IDENTIFICATION OF DUPLICATES IN THE GERMPLASM OF SWEET POTATO (*Ipomoea batatas* (L.) LAM.) USING MORPHOLOGICAL AND MOLECULAR MARKERS

by

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DECLARATION

I hereby declare that this thesis entitled **"(Identification of duplicates in the germplasm of sweet potato (*Ipomoea batatas* (L.) Lam.) using morphological and molecular 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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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
CTAB	Cetyltrimethyl ammonium bromide
DNA	Deoxyribonucleic acid
DAF	DNA Amplification Fingerprinting
dNTPs	Deoxy nucleotide tri phosphates
EDTA	Ethylene diamine tetra acetic acid
EST	Expressed Sequence Tag
FAOSTAT	Food and Agriculture Organization Statistical Database
g	gram
GCDT	Global Crop Diversity Trust
h	Hour
ha	Hectare
ICAR-CTCRI	ICAR- Central Tuber Crops Research Institute
IPGRI	International Plant Genetic Resources Institute

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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
N	North
NAGS	National Active Germplasm Site
NaCl	Sodium chloride
ng	Nanogram
nm	Nanometer
^{o}C	Degree Celsius
OD	Optical density
PC	Principal component
PCA	Principal component analysis

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PCR	Polymerase chain reaction
PVP	Polyvinylpyrrolidine
RAPD	Random amplified polymorphic DNA
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	*Thermusaquaticus*
TBE	Tris-borate EDTA buffer
TE	Tris-EDTA buffer
Tris HCl	Tris (Hydroxy methyl) aminomethane hydrochloride
U	Enzyme units
UPSG	US Potato Gene bank
UV	Ultra violet
V	Volt
v/v	volume/volume
W	Watt
w/v	weight/volume
µg	Microgram

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µl	Microlitre
µM	Micromolar

INTRODUCTION

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1. INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is one of the most versatile tuberous root crop in the world. About 600 to 700 species are coming under the genus *Ipomoea* and *batatas* is the only species with edible roots. Now this single species itself satisfies the hunger of millions as the staple food of most of the developing countries. The developing countries alone contribute to 95% of the total sweet potato production (Veasey *et al.*, 2008). As per the report of FAOSTAT (2014), the total sweet potato production in the world is estimated as 106 MT in 8,352,323 hectares. In this, 85.9% of production is from Asia. Others contributions include as 2.3% from Americas, 0.1% from Europe, 0.6% from Oceania and 11.1% from Africa. In India, the average sweet potato yield is 10.2 t/ ha.

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

Sweet potato is an economically important crop in tropical, subtropical and warm temperate regions (Mervat *et al.*, 2009) and there are several varieties cultivated all over the world with large morphological variation. The variability is due to the out-crossing nature, self-incompatibility and hexaploidy as 2n = 6x = 90 (Ozias-Akins and Jarret, 1994) with basic chromosome number x = 15. Other species in this genus shows different ploidy levels: diploid (2x) to hexaploid (6x), with mostly tetraploid (4x) ones (Jones, 1968).

Sweet potato can adapt to a wide range of environmental condition including soil of poor fertility and comparatively low pH and also it has a very good drought tolerance capacity. It holds the highest solar energy fixing capacity

among the other food crops mainly due to its enormous capacity to produce dry matter for a long duration (Hahn, 1977).

Worldwide, sweet potato varieties and accessions are collected and maintained in germplasm collections. Presence of duplicates is one of the universal problems in every germplasm. The duplicated accessions may arise due to different accessions given similar names or same accessions having different names. However the problem can happen any time as there is a wide spread exchange of propagules all over the world (Ahikpa *et al.*, 2013). Often it leads to unnecessary expense in terms of labour, area and maintenance of germplasm. So duplicates must be brought into a reference status by the elimination of their copies (Naik *et al.*, 2006). These reference or core accession can be taken into the core collection and thus germplasm can be rationalised with seldom redundant material (Brown, 1989).

Globally, 36 collections have been identified holding 29,016 accessions of sweet potato genetic resources. Half (18 collections) include the bulk of the accessions analyzed; the other half correspond to smaller collections, representing 14% of the total accessions. One third of these are found in the global collection that CIP maintains in its genebank in Peru; the remaining two thirds, are shared by five Asian-Oceanian, one African, and one North American collections. The 36 collections analyzed maintain in total 29,016 accessions, including landraces, improved material and wild Ipomoea species (GCDT, 2007).

ICAR- Central Tuber Crops Research Institute, Sreekariyam is the National repository of tropical tuber crops germplasm or it is called the National Active germplasm site (NAGS) of tuber crops other than potato. At ICAR-CTCRI a total of 6000 accessions of various tropical tuber crops are being maintained. A total of 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 Characterisation of tuber crops germplasm is one of the major mandates of the Institute.

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Sweet potato germplasm maintained at ICAR-CTCRI consists of huge number of accessions acquired from various parts of the world through various sources and donors and collectors. A lot of variability exists in the leaves, stem and storage root characteristics. A lot of morphologically similar accessions also exist in the germplasm.

This merging of germplasm from different parts of the country often creates the possibility of duplicates in the germplasm. In order to identify the duplicates, it is essential to know about the genetic similarity and variability within the genotypes and genetic diversity studies can assist in duplicate identification and specific selection of parents for plant breeding purposes (Martins *et al.*, 2012). The need of genetic diversity analysis for estimating genetic similarity and for duplicate identification is also been reported by Chavarriaga-Aguirre *et al.* (1999).

To start with, the present study used the International Plant Genetic Resources Institute (IPGRI) descriptors for morphologically characterizing the sweet potato accessions to detect morphological similarity. The duplicates identified through morphological characterization alone may not be correct, as the expression of quantitative traits are often prone to environmental influences (Rao, 2004). So the molecular markers often need to accompany morphological and agronomic characterizations as they wrap the whole genome and are not environmentally influenced (Goulao and Oliveira, 2001). 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). A preliminary study using ISSR markers has revealed duplicates in a small collection of sweet potato germplasm (ICAR-CTCRI, 2015). So in this study, morphological characterization complemented with molecular characterization using ISSR primers was carried out.

REVIEW OF LITERATURE

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2. REVIEW OF LITERATURE

2.1 SWEET POTATO AS A CROP

Sweet potato is a dicotyledonous, perennial root crop from the family Convolvulaceae (morning glory family) grown widely in tropical, subtropical and temperate regions of the world and nowadays extensively known for its ability of high productivity per unit time which makes it inevitable during food scarcity (Walker *et al.*, 2011). Once Solomon islands had the most per capita production of sweet potato which was estimated to be 160Kg/person/year (Padmaja, 2009).

In Latin America, the crop has so many common names as camote, batata, batata doce, boniato, kumara and apichu. Systematic classification of sweet potato is as follows: it is included in the family, Convolvulaceae of the tribe, *Ipomoeae*. As in the name, it belongs to the genus *Ipomoea*. Eriospermum is both the sub-genus and section to which the crop belongs. Inside this section, it is coming under the same series and species namely Batatas (Huaman, 1999).

There are 13 closely related wild relative species of sweet potato. Among these, it was suggested that *I. trifida* is the most closely related one by Austin (1988). Huang and Sun (2000) attempted to prove this after restriction analysis of chloroplast DNA and characterisation using ISSR primer and found that *I. trifida* (diploid) is the closest relative of cultivated *I. batatas* and also they suggested that it might be the progenitor of sweet potato. Srisuwan *et al.* (2006) also came up with the same postulate by studying the organization and distribution of rDNA of its related wild species by using FISH technique. Several molecular techniques such as microsatellites (Buteler *et al.*,1999) and Restriction Fragment Length Polymorphisms (RFLPs) (Jarret *et al.*, 1992) also disclosed the close connection of *I. trifida* with *I. batatas.* Despite most of the techniques indicate the close relation between the two species, the exact close wild relative or progenitor is yet to be found out.

2.2 AGRO CLIMATIC CONDITIONS

Sweet potatoes are mainly grown between latitudes 42° N and 35° S on the equator and at an altitude of 3000 m from the sea level. Vine cuttings or rooted sprouts are used as propagating material in the tropics and temperate regions respectively and these are planted on mounds or ridges in the field. Mounds are widely used in tropics, mainly in soil moisture rich areas which enable water to easily drain out. This is beneficial as the plant does not thrive in water logging condition and the top soil quantity will also increase. Every mound will possess a height of 60cm at 90-120 cm distance between each one. In the case of ridges, height will be of 45 cm with an interval same as that of mounds (Woolfe, 1992).

If it is above the temperature of 24°C, excellent growth occurs. Adverse growth retardation will happen when the temperature is below 10°C. It grows best in a relative high light intensity while root formation and flowering are enhanced in short day lengths as in tropics. Annually it needs an optimum rainfall of 75-100 cm while in the growing season, the requirement is 50 cm. The remaining rain that gets in non-growing season is used up for the propagation and preservation of vine growth which is the planting material for next generation.

Organic matter rich sandy-loam soil along with a permeable sub soil is the most preferable one for the high growth of sweet potato. As the plant do not survive in water logging condition, good drainage is necessary. Dense soil characterised with low aeration will adversely affect the storage root formation and leads to very low quantity 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). Drought resistance is the other fine character exhibited by sweet potato and it can be cultivated on heavy clay as well as peat soils. It will grow on slopes of till 40°. As it is resistant to dryness, green vines can be seen even during the drought season. In Eastern Africa, animals are supplied with these healthy vines when there is a well-established drought condition (Jana, 1982).

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2.3 ORIGIN AND DISTRIBUTION

Although sweet potato is world renowned for its economic importance, the origin, timing and geographical location(s) are remaining uncertain (Roullier *et al.*, 2013). Scientists points towards somewhere near the Yucatan Peninsula of Mexico and the south of Ornico river in Venezuela as the centre of origin of sweet potato based on its morphological similarity with other *Ipomoea* species in the wild (Austin, 1988; Srisuwan *et al.*, 2006; O'Brien (1972). The place where the domestication of sweet potato started is believed to be in Central America at least 5000 years ago. Sweet potato remnants were obtained from Peruvian caves of South America in 8000 BC. Still a debate is going on about its origin (Ugent *et al.*, 1988). Several studies have been put forward by scientific community to identify the most likely primary centre of origin and most of them lead towards central America. One such revealing was done by Huang and Sun (2000) when they adopted molecular techniques for analysing diversity of *Ipomoea* species of Central America in view of abundance of wild relatives of sweet potato there. The secondary centres of origin other than America hold to be New Guinea, South East Asia, East Africa and China (Yen, 1982 and Austin 1988).

The dispersal of sweet potato throughout the world has been reported as Polynesian, Columbian and European introductions. However the best known and largely documented is of European voyagers. From West Indies, Columbus introduced the sweet potato (at that time known as "batata") into Europe in 1492 when his first voyage completed. Then the sweet potato travelled to Africa, Brazil, and India via Portuguese voyagers during the 16th century. At the same time, Spanish traders transferred it to Philippines from Mexico directly. This is evident from the linguistic word "camote line", the name came through its Mayan roots when the Mexican sweet potatoes are transferred between Acapulco, Mexico, and Manila in the 16th century itself. From there further dispersal occurred towards East Asian countries such as China and Japan (O'Brien, 1972 and Yen, 1982). The information regarding the pre historic dispersal of sweet potato is quite less. So several studies are going on and in a diversity assessment

done by Rossel *et al.,* (2001) using AFLP, it is suggested that the introduction of sweet potato into Oceania, could be from Meso American sweet potato via natural dispersal.

2.4. GENERAL PLANT MORPHOLOGY

In the genus *Ipomoea*, *batatas* is the only species that produces starchy thickened storage roots that is fit to be eaten. This vegetatively propagated crop exhibits growth habit predominantly as creepy nature with a vine system that spreads out very rapidly parallel to the ground in erect, semi erect, spreading and very spreading modes. The major types of root system include fibrous roots and the main storage roots (lateral roots) which are the store house of photosynthetic products. At the initial stage, fibrous roots are developing into lateral roots. Upon maturing lignified roots (pencil roots) and fleshy bulged unlignified storage roots are arised from the lateral roots (Huaman, 1999).

The general outline of leaves can be of rounded, reniform, triangular, hastate, lobed cordate and almost divided. About 3 to 7 lobes will be there in a leaf. Some cultivar exhibit different leaf shapes in same plant. Varying colours are possessed by both mature leaves and immature leaves that ranges between green, yellowish green and purple and some cultivar will be with mature green leaves and purple immature leaves.

The flowering ability differed among the cultivars and exihibits few flowering, non-flowering and copiously flowering types under regular conditions. The flower is bisexual with a cyme inflorescence after the division of peduncle into two axillary peduncles, each one is dividing again after the flower is formed thus it is biparous cyme. Sometimes, single flowers are also there. The calyx usually consists of 5 sepals as 2 outer and 3 inner and the corolla contains 5 petals fused to form a funnel with reddish to purple on the inner side and pale purple or lilac limb and as whole flowers can be seen in white or purple (Bovell-Benjamin, 2007).

Fruits are often capsule. The outer surface will be pubescent or glabrous. Upon maturity, the capsule attains brown colour. One to four seeds will be there

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in each capsule. The seed colour will be brown to black with varying shape that ranges from rounded, angular or irregular. Seed size is approximately 3mm. In sweet potato seed germination is very difficult. The commercial part of sweet potato is its storage root. It forms as clusters around the stem. Adventitious buds are formed on the surface of storage roots from where the sprouting is initiated.

Depending on the agro climatic conditions as well as the cultivar, the shape, skin colour and flesh colour can be varied. Shape of tuber will be of elliptic, round, obovate, ovate, oblong round-elliptic, long oblong, long irregular, curved and long elliptic. It exhibits different skin colors as whitish, yellow, cream, brown-orange, orange, red-purple, pink and very dark purple. White, cream, yellow, or orange coloured flesh can be seen in a variety of cultivars. Some shows purple throughout the whole flesh due to increased anthocyanin content (Huaman, 1999).

2.5. NUