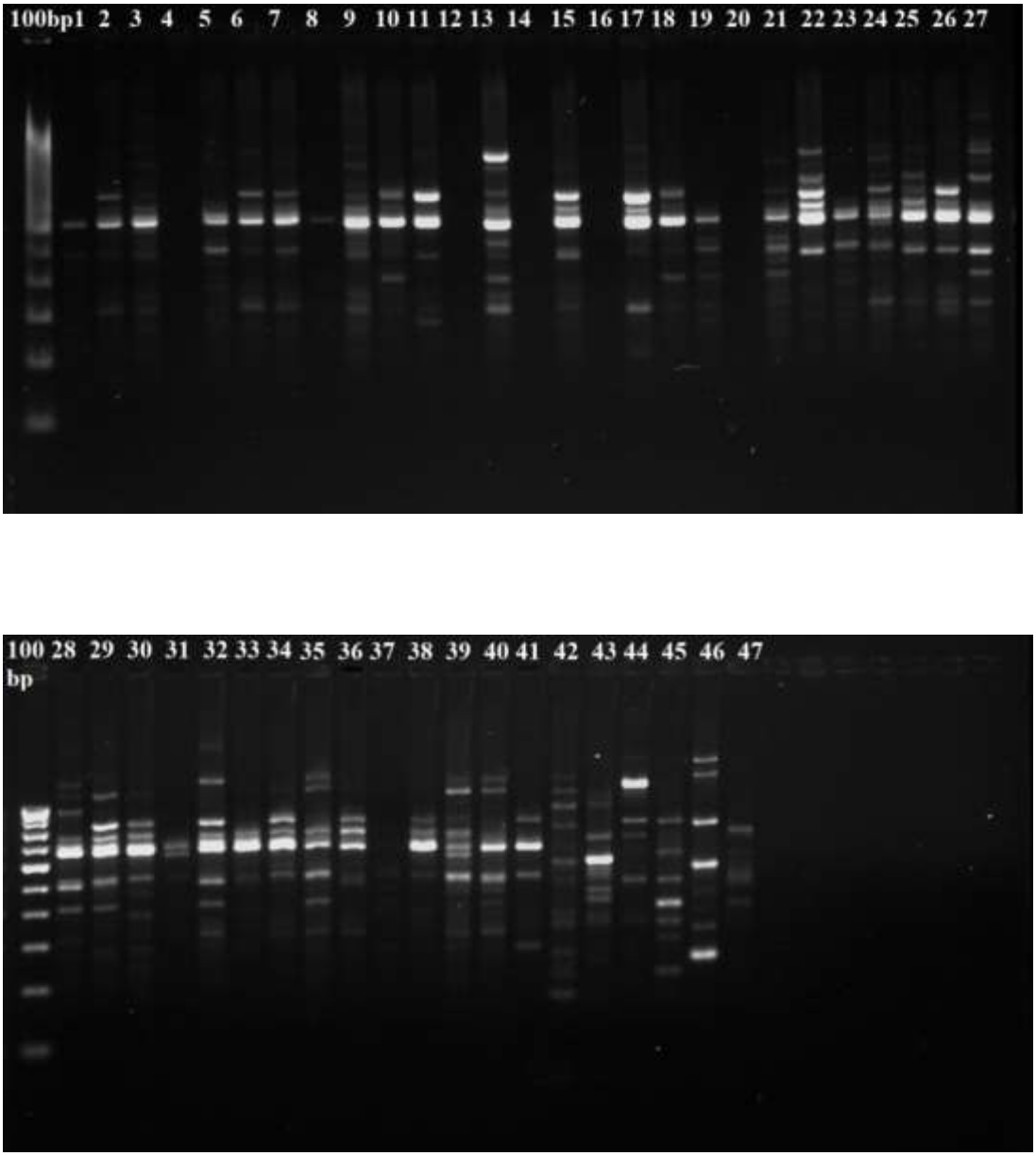


Plate 10: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC 809

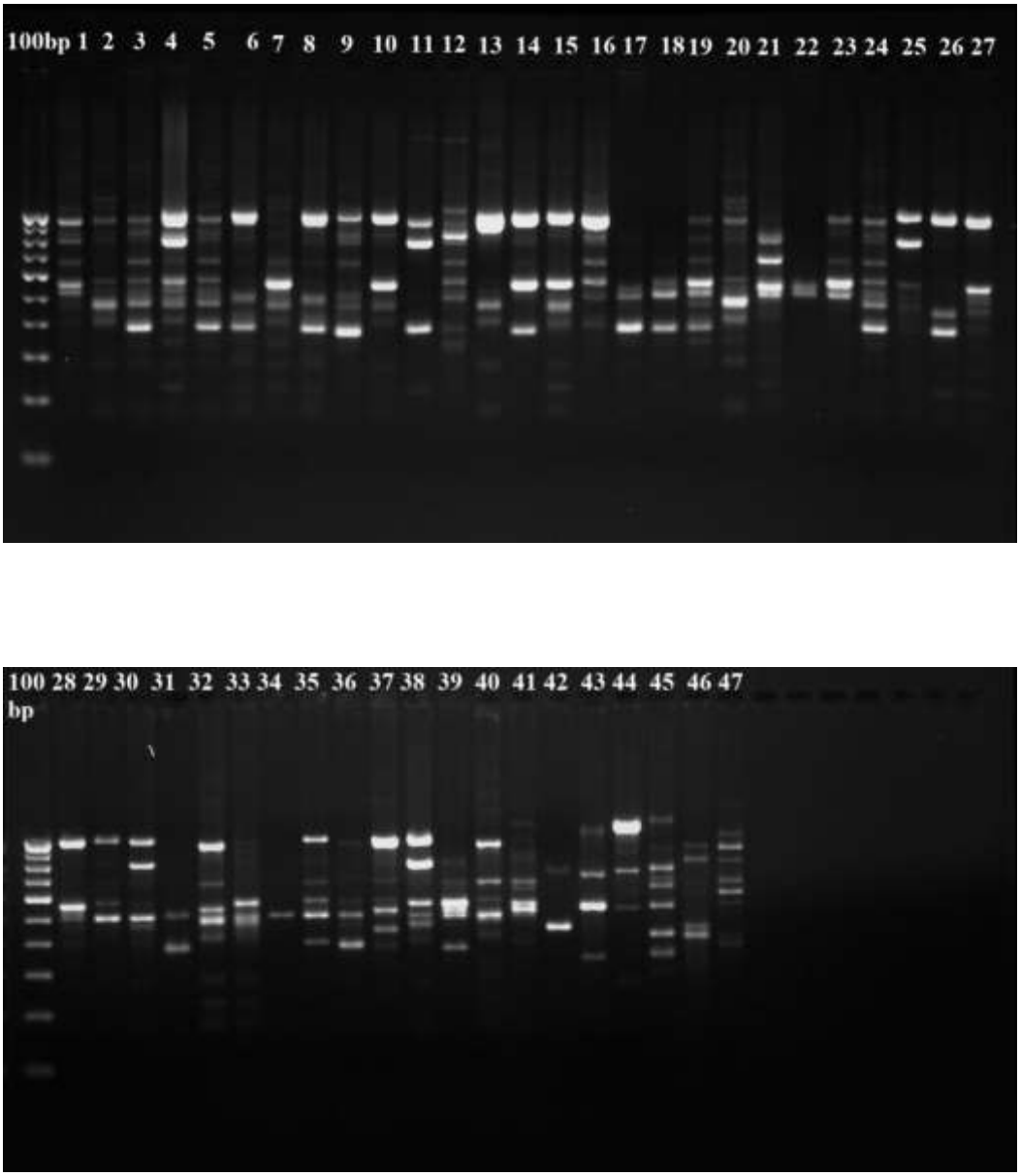
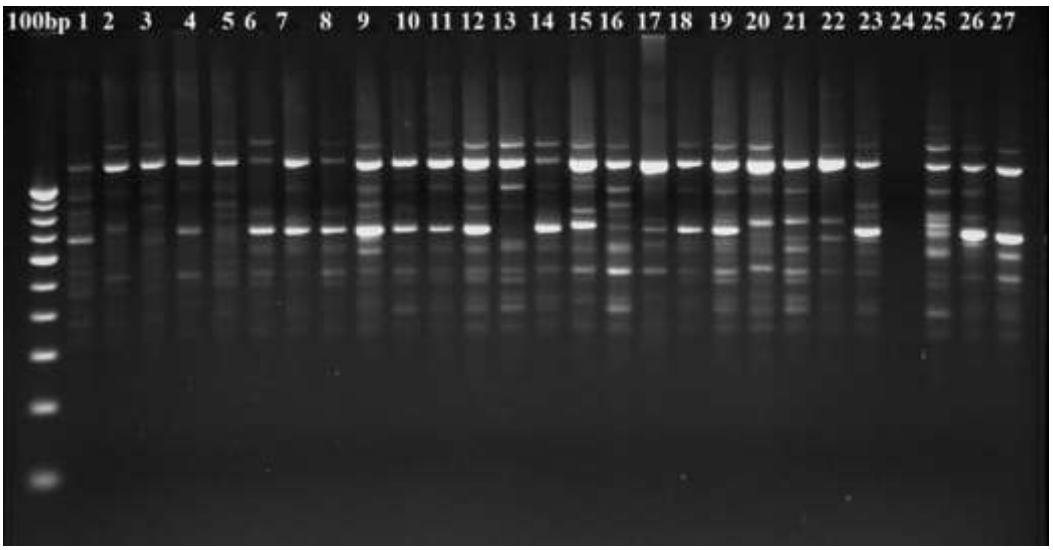


Plate 11: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC 825



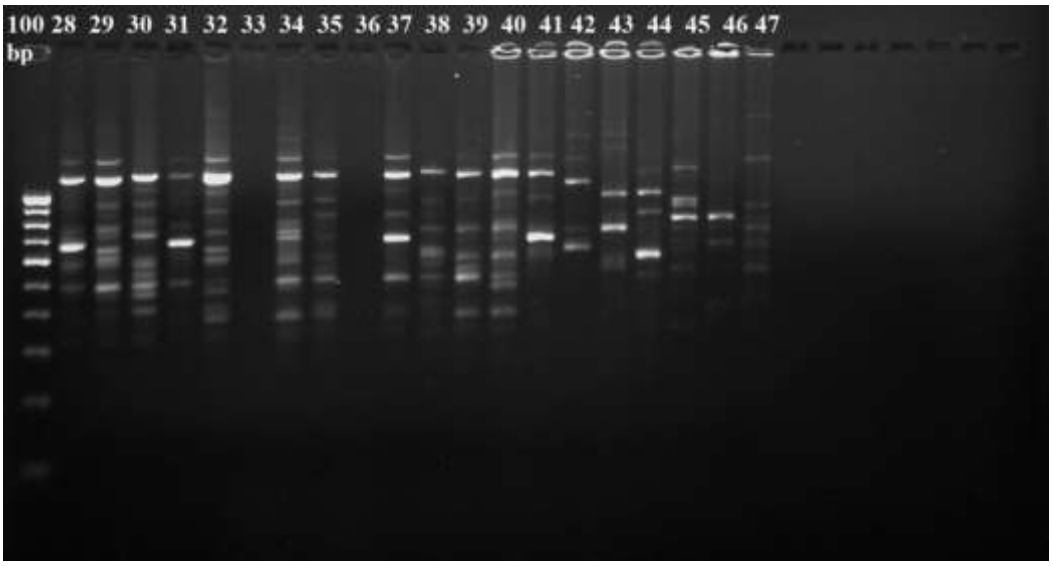
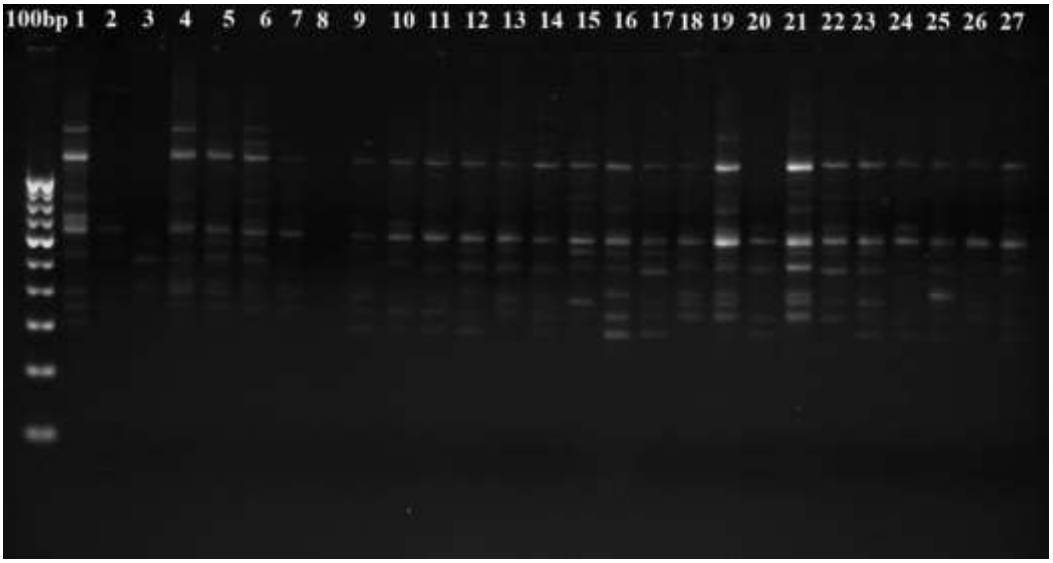


Plate 12: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC 827



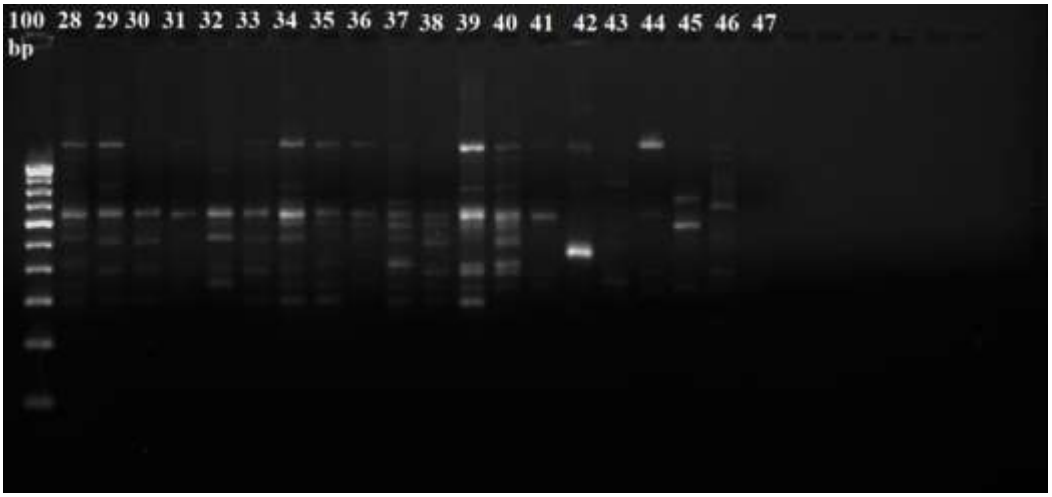
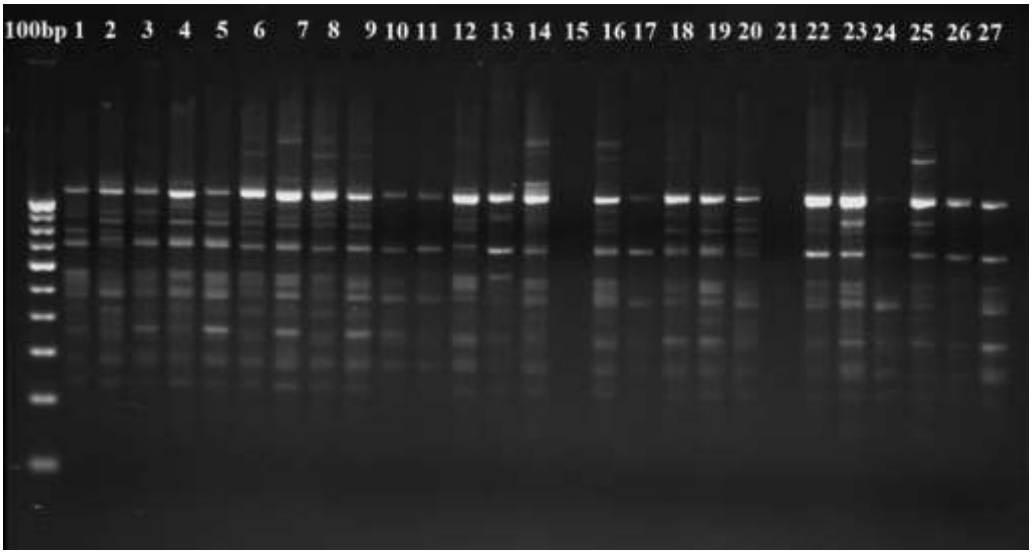


Plate 13: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC 847



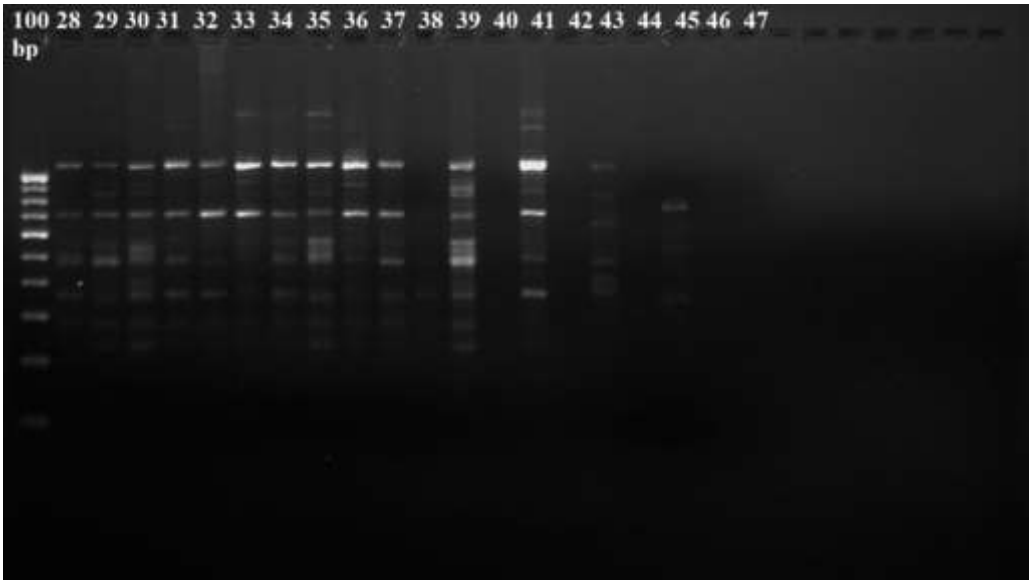
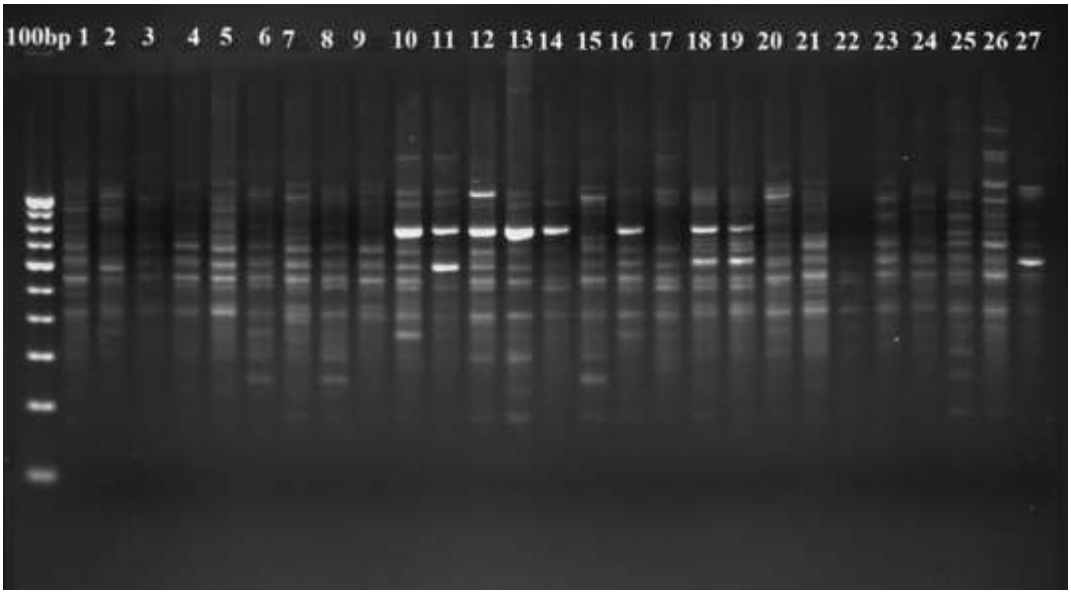


Plate 14: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer UBC 808



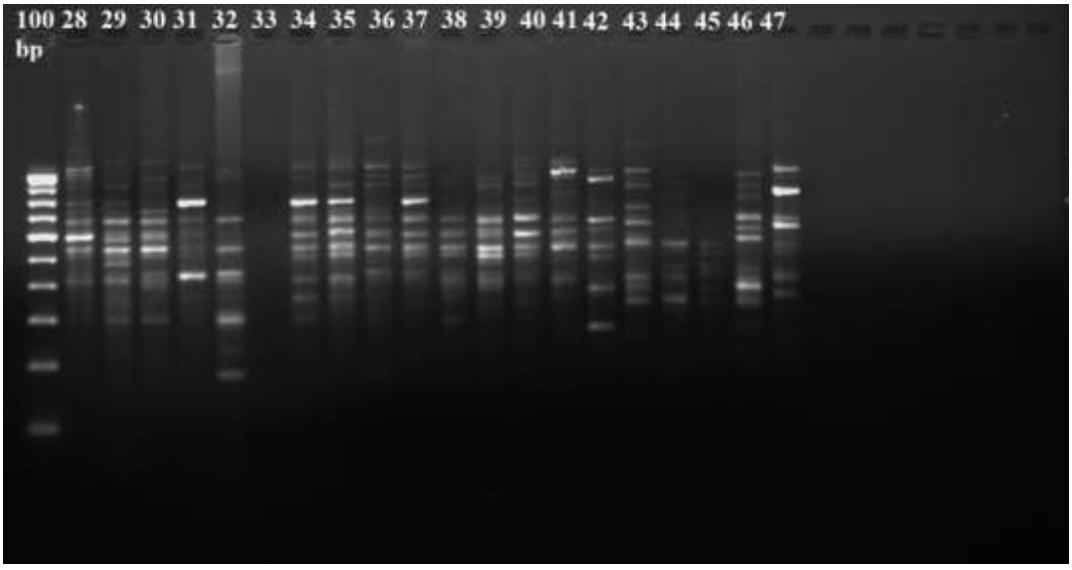


Plate 15: ISSR profile of 47 accessions of *Ipomoea* resolved on 2% agarose gel using primer $(GA)_9AC$

4.3.2. Cluster analysis

The UPGMA dendrogram created using Jaccard's similarity coefficient separated the accessions into two principal clusters at a coefficient 0.58 (Figure 2). All 41 sweet potato accessions were included in the first principal cluster. This principal cluster was divided into 7 sub clusters at a distance of 0.72 (Table 10) which is again sub-divided into small clusters indicating a lot of intraclusteral variability between the accessions. All the wild species were clustered together in the second principal cluster. The second principal cluster consisted of one outlier (2A) which is *I. palmata* and a subcluster 2B which included the wild species *I. obscura, I. triloba, I. aquatica, I. vitifolia and I. nil*. In the first principal cluster Iba3 and Iba5 (S 623 and S 632) were grouped together with 85% similarity. This set can be considered as the most similar accessions in the study.

Table 10. Accessions grouped on the basis of ISSR markers

Clusters	**Sub- Clusters**	**Accessions**
Cluster 1	1A	Iba1, Iba3, Iba5, Ib21, Iba37, Iba40, Iba28, Iba29, Iba40
	1B	Iba10, Iba11, Iba17, Iba15, Iba25, Iba26, Iba27
	IC	Iba13, Iba32, Iba33, Iba36, Iba34, Iba35
	ID	Iba4, Iba18, Iba19, Iba12, Iba16, Iba14, Iba6, Iba8, Iba7
	1E	Iba2, Iba41, Iba24, Iba31, Iba9, Iba39, Iba38
	1F	Iba22, Iba23
	1G	Iba20
Cluster 2	2A (outlier)	*I. palmata*,
	2B	*I.obscura, I.triloba, I.aquatica, I.vitifolia. I.nil*

4.3.3. Genetic relationships based on similarity matrix

An estimate of genetic relationships were depicted from the marker data using Jaccard's similarity coefficient (Table 11). Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 85% to a minimum of 50%. The maximum similarity of 85% was observed between Iba3 and Iba5 (S 623 and S 632) (Plate 16). The least similarity of 50% was observed between Iba19 (AR-2017-2) and *I. vitifolia*. Iba38 (S 823) was the most similar sweet potato accession to *I.nil* (69%), *I.aquatica* (66%) and *I.vitifolia* (63%) than all the other accessions. Iba31 (SD-11) was similar to both *I. palmata* (66%) and *I. triloba* (72%). Iba24 (S 663) was the most similar sweet potato accession to *I.obscura* by 62%. *I. palmata* was similar to *I. triloba and I.nil* by 64%. *I.obscura* showed 64% similarity with with *I.nil.* A similarity coefficient of 0.62 was the least similarity observed between *I.obscura* and *I.vitifolia.* The most similar wild species as indicated by ISSR markers were *I. triloba* and *I.aquatica* (72%). The six wild species showed a similarity ranging between 59-66% between each other (Table 11). *I.batatas* were similar to these wild species with a similarity coefficient ranging between 0.50-0.72.

Table 11. Jaccard's similarity coefficient between 6 wild accessions

	I.palmata	*I.obscura*	*I. triloba*	*I.aquatica*	*I.vitifolia*	*I.nil*
I.palmata	1.00					
I.obscura	0.62	1.00				
I. triloba	0.64	0.63	1.00			
I.aquatica	0.59	0.63	0.72	1.00		
I.vitifolia	0.59	0.62	0.66	0.61	1.00	
I.nil	0.64	0.64	0.66	0.65	0.63	1.00

A

Iba 3 (S 623)

Iba 5 (S 632)

B

I.triloba

I.aquatica

Plate 16: (A) Sweet potato accessions showing highest (85%) molecular similarity

(B) Wild accessions showing maximum similarity (71%) through molecular analysis

4.3.4. Mantel test

The Mantel statistic (r) value based on Spearman's rank correlation obtained as 0.4365 with a significance value (p) of 0.0001 indicated significant correlation between morphological and molecular marker information. Significant values obtained by Mantel test indicated that the morphological variability observed is genetically fixed and that the ISSR markers used could identify the variability.

Table 12. NTSYS-pc similarity coefficients between 47 accessions (Jaccard's similarity coefficient)

	Iba1	Iba2	Iba3	Iba4	Iba5	Iba6	Iba7	Iba8	Iba9	Iba10	Iba11	Iba12	Iba13	Iba14	Iba15	Iba16	Iba17	Iba18	Iba19	Iba20	Iba21	Iba22	Iba23
Iba1	1.00																						
Iba2	0.70	1.00																					
Iba3	0.73	0.76	1.00																				
Iba4	0.73	0.74	0.71	1.00																			
Iba5	0.77	0.77	0.85	0.76	1.00																		
Iba6	0.71	0.73	0.72	0.73	0.77	1.00																	
Iba7	0.69	0.74	0.72	0.75	0.79	0.77	1.00																
Iba8	0.69	0.71	0.69	0.71	0.72	0.81	0.79	1.00															
Iba9	0.68	0.70	0.71	0.70	0.74	0.73	0.75	0.70	1.00														
Iba10	0.71	0.66	0.67	0.69	0.73	0.71	0.70	0.66	0.69	1.00													
Iba11	0.69	0.68	0.70	0.67	0.71	0.74	0.71	0.72	0.70	0.79	1.00												
Iba12	0.71	0.70	0.69	0.71	0.73	0.71	0.74	0.74	0.72	0.74	0.77	1.00											
Iba13	0.69	0.72	0.68	0.69	0.72	0.72	0.73	0.69	0.66	0.75	0.72	0.74	1.00										
Iba14	0.69	0.72	0.67	0.74	0.70	0.71	0.67	0.70	0.70	0.73	0.79	0.79	0.67	1.00									
Iba15	0.69	0.75	0.71	0.67	0.77	0.76	0.69	0.66	0.67	0.73	0.73	0.68	0.72	0.66	1.00								
Iba16	0.69	0.72	0.68	0.75	0.74	0.74	0.73	0.71	0.76	0.70	0.73	0.80	0.67	0.76	0.70	1.00							
Iba17	0.67	0.73	0.70	0.69	0.72	0.70	0.68	0.64	0.72	0.73	0.78	0.68	0.68	0.71	0.75	0.69	1.00						
Iba18	0.67	0.70	0.66	0.78	0.69	0.73	0.73	0.69	0.76	0.71	0.73	0.72	0.69	0.75	0.67	0.73	0.76	1.00					
Iba19	0.74	0.69	0.70	0.77	0.76	0.73	0.73	0.69	0.71	0.74	0.73	0.72	0.68	0.79	0.68	0.73	0.73	0.81	1.00				
Iba20	0.71	0.73	0.67	0.70	0.72	0.68	0.73	0.70	0.64	0.73	0.64	0.75	0.67	0.67	0.71	0.74	0.65	0.61	0.68	1.00			
Iba21	0.70	0.66	0.70	0.70	0.73	0.70	0.66	0.64	0.66	0.71	0.64	0.67	0.71	0.65	0.71	0.64	0.68	0.72	0.77	0.62	1.00		
Iba22	0.60	0.68	0.63	0.66	0.64	0.69	0.68	0.64	0.71	0.69	0.73	0.69	0.73	0.69	0.69	0.68	0.75	0.73	0.66	0.64	0.69	1.00	
Iba23	0.64	0.69	0.64	0.70	0.67	0.71	0.66	0.64	0.71	0.69	0.70	0.70	0.65	0.71	0.68	0.72	0.74	0.79	0.71	0.59	0.68	0.73	1.00

	Iba1	Iba2	Iba3	Iba4	Iba5	Iba6	Iba7	Iba8	Iba9	Iba10	Iba11	Iba12	Iba13	Iba14	Iba15	Iba16	Iba17	Iba18	Iba19	Iba20	Iba21	Iba22	Iba23
Iba24	0.65	0.75	0.70	0.67	0.71	0.71	0.68	0.64	0.74	0.68	0.69	0.66	0.63	0.63	0.72	0.70	0.72	0.65	0.63	0.65	0.63	0.68	0.64
Iba25	0.64	0.71	0.73	0.67	0.73	0.75	0.73	0.74	0.72	0.70	0.73	0.70	0.71	0.68	0.72	0.70	0.73	0.69	0.72	0.63	0.72	0.71	0.69
Iba26	0.65	0.69	0.66	0.65	0.70	0.74	0.68	0.71	0.68	0.75	0.76	0.68	0.68	0.64	0.77	0.69	0.77	0.66	0.69	0.67	0.67	0.70	0.68
Iba27	0.73	0.68	0.67	0.66	0.66	0.73	0.67	0.66	0.71	0.70	0.72	0.69	0.71	0.66	0.73	0.67	0.69	0.65	0.68	0.64	0.71	0.73	0.65
Iba28	0.71	0.73	0.73	0.69	0.75	0.66	0.70	0.68	0.66	0.67	0.70	0.73	0.72	0.68	0.73	0.65	0.73	0.65	0.69	0.68	0.73	0.70	0.64
Iba29	0.74	0.78	0.74	0.73	0.76	0.70	0.71	0.68	0.70	0.68	0.67	0.70	0.72	0.70	0.73	0.70	0.70	0.66	0.68	0.68	0.76	0.68	0.66
Iba30	0.66	0.75	0.72	0.68	0.70	0.67	0.69	0.67	0.65	0.67	0.64	0.68	0.73	0.67	0.69	0.63	0.69	0.69	0.67	0.64	0.69	0.63	0.67
Iba31	0.65	0.69	0.65	0.70	0.69	0.71	0.64	0.70	0.69	0.67	0.74	0.69	0.68	0.72	0.67	0.74	0.71	0.69	0.64	0.59	0.61	0.68	0.67
Iba32	0.66	0.68	0.69	0.66	0.69	0.67	0.70	0.65	0.67	0.69	0.70	0.66	0.79	0.63	0.69	0.64	0.68	0.67	0.69	0.63	0.67	0.69	0.62
Iba33	0.65	0.65	0.63	0.63	0.64	0.72	0.69	0.69	0.63	0.69	0.72	0.66	0.67	0.69	0.72	0.69	0.73	0.66	0.65	0.66	0.59	0.70	0.64
Iba34	0.70	0.71	0.68	0.65	0.73	0.79	0.71	0.69	0.69	0.73	0.77	0.71	0.75	0.72	0.73	0.73	0.71	0.71	0.71	0.70	0.67	0.72	0.66
Iba35	0.72	0.70	0.71	0.71	0.75	0.73	0.71	0.68	0.67	0.65	0.72	0.71	0.71	0.73	0.68	0.75	0.72	0.72	0.71	0.68	0.65	0.69	0.69
Iba36	0.66	0.74	0.74	0.68	0.73	0.73	0.73	0.72	0.65	0.68	0.72	0.65	0.72	0.68	0.71	0.69	0.73	0.71	0.70	0.65	0.65	0.70	0.62
Iba37	0.72	0.69	0.71	0.70	0.73	0.67	0.65	0.64	0.68	0.72	0.74	0.71	0.69	0.75	0.72	0.73	0.73	0.70	0.72	0.68	0.72	0.66	0.66
Iba38	0.64	0.67	0.65	0.69	0.67	0.71	0.67	0.66	0.70	0.68	0.70	0.63	0.69	0.62	0.72	0.66	0.70	0.68	0.62	0.60	0.64	0.67	0.68
Iba39	0.69	0.69	0.71	0.65	0.73	0.68	0.66	0.63	0.76	0.68	0.70	0.69	0.66	0.68	0.72	0.70	0.73	0.69	0.69	0.64	0.70	0.73	0.66
Iba40	0.73	0.72	0.74	0.72	0.75	0.72	0.72	0.65	0.68	0.71	0.73	0.68	0.72	0.69	0.76	0.69	0.72	0.71	0.76	0.67	0.77	0.69	0.67
Iba41	0.67	0.78	0.64	0.67	0.71	0.67	0.69	0.65	0.69	0.70	0.67	0.67	0.66	0.72	0.71	0.71	0.69	0.69	0.71	0.69	0.67	0.68	0.64
I.palmata	0.52	0.59	0.52	0.55	0.53	0.55	0.52	0.56	0.56	0.55	0.60	0.51	0.56	0.55	0.59	0.57	0.59	0.55	0.50	0.54	0.55	0.59	0.56
I.obscura	0.51	0.60	0.57	0.53	0.57	0.55	0.54	0.55	0.57	0.54	0.55	0.57	0.55	0.51	0.58	0.56	0.56	0.57	0.51	0.55	0.55	0.58	0.59
I.triloba	0.53	0.67	0.57	0.60	0.59	0.62	0.62	0.57	0.60	0.60	0.65	0.58	0.62	0.58	0.66	0.63	0.65	0.58	0.54	0.55	0.54	0.62	0.61
I.aquatica	0.55	0.60	0.53	0.57	0.56	0.56	0.56	0.55	0.55	0.54	0.56	0.55	0.55	0.51	0.60	0.57	0.60	0.57	0.51	0.58	0.51	0.60	0.54
I.vitifolia	0.52	0.59	0.52	0.54	0.53	0.56	0.55	0.55	0.53	0.54	0.61	0.61	0.56	0.54	0.59	0.58	0.55	0.54	0.50	0.55	0.54	0.57	0.55
I.nil	0.59	0.60	0.58	0.57	0.61	0.55	0.55	0.61	0.56	0.58	0.59	0.61	0.57	0.60	0.64	0.60	0.59	0.55	0.56	0.56	0.57	0.58	0.59

	Iba24	Iba25	Iba26	Iba27	Iba28	Iba29	Iba30	Iba31	Iba32	Iba33	Iba34	Iba35	Iba36	Iba37	Iba38	Iba39	Iba40	Iba41	I.palmata	I.obscura	I.triloba	I.aquatica	I.vitifolia	I.nil
Iba24	1.00																							
Iba25	0.72	1.00																						
Iba26	0.73	0.81	1.00																					
Iba27	0.71	0.78	0.74	1.00																				
Iba28	0.71	0.76	0.69	0.75	1.00																			
Iba29	0.69	0.70	0.66	0.73	0.82	1.00																		
Iba30	0.68	0.72	0.65	0.68	0.79	0.80	1.00																	
Iba31	0.73	0.70	0.73	0.66	0.70	0.68	0.67	1.00																
Iba32	0.62	0.70	0.66	0.71	0.74	0.72	0.72	0.65	1.00															
Iba33	0.72	0.70	0.70	0.66	0.70	0.69	0.69	0.72	0.69	1.00														
Iba34	0.70	0.73	0.74	0.73	0.73	0.74	0.70	0.71	0.75	0.73	1.00													
Iba35	0.69	0.73	0.70	0.70	0.76	0.74	0.72	0.73	0.69	0.73	0.81	1.00												
Iba36	0.76	0.73	0.75	0.69	0.75	0.73	0.74	0.70	0.73	0.80	0.78	0.76	1.00											
Iba37	0.66	0.67	0.71	0.69	0.73	0.75	0.72	0.70	0.70	0.70	0.73	0.74	0.75	1.00										
Iba38	0.70	0.66	0.69	0.70	0.67	0.71	0.73	0.72	0.65	0.70	0.67	0.65	0.70	0.69	1.00									
Iba39	0.71	0.70	0.69	0.73	0.73	0.79	0.73	0.69	0.66	0.68	0.73	0.75	0.69	0.71	0.75	1.00								
Iba40	0.66	0.69	0.70	0.69	0.76	0.77	0.71	0.65	0.73	0.67	0.71	0.72	0.70	0.78	0.69	0.72	1.00							
Iba41	0.69	0.67	0.73	0.64	0.69	0.73	0.70	0.72	0.61	0.66	0.73	0.73	0.74	0.72	0.68	0.75	0.69	1.00						
I.palmata	0.64	0.55	0.57	0.58	0.57	0.59	0.57	0.66	0.57	0.62	0.60	0.55	0.59	0.56	0.65	0.59	0.53	0.57	1.00					
I.obscura	0.62	0.57	0.58	0.57	0.60	0.56	0.55	0.59	0.53	0.55	0.57	0.59	0.59	0.57	0.59	0.61	0.56	0.59	0.62	1.00				
I.triloba	0.70	0.61	0.69	0.60	0.62	0.63	0.62	0.72	0.61	0.66	0.62	0.60	0.64	0.59	0.68	0.61	0.64	0.63	0.64	0.63	1.00			
I.aquatica	0.64	0.50	0.62	0.53	0.60	0.59	0.59	0.64	0.58	0.63	0.59	0.61	0.61	0.59	0.66	0.61	0.58	0.61	0.59	0.63	0.72	1.00		
I.vitifolia	0.62	0.54	0.59	0.53	0.55	0.55	0.54	0.61	0.55	0.60	0.56	0.53	0.58	0.55	0.63	0.58	0.57	0.61	0.59	0.62	0.66	0.61	1.00	
I.nil	0.63	0.58	0.61	0.63	0.65	0.65	0.61	0.68	0.56	0.62	0.57	0.60	0.59	0.62	0.69	0.65	0.60	0.64	0.64	0.64	0.66	0.65	0.63	1.00

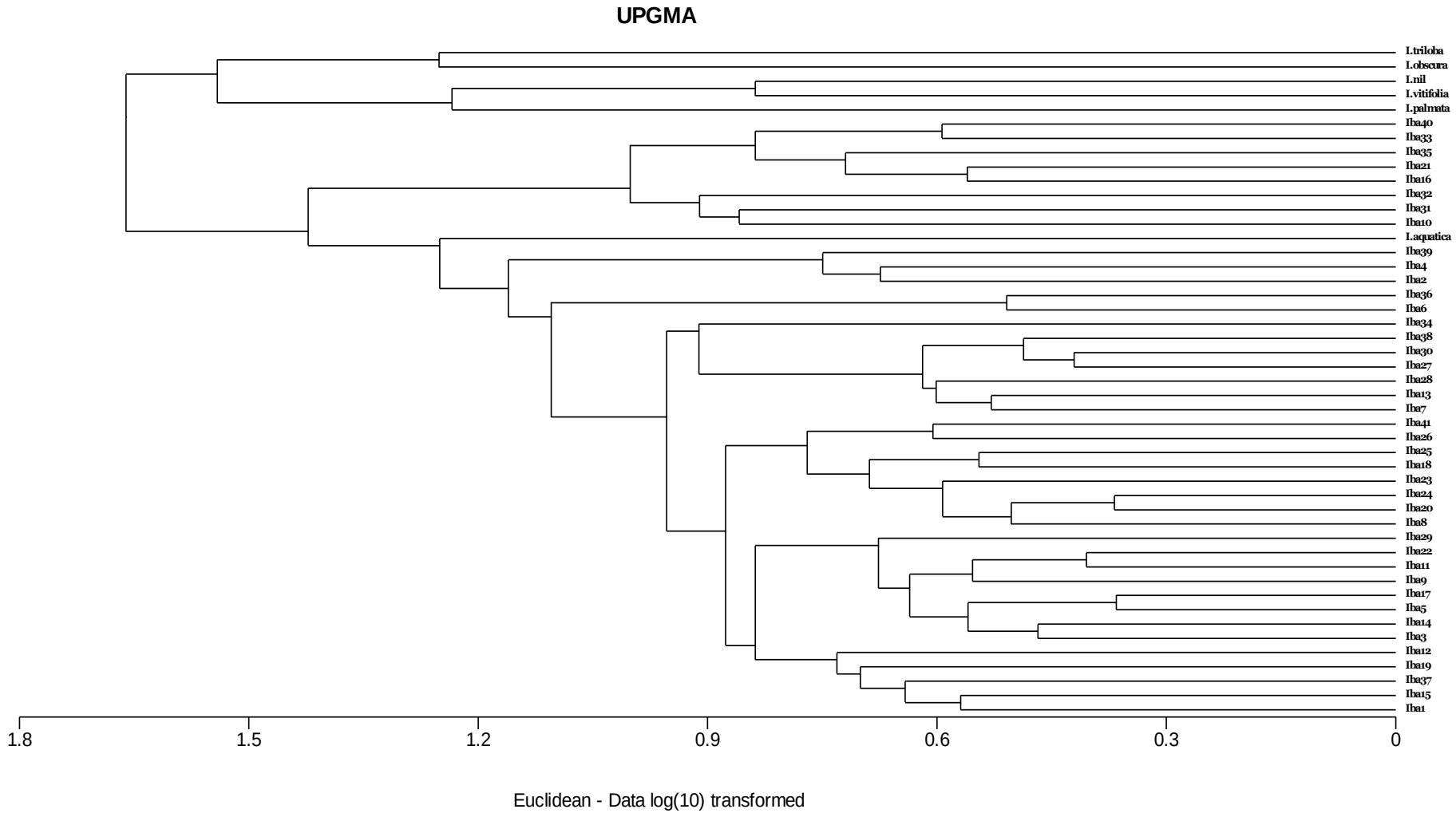


Figure 1: Dendrogram grouping of accessions based on morphological Characters

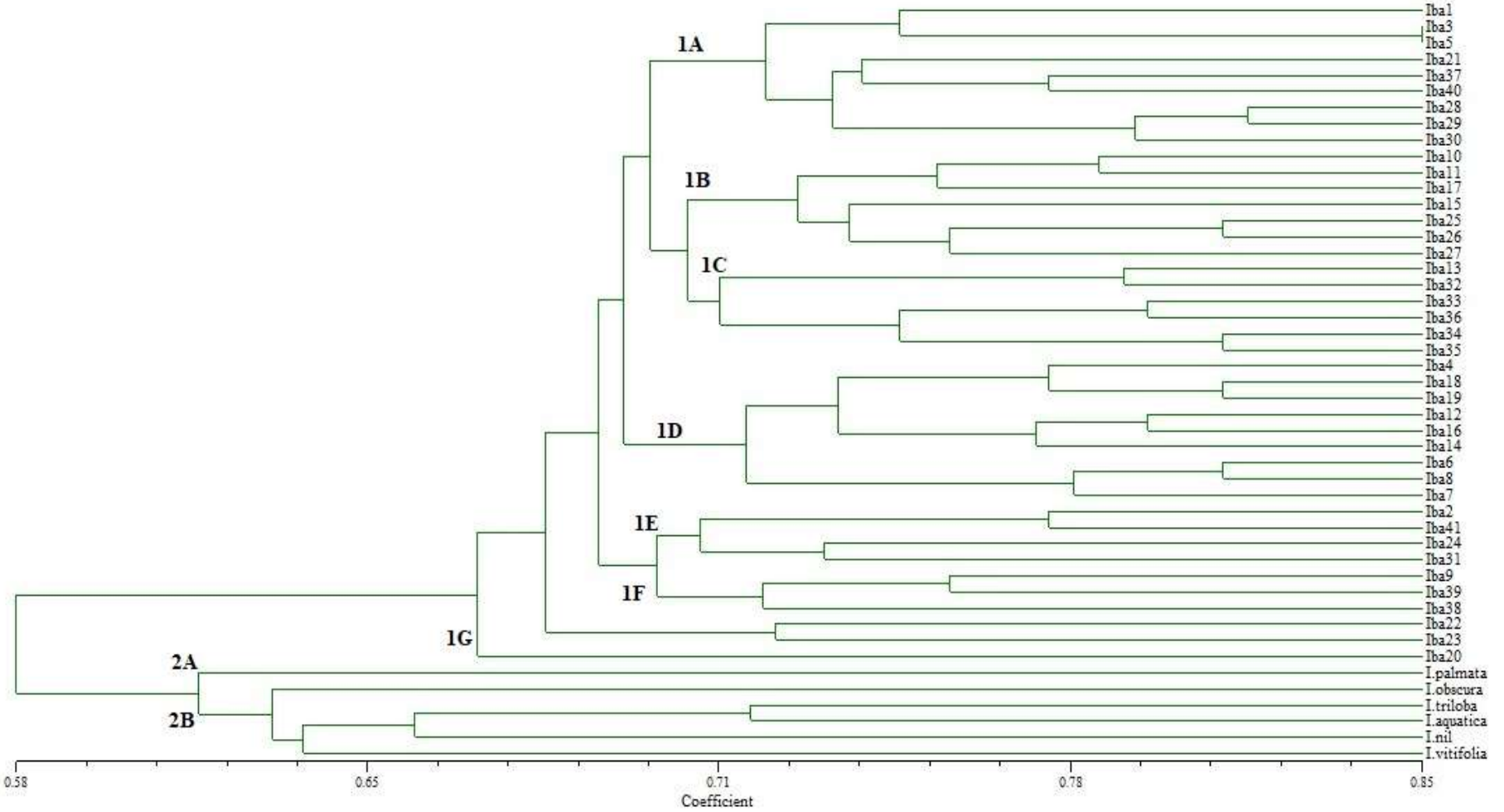


Figure 2: Cluster dendrogram of 47 accessions based on Hierarchical clustering

DISCUSSION

5. DISCUSSION

Sweet potato (*Ipomoea batatas* (L.) Lam) is a herbaceous dicotyledonous perennial plants belonging to family morning glory also called Convolvulaceae. It is one of the most versatile tuberous root crops in the world. About 600 to 700 species are coming under the genus *Ipomoea* and *I. batatas* is the only species with edible roots. High starch levels in storage roots and an array of minerals makes them as a staple food in developing countries.

Collections of 1400 accessions of sweet potato from different parts of the world are maintained in the National Active Germplasm Site on sweet potato at ICAR-CTCRI. Genetic diversity that exists within the sweet potato germplasm is helpful for the future crop improvement programmes. To achieve this goal there is need for better understanding and reliable information about the available sweet potato germplasm.

In the present study, intraspecific variation in sweet potato accessions and interspecific divergence in *Ipomoea* spp. were carried out using morphological and molecular markers and the characterization highlighted the diversity of the crop.

5.1 MORPHOLOGICAL CHARACTERIZATION

Eighteen morphological characters were recorded using IPGRI descriptors. Six wild species along with 41 sweet potato accessions were selected for the study. High variability was observed among the accessions. Predominant vine colour, immature leaf colour, leaf lobes type, shape of central leaf lobe, and petiole pigmentation were the main variable characters observed within the accessions and these could be used in distinguishing the accessions. The accessions showed similarity in characters such as twining, vine internode diameter, general outline of leaves and mature leaf colour. Fongod *et al*. (2012) in his study with 19 genotypes of sweet potato observed that vine tip pubescence, type of leaf lobes, and number of leaf lobes, mature leaf colour, immature leaf colour, predominant skin colour, and predominant flesh colour were the characters which allowed separation of accessions.

5.1.1 Statistical analysis

In the present study, the UPGMA cluster analysis using 18 morphological descriptors separated the selected forty seven sweet potato accessions into two principal clusters at a Euclidean distance of 1.7. While comparing the two principal clusters, second principal cluster having the maximum number of accessions. In the present study 100 % similarity was not obtained between any two accessions. The maximum similarity was obtained between 2 pairs of accessions, Iba5 (S632) & Iba17 (SP-11) and Iba20 (S629) & Iba24 (S663) at a Euclidean distance of 0.4 (Log 10 transformed).

Karuri *et al*. (2010) studied morphological data of 89 sweet potato accessions, dendrogram obtained with two major clusters at a Euclidean distance of 6.98 which is high in contrast to that obtained in this study. Among the 34 characters studied, the major characters that separated the accessions into two major clusters in their study were the shape of the central leaf lobe and the general outline of leaf.

The PCA revealed a very high value for leaf predominant vine colour which indicates that this character has played a major role in clustering of the accessions studied.

5.2 MOLECULAR CHARACTERIZATION

5.2.1 Primer screening for pcr

Dellaporta method (Dellaporta *et al*., 1983) was used to isolate DNA. This method is useful for obtaining high quality DNA which is free from phenolic compounds. The same method is used by Krishna *et al*. (2015) to yield high-quality genomic DNA from the leaves of *Gymnema sylvestre*.

ISSR primers were screened in the sweet potato accessions for selecting primers to get high polymorphism and obtaining clear and good gel profile. A similar screening of 100 ISSR primers was done by Liu *et al*. (2019) before the analysis. They selected a total of 129 sweet potato accessions and the selected 20 ISSR primers and produced 230 polymorphic bands. Zhang *et al.* (2014) also screened 100 ISSR primers and they selected only 17 primers as they gave sharp, reproducible and polymorphic banding pattern. In this study, a total of 17 ISSR

primers were screened. Out of which, only 11 primers were found suitable for characterization as they provided good and clear banding patterns which were only chosen for further analysis.

5.2.2 ISSR analysis of sweet potato accessions

ISSR analysis is simpler and quick than most of other marker systems. PCR amplification involves single primer and binds randomly to sequence repeats with 1-3 bases that anchor the primer at 3′ and 5′ end. It does not need the prior flanking sequence information as well as high expenditure of other marker systems. In this study the ISSR primers used were UBC 808, UBC 818, UBC 807, UBC 825, UBC 809, UBC 811, UBC 827, UBC 847, $(GA)_9AT$, $(ACC)_6Y$ and (GA)9AC.

Compared to other marker systems, ISSRs can detect high level of polymorphism (Kafkas *et al*., 2006). The present study revealed high level of polymorphism of ISSR primers as the selected ISSR primers provided 100% polymorphic gel profile and obtained an average number of 19.2 polymorphic bands per primer. A total of 211 polymorphic bands were obtained with 11 primers and this is high when compared to those obtained in a study conducted in sweet potato by Sabarinath *et al*. (2018) where they obtained total of 80 polymorphic bands and 7.3 bands on an average as well as only 89.8% of polymorphism using 11 ISSR primers. The polymorphism obtained can be considered similar with the study of Liu *et al*. (2019) done on 129 sweet potato. Using 20 primers they obtained 232 bands in which 230 bands show polymorphism, with a polymorphic ratio of 99.1% and each primer generated a mean of 11.6 polymorphic fragments.

In the present study, the highest band number (23) was obtained from UBC 825, UBC 827 and $(GA)_9AC$. The lowest number of bands (12) was obtained using the primer UBC 807. So the number of polymorphic bands in the study ranged from 12 to 23. Total of 211 polymorphic bands were generated with 100% polymorphism.

5.2.2 Cluster analysis

Using the molecular scoring data, using Jaccard's similarity coefficient based on UPGMA, hierarchical clustering was done for forty seven accessions.

The dendrogram with two principal clusters were obtained at a similarity coefficient of 0.58. Both principal clusters were further divided into sub clusters.The first principal cluster consisted of 41 accessions and subdivided into 7 sub clusters which indicate high intra-clusteral variability indicating the variability in the sweet potato accessions selected for the study. The second principal cluster comprised of all the 6 wild species and divided into one outlier which is *Ipomoea palmata* and a sub cluster, which included all the other the species *I.obscura, I.triloba, I.aquatica, I.vitifolia. I.nil*.

Hu *et al*. (2003) employed a genetic diversity study to observe the relationship between 38 sweet potato accessions with its wild species and for constructing a linkage map of sweet potato. The dendrogram showed three principal clusters and all the sweet potato accessions grouped into the first principal cluster and all the other wild species are grouped into 2 clusters, *I.trifida* (2 accessions) belongs to one cluster and the other cluster comprise of *I.triloba* (2 accessions), *I.tiliacea, I.lacunosa*. It indicated that the sweet potato accessions were genetically quite dissimilar from its wild species. Dissimilarity was obtained within the genotypes of sweet potato. The occurrence of these dissimilar accessions may be attributable to the outcrossing of the cultivated genotypes with sweet potato. These arguments strongly supports the cluster data obtained in the present study.

Huang and Sun (2000) while analyzing the genetic diversity and relationships of 40 accessions of *Ipomoea*, representing ten species of series Batatas based on 15 ISSR markers and restriction-site variation in four non-coding regions of chloroplast DNA, could obtain a total of 2071 ISSR fragments were generated with an average of 52 bands per accession. Of the species examined, *I. trifida* was found to be the most closely related to cultivated sweetpotato, the hexaploid *I. batatas*.

ISSR markers were not used earlier extensively for the studies pertaining to relationship at the interspecific levels mainly because of the concerns of non-homology in co migration of DNA bands which are specific to fast evolving markers such as RAPD (Rieseberg, 1996). But in recent years comparative studies of genetic relationships have shown that ISSR markers can provide the same level of accuracy

as RFLP markers (Nagaoka and Ogihara, 1997). The utility of ISSRs in resolving relationships at both intra- and interspecific levels were done in the following years. Huang and Sun (2000) and Hu *et al*. (2003) used inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA to investigate the genetic relationships between cultivated sweet potato and its wild relatives.

5.2.3 Similarity matrix

Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 85% to a minimum of 50%. This means that dissimilarity or variability ranged from 15-50 %. This indicates that high variability exist between the selected accessions.

Among the 47 accessions studied, the highest similarity index of 0.85 was obtained between S 623 and S 632 whereas the lowest similarity index (0.50) was obtained between AR-2017-2 and *I.vitifolia*. Iba38 (S 823) was the most similar sweet potato accession to *I.nil* (69%), *I.aquatica* (66%) and *I.vitifolia* (63%) than all the other accessions. The sweet potato accession Iba31 (SD-11) showed a similarity of 72% to *I. triloba.* *I.obscura* was similar to *I.nil* by 64% . A similarity coefficient of 0.62 was the least similarity observed between *I.obscura* and *I.vitifolia. I. triloba* has a maximum similarity with *I.aquatica* by 72%. Among all wild species used in the present study *I. triloba* is more related to sweet potato than *I. aquatica*. Within the Series Batatas there are 13 wild species that are considered to be related to the sweetpotato which includes *I. triloba* (Austin and Huaman; 1996Austin, 1978; 1979).

5.2.4 Mantel test

The Mantel statistic (r) value based on Spearman's rank correlation obtained as 0.4365 with a significance value (p) of 0.0001 indicated significant correlation between morphological and molecular marker information. Similar result was obtained by Sabarinath *et al*. (2018). This indicates that the observed phenotypic variation was at least partly caused by genetic factors as suggested by Beyene *et al*. (2006) in maize. The significance of the correlation between the two matrices (genetic and morphological) may suggest that there is some linkage between the ISSR markers used

and morphological characters chosen, as suggested by Louati *et al.* (2019) in *Argania spinosa*. Thus, ISSR markers could be successfully used for gene tagging or used in marker-assisted selection for agronomic traits (Reddy *et al*., 2002).

The significant correlations indicate that these sets of data likely reflect the same pattern of genetic diversity. This congruence indicates that both marker systems are equally suited for genetic diversity study as seen in maize accessions (Beyene *et al*., 2005). Integration between all markers will be useful for distinguishing new accessions and genotyping local varieties. Moreover, the Mantel statistic value in this study is higher in contrast to that obtained by Karuri *et al*. (2010). In his study comparison between morphological and molecular data using the Mantel test revealed a low correlation ($r = -0.05$) between both data sets. Despite the poor correlation a high degree of variation was observed among the genotypes that can be utilized in breeding programs.

SUMMARY

6. SUMMARY

The study entitled "Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp." was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020. The objective of the study was to analyse the intraspecific variation in sweet potato and interspecific divergence in *Ipomoea* spp. based on morphological and molecular markers. Forty seven accessions which included 41 sweet potato accessions and six wild species of *Ipomoea* were selected from the National Active sweet potato germplasm site at ICAR-CTCRI for the study.

The study was divided into two parts; morphological and molecular characterization. Morphological characterization comprised of recording the observations of all the aerial parts of sweet potato based on the IPGRI descriptors (CIP *et al*., 1991) for sweet potato. The percentage distribution of each trait for a particular character was obtained. The recorded data were subjected to statistical analysis using Multivariate statistical package (MVSP 3.22). The dendrogram resulted in the separation of accessions into two principal clusters at a Euclidean distance of 1.7. The first three principal components (PC) accounted for 60.9% of the variability and the highly loaded characters were predominant vine colour, secondary vine colour, leaf lobes type, leaf lobe number, shape of central leaf lobe and petiole pigmentation in PC1, twining, ground cover, vine internode length, petiole pigmentation, abaxial leaf vein pigmentation and immature leaf colour in PC2 , predominant vine colour, leaf lobe type, shape of central leaf lobe, immature leaf colour, petiole length and petiole pigmentation in PC3 that contributed to the clustering of the sweet potato accessions. The trait included in PC1, PC2 and PC3 was petiole pigmentation. The trait included in PC1 and PC3 was predominant vine colour, shape of central leaf lobe and leaf lobe shape. Immature leaf colour was the trait included in both PC2, PC3 and PC4. Hence predominant vine colour, leaf lobes type, shape of central leaf lobe, immature leaf colour and petiole pigmentation was important in distinguishing the accessions.

For molecular characterization, DNA was isolated from fresh young leaves from the germplasm. DNA was isolated using Dellaporta method (Dellaporta *et al*., 1983). The DNA obtained had an A260/A280 ratio of 1.90-2.35.

A total of 17 ISSR primers were used for the screening the accessions. 11 primers which gave clear and reproducible bands were selected for further screening of accessions. All the primers amplified at an annealing temperature of 56.3 ^{O}C. The PCR products were resolved in 2% agarose gel and clear polymorphic bands were obtained. Primers together showed 100% polymorphism and a total of 211 polymorphic bands were obtained. The number of bands ranged from 12 to 23 with an average mean value of 19.2 polymorphic bands per primer. Primers UBC 825, (GA)9AC and UBC 827 produced the highest number of bands (23). NTSYS PC Version 2.02 program was used to generate a dendrogram using ISSR marker data, which grouped the accessions on the basis of Jaccard's similarity coefficient. Thus the forty seven accessions were grouped into two principal clusters at 0.58 on similarity index scale. All the sweet potato accessions were grouped together in this principal cluster. This cluster was sub divided into seven sub-clusters and there was lot of intra-clusteral variation. The second principal cluster comprised of all the six wild species with *I. palmata* as the outlier. To determine the similarity and diversity between two accessions, pair-wise distance (similarity) matrices was computed using sequential, agglomerative, hierarchical and nested (SAHN) clustering option of the NTSYS-PC. In this, the maximum similarity was observed between S 623 and S 632 (Iba 3 and Iba 5) (0.85) and least similarity coefficient of 0.35 was observed between AR-2017-2 and *I. vtifolia* (Iba 19 and I. *vitifolia*). The morphological and molecular data were found to be positively correlated based on Spearman rank correlation coefficient using Mantel's test indicating that the morphological characters are genetically fixed. The molecular clustering of the accessions showed a very high range of intra-clusteral variability between accessions. Although a clonally propagated crop, the self-incompatibility, out crossing nature and hexaploidy exhibited by sweet potato might have contributed to the high diversity.

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APPENDICES

APPENDIX I

DNA Extraction buffer

Tris HCl (pH 8.0)	100 mM
EDTA (pH 8.0)	20 mM
NaCl	2 M
β-Mercaptoethanol	0.2% (v/v)
PVP	2% (w/v)
Distilled water	

APPENDIX II

TE buffer (10 X)

Tris HCl (pH 8.0)	10mM
EDTA	1mM

APPENDIX III

TBE buffer (10X)

Tris base	107g
Boric acid	55g
0.5M EDTA (pH 8.0)	40ml

Final volume made up to 1000ml with distilled water and autoclave before use.

APPENDIX IV

Chloroform:Isoamyl alcohol

Chloroform	24ml

Isoamyl alcohol	1ml

Mix 24 parts of chloroform with one part of isoamyl alcohol and store in a tightly sealed container.

APPENDIX V

70% Ethanol

100% Ethanol	70ml
Distilled water	30ml

ABSTRACT

MORPHOLOGICAL AND MOLECULAR ANALYSIS FOR ASSESSING INTRASPECIFIC VARIATION IN SWEET POTATO (*Ipomoea batatas* (L.) Lam.) AND INTERSPECIFIC DIVERGENCE IN *Ipomoea* spp.

by

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(2015-09-003)

Abstract of thesis

Submitted in partial

fulfillment of the requirement

for the degree of

B. Sc. - M. Sc. (INTEGRATED) BIOTECHNOLOGY

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF PLANT BIOTECHNOLOGY
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KERALA, INDIA 2020

ABSTRACT

The study entitled "Morphological and molecular analysis for assessing intraspecific variation in sweet potato (*Ipomoea batatas* (L.) Lam.) and interspecific divergence in *Ipomoea* spp." was carried out in the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2019-2020 with an objective to analyse the intraspecific variation in sweet potato and interspecific divergence in *Ipomoea* spp. based on morphological and molecular markers.

Forty one *Ipomoea batatas* accessions from the National Active sweet potato germplasm site at ICAR-CTCRI and 6 wild species collected from ICAR-CTCRI campus and KAU, Vellayani (*Ipomoea aquatica, Ipomoea palmata, Ipomoea obscura, Ipomoea vitifolia, Ipomoea nil* and *Ipomoea triloba*) were used for the study.

Morphological analysis was performed by using eighteen sweet potato descriptors as provided by IPGRI (CIP *et al*., 1991). The recorded data were analyzed statistically by various tools such as PCA and cluster dendrogram using Multivariate statistical package (MVSP 3.22). The dendrogram separated the accessions into two principal clusters at a Euclidean distance of 1.7. The PCA analysis revealed predominant vine colour, leaf lobes type, shape of central leaf lobe, immature leaf colour and petiole pigmentation as the major variables that contributed to the clustering of the sweet potato accessions.

. Molecular characterisation was performed using ISSR markers. The genomic DNA was isolated from young leaves using Dellaporta *et al.* (1983) method. 11 ISSR primers were used for screening of forty seven accessions. PCR amplicons obtained using selected primers were resolved on 2% agarose and polymorphic bands were scored. The selected primers showed 100% polymorphism and the number of bands ranged from 12 to 23 with a mean value of 19.2 polymorphic bands per primer. A total of 211 polymorphic bands were obtained. The data analysed using NTSYS PC 2.02 program generated a dendrogram, which grouped the forty seven accessions into two principal clusters based on Jaccard's similarity coefficient. All the sweet potato accessions were included in the first principal cluster which was subdivided into seven

sub-clusters and there was a lot of intra-cluster variation. The second principal cluster consisted of all the 6 wild species with *Ipomoea palmata* as an outlier. The clustering was also found similar in morphological characterization. The similarity between the different accessions ranged between 50-85%. The accessions S623 and S632 (Iba 3 and Iba 5) were 85% similar. The least similar accessions were AR-2017-2 (Iba 19) and *I.vitifolia* (35%). Three *Ipomoea batatas* [S 823 (Iba 38), S 663(Iba 24) and SD-11 (Iba 31)] showed maximum similarity (72%) with the wild *Ipomoea* spp. *Ipomoea triloba* and *Ipomoea aquatica* were the most similar ones among all the wild species by 72%. The least similar accessions among wild species were *Ipomoea palmata, I. aquatica* and *Ipomea vitifolia* (59%). Among all the wild species *I.triloba* showed maximum similarity (72%) with sweet potato (Iba 31). Mantel's test also showed a significant correlation ($r = 0.4365$; $p = 0.0001$) between the molecular and morphological distance matrices indicating that the morphological characters that were used in this study are genetically controlled and not affected by the environment. The hexaploid nature of the crop, self-incompatibility, along with the outcrossing nature together might have contributed to the high variation observed among the accessions.