GENETIC DIVERSITY ANALYSIS OF SWEET POTATO (*Ipomoea batatas* (L.) LAM.) GERMPLASM USING MORPHOLOGICAL AND ISSR MARKERS

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THESIS

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DEPARTMENT OF PLANT BIOTECHNOLOGY

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DECLARATION

I, hereby declare that this thesis entitled **"Genetic diversity analysis of sweet potato (*Ipomoea batatas* (L.) Lam.) germplasm using morphological and ISSR markers"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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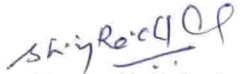
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Dedicated to my dear parents

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LIST OF ABBREVIATIONS

%	Percentage
A_{230}	Absorbance at 230 nm wavelength
A_{260}	Absorbance at 260 nm wavelength
A_{280}	Absorbance at 280 nm wavelength
AFLP	Amplified fragment length polymorphism
bp	Base pair
CGN	Center for Genetic resources, the Netherlands
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	gram
h	Hour
ha	Hectare
ICAR-CTCRI	ICAR- 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
MT	Metric Ton
MVSP	Multi Variate Statistical Package
N	North
NaCl	Sodium chloride
NAGS	National Active Germplasm Site
ng	Nanogram
nm	Nanometer
^{o}C	Degree Celsius
OD	Optical density
PC	Principal component
PCA	Principal component analysis
PCR	Polymerase chain reaction
POP	Package of Practices
PVP	Polyvinylpyrrolidine
RAPD	Random amplified polymorphic DNA
RDA	Recommended daily allowance
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RNase	Ribonuclease
rpm	revolutions per minute

s	second
S	South
sp.	Species
spp.	Species (plural)
SSR	Simple sequence repeat
Taq	*Thermus aquaticus*
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
TM	Trademark
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UV	Ultra violet
V	Volt
v/v	volume/volume
W	Watt
w/v	weight/volume
μg	Microgram
μl	Microlitre
μM	Micromolar

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam), an autohexaploid species ($2n=6x=90$) belonging to Convolvulaceae family, is normally propagated by asexual means and is a native of tropical America (Chen *et al.*, 1992). The exact location of the botanical origin of sweet potato is unknown, but Central America is considered as the primary centre of diversity, while South America (Peru, Ecuador) (Zhang *et al.*, 2000), as well as the Brazilian territory (Austin, 1988) is considered as the secondary center of diversity of the crop. Presently this single species itself fulfills the hunger of millions of people as the staple food of most of the developing nations. The developing nations alone contribute to 95% of the aggregate sweet potato production of the world (Veasey *et al.*, 2008). The total sweet potato production in the world in 8,352,323 hectares is estimated as 106 Metric tonnes (MT) as per FAOSTAT (2014). In this, 85.9% of production is from Asia, 2.3% from Americas, 0.1% from Europe, 0.6% from Oceania and 11.1% from Africa. The average sweet potato yield in India is 10.2 t/ ha.

Carl Linnaeus described the *Ipomoea* species for the first time in 1753 as *Convolvulus batatas*. Later in 1791, Lamarck included this species by classification within the genus *Ipomoea*. Surface of pollen grain and shape of the stigma was the basis of this classification. Thus the name was modified as *Ipomoea batatas* (L.) Lam. (Huaman, 1999).

Sweet potato varieties are classified into white, yellow, cream, orange and purple coloured depending on the tuber flesh colour. Among these purple and orange fleshed varieties are considered as the dietary antioxidants. Orange fleshed sweet potato is the beta-carotene rich variety that help to alleviate vitamin A deficiency (Ravi and Indira, 1999). Sweet potatoes having purple-fleshed tubers contain a lot of anthocyanins, with an anthocyanin content reported to be around 802-1747 mg/kg of fresh weight (Steed and Truong, 2008).

ICAR-Central Tuber Crops Research Institute located in Sreekariyam is

the National repository of tropical tuber crops germplasm. It is also known as the National Active germplasm site (NAGS) of tuber crops other than potato. A total of 6000 accessions of various tropical tuber crops are being maintained at ICAR-CTCRI out of which, 1400 accessions of sweet potato accession are maintained in the NAGS at ICAR-CTCRI, Sreekariyam and in its regional station at Bhubaneswar. Collection, Conservation and characterization of tuber crops germplasm is one of the major mandates of the Institute. Sweet potato germplasm maintained here contains a huge number of accessions acquired from different parts of the world through various sources, donors and collectors. A lot of variability exists in the leaves, stem and storage root characteristics of the accessions maintained. In addition, a lot of morphologically similar accessions also exist in the germplasm.

Morphological descriptors are primarily and essentially used to identify the genetic diversity in a germplasm. Morphological characterization of accessions make available information on the conserved germplasm, placing it in the most effective form for use in future, and it is also important to note that the value of the germplasm increases as it becomes known and documented (Painting *et al.*, 1995; Sudré *et al.*, 2010). Morphological characterizations have been done in the sweet potato germplasm collections all over the world for diversity analysis (Afuape *et al.*, 2011; Laurie *et al.*, 2013., Placide *et al.,* 2015) and duplicate identification.

Response to changes in environmental factors, mislabeling or wrong naming of some accessions as a result of close resemblance to some others (Asare *et al.*, 2011) are the major limitation of morphological descriptors. Thus the studies of genetic diversity are considered more reliable when they utilize molecular markers because they cover a large part of the genome and are not influenced by the environment (Goulão and Oliveira, 2001; Rao, 2004; Morales *et al.,* 2011). In sweet potato, the most widely used molecular markers are random amplified polymorphic DNA (RAPD), amplified fragment length

polymorphism (AFLP), simple sequence repeats (SSR) and inter-simple sequence repeats (ISSR). Among these, ISSR markers have high repeatability compared with RAPD, require no prior knowledge of the genome as do SSR markers (Joshi *et al.*, 2000). ISSR is simpler to perform than AFLP markers, where they are based solely on PCR and resolvable on an agarose gel.

It has been reported that ISSR primers can differentiate between clones as well as closely related cultivars and are widely used because of the low cost, high reproducibility and reliability (Zietkiewicz *et al.,* 1994). With this background, the present study entitled, **"Genetic diversity analysis of sweet potato (*Ipomoea batatas* (L.) Lam.) germplasm using morphological and ISSR markers"** was undertaken with the following objectives -

1. To study the morphological variability in sweet potato germplasm from the eastern states of India using IPGRI descriptors

2. To characterize these sweet potato germplasm using Inter Simple Sequence Repeat (ISSR) markers

3. To analyse the genetic diversity based on the morphological and ISSR markers

2. REVIEW OF LITERATURE

2.1 SWEET POTATO

Sweet potato, *Ipomoea batatas* (L.) Lam. is a dicotyledonous, perennial root crop from the family Convolvulaceae (morning glory family). It is an important food crop widely cultivated in the world with unique properties and used in numerous food and industrial applications such as processed foods, starch and bio-energy production (Gichuki *et al.*, 2003). The sweet potato plant which is frequently developed as an annual from the effortlessly established vegetative cuttings is a trailing vine with alternate leaves up to 30 cm (12 inches) in length, which go from heart-shaped to lobed, depending upon the cultivar. Flower petals are combined into a radially symmetrical funnel-shaped corolla, pink to purple in shading and even white. Different *Ipomoea* species are cultivated as ornamental morning glories and despite the fact that the flowers of *I. batatas* are not as showy as a few, it is the wellspring of ornamental and edible cultivars, including some with multi coloured foliage. Tubers of sweet potato are long and tapered, with a smooth skin whose colour ranges between purple, red, brown and beige. The flesh ranges from white through yellow, orange, and purple, and might be dry and mealy (as in the yellowish types referred to as in yams) or moist and smooth (Walker *et al.*, 2011).

Family: Convolvulaceae

Tribe: Ipomoeae

Genus: *Ipomoea*

Sub-genus: Eriospermum

Section: Eriospermum

Series: Batatas

Species: *Ipomoea batatas* (L.) Lam.

2.1.1 Origin

In view of examination of morphological characters of sweet potato and the wild *Ipomoea* species, the centre of origin of *Ipomoea batatas* was believed to be somewhere

between the Yucatan landmass of Mexico and the mouth of the Orinoco river in Venezuela more than 10,000 years ago (Burri *et al.*, 2011). As of late, the highest diversity in Central America uncovered using molecular markers provides evidence that Central America is the primary centre of diversity and undoubtedly the centre of origin, considering the wealth of the wild relatives of sweet potato (O'Brien *et al.*, 1972). Sweet potato was acquainted with Western Europe from West Indies after the first expedition of Columbus in 1492. In the 16th century, Portuguese adventurers transferred sweet potato to Africa, South East Asia, India and the East Indies, while coordinate exchange of the plant was done by Spanish exchanging ships from Mexico to the Philippines.

Among the species within the genus *Ipomoea* series Batatas, 13 are thought to be closely related to sweet potato (Zhang *et al.*, 2004). In any case the wild ancestor of this plant is not still distinguished, yet several hypotheses have been advanced to clarify the origin of sweet potato. Nishiyama in 1971 stated that sweet potato was originated from diploid *I. leucantha* Jacq., which by polyploidization derived into tetraploid *I. littoralis* (Keegan *et al.*, 1995). The hybridization between these two species might have generated triploid *Ipomoea trifida*, which was brought to hexaploid status by doubling of the triploid chromosome set. Later the selection and domestication of these wild plants might have given rise to hexaploid *I. batatas*.

2.1.2 Distribution

Around the world, the biggest producers of sweet potatoes are China, India, Indonesia and Japan. Brazil is the key producer in Latin America, with approximately three million tons for every year (Cavalcante *et al.*, 2009). Sweet potatoes originated in Central America and South America, and east African varieties exhibit characteristics totally different to American varieties (Gichuki *et al.*, 2003). Countries like China and those in Sub-Saharan Africa have increased the area under sweet potato cultivation and its production during the most recent decades. Both regions represent around 87% of cultivated area of sweet potato in the developing world. China, already the largest producer in 1990, continued to witness declining use of sweet potato as

a staple food but increased utilization for animal feed and industrial starch (Fuglie *et al.*, 2007). Sub-Saharan Africa added roughly two million hectares of sweet potato production in the most recent decade.

Today sweet potato, with more than 130 million tons in annual production, is on the fifth rank of the most important food crop in emerging nations after rice, wheat, rice, maize, and cassava (Zhang *et al.*, 2004). Its wide versatility on marginal land and rich nutritional content provide potential for preventing malnutrition and upgrading food security to the world.

2.2 AGRO CLIMATIC CONDITIONS

Sweet potato, the herbaceous perennial is domesticated as an annual and grows best in tolerably warm atmosphere and temperature of 21-26 °C. These plants require light textured soil with the ideal pH of 5.5-6.5. The crop is grown on ridges, mounds and flat beds relying on the soil and agro-climatic conditions. As sweet potato removes considerable amounts of plant nutrients, incorporation of 5 tonnes/ha of natural compost and a moderate dose of inorganic manures (50-75 kg N, 25-50 kg P_2O_5 and 75-100 kg K_2O/ha) is prescribed. Sweet potato requires maybe a couple weeding followed by earthing up for facilitating storage root bulking. Dry season planting dependably delivers higher storage root yield than wet season planting however, it requires supplemental water. Sweet potato weevil, which is causing production losses in certain parts of the world, can be lessened by following integrated pest management (IPM) techniques. Virus causing diseases can be avoided by choosing disease free quality planting materials (Nedunchezhiyan *et al.*, 2007).

If the temperature is above 24 °C, excellent development occurs. Adverse growth retardation will occur when the temperature is under 10 °C. The crop grows best in a relatively high light intensity while flowering and root formation are enhanced in short day lengths as in tropics. Yearly it needs an ideal rainfall of 75-100 cm while in the growing season, the requirement is 50 cm. The rest of the rain that gets in non-growing season is used up for the spread and preservation of vine which is the planting material for next generation. Organic matter rich sandy topsoil alongside a permeable sub soil

is the most preferable one for the rapid development of sweet potato. As water logging condition is not suitable for the plant, good drainage is necessary. Soil characterized with low air circulation will adversely affect root development and leads to low yield. The crop is more sensitive to saline and alkaline conditions and so an acidic soil pH of 5.6-6.6 is favoured (Onwueme, 1978). The ample genetic base is the major reason for the adaptability to diverse agro climatic condition (Bourke, 1985).

2.3 GENERAL PLANT MORPHOLOGY

Ipomoea batatas is grown by vegetative propagation as an annual plant using both storage roots and stem cuttings. The creeping vines, with the younger portions twining and ascending, will be of 3-4 m in length and contains abundant milky latex. Stems of the plant are cylindrical, slender and flexible. These stems may be free from hair or pubescent, usually producing aerial roots in the zone of the nodes. Roots of the plant are tuberous and edible in nature. The major types of root system include main storage roots (lateral roots) and the fibrous roots. Lateral roots help in the storage of photosynthetic products. Fibrous roots develop into lateral roots at the initial stage. Upon maturing, these lateral roots develop into lignified roots (pencil roots) and fleshy bulged storage roots which are unlignified (Huaman, 1999).

Fruits of the plant are often capsule. The outer surface of the capsule will be pubescent or free from hair on the outer surface. Upon development, the capsule achieves brown colour. 1 to 4 seeds will be contained in each capsule. The seed shape varies from rounded, angular or irregular and colour varied from brown to black. Seed size is approximately 3mm. In sweet potato seed germination is difficult. The commercial part of the plant is its storage root. It is formed as clusters around the stem. Adventitious buds formed on the surface of storage roots helps in the initiation of sprouting (Loebenstein *et al.*, 2009).

In sweet potato the leaves are simple and are spirally arranged alternatively on the cylindrical shaped stem. There will be about 3 to 7 lobes in a leaf. The general outline of leaves can be mainly of 7 types, namely rounded, reniform, triangular, hastate, lobed, cordate and almost divided. Some varieties exhibit different leaf shapes

in the same plant. Both mature leaves and immature leaves possess varying colours that ranges between green, yellowish green and purple and some accessions show purple mature leaves and green immature leaves and plants with variegated leaves are seen in ornamental varieties.

2.4 NUTRITIONAL AND HEALTH IMPORTANCE

Sweet potato is utilized as a staple food, a root vegetable (including its fleshy roots, tender leaves, and petioles), animal feed, a snack food, a source for industrial fermentation and starch extraction, and for various processed products. (Bouwkamp, 1985; Kays, 1985; Lin *et al.*, 1985; Sakamoto *et al.*, 1985). Sweet potato has high nutritional value, with the exception of both protein and niacin. The crop provides over 92% of nutrients per calorie required for people (Food and Nutrition Board, 1980; Watt *et al.*, 1975). Roots are a valuable source of carbohydrates, vitamins (provides 49% of the recommended daily allowance (RDA) for vitamin C and 100% of the RDA for retinol), and minerals (providing 15% of the RDA for potassium and 10% of the RDA for iron (Food and Nutrition Board, 1980; Watt *et al.*, 1975; Anon *et al.*, 1980). Besides simple starches, sweet potatoes are rich in complex sugar, iron, dietary fiber and vitamin content such as beta-carotene (a pro-vitamin A carotenoid), vitamin C, vitamin B_2 and vitamin E (Ishida *et al.*, 2000). Pink, yellow, and green varieties are also high in beta-carotene. The nutritional value of sweet potato is represented in Table-1.

Table 1 Nutritional Value of Sweet Potato (per 100 g)

Nutritional value per 100 g	
Energy	360 kJ (86 kcal)
Protein	1.6 g
Carbohydrates	20.1 g
Starch	12.7 g
Sugars	4.2 g
Dietary fiber	3.0 g
Fat	0.1 g

Vitamin A equivalent	0.709 g (89%)
beta-carotene	0.8509 g (79%)
lutein and zeaxanthin	0 g
Thiamine (vitamin B1)	0.1 mg (9%)
Niacin (vitamin B3)	0.61 mg (4%)
Pantothenic acid (vitamin B5)	0.8 mg (16%)
Vitamin B6	0.2 mg (15%)
Folate (vitamin B9)	11.1 mg (3%)
Vitamin C	2.4 mg (3%)
Vitamin E	0.26 mg (2%)
Calcium	30.0 mg (3%)
Iron	0.6 mg (5%)
Magnesium	25.0 mg (7%)
Phosphorus	47.0 mg (7%)
Potassium	337 mg (7%)
Sodium	55 mg (4%)
Zinc	0.3 mg (3%)

Orange-fleshed sweet potatoes may be one of nature's unsurpassed beta-carotene sources. Several recent studies have demonstrated that the sweet potato has better capacity to raise blood levels of vitamin A. This advantage may be particularly valid for children. In a few examinations from Africa, sweet potatoes were found to contain between 100-1,600 micrograms of RAE (retinol activity equivalents) of vitamin A in every 3.5 ounces and it is enough, on an average, to meet vitamin A needs of at least 35%. In many cases, sweet potatoes contain enough RAE to meet over 90% of vitamin A needs. For those who are involved in hard jobs, sweet potato is a good source of carbohydrates, vitamins and minerals. Orange-fleshed sweet potato (OFSP) bio-fortified with vitamin A was the first bio-fortified crop released by HarvestPlus and

its partners (Gruneberg *et al.*, 2015). A diet based on the sweet potato is beneficial for those suffering from stomach cancer (You *et al.*, 1988). Research has additionally demonstrated that phytonutrients in sweet potatoes might have the capacity to help lower the potential health hazard posed by free radicals (Huang *et al.*, 2004). The other bio-fortified genotypes are the purple fleshed sweet potatoes, which are a rich source of anthocyanins and have medicinal value as anti- oxidant and cancer preventing agent. Besides, in Japan the coloured roots are used for extracting the pigment, which is further used in various food products. Suda *et al.* (2003) reported that the sweet potato anthocyanins have multiple physiological functions such as radical scavenging, anti-mutagenic, hepatoprotective, anti-hypertensive and hypoglycaemic activities.

2.5 GERMPLASM COLLECTION AND CONSERVATION

Sweet potato is normally vegetatively propagated because of the heterozygous nature of the seeds. Attempts have been made in the recent past to introduce new sources of genetic diversity because of narrow genetic base of germplasm in most of the breeding programmes. However, in spite of this, changing farming systems and rising population pressure has become a reason for rapid loss of plant genetic resources. Many factors such as the introduction of modern agricultural techniques, change in land use, use of pesticides, fertilisers and fungicides has made traditional cultivars backward due to their replacement with improved cultivars (Rao and Riley, 1994). Land races and farmer varieties of sweet potato have unique properties such as disease, taste and pest resistance and hence a need is there to conserve them as conserved seed or as vegetative materials in the field. The great abundance of genetic diversity existing in gene pools holds tremendous potential for the current and future uses for the benefit of mankind. The gene pool available could be used either through conventional practices or by the use of biotechnology. Conservation can be either *in-situ* or *ex-situ*. *Ex-situ* conservation keeps up germplasm outside its natural habitats, in five facilities that have specifically created such as field, in vitro banks or botanical gardens, seed, pollen and DNA libraries. On the other hand, *in-situ* conservation relies on natural

habitats and natural ecosystems (Perino, 1992; Rao and Riley, 1994). The fundamental objective of plant genetic resources is to capture the maximum of genetic variation in the least number of samples (Marshall and Brown, 1975).

2.6 GERMPLASM CHARACTERIZATION

A germplasm collection of crop cultivars with varying environmental adaptive capacity can be utilized as a critical resource for farmers, as well as a source of genes for future crop improvement. The major goals of germplasm characterization are (1) to describe accessions and establish their diagnostic characters and identify duplicates (2) to classify group of accessions using sound criteria (3) to identify accessions with desired agronomic traits and select entries for more precise evaluation (4) to develop interrelationships between or among traits and between geographic groups of cultivars (5) To estimate the extent of variation in the collection.

Knowledge about the structure of existing cultivars and genetic diversity can help making better decisions in conservation, and support direct breeding projects. Characterization of crop genetic diversity can be achieved through both morphological and molecular tools. Morphological characterization is the initial step in diversity assessment; anyway there are major limitations in depending only on morphological characterization including low repeatability, low levels of polymorphism, late expression for specific traits; phenotypic plasticity and parallel evolution (Karuri et al., 2010; Yada et al., 2010). A number of molecular markers including restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphism (AFLP), single nucleotide polymorphisms (SNPs) and microsatellites or simple sequence repeats (SSRs), have been developed and used to complement the disadvantages of morphological characterization.

2.7 GENETIC DIVERSITY OF SWEET POTATO

Over a period of time, natural hybridization and selection have resulted in evolvement of hundreds of native sweet potato lines in South and Central America, Asia, Africa and Oceania. Central and South America has been recognized as the primary centre of diversity of sweet potato with Asia, East Africa and Oceania suggested as secondary centers of diversity.

The International Potato Center (CIP) has carried out numerous sweet potato-gathering expeditions in Latin America and the Caribbean since 1985 (Huamán *et al.*, 1997). Donations from different nations and the exchange of sweet potato collections previously maintained in other international research centres further expanded this collection. The CIP sweet potato gene bank now contains the largest germplasm holding of 5526 cultivated accessions acquired from 57 countries from America, Asia, Africa and Oceania (Huamán *et al.*, 1999). Among this, 2589 are from Latin America and 1671 accessions are from 30 countries of Asia and the Pacific transferred from the AVRDC (Asian Vegetable Research and Development Centre) after 1991. Peru alone contributed 1099 accessions (Huamán *et al.*, 1997). Several national projects in the major sweet potato producing countries hold smaller germplasm collections. Characterization of this germplasm based on determination of amount and distribution of genetic diversity is necessary for proper utilization and conservation.

2.8 MORPHOLOGICAL CHARACTERIZATION

Morphological characterization has been used widely since time immemorial for different purpose such as diversity analysis (Khalik *et al.*, 2013, Fongod *et al.*, 2012 and Tsegaye *et al.*, 2007), taxonomic classification of plants (Aguoru *et al.*, 2015) and to identify and eliminate duplicates (Yada *et al.*, 2010).

Morphological diversity among sweet potato accessions were calculated among different geographical areas in Malawi based on seven descriptors of sweet potato (IBPGR, 1985). In this study a total 260 sweet potato germplasm accessions were collected for characterization of morphological traits from the south-eastern, northern,

and southern regions of Malawi at 80 to 100 days after planting (Mok *et al.*, 1998). Mean Nei' gene diversity (h = 0.30) and Shannon information index (I = 0.43) of landraces showed moderate genetic diversity compared to that exhibited by introductions (h = 0.25; I = 0.38). Therefore, the morphological study concluded that landraces could be used in breeding programs than introductions in Malawi (Chipungu *et al.*, 2017).

Genetic diversity of 89 sweet potato genotypes was evaluated using morphological and molecular markers among Kenyan sweet potato genotypes.18 aerial and 16 storage root characters (IBPGR, 1985) were used in the morphological characterization. Analysis of variance (ANOVA) showed that all the characters were significantly different (P <0.01) between the genotypes evaluated. The genotypes exhibited high morphological diversity in aerial and storage root characters. An analysis of variance showed that all the characters evaluated were significantly different (P<0.01) between the genotypes (Karuri *et al.*, 2010).

2.9 MOLECULAR MARKERS

Genetic divergence can be analyzed based on morphological, molecular, agronomic, biochemical, physiological and other characteristics. Among these, studies with molecular markers have made significant contributions to our understandings, when compared with other types of markers in studying genetic diversity. Molecular markers present a greater number of polymorphic loci, which helps in distinguishing between accessions that may have similar morphological and agronomical traits (Gonçalves *et al.*, 2008). Different classes of molecular markers are being studied for a wide range of applications including mapping of specific genes, selecting genotypes in breeding programs using marker-assisted selection and in establishing genetic resources.

Number of molecular markers including amplified fragment length polymorphism (AFLP), random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLPs), single nucleotide polymorphisms, (SNPs)

microsatellites or simple sequence repeats (SSRs) have been developed and utilized to complement morphological characterization. In a study done on yams in Ghana using SSR markers Otoo *et al.*(2009) remarked that the selection of any particular DNA marker in a crop depends largely on the objective of the research, available resources and technical skills.

ISSR-PCR is a technique that evades the majority of limitations of the other marker techniques such as high cost of AFLP, poor reproducibility of RAPD and prior sequence knowledge requirement of SSR (Zietkiewicz *et al.*, 1994, Gupta *et al.*, 1994, Wu *et al.*, 1994) and generates highly polymorphic bands (Reddy *et al.*, 2002).

2.9.1 ISSR markers

ISSR markers are rapidly being utilized by the research community in different fields of plant improvement (Godwin *et al.*, 1997). ISSR marker technique is useful in areas of genetic diversity, gene tagging, phylogenetic studies, genome mapping and evolutionary biology in a wide variety of crop species. In this method simple sequence repeats (SSRs) are used as primers to amplify mainly the inter-SSR regions.

Inter simple sequence repeat (ISSR) strategy is a PCR based technique, which includes amplification of DNA segment present at an amplifiable distance in the middle of two identical microsatellite repeat regions oriented in opposite direction. The technique utilizes microsatellites, generally 16-25 bp long, as primers in a single primer PCR reaction focusing multiple genomic loci to amplify mainly the inter-SSR sequences of various sizes. The microsatellite repeats used as primers can be repeats of di-nucleotide, tri-nucleotide, tetra-nucleotide or penta-nucleotide. The primers used can be either unanchored (Wu *et al.*, 1994) or more usually anchored at 3' or 5' end with 1 to 4 degenerate bases extended into the flanking sequences (Zietkiewicz *et al.*, 1994).

2.9.2 ISSR in sweet potato

ISSR does not require prior knowledge of the genome sequence and exhibits high levels of polymorphism (Reddy *et al.*, 2002; Li *et al.*, 2008), making it reasonable for investigations in sweet potato. ISSR markers have successfully been used to study the genotype and genetic diversity identity of many crops.

In a study made by Camargo *et al.* (1994) on sweet potato accessions collected from highlands of Paraná State, Brazil, the data obtained from all 67 polymorphic loci generated by the 10 ISSR primers were used to estimate the genetic similarity between sweet potato genotypes. The proportion of polymorphic loci amplified by 10 ISSR primers, 67 of 81 loci, was found similar to those reported in the literatures (Camargo *et al.*, 1994). They identified that three ISSR primers, UBC 807 and UBC 808 and UBC 836 gave the best amplification results for all attributes, and thus these primers could be used as representative ISSR primers for the genetic analysis among sweet potato lines.

A core collection of 240 sweet potato accessions of an elite germplasm collection of sweet Potato (*Ipomoea batatas* L.) in China were characterized using ISSR markers by Zhang *et al.* (2014). The accessions were composed of 220 cultivars or breeding lines from 13 major sweet potato producing regions in China. Among the 100 ISSR primers tested, 17 yielded sharp, polymorphic, and reproducible band patterns which were selected to evaluate sweet potato genetic diversity. One hundred and ninety six polymorphic and distinct bands were generated in the ISSR analysis. The number of polymorphic bands obtained from each primer varied from 1 to 30, with a mean of 11.53. Using PCR amplification data based on the 17 ISSR primers, the genetic similarity matrix among all sources used in this study was calculated. The pair wise similarity coefficient detected by ISSR markers varied from 0.4745 to 0.9337, with an average of 0.7302, indicating a low level of polymorphism in this sweet potato collection (Zhang *et al.*, 2014).

Sweet potato roots collected from rural lands and local markets in Rio de Janeiro State, Brazil were characterized using RAPD and ISSR molecular markers by Monique *et al.*, (2000) to compare the efficiency in discrimination of these two types of molecular markers and estimate the genetic variability of the population The RAPD and ISSR marker results corresponded well, with a correlation of 0.55. All of the accessions were considered distinct, which demonstrates that traditional farmers maintain sweet potato genotypes that have good genetic diversity.

3. MATERIALS AND METHODS

The present study was carried out at the Division of Crop Improvement, ICAR-CTCRI, Sreekariyam, Thiruvananthapuram during 2017-2018. This section accounts for procedures and experimental materials used in the study.

3.1 PLANT MATERIALS

Ipomoea batatas (L.) Lam. accessions collected from various geographic locations are conserved in the sweet potato germplasm repository of ICAR-CTCRI which is the National Active germplasm site (NAGS) of sweet potato (Plate 1). Fifty-two accessions collected from the eastern states of India were selected from this repository along with two wild species of *Ipomoea* for the present study making a total of 54 samples. The plants were raised in the field with two plants per accession spaced at 90 cm x 90 cm spacing. The vines were planted during Jan 2018. Weeding and intercultural operations were carried out as per standard POP. The details of the accessions and their place of collection are given in Table 2.

Table 2 Passport data of the sweet potato accessions used for the study

Local collection name	Institute accession no.	Place of collection
IKSP-22	S1404	Chhattisgarh
IKSP-23	S1405	Chhattisgarh
IKSP-34	S1408	Chhattisgarh
IKSP-36	S1409	Chhattisgarh
IGSP2	S1437	Chhattisgarh
IGSP8	S1438	Chhattisgarh
IGSP10	S1439	Chhattisgarh

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

IGSP11	S1440	Chhattisgarh
Agarthala 1	S1498	Meghalaya
Agarthala 2	S1499	Meghalaya
IGSP14	S1442	Chhattisgarh
IGSP13	S1441	Chhattisgarh
Gul Bazeer-1	S1500	Meghalaya
Gul Bazeer-3	S1502	Meghalaya
Nagaland-1	S1503	Nagaland
Nagaland-2	S-1509	Nagaland
Kamalghat-1	S1504	Meghalaya
Kamalghat-2	S1505	Meghalaya
Cherrapunchi-1	S1506	Meghalaya
Cherrapunchi-2	S1507	Nagaland
White/Red	S1508	Meghalaya
Nongpon-1	S1510	Tripura
Nongpon-2	S1511	Tripura
Nongpon-3	S1512	Tripura
IGSP-10-9	S1666	Kerala
Nongpon5	S1514	Tripura
Lembucherra-1	S1515	Tripura
Lembucherra-3	S1527	Tripura
Jowai-1	S1516	Tripura
Jowai-2	S1517	Tripura
Jowai-3	S1518	Tripura
Jowai-4	S1519	Tripura
Barrabazeer-1	S1522	Tripura
Barrabazeer-2	S1523	Tripura

Barrabazeer-3	S1524	Tripura
Udaipur-1	S1525	Tripura
Srini-1	S1600	Meghalaya
Ori-2 (Siva)	S1565	Orissa
WB-2	S1569	West Bengal
Shillong-1	S1598	Meghalaya
WB-5	S1572	West Bengal
Bihar-2	S1574	Bihar
Bihar-4	S1576	Bihar
Kissan AP Farm	S1665	Arunachal Pradesh
JASM3B	S1662	Arunachal Pradesh
JASM2B	S1660	Arunachal Pradesh
JASM 3A	S1661	Arunachal Pradesh
SWARNA AP	S1656	Arunachal Pradesh
JASM 17	S-1658	Arunachal Pradesh
VLS 6	S1433	Bihar
JASM 5A	S1663	Arunachal Pradesh
JASSM 2	S-1659	Arunachal Pradesh
I. triloba	I-t	Kerala
I. aquatica	I-a	Kerala

3.2 MORPHOLOGICAL OBSERVATION

Descriptors listed by the International Plant Genetic Resources Institute (IPGRI) were used to take morphological observations (CIP *et al.*, 1991). Eighteen morphological descriptors related to vine and leaf characters were selected for the present study. The observations on leaf and vine 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 3. All the recorded morphological data were tabulated in excel sheet for further statistical analysis.

Table 3 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 (7-9 mm)-5,Thick (10-12 mm)-7, Very thick (>12 mm)-9
5	Internode length	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 purple-9
7	Secondary vine color	Absent-0, Green base-1, Green tip-2, Green nodes-3, Purple 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-1, 3 Lateral lobes- 3, 5 Lateral lobes-5, 7 lateral lobes-7, 9 lateral lobes-9
12	Shape of central 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 size	Small (<8 cm)-3, Medium (8-15 cm)-5, Large (16-25 cm)-7, Very large (>25 cm)-9
14	Abaxial leaf vein 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 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 purple 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 purple lower-8, Purple both surfaces-9

17	Petiole pigmentation	Green-1, Green with purple near stem-2, Green with purple 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, Intermediate (21-30 cm)-5, Long (31-40 cm)-7, Very long (>40 cm)-9

3.2.2 Diversity parameters

Percentage distribution of traits were calculated. Percentage distribution of traits across the 54 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 (Sneath and Sokal, 1973; Ariyo and

Odulaja, 1991).

3.3 MOLECULAR CHARACTERIZATION

3.3.1 DNA extraction

3.3.1.1 Sample collection

Fresh and tender young leaves of sweet potato accessions were collected from the germplasm of ICAR-CTCRI, Thiruvananthapuram. The leaf samples were collected in small plastic bags and brought to the lab in an ice box. DNA was extracted from fresh tender and young leaf samples using Dellaporta *et al.* (1983) method of DNA extraction for sweet potato.

Leaves collected were weighed out (1g) and ground in a pre-cooled mortar and pestle to a fine powder using liquid nitrogen. Pre-warmed extraction buffer (15ml) containing β-mercaptoethanol (0.2 %) and 2 % PVP was added to the powdered mixture. The homogenized lysate was transferred into 30 ml oakridge tubes and kept at 4 °C for 5 minutes. 1 ml of 20 % SDS was added to the lysate and incubated at 65 °C in waterbath for 1 hour with intermittent shaking. 5 ml of 5M potassium acetate was added and mixed by inversion. It was kept for incubation at 4 °C for 20 minutes. The mixture was then centrifuged at 12,000 rpm for 20 minutes at 4 °C. The resultant supernatant was transferred to another fresh 30 ml oakridge tube. Equal volume of ice-cold isopropanol was added and mixed by gentle inversion. The mixture was then incubated at -20 °C for 1 hour or at 4 °C overnight. The precipitated DNA was pelletized at 12,000 rpm for 10 minutes and the supernatant was discarded without disturbing the pellet. The pellet was dissolved in 1 ml sterile distilled water. In order to remove RNA contamination 5 μl of RNase A was added and the mixture was incubated at 37° C for 1hr. After incubation an equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed by repeated inversion. The mixture was centrifuged at 12000 rpm for 15 minutes at 4 °C. The supernatant was then transferred into fresh microfuge tubes. Two volumes of absolute ethanol and 1/10 [th] volume 3M Sodium acetate were added to the tubes for precipitation of DNA. The tubes were

incubated at -20 °C for 1 hour or at 4 °C overnight after given a gentle inversion. The supernatant was discarded and pellet saved. The pellet was washed with 500 µl 70% ethanol (10000 rpm for 8 minutes). Pellet thus obtained was saved and air dried completely. Finally the air dried pellet was dissolved in TE buffer (300-500 µl) and stored at -20° C until use. The presence of impurities 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 gel. Dry and clean gel casting tray was set up with combs have adequate number of wells to make a mold and placed on a horizontal flat surface for uniform size. Agarose was dissolved in 1X TBE freshly prepared in a conical flask and melted by boiling for 1-2 minutes to make 0.8% gel. About 0.5µl of ethidium bromide (EtBr) from a stock of 10mg/ml was added to the molten gel immediately after the flask attained a hand bearable temperature. After the addition of EtBr, the molten gel was poured into the casting tray immediately and was allowed to solidify. After solidification, the combs were removed and the gel along with tray was placed in gel electrophoresis system. 1X TBE was added to the electrophoresis unit until the gel got fully submerged. 4µl of extracted DNA sample was mixed with 2µl of 1X loading dye 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. After sufficient run, the bands in the gel were visualized under the ultra violet light using the gel documentation system (G: Box, Syngene) and the image is captured.

3.3.2.2 Quantification of DNA

The extracted DNA was quantified using Thermo Scientific NanoDrop™ 1000 Spectrophotometer. Both the quantity and quality were assessed with the help of this equipment with high accuracy. NanoDrop™ requires only 1µl of sample to calibrate different measurements needed such as the concentration of isolated DNA (ng/µl),

absorbance at 260 nm, 280 nm and the ratio of absorbance at 260 nm to 280 nm. These above mentioned measurements were taken and recorded. According to the absorbance/OD value appropriate samples were selected.

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

Primers were ordered and obtained in the lyophilized form. It was subjected to centrifugation before the first opening. The oligos were first dissolved in nuclease free water. Initially the main primer stock was made at 100 µM 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 µM by diluting 10 µl of main stock with 90 µl of nuclease free water and stored at -20 °C in refrigerator.

3.4.1   PCR amplification

The diluted samples were amplified for screening using the above mentioned 17 ISSR primers at standardized conditions of temperature and time. The components of PCR (Table 4) and PCR cycle used for amplification are given below.

Table 4 PCR

Components	Stock concentration	Required concentration	Volume for one reaction (20 µl)
Taq buffer (with 15 mM MgCl₂)	10x	1x	2 µl
MgCl₂ (additional)	25 mM	1 mM	0.8 µl
dNTP mix	10 mM	0.2 mM	0.4 µl
Primer (ISSR)	10 µM	0.25 µM	0.5 µl
Taq DNA polymerase	5U/µl	1U	0.2 µl
DNA template	10 ng/µl	40 ng	4 µl
SDW	(For making the final volume to 20 µl)		12.1 µl
Total			**20 µl**

3.4.2 PCR conditions

PCR was carried out in Proflex™ thermalcycler programmed for an initial denaturation at 94 °C for 5 minutes followed by 35 cycles with denaturation at 94 °C for 30 seconds, primer annealing at 56.3 °C for 1 minute and extension at 72 °C for 1 minute. The final extension was performed at 72 °C for 10 minutes followed by hold at 4 °C.

To select suitable and efficient primers, the amplification of PCR products was checked by agarose gel electrophoresis. The amplified products were resolved in a 2 % agarose gel along with 100 bp and 1 kb ladders and the bands on gel were visualized under UV light of G: Box gel documentation system using GeneSyS software (Syngene). The quality of bands obtained was validated by visual observation and primers with scorable bands were selected for amplifying the 50 sweet potato accessions. The details of the selected primers are depicted in Table 5.

Table 5: List of selected ISSR primers for sweet potato characterization

Sl. No.	Primer Name	Sequence	Annealing temperature (°C)
1	UBC 807	AGAGAGAGAGAGAGAGT	56.3 °C
2	UBC 808	AGAGAGAGAGAGAGAGC	56.3 °C
3	UBC 809	AGAGAGAGAGAGAGAGG	56.3 °C
4	UBC 818	CACACACACACACACAG	56.3 °C
5	UBC 825	ACACACACACACACACT	56.3 °C
6	UBC 827	ACACACACACACACACG	56.3 °C
7	UBC 847	CACACACACACACACARC	56.3 °C
8	UBC 860	TGTGTGTGTGTGTGTGRA	56.3 °C
9	(ACC)$_6$Y	ACCACCACCACCACCACCY	56.3 °C
10	(GA)$_9$AT	GAGAGAGAGAGAGAGAGAT	56.3 °C
11	(GA)$_9$AC	GAGAGAGAGAGAGAGAGAC	56.3 °C

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 54 accessions was subjected to molecular characterization with the selected ISSR primers. The PCR products were resolved in 2% agarose gel along with 100 bp and 1 kb ladders to identify the molecular weight of obtained bands and for polymorphism studies.

3.5.1 Agarose Gel Electrophoresis

Agarose gel electrophoresis is being used for DNA analysis and also for DNA separation, which is a mile stone of marker analysis. Agarose gel can also be used for isolating a particular band. The DNA molecules will separate based on their size in the gel through sieving effect. The sieving effect will increase as the agarose concentration

increases.

3.5.2 Gel preparation

2.4 g of agarose was weighed out and transferred into a 250 ml conical flask. 120 ml 1X TBE buffer was added to it and the solution was boiled in microwave oven till the agarose completely dissolved in buffer. The solution was allowed to cool to which 1µl of ethidium bromide (10 mg/ml) was added with at most care in order to avoid the spilling as well as bubble formation. The casting tray was prepared and combs were kept in position. The warm gel was poured into the casting tray and allowed to solidify for 20 min.

3.5.3 Gel loading and running

The horizontal electrophoresis tank was filled with 1X TBE buffer. The solidified gel was transferred to electrophoretic apparatus and confirmed submergence of gel completely in the buffer. The combs from the solidified gel were removed slowly without disturbing the wells. 8µl of each sample was loaded into the wells, along with 3µl each of 100 bp and 1kb ladders at both the end wells for reference. The gel was allowed to run at 100 V for about 1 to 2 hr. The bands were visualized under UV transilluminator and documented using gel documentation system.

3.5.4 Visualization of gel profile

The gel including the tray was taken out and was viewed under the ultraviolet light of G: Box gel documentation system (Syngene) using GeneSYS software. 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 scored. 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

Polymorphic marker ratio was calculated based on the scoring data obtained. A

band is said to be polymorphic, when a particular band is present in one accession and absent in any of the other accessions. The percentage of polymorphism was calculated. The equation for calculating percentage of polymorphism is given below.

Percentage of polymorphism= (No. of polymorphic bands/ Total number of bands) 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 54 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..

4. RESULTS

The results of the study entitled "Genetic diversity analysis of sweet potato (*Ipomoea batatas* (L.) Lam.) germplasm using morphological and ISSR markers" carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2017-2018 are described in this chapter.

4.1 MORPHOLOGICAL CHARACTERIZATION

Fifty four 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 52 sweet potato varieties from the eastern states of India and 2 wild species (*Ipomoea aquatica* and *Ipomoea triloba*) from the sweet potato germplasm repository of ICAR-CTCRI. Habit of accessions selected for the study is shown in Plate 2-6. Leaf lobe variability and abaxial vein pigmentation of accessions is depicted in plate 7.

4.1.1 Percentage distribution

Percentage distribution of traits across the selected accessions was calculated and the table is shown below (Table 6, Plate 8).

Table 6. Frequency distribution of morphological traits

Sl. No.	Characters	Observed Traits	Frequency distribution
1	Twining	Non- twining	98%
		Very-twining	2%
2	Plant type	Erect	30%
		Semi-erect	66%
		Extremely spreading	4%
3	Ground cover	Small	31%
		Medium	52%
		High	17%

S 1404   S 1405   S 1408

S 1409   S 1437   S 1438

S 1439   S 1440   S 1441

S 1442   S1498   S1499

Plate - 2 Habit of different accessions used in the study

S1500

S1502

S1503

S1509

S1504

S1505

S1506

S1507

S1508

S1510

S1511

S1512

Plate - 3 Habit of different accessions used in the study

S1666

S1514

S1515

S1527

S1516

S1517

S1518

S1519

S1522

S1523

S1524

S1525

Plate - 4 Habit of different accessions used in the study

S1600	S1565	S1569
S1598	S1572	S1574
S1576	S1665	S1659
S1662	S1660	S1661

Plate - 5 Habit of different accessions used in the study

S1663
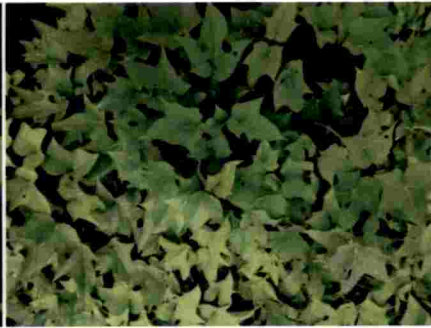
S1433

S1656

S1658

I. aquatica

I. triloba

Plate - 6 Habit of different accessions used in the study
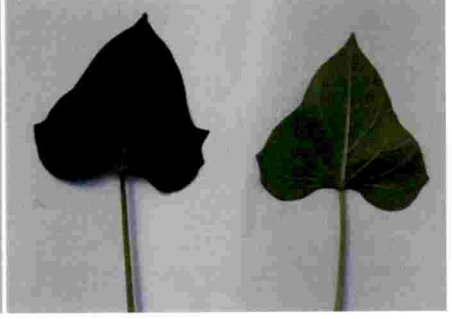
S1405 S1408 S1409
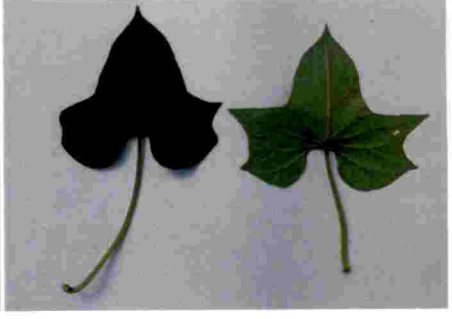
S1439 S1438 S1499

S1505 S1517 S1523

Plate - 7 Leaf variability and abaxial vein pigmentation

Predominant vine colour

Legend:
- Green
- Green with few purple spots
- Green with many purple spots
- Green with many dark-purple spots
- Mostly purple
- Mostly dark purple
- Totally purple
- Totally dark purple

Values: 6%, 2%, 5%, 11%, 4%, 11%, 41%, 20%

Type of leaf lobes

Legend:
- No lateral lobes
- Very slight (teeth)
- Slight
- Moderate
- Deep
- Very deep

Values: 4%, 1%, 20%, 17%, 20%, 28%

Petiole Pigmentation

Legend:
- Green
- Green with purple near stem
- Green with purple near leaf
- Green with purple at both ends
- Green with purple spots throughout petiole
- Green with purple stripes

Values: 7%, 7%, 35%, 27%, 9%, 13%, 2%

Plate - 8 Frequency distribution of major variables

4	Vine internode length	Very short	11%	
		Short	89%	
5	Vine internode diameter	Very-thin	17%	
		Thin	83%	
6	Predominant vine colour	Green	41%	
		Green with few purple spots	20%	
		Green with many purple spots	11%	
		Green with many dark- purple spots	4%	
		Mostly purple	11%	
		Mostly dark purple	6%	
		Totally purple	2%	
		Totally dark purple	6%	
7	Secondary vine colour	Absent	33%	
		Green base	4%	
		Green tip	20%	
		Green nodes	2%	
		purple base	4%	
		Purple tip	7%	
		Purple nodes	30%	
8	Vine tip pubescence	None	22%	
		Sparse	33%	
		Moderate	30%	
		Heavy	15%	
9	General leaf outline	Rounded	2%	
		Cordate	6%	
		Triangular	31%	
		Hastate	24%	
		Lobed	30%	
		Almost divided	7%	
10	Type of leaf lobes	No lateral lobes	11%	
		Very slight (teeth) Slight	20% 28%	
		Moderate	20%	
		Deep	17%	

		Very deep	4%
11	Number of leaf lobes	Central tooth without lateral lobe	24%
		3 Lateral lobes	35%
		5 Lateral lobes	37%
		7 lateral lobes	4%
12	Shape of central leaf lobe	Toothed	11%
		Triangular	28%
		Semi-circular	2%
		Semi-elliptic	39%
		Elliptic	9%
		Lanceolate	2%
		Linear (narrow)	9%
13	Mature leaf size	Small	6%
		Medium	94%
14	Abaxial leaf vein pigmentation	Yellow	2%
		Green	43%
		Purple spot at base of main rib	18%
		Purple spots in several veins	4%
		Main rib partially purple	11%
		Main rib mostly or totally purple	2%
		All veins partially purple	7%
		All veins mostly or totally purple	13%
15	Mature leaf colour	yellow-Green	2%
		Green	70%
		Green with purple edge	19%
		green with purple veins on upper surface	7%

		green upper, purple lower	2%
16	Immature leaf color	Green	7%
		Green with purple edge	49%
		green with purple veins on upper surface	9%
		Slightly purple	13%
		Mostly purple	20%
		Purple both surfaces	2%
17	Petiole length	Very short	43%
		Short	57%
18	Petiole pigmentation	Green	35%
		Green with purple near stem	2%
		Green with purple near leaf	13%
		Green with purple at both ends	9%
		Green with purple spots throughout petiole	27%
		Green with purple stripes	7%
		Totally or mostly purple	7%

4.1.2 Cluster analysis

Cluster analysis using Multivariate Statistical Package (MVSP 3.22) generated a dendrogram (Figure 1). UPGMA Cluster analysis using 18 variables separated all the 54 accessions into two major clusters and one outlier and at a Euclidean distance of 1.2. The pattern of clustering of accessions is described in Table 7. Accessions showing highest similarity are depicted in plate 9.

A. Similar accessions

S 1439

S 1442

B. Dissimilar accessions

S 1408

S 1659

Plate - 9 Accessions showing highest morphological similarity (A) and dissimilarity (B)

57
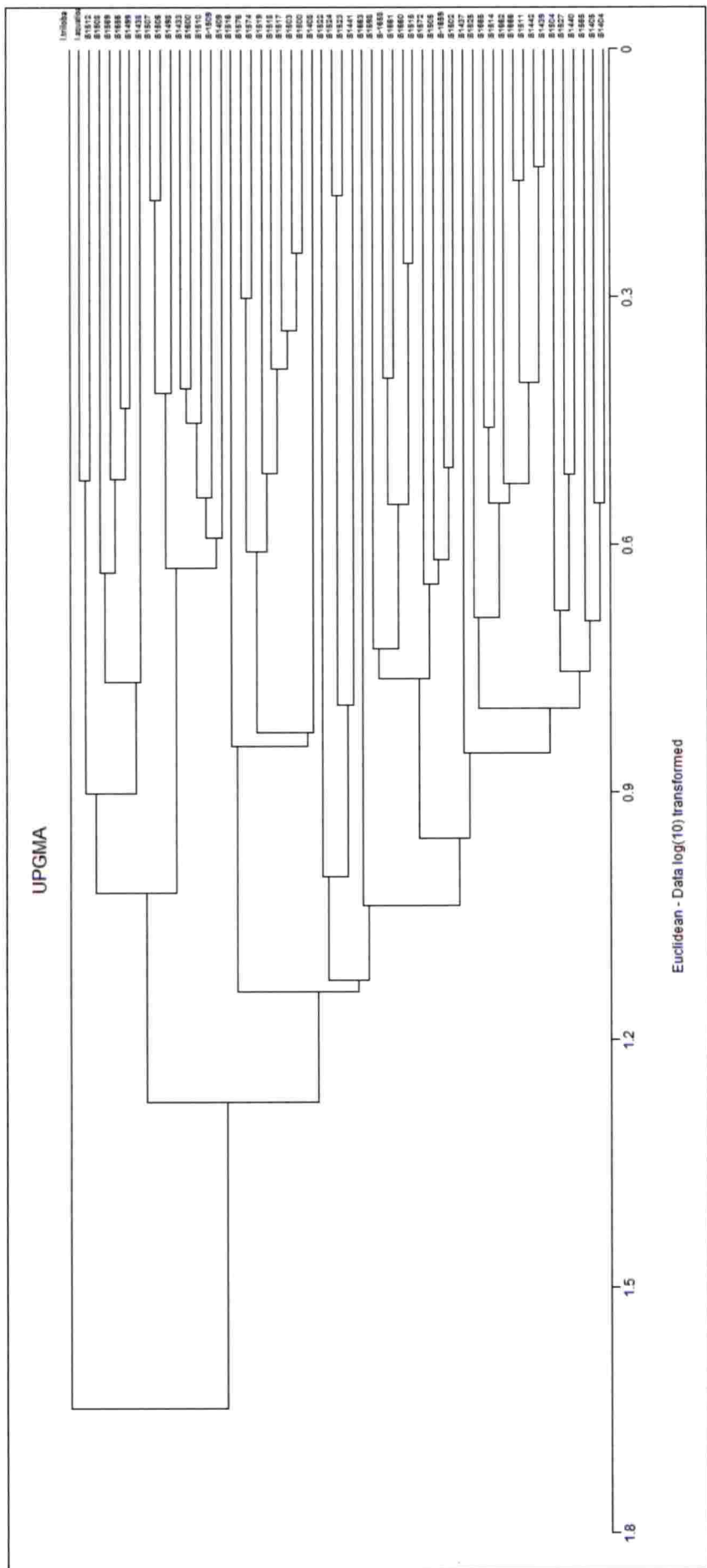
Figure – 1 Dendrogram grouping of accessions based on morphological Characters

Table 7 The clustering pattern of different accessions

Clusters	Sub-Clusters	Accessions
Outlier		*I. triloba*
Cluster 1	1A	*I. aquatica*, S 1512, S 1508, S 1569, S 1656, S 1499,S 1438
	1B	S 1507, S 1506, S 1498, S 1433, S 1600, S 1510, S 1509, S 1409
Cluster 2	2A	S 1516, S 1576, S 1574, S 1519, S 1518, S 1517, S 1503, S 1500, S 1408
	2B	S 1522
	2C	S 1524, S 1523, S 1441
	2D	S 1663
	2E	S 1598, S 1658, S 1661, S 1660, S 1550, S 1572, S 1505, S 1659, S 1502
	2F	S 1437, S1525, S 1665, S 1514, S 1662, S 1666, S 1611, S 1442, S 1439 S 1504, S1527, S 1440, S 1565, S 1405, S 1404

4.1.3 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 67.5% of the variability among the characters studied (Table 8).

PC1 accounted for 36.3% of the variation. The first principal component had predominant vine colour, leaf lobes type, abaxial vein pigmentation and petiole pigmentation with high values. The second principal component accounted for 19% of

the variation which included vine tip pubescence, leaf lobes type and immature leaf colour. The third principal component accounted for 12.3% of the variation which included predominant vine colour, secondary vine colour and petiole pigmentation. The trait included in PC1 and PC2 was the leaf lobes type. Predominant vine colour was the trait included in both PC1 and PC3. Hence predominant vine colour and leaf lobes type was important in distinguishing the accessions.

Table 8 Principal component analysis in 54 accessions of sweet potato

(Highly loaded variables in combined analysis given in boldface)

Variables	PC1	PC2	PC3	PC4
Twining	-0.02	-0.145	0.013	0.16
Plant type	0.01	-0.152	-0.027	0.255
Ground cover	-0.045	-0.013	0.141	**0.507**
Vine internode length	0.003	-0.045	0.015	0.095
Vine internode diameter	0.007	0.036	0.027	0.052
Predominant vine colour	**0.457**	0.173	**0.345**	0.053
Secondary vine colour	0.175	0.275	**-0.819**	-0.09
Vine tip pubescence	0.152	**0.585**	0.082	0.165
General outline of leaf	-0.161	0.155	0.143	-0.137
Leaf lobes type	**-0.443**	**0.433**	0.143	-0.139
Leaf lob number	-0.286	0.266	0.077	-0.139
Shape of central leaf lobe	-0.265	0.254	0.099	-0.102
Mature leaf size	-0.006	0.044	0.007	-0.022
Abaxial leaf vein pigmentation	**0.377**	0.133	0.034	**-0.376**
Mature leaf colour	0.121	0.042	-0.098	-0.08
Immature leaf colour	0.035	**0.319**	-0.198	**0.556**
Petiole length	-0.005	0.081	0.063	0.271
Petiole Pigmentation	**0.45**	0.169	**0.267**	-0.061

4.2 MOLECULAR DATA ANALYSIS

4.2.1 DNA isolation and quantification

DNA of 54 accessions was isolated using the method described by Dellaporta *et al.* (1983). The quality of isolated DNA was checked in 1% agarose gel (Plate 10). The quantity as well as purity of the DNA obtained from spectrophotometric readings are included in Table 9. The concentration of the isolated DNA ranged from 106.6 ng/µl to1827.9 ng/µl.

Table 9 Spectrophotometric readings of DNA isolated using Dellaporta method (Dellaporta *et al.*, 1983).

Sl. No.	Sample Name	A_{260}/A_{280}	Concentration (ng/µl)
1	S1404	1.84	1136.237
2	S1405	1.98	1178.823
3	S1408	1.76	813.087
4	S1409	1.86	814.918
5	S1437	1.92	513.598
6	S1438	2.09	793.06
7	S1439	2.23	304.256
8	S1440	2.08	297.25
9	S1498	1.98	1827.964
10	S1499	1.96	603.163
11	S1442	2.03	328.324
12	S1441	2.03	1047.392
13	S1500	2.08	753.43
14	S1502	1.94	703.763
15	S1503	1.88	1466.43
16	S1509	1.92	1530.62
17	S1504	1.76	701.79
18	S1505	1.83	1358.068
19	S1506	1.84	540.543
20	S1507	1.98	694.16
21	S1508	1.76	329.276
22	S1510	2.03	330.556
23	S1511	1.87	732.296
24	S1512	2.01	860.076

25	S1666	2.05	754.895
26	S1514	2.08	928.108
27	S1515	1.87	1656.658
28	S1527	1.76	722.027
29	S1516	1.86	753.182
30	S1517	1.92	734.87
31	S1518	1.88	520.253
32	S1519	2.11	508.253
33	S1522	2.12	1024.778
34	S1523	2.05	992.567
35	S1524	2.05	1054.224
36	S1525	1.98	476.527
37	S1600	1.96	481.786
38	S1565	2.08	554.885
39	S1569	1.94	287.593
40	S1598	2.02	353.077
41	S1572	1.84	106.633
42	S1574	1.98	773.888
43	S1576	1.96	342.798
44	S1665	2.08	455.348
45	S1662	2.01	954.674
46	S1660	2.05	792.173
47	S1661	1.97	448.679
48	S1656	2.03	509.843
49	S-1658	2.05	620.626
50	S1433	1.98	449.808
51	S1663	1.96	227.957
52	S-1659	2.01	213.277
53	I-t	1.87	302.787
54	I-a	2.01	449.878

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 11 to Plate 17.

4.2 ANALYSIS OF MOLECULAR MARKER DATA

4.2.3 ISSR profile

Using 11 ISSR primers (Plates 11-17), a total of 89 bands were generated of which 80 bands were polymorphic. An average of 7.3 polymorphic bands per primer was obtained. Total number of bands per primer ranged from 5 (UBC 827 and UBC 860) to 16 (UBC 808). A total of 89.8% polymorphism was obtained (Table 10).

Table 10 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	6	6	100
2	UBC-808	16	13	81.25
3	UBC-809	6	6	100
4	UBC-818	8	8	100
5	UBC-825	10	7	70
6	UBC-827	5	4	80
7	UBC-860	5	5	100
8	$(GA)_9AC$	12	12	100
9	UBC-811	7	7	100
10	UBC-817	6	5	83.3
11	UBC-810	8	7	87.5
	Total	**89**	**80**	**89.8**

4.2.4 Cluster analysis

The UPGMA dendrogram created using Jaccard's similarity coefficient separated the accessions into three principal clusters at a coefficient 0.56 (Figure 2).
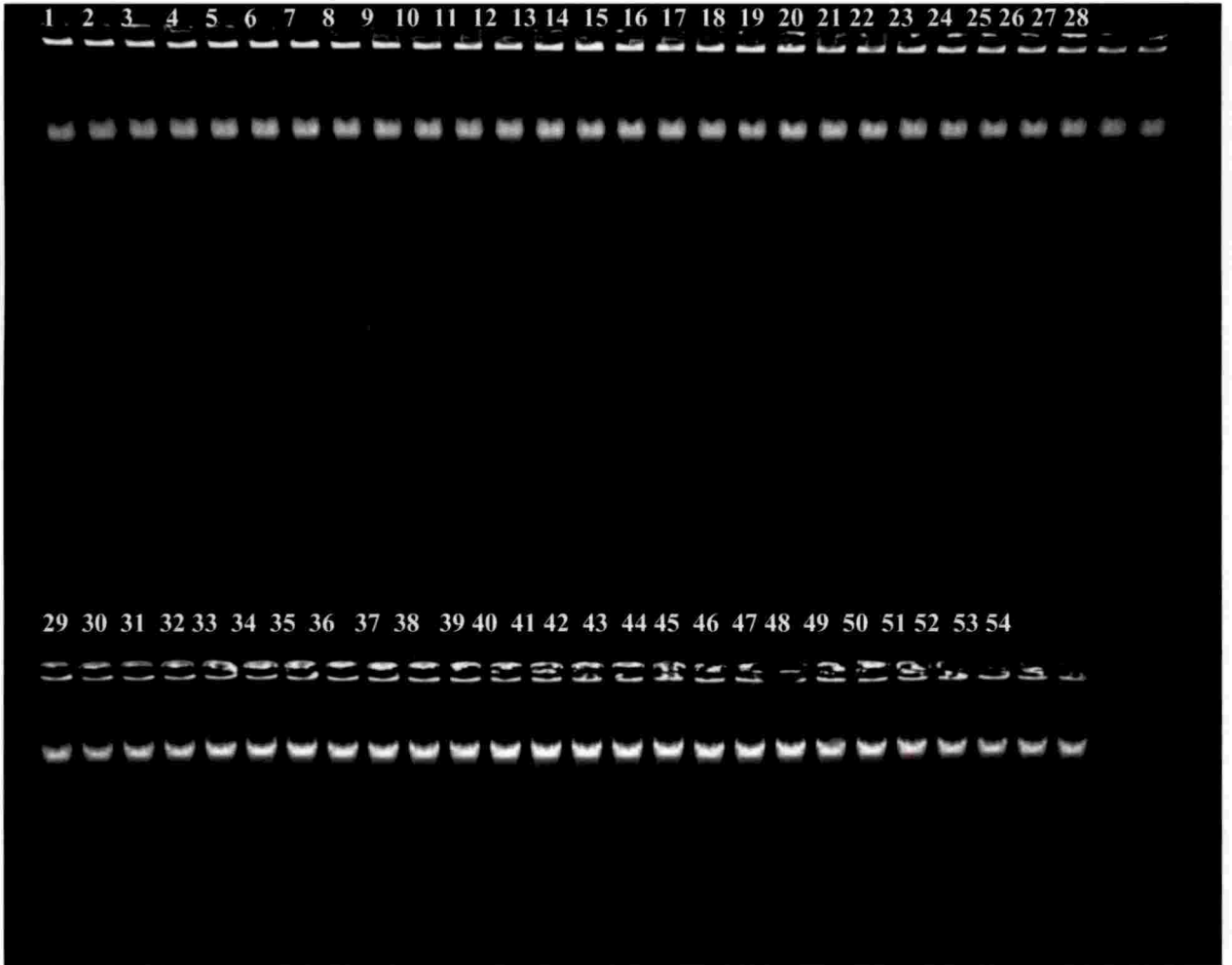
Plate - 10 Qualitative analysis of DNA of 54 accessions of Sweet potato using agarose gel electrophoresis (Dellaporta *et al.*, 1983)
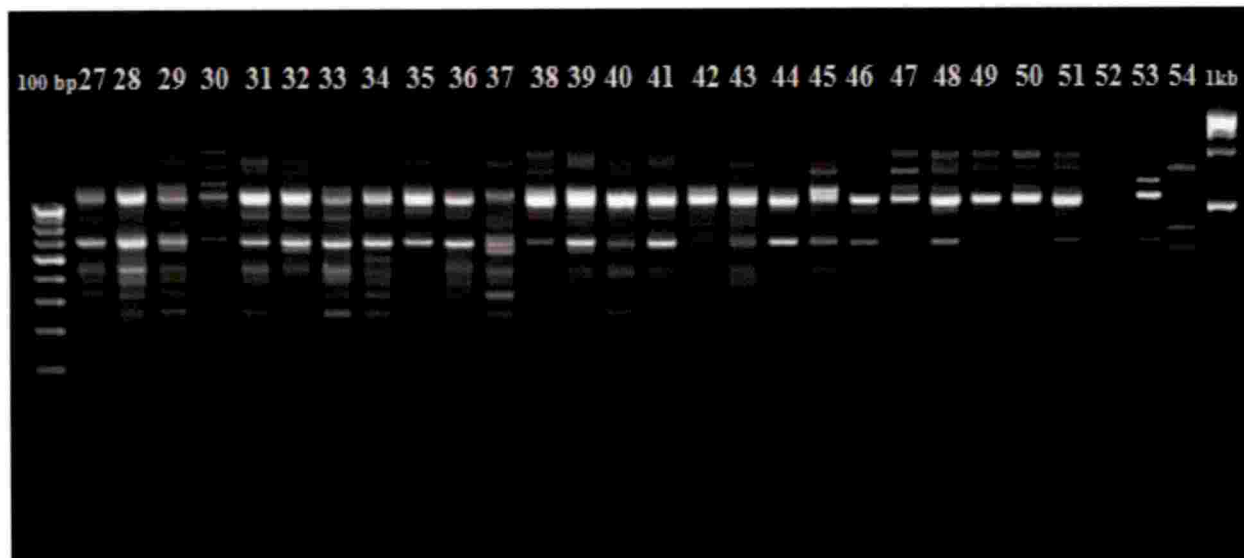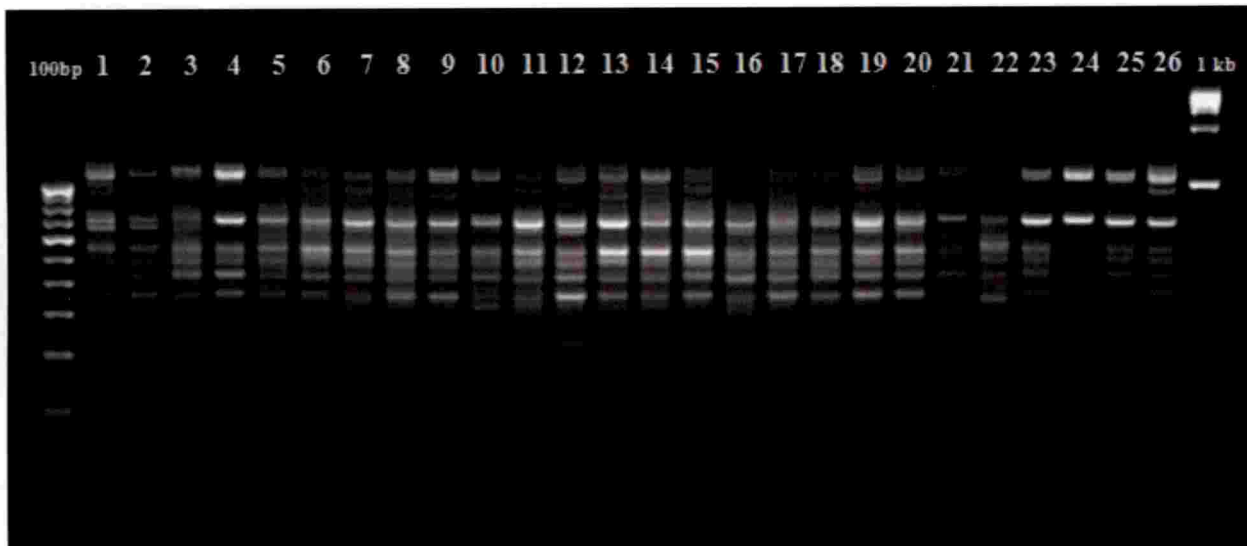
Plate - 11 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 808
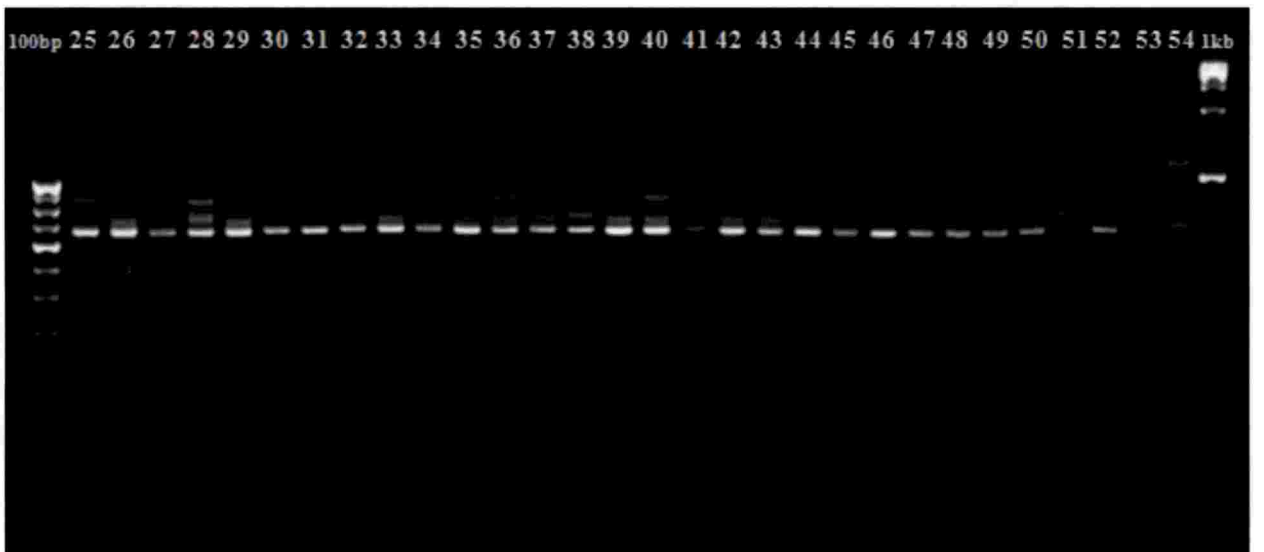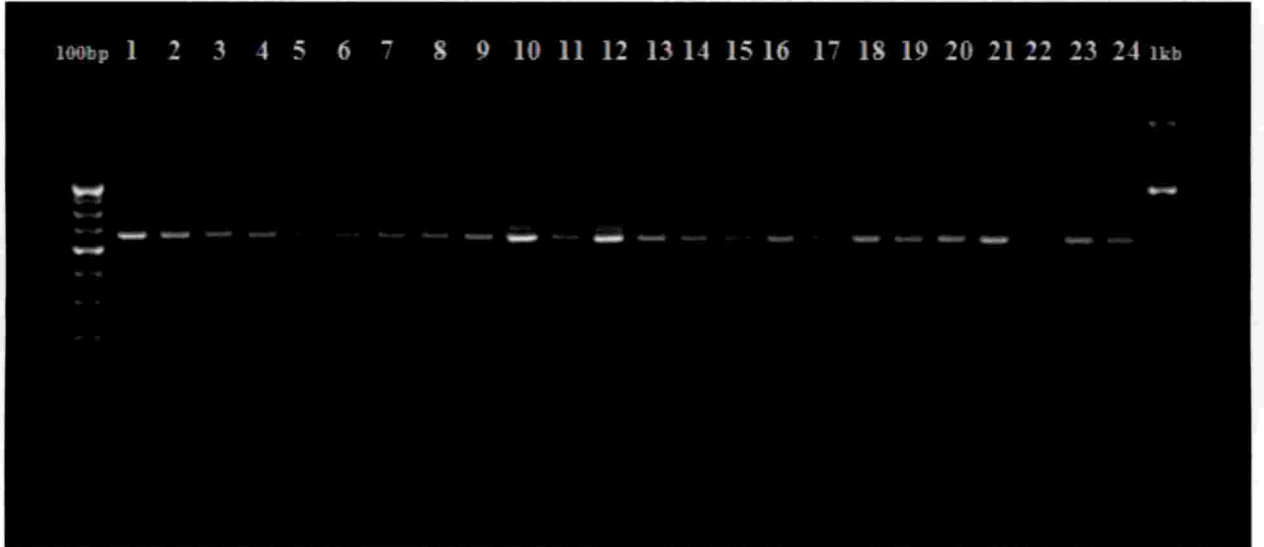
Plate - 12 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 809
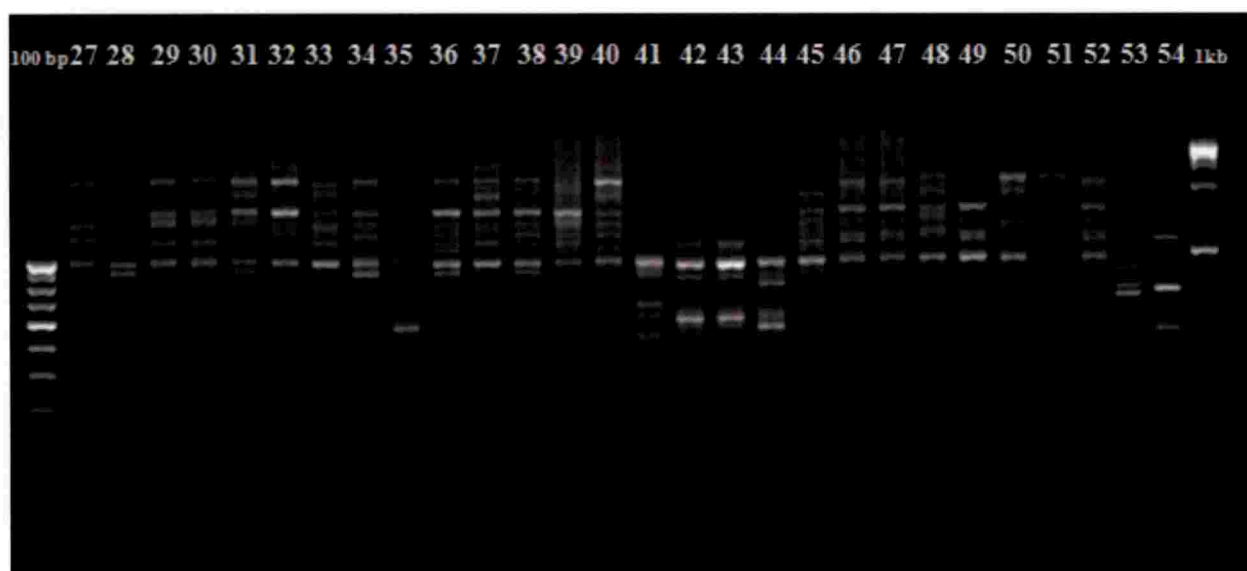
Plate - 13 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 825
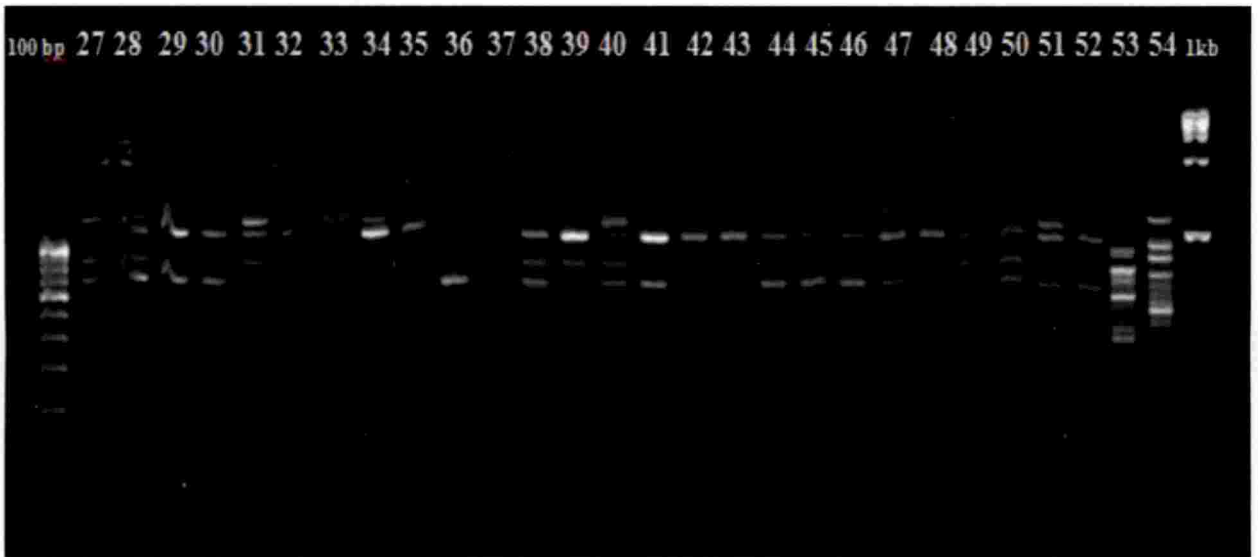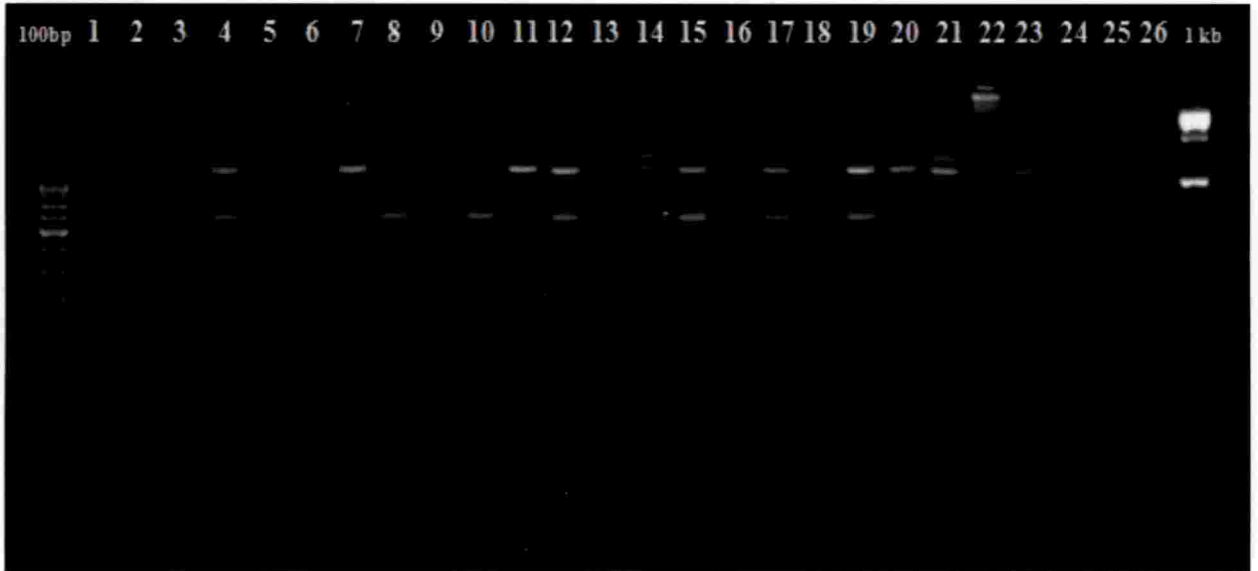
Plate - 14 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 827
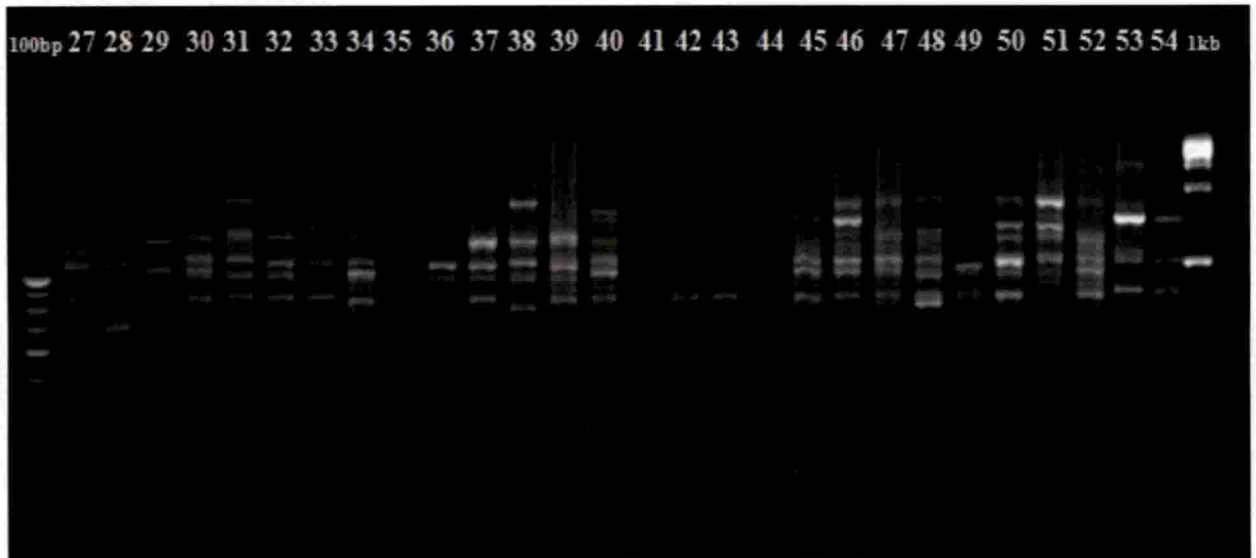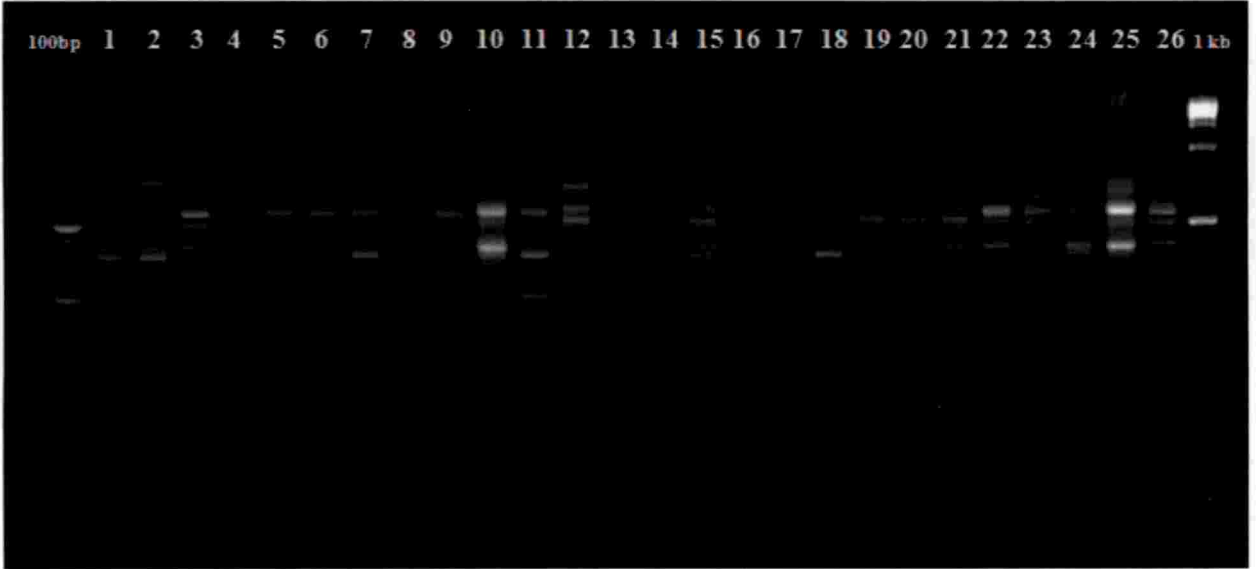
Plate - 15 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer (GA)9AC
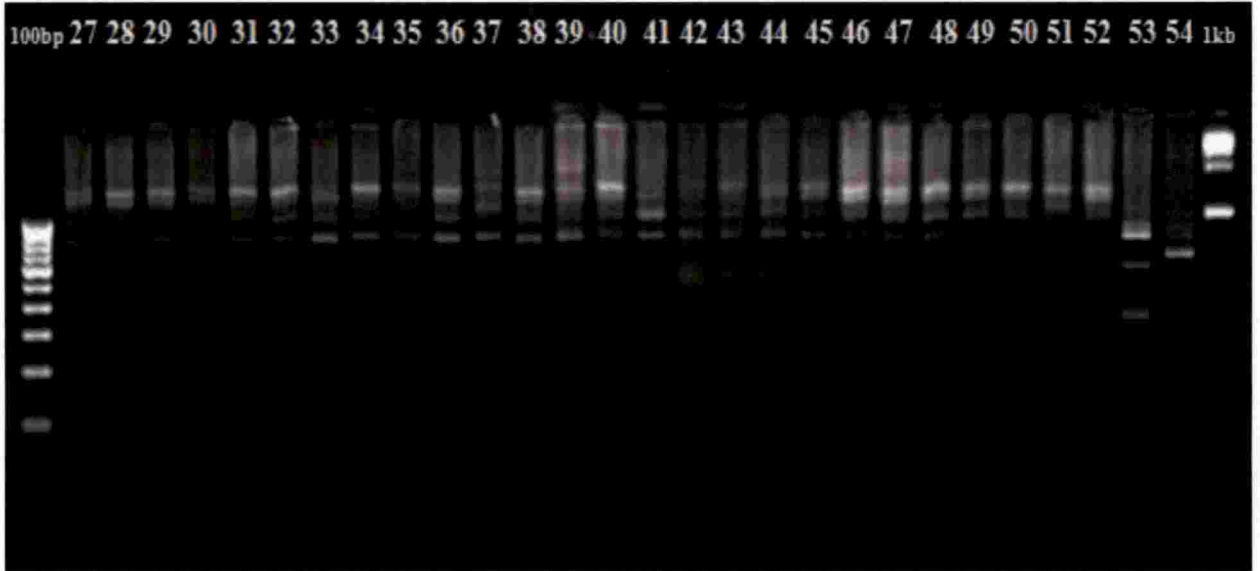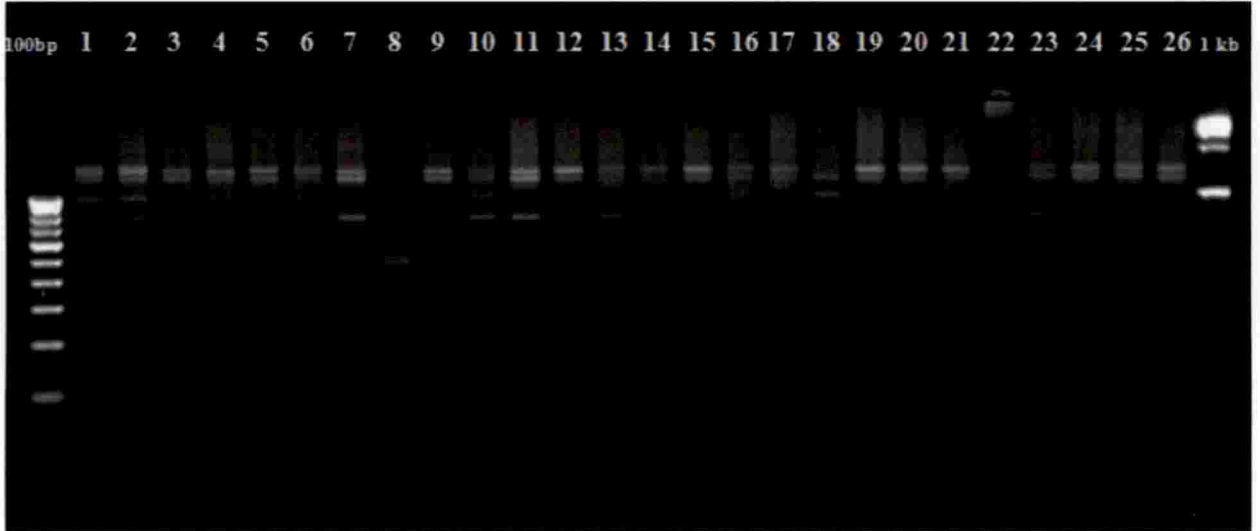
Plate - 16 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 817

Plate - 17 ISSR profile of 54 accessions of *Ipomoea* resolved on 2 % agarose gel using primer UBC 810
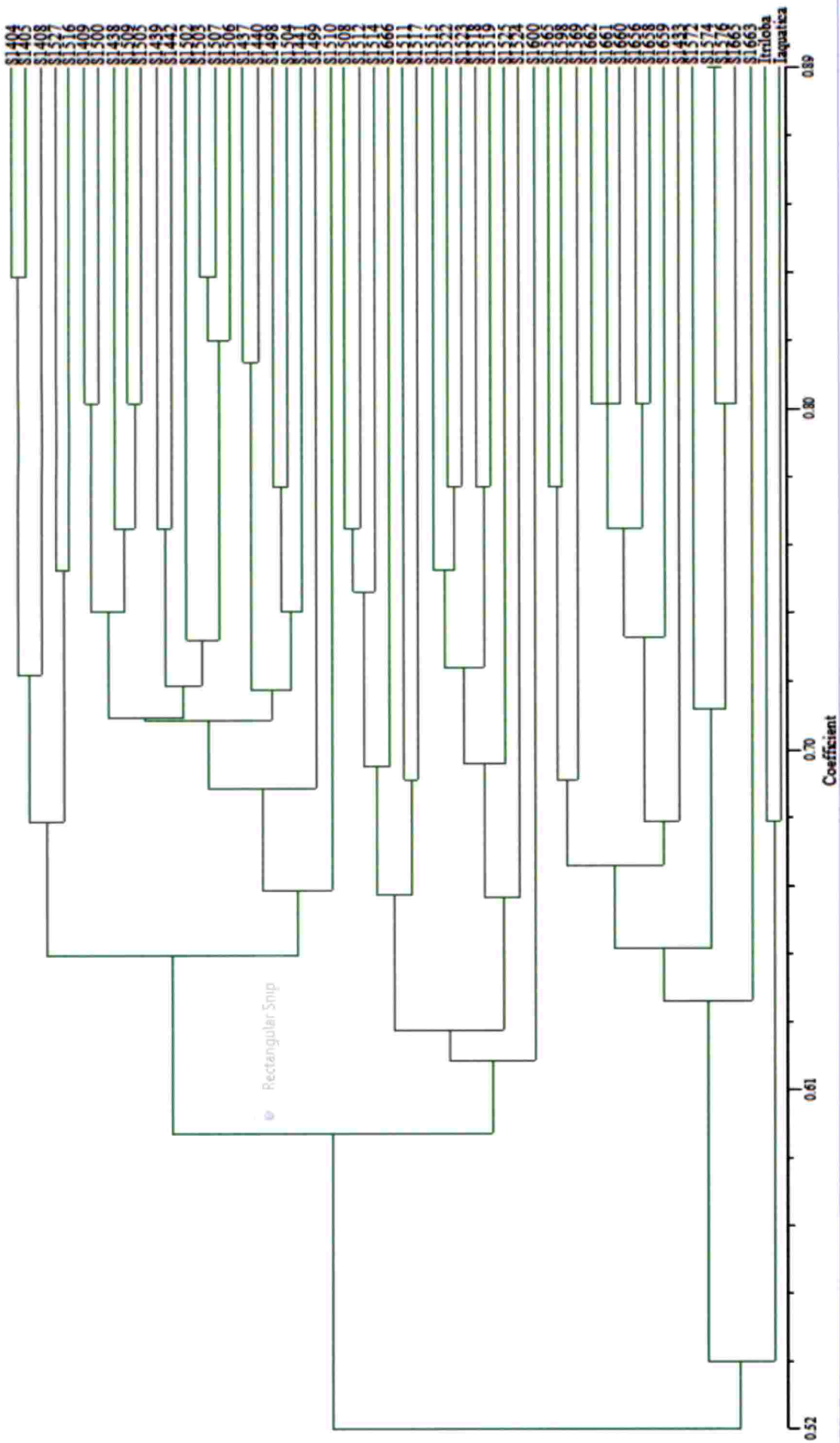
Figure - 2 Cluster dendrogram of 50 accessions based on Hierarchical clustering

The first principal cluster comprised of 8 major sub clusters which included a total of 37 accessions. The second principal cluster consisted of 15 accessions in 3 sub clusters. In this principal cluster S-1574 and S-1576 were grouped together with 89% similarity. This set can be considered as the most similar accessions in the study. The third principal cluster comprised of the two wild species, *Ipomoea triloba and Ipomoea aquatica*. There was lot of intra-clusteral variability within each principal cluster. Within first cluster the intra-clusteral variation ranged from 0.58-0.84. Within principal cluster 2, variability ranged from 0.63-0.89.

Table 11. Accessions grouped based on ISSR markers

Clusters	Sub-Clusters	Accessions
Cluster 1	1A	S 1404, S 1405, S 1408, S 1527, S 1516
	1B	S 1409, S 1500, S 1438, S 1509, S 1505, S 1439, S 1442, S 1502, S 1503, S 1507, S 1506, S 1437, S 1440, S 1498, S 1504, S 1441, S 1499
	IC	S 1510
	ID	S 1508, S 1512, S1514, S 1666
	1E	S 1511, S 1517
	1F	S 1515, S 1522, S 1523, S 1518, S 1519, S 1525
	1G	S 1524
	1H	S 1600
Cluster 2	2A	S 1565, S 1598, S 1569
	2B	S 1662, S 1661, S 1660, S 1656, S 1658, S 1659, S 1433
	2C	S 1572, S1574, S 1576, S 1665
	2D	S 1663
Cluster 3		*I. triloba, I. aquatica*

4.2.5 Genetic relationships based on similarity matrix

An estimate of genetic relationships were depicted from the marker data using

Jaccard's similarity coefficient (Table 12). Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 89% to a minimum of 37%. The maximum similarity of 89% was observed between S1574 and S1576 (Bihar-2 and Bihar- 4) (Plate 24). The least similarity of 37% was observed between S1408 (IKSP-34) and S1572 (WB-5), and S1527 (Lembucherra-3) and S1572 (WB-5) (Plate 18). A similarity coefficient of 0.39 was observed between S 1408 (IKSP-34) and S1574 (Bihar-2), S1409 (IKSP-36) and S1663 (JASM 5A), S1404 (IKSP-22) and S1572 (WB-5). *I. triloba* and *I. aquatica* was similar by 69%. S1517 (Jowai-2) was the most similar sweet potato accession to *I. triloba* (63%) than all the other accessions. S1512 (Nongpon-3) was similar to both *I. triloba* and *I. aquatica* by 57%. S1662 and S1661 were similar to *I. aquatica* by 57% which is the maximum similarity observed between *I. aquatica* and sweet potato accessions in the present study.

4.3 MANTEL TEST

The Mantel statistic (r) value based on Pearson's correlation coefficient obtained as 0.0985 with a significance value (p) of 0.0003 indicated significant correlation between morphological and molecular marker information.

A. Similar accessions

S1574

S1576

B. Dissimilar accessions

S1572

S 1408

S1572

S1527

Plate - 18 (A) Accessions showing maximum (89%) similarity through molecular analysis

(B) Accessions showing least (37%) similarity through molecular analysis

Table 12 NTSYS-pc similarity coefficients between 54 accessions

	S1404	S1405	S1408	S1409	S1437	S1438	S1439	S1440	S1498	S1499	S1442	S1441	S1500	S1502	S1503	S1509	S1504	S1505	S1506	S1507	S1508	S1510	S1511	S1512	S1666	S1514	S1515
S1404	1.00																										
S1405	0.83	1.00																									
S1408	0.73	0.72	1.00																								
S1409	0.65	0.69	0.67	1.00																							
S1437	0.75	0.74	0.71	0.72	1.00																						
S1438	0.64	0.61	0.69	0.76	0.75	1.00																					
S1439	0.69	0.61	0.69	0.72	0.71	0.75	1.00																				
S1440	0.65	0.64	0.61	0.71	0.81	0.72	0.72	1.00																			
S1498	0.66	0.67	0.69	0.72	0.73	0.69	0.73	0.76	1.00																		
S1499	0.64	0.70	0.62	0.72	0.66	0.66	0.66	0.76	0.73	1.00																	
S1442	0.70	0.64	0.67	0.71	0.72	0.72	0.76	0.73	0.65	0.70	1.00																
S1441	0.66	0.67	0.73	0.70	0.71	0.73	0.69	0.70	0.71	0.71	0.67	1.00															
S1500	0.58	0.64	0.63	0.80	0.70	0.74	0.65	0.69	0.65	0.72	0.72	0.70	1.00														
S1502	0.65	0.62	0.65	0.69	0.70	0.72	0.72	0.73	0.67	0.70	0.69	0.67	0.66	1.00													
S1503	0.72	0.67	0.62	0.67	0.70	0.72	0.73	0.74	0.74	0.69	0.71	0.71	0.67	0.74	1.00												
S1509	0.72	0.75	0.72	0.73	0.71	0.72	0.73	0.75	0.64	0.67	0.74	0.70	0.73	0.64	0.74	1.00											
S1504	0.66	0.70	0.71	0.61	0.74	0.66	0.67	0.72	0.58	0.62	0.75	0.78	0.67	0.70	0.75	0.74	1.00										
S1505	0.56	0.62	0.58	0.64	0.64	0.63	0.66	0.71	0.72	0.65	0.70	0.70	0.80	0.61	0.72	0.73	0.65	1.00									
S1506	0.58	0.62	0.67	0.71	0.67	0.58	0.57	0.73	0.58	0.61	0.71	0.78	0.66	0.61	0.81	0.65	0.65	0.78	1.00								
S1507	0.61	0.64	0.65	0.69	0.76	0.65	0.60	0.66	0.52	0.63	0.73	0.72	0.73	0.61	0.83	0.62	0.63	0.75	0.82	1.00							
S1508	0.72	0.64	0.65	0.64	0.74	0.52	0.55	0.61	0.66	0.58	0.66	0.74	0.70	0.54	0.64	0.69	0.54	0.72	0.70	0.72	1.00						
S1510	0.58	0.64	0.71	0.63	0.64	0.60	0.58	0.66	0.51	0.62	0.61	0.67	0.73	0.69	0.70	0.66	0.64	0.65	0.69	0.71	0.72	1.00					
S1511	0.66	0.65	0.69	0.67	0.71	0.64	0.61	0.61	0.66	0.55	0.63	0.67	0.62	0.64	0.61	0.67	0.57	0.56	0.64	0.73	0.71	0.70	1.00				
S1512	0.64	0.67	0.69	0.67	0.73	0.62	0.67	0.64	0.51	0.62	0.63	0.67	0.62	0.63	0.61	0.65	0.58	0.58	0.69	0.64	0.73	0.67	0.69	1.00			
S1666	0.58	0.64	0.71	0.67	0.73	0.67	0.58	0.70	0.62	0.60	0.70	0.60	0.65	0.67	0.64	0.70	0.65	0.63	0.57	0.62	0.66	0.70	0.69	0.73	1.00		
S1514	0.66	0.65	0.69	0.67	0.71	0.67	0.61	0.65	0.66	0.62	0.67	0.71	0.67	0.62	0.69	0.67	0.64	0.56	0.67	0.67	0.61	0.66	0.67	0.65	0.67	1.00	
S1515	0.64	0.67	0.69	0.67	0.73	0.69	0.64	0.67	0.51	0.55	0.65	0.69	0.64	0.67	0.64	0.65	0.57	0.51	0.58	0.64	0.64	0.65	0.65	0.72	0.61	0.60	1.00
S1527	0.71	0.70	0.71	0.67	0.73	0.71	0.64	0.70	0.62	0.60	0.70	0.73	0.65	0.65	0.64	0.67	0.60	0.60	0.65	0.70	0.60	0.65	0.63	0.58	0.61	0.69	0.73

	S1404	S1405	S1408	S1409	S1437	S1438	S1439	S1440	S1498	S1499	S1442	S1441	S1500	S1502	S1503	S1509	S1504	S1505	S1506	S1507	S1508	S1510	S1511	S1512	S1666	S1514	S1515
S1516	0.66	0.67	0.66	0.65	0.60	0.64	0.57	0.61	0.60	0.55	0.61	0.64	0.58	0.63	0.64	0.56	0.69	0.61	0.65	0.72	0.71	0.61	0.67	0.58	0.56	0.64	0.71
S1517	0.57	0.56	0.57	0.58	0.55	0.48	0.57	0.58	0.57	0.48	0.56	0.60	0.56	0.61	0.62	0.56	0.62	0.54	0.54	0.58	0.64	0.65	0.70	0.63	0.65	0.69	0.61
S1518	0.57	0.56	0.60	0.54	0.60	0.55	0.53	0.52	0.48	0.51	0.49	0.55	0.63	0.58	0.53	0.49	0.60	0.52	0.49	0.58	0.62	0.63	0.52	0.58	0.63	0.62	0.73
S1519	0.55	0.56	0.60	0.56	0.57	0.66	0.66	0.58	0.57	0.57	0.65	0.62	0.67	0.67	0.62	0.58	0.66	0.65	0.56	0.63	0.69	0.67	0.61	0.63	0.61	0.66	0.69
S1522	0.47	0.53	0.56	0.57	0.56	0.61	0.56	0.53	0.54	0.47	0.55	0.49	0.60	0.57	0.54	0.57	0.63	0.60	0.53	0.60	0.63	0.60	0.62	0.64	0.60	0.63	0.74
S1523	0.52	0.60	0.52	0.57	0.58	0.58	0.52	0.62	0.56	0.58	0.57	0.56	0.62	0.64	0.58	0.60	0.63	0.55	0.55	0.60	0.65	0.62	0.62	0.60	0.62	0.58	0.76
S1524	0.52	0.53	0.49	0.53	0.49	0.54	0.52	0.48	0.49	0.58	0.51	0.54	0.57	0.62	0.52	0.53	0.52	0.51	0.53	0.57	0.58	0.62	0.60	0.60	0.64	0.52	0.66
S1525	0.54	0.55	0.56	0.55	0.58	0.58	0.56	0.60	0.58	0.54	0.53	0.58	0.53	0.60	0.49	0.57	0.63	0.60	0.60	0.60	0.65	0.62	0.66	0.69	0.66	0.67	0.67
S1600	0.61	0.66	0.58	0.62	0.56	0.54	0.58	0.60	0.67	0.63	0.55	0.63	0.55	0.57	0.61	0.55	0.63	0.55	0.55	0.64	0.63	0.60	0.64	0.55	0.55	0.67	0.57
S1565	0.57	0.58	0.57	0.52	0.55	0.51	0.55	0.56	0.57	0.51	0.52	0.55	0.49	0.63	0.55	0.58	0.55	0.56	0.54	0.56	0.66	0.56	0.58	0.67	0.63	0.62	0.58
S1569	0.58	0.55	0.47	0.48	0.49	0.49	0.52	0.48	0.43	0.52	0.53	0.40	0.51	0.53	0.56	0.55	0.54	0.53	0.46	0.53	0.52	0.51	0.51	0.55	0.64	0.58	0.62
S1598	0.62	0.58	0.55	0.52	0.53	0.51	0.62	0.54	0.55	0.55	0.61	0.57	0.49	0.58	0.62	0.54	0.60	0.56	0.56	0.58	0.57	0.54	0.58	0.58	0.52	0.60	0.61
S1572	0.39	0.43	0.37	0.43	0.44	0.46	0.48	0.52	0.53	0.53	0.40	0.53	0.40	0.49	0.51	0.47	0.51	0.45	0.47	0.45	0.48	0.58	0.49	0.49	0.52	0.46	0.57
S1574	0.46	0.49	0.39	0.47	0.48	0.44	0.48	0.49	0.42	0.51	0.45	0.51	0.45	0.49	0.53	0.49	0.46	0.47	0.49	0.52	0.46	0.63	0.52	0.52	0.63	0.46	0.46
S1576	0.51	0.54	0.44	0.49	0.51	0.51	0.55	0.56	0.48	0.55	0.49	0.57	0.47	0.52	0.57	0.56	0.55	0.54	0.56	0.56	0.55	0.58	0.54	0.56	0.63	0.53	0.53
S1665	0.46	0.47	0.42	0.43	0.44	0.48	0.48	0.56	0.42	0.55	0.47	0.48	0.47	0.56	0.53	0.56	0.51	0.52	0.45	0.47	0.55	0.54	0.40	0.52	0.61	0.56	0.55
S1662	0.58	0.55	0.52	0.48	0.54	0.54	0.54	0.55	0.49	0.54	0.51	0.56	0.55	0.53	0.56	0.60	0.56	0.55	0.53	0.55	0.56	0.51	0.53	0.55	0.55	0.48	0.53
S1660	0.54	0.53	0.47	0.55	0.47	0.54	0.56	0.53	0.52	0.52	0.53	0.54	0.53	0.55	0.58	0.55	0.58	0.55	0.53	0.57	0.56	0.55	0.55	0.53	0.53	0.56	0.53
S1661	0.52	0.51	0.47	0.48	0.43	0.47	0.52	0.51	0.45	0.47	0.46	0.49	0.51	0.46	0.54	0.51	0.52	0.51	0.51	0.53	0.54	0.53	0.57	0.48	0.55	0.50	0.52
S1656	0.54	0.53	0.49	0.46	0.49	0.47	0.47	0.48	0.47	0.47	0.46	0.52	0.51	0.48	0.56	0.51	0.49	0.44	0.51	0.55	0.54	0.51	0.53	0.51	0.55	0.47	0.58
S1658	0.49	0.48	0.52	0.51	0.49	0.54	0.52	0.53	0.52	0.56	0.46	0.56	0.60	0.53	0.49	0.55	0.54	0.53	0.48	0.51	0.58	0.51	0.55	0.53	0.55	0.47	0.58
S1433	0.54	0.55	0.49	0.48	0.47	0.52	0.52	0.51	0.43	0.52	0.48	0.47	0.53	0.60	0.61	0.51	0.49	0.48	0.55	0.55	0.54	0.51	0.44	0.51	0.60	0.56	0.54
S1663	0.49	0.51	0.45	0.39	0.52	0.54	0.47	0.57	0.52	0.45	0.51	0.56	0.44	0.51	0.54	0.55	0.58	0.53	0.55	0.51	0.52	0.53	0.42	0.53	0.44	0.52	0.56
S1659	0.45	0.46	0.45	0.48	0.47	0.52	0.49	0.55	0.47	0.49	0.42	0.47	0.51	0.48	0.47	0.46	0.49	0.48	0.44	0.44	0.47	0.53	0.51	0.48	0.51	0.47	0.47
l. triloba	0.52	0.48	0.52	0.48	0.43	0.52	0.47	0.46	0.49	0.47	0.51	0.54	0.51	0.53	0.47	0.57	0.54	0.51	0.53	0.51	0.58	0.53	0.60	0.57	0.57	0.52	0.49
l. aquatica	0.49	0.53	0.43	0.42	0.43	0.43	0.40	0.44	0.43	0.54	0.51	0.47	0.48	0.46	0.52	0.51	0.45	0.42	0.48	0.48	0.47	0.51	0.53	0.57	0.53	0.52	0.49

	S1527	S1516	S1517	S1518	S1519	S1522	S1523	S1524	S1525	S1600	S1565	S1569	S1598	S1572	S1574	S1576	S1665	S1662	S1660	S1661	S1656	S1658	S1433	S1663	S1659	Ltri	Laq
S1527	1.00																										
S1516	0.75	1.00																									
S1517	0.62	0.69	1.00																								
S1518	0.57	0.64	0.69	1.00																							
S1519	0.57	0.60	0.62	0.78	1.00																						
S1522	0.58	0.67	0.67	0.72	0.76	1.00																					
S1523	0.61	0.65	0.65	0.76	0.70	0.78	1.00																				
S1524	0.54	0.58	0.58	0.70	0.63	0.62	0.73	1.00																			
S1525	0.58	0.67	0.67	0.67	0.67	0.75	0.73	0.64	1.00																		
S1600	0.56	0.67	0.67	0.63	0.63	0.62	0.64	0.57	0.69	1.00																	
S1565	0.55	0.60	0.64	0.53	0.51	0.61	0.56	0.54	0.61	0.61	1.00																
S1569	0.52	0.52	0.61	0.61	0.58	0.66	0.62	0.53	0.57	0.55	0.70	1.00															
S1598	0.60	0.60	0.60	0.48	0.57	0.61	0.52	0.49	0.52	0.58	0.78	0.70	1.00														
S1572	0.37	0.44	0.53	0.51	0.55	0.52	0.52	0.52	0.54	0.56	0.62	0.58	0.57	1.00													
S1574	0.44	0.48	0.57	0.53	0.46	0.49	0.49	0.56	0.56	0.56	0.66	0.63	0.64	0.73	1.00												
S1576	0.48	0.53	0.60	0.53	0.53	0.56	0.52	0.54	0.63	0.61	0.69	0.63	0.69	0.73	0.89	1.00											
S1665	0.53	0.51	0.57	0.53	0.51	0.52	0.58	0.61	0.54	0.56	0.69	0.65	0.62	0.69	0.80	0.80	1.00										
S1662	0.56	0.58	0.58	0.54	0.54	0.55	0.53	0.53	0.60	0.55	0.72	0.73	0.72	0.58	0.65	0.65	0.72	1.00									
S1660	0.52	0.63	0.70	0.52	0.52	0.62	0.51	0.48	0.55	0.55	0.74	0.66	0.76	0.58	0.67	0.70	0.70	0.80	1.00								
S1661	0.58	0.67	0.67	0.52	0.52	0.60	0.55	0.57	0.60	0.55	0.72	0.64	0.67	0.58	0.67	0.65	0.70	0.80	0.80	1.00							
S1656	0.52	0.56	0.63	0.52	0.43	0.60	0.51	0.53	0.55	0.57	0.72	0.66	0.70	0.65	0.72	0.72	0.70	0.80	0.73	0.80	1.00						
S1658	0.52	0.52	0.63	0.54	0.52	0.60	0.53	0.57	0.55	0.53	0.67	0.60	0.65	0.58	0.65	0.67	0.67	0.75	0.78	0.73	0.80	1.00					
S1433	0.47	0.52	0.49	0.54	0.56	0.57	0.53	0.57	0.46	0.48	0.63	0.62	0.61	0.63	0.63	0.58	0.67	0.64	0.64	0.69	0.75	0.69	1.00				
S1663	0.43	0.56	0.54	0.45	0.52	0.51	0.48	0.48	0.51	0.48	0.65	0.51	0.58	0.61	0.63	0.61	0.65	0.66	0.69	0.66	0.66	0.64	0.66	1.00			
S1659	0.45	0.52	0.65	0.52	0.49	0.60	0.55	0.44	0.53	0.55	0.67	0.66	0.58	0.67	0.67	0.61	0.67	0.69	0.78	0.75	0.71	0.75	0.71	0.71	1.00		
I. triloba	0.43	0.49	0.63	0.54	0.47	0.57	0.57	0.62	0.57	0.51	0.61	0.53	0.56	0.54	0.52	0.54	0.52	0.55	0.62	0.57	0.55	0.60	0.53	0.51	0.57	1.00	
I. aquatica	0.40	0.43	0.49	0.52	0.49	0.46	0.55	0.53	0.51	0.46	0.54	0.55	0.49	0.54	0.54	0.45	0.47	0.57	0.53	0.57	0.55	0.53	0.51	0.51	0.55	0.69	1.00

5. DISCUSSION

Sweet potato (*Ipomoea batatas* (L.) Lam) is a herbaceous perennial plants belonging to Convolvulaceae family. *Ipomoea batatas* is the only species with edible roots in this genus. Its high nourishing ability makes it an important food crop mainly because of its high starchy roots. Sweet potato makes a significant contribution in the diets of developing countries.

The National Active Germplasm Site on sweet potato at ICAR-Central Tuber Crop Research Institute conserves about 1400 accessions of sweet potato collected from different parts of the world. Comprehensive information concerning locally available sweet potato germplasm is of vital importance for advancement of breeding works. To achieve this goal there is need for better understanding and reliable information about the genetic diversity that exists within the locally available sweet potato germplasm.

In the present study, the genetic diversity in the sweet potato accessions 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. Two wild species along with 52 sweet potato accessions were selected for the study. High variability was observed among the accessions. Predominant vine colour, leaf lobes type, abaxial leaf vein pigmentation, 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, plant type, mature leaf colour, vine internode diameter and vine internode length. Karuri *et al.* (2010) in his study with 89 genotypes of sweet potato observed that the general outline of leaf and the shape of central leaf lobe were the characters which allowed separation of accessions.

5.1.1 Statistical analysis

In the present study, the UPGMA cluster analysis using 18 variables separated the selected fifty four sweet potato accessions into two principal clusters at a Euclidean distance of 1.2. Among the two principal clusters, 2nd principal cluster was 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 S 1439 and S 1432 at a Euclidean distance of 0.2 (Log 10 transformed). General outline of leaf was the only character which separated these two accessions. Thus the study did not identify any duplicates. This is very low compared to Koussao et al. (2014) where, eight duplicates were obtained among 112 sweet potato germplasm collections taken from Burkina Faso in a diversity study.

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

5.2 PRIMER SCREENING FOR PCR

The DNA was isolated using Dellaporta method (Dellaporta et al., 1983) to obtain high quality DNA which is free from phenolic compounds. Angeles et al. (2015) used the same method to yield high-quality genomic DNA from coconut leaves.

17 ISSR primers were screened in the sweet potato accessions for selecting primers that gave a clear and good gel profile. A similar screening was done by Costa et al. (2000) before the analysis. They surveyed a total of 100 UBC primers using the gradient PCR and selected only 11 primers which produced four or more bands. Basha and Sujatha (2007) also screened ISSR primers. They investigated 100 ISSR primers and they selected only 48 out of the total primers as they gave considerable clear and good bands. 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.

5.2.1 ISSR analysis of sweet potato accessions

ISSR analysis is quick and simpler than most of the marker systems. It involves single primer for PCR amplification and binds to sequence repeats randomly with 1-3 bases that anchor the primer at 3' and 5' end. It circumvents the requirement of prior flanking sequence information as well as high expenditure of other marker systems. In this study the ISSR primers used were UBC 807, UBC 808, UBC 818, UBC 825, UBC 809, UBC 817, UBC 810, UBC 811, UBC 860, UBC 827 and (GA)$_9$AC.

ISSR will detect high level of polymorphism compared to other marker system (Nagaoka and Ogihara, 1996, Goulão *et al.*, 2001). The present study revealed moderate level of polymorphism of ISSR primers as the selected ISSR primers provided 89.8% polymorphic gel profile and obtained an average number of 7.3 polymorphic bands per primer. A total of 80 polymorphic bands were obtained with 11 primers, in the present study. The polymorphism obtained can be considered similar with the study of Moulin *et al.* (2012) done on sweet potato landraces. Using 19 primers they obtained 146 bands, 92.4% of which were polymorphic and each primer generated a mean of 7.1 polymorphic fragments. A higher value of mean band number per primer was obtained by Qiang *et al.* (2009). They used 10 ISSR primers and obtained a mean band number of 13.8 per primer and a total of 124 polymorphic bands were generated.

In the present study, the highest band number of 16 was obtained from UBC 808. The lowest number of bands (5) was obtained using the primers UBC 827 and UBC 860. So the number of polymorphic bands in the study ranged from 4 to 13 which are in contrast to that obtained in the study conducted by Qiang et al. (2009) among sweet potato accessions. The value ranged from 7 to 17 by using 10 ISSR primers and generated a total of 138 bands which is also high when compared to the present study.

5.2.2 Cluster analysis

Using the molecular scoring data, hierarchical clustering was done based on UPGMA using Jaccard's similarity coefficient and the fifty four accessions were

divided into two principal clusters and one outlier at a similarity coefficient of 0.56. Both principal clusters were further divided into sub clusters.

The first principal cluster consisted of 37 accessions in many sub clusters which indicates high intra-clusteral variability indicating the variability in the sweet potato accessions selected for the study. Accessions collected from the same geographical area were grouped together in a single cluster. However, a few accessions collected from the same locality are also separated apart. Fajardo *et al.* (2002) observed a high level of genetic similarity between accessions collected from the same locality. The dendrogram also indicated that some genotypes were genetically quite dissimilar from other genotypes collected from the same locale. The occurrence of these dissimilar accessions may be attributable to the outcrossing of the cultivated genotypes with sweet potato growing in the nearest area or the occurrence of an occasional off-type (introduction) within the locale. These arguments strongly supports the cluster data obtained in the present study.

The second principal cluster comprised of one set of two accessions with 89% similarity, both are collected from Bihar. This grouping was similar to that obtained with morphological data. *Ipomoea triloba* and *Ipomoea aquatica* were grouped as a third cluster because of their difference in banding patterns compared to all other sweet potato accessions and they showed their species specificity.

5.2.3 Similarity matrix

Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 89% to a minimum of 37%. This means that dissimilarity or variability ranged from 11-63 %. This indicates that high variability exist between the selected accessions.

Among the 54 accessions studied, the highest similarity index of 0.89 was obtained between S1574 and S1576 whereas the lowest similarity index (0.37) was obtained between S1408 and S1572 and between S1527 and S1572. S1408 and S1572 were collected from Chhattisgarh and West Bengal respectively and S1527 and S1572

were accessions collected from Tripura and West Bengal respectively. *I. triloba* and *I. aquatica* was similar by 69%. S1517 (Jowai-2) was the most similar sweet potato accession to *I. triloba* (63%) than all the other accessions. S1662 and S1661 were similar to *I. aquatica* by 57% which is the maximum similarity observed between *I.aquatica* and sweet potato accessions in the present study. This indicates that *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, 1978; 1979; Austin and Huaman, 1996).

Mantel test

Mantel's test (r =0.0985; p=0.0003) indicated significant correlation between morphological and molecular distance matrices. Thus both the data can be considered reliable in developing a core collection. ISSR markers have been used in combination with morphological descriptors to analyse genetic diversity in sweet potato germplasm and useful core collections have been developed using this combination (Li *et al.*, 2009; Karuri *et al.*, 2010).

6.   SUMMARY

The study entitled "Genetic diversity analysis of sweet potato (*Ipomoea batatas* (L.) Lam.) germplasm using morphological and ISSR markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2017-2018. Characterization of crop germplasm based on determination of amount and distribution of crop genetic diversity is necessary for proper utilization and conservation. Fifty four accessions which included two wild species of *Ipomoea* and 52 sweet potato accessions 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 descript tors (CIP *et al.*, 1991) for sweet potato. The percentage distribution of each trait for a particular character was obtained as it can be used to distinguish within species and among species differences in later studies. 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 and an outlier at a Euclidean distance of 1.2. No duplicates were observed. The first three principal components (PC) accounted for 67.5% of the variability and the highly loaded characters in the three principal components were predominant vine colour, leaf lobes type, abaxial vein pigmentation and petiole pigmentation (PC1), vine tip pubescence, leaf lobes type and immature leaf colour (PC2) , vine colour, secondary vine colour and petiole pigmentation (PC3) that contributed to the clustering of the sweet potato 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 A_{260}/A_{280} ratio of 1.76-2.11.

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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 °C. The PCR products were resolved in 2% agarose gel and clear polymorphic bands were obtained. Primers together showed 89.8% polymorphism and a total of 80 polymorphic bands were obtained. The number of bands ranged from 5 to 16 with an average mean value of 8.1 bands per primer and 7.3 polymorphic bands per primer. Primer UBC 808 produced the highest number of bands (16). 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 fifty four accessions were grouped into three principal clusters at 0.56 on similarity index scale. The first principal cluster included 37 accessions which were grouped in many sub-clusters and there was lot of intra-clusteral variation. The second principal cluster comprised of fifteen accessions, in which two accessions S1574 and S1576 were grouped together with 89% similarity. The third principal cluster comprised of the two wild species of *Ipomoea*. 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 S1574 and S1576 (0.89) and least similarity coefficient of 0.37 was observed between S1408 and S1572 and between S1527 and S1572. The morphological and molecular data were found to be positively correlated using the Pearson correlation coefficient. The molecular clustering of the accessions showed a very high range of intraclusteral 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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APPENDIX I

CTAB 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)	10 mM
EDTA	1 mM

APPENDIX III

TBE buffer (10 X)

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

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

APPENDIX IV

Chloroform: Isoamyl alcohol

Chloroform	24 ml
Isoamyl alcohol	1 ml

Mix 24 parts of chloroform with 1 part of isoamyl alcohol and store in a tightly

APPENDIX V

70% ethanol

100% ethanol	70 ml
Distilled water	30 ml

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GENETIC DIVERSITY ANALYSIS OF SWEET POTATO
(*Ipomoea batatas* (L.) LAM.) GERMPLASM USING
MORPHOLOGICAL AND ISSR MARKERS

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SABARINATH V.B.

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Abstract of Thesis

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Faculty of Agriculture
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DEPARTMENT OF PLANT BIOTECHNOLOGY
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ABSTRACT

Characterization of crop germplasm based on determination of amount and distribution of crop genetic diversity is necessary for proper utilization and conservation. This could be achieved through both morphological and molecular tools. This study entitled "Genetic diversity analysis of sweet potato (*Ipomoea batatas* (L.) Lam.) germplasm using morphological and ISSR markers" was carried out in the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2017-2018 with an objective to identify genetic diversity in the sweet potato germplasm based on morphological and molecular markers.

ICAR-CTCRI is the National Active germplasm site (NAGS) of tropical tuber crops which maintains 1400 accessions of sweet potato at Sreekariyam and its regional Centre at Bhubaneswar. 54 accessions of sweet potato including 52 accessions from eastern states of India and two wild species *I. triloba* and *I. aquatica* were selected from this collection.

The study consisted of two parts -morphological and molecular characterization. Morphological analysis was performed by using eighteen sweet potato descriptors as provided by IPGRI (CIP *et al.*, 1991). The recorded data was analyzed statistically by various tools such as PCA and cluster dendrogram using Multivariate statistical package (MVSP 3.22). The dendrogram separated into the accessions into two principal clusters and one outlier at a Euclidean distance of 1.2. The PCA analysis revealed predominant vine colour, leaf lobes type as the major variables that contributed to the clustering of the sweet potato accessions.

Molecular analysis 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 fifty four accessions. After the final PCR using selected primers, the product was resolved in 2% agarose and polymorphic bands were obtained. Primers showed 89.8% polymorphism and the number of bands ranged from 5 to 16 with a mean value of 7.3 polymorphic bands per primer. A total

of 80 polymorphic bands were obtained. The data analysed using NTSYS PC 2.02 program generated a dendrogram, which grouped the accessions based on Jaccard's similarity coefficient which separated the fifty four accessions into three principal clusters. The first principal cluster comprised of 37 accessions which were grouped into many subclusters and there was lot of intra-clusteral variation. The second principal cluster consisted of 15 accessions and this principal cluster comprised of two accessions with 89% similarity which were also found similar in morphological characterization. The third principal cluster comprised of the two wild species, *Ipomoea troloba* and *Ipomoea aquatica*. The similarity between the different accessions ranged between 37-89%. The accessions S1574 and S1576 were 89% similar. The least similar accessions were S1408 and S1572, S1527 and S1572 (37%). A high diversity of 63% existed within the selected accessions. Mantel's test also showed significant correlation (r = 0.0985; p = 0.0003) between the molecular and morphological distance matrices.

The hexaploid nature of the crop, self-incompatibility, along with the out crossing nature together might have contributed to the high variation observed among the accessions.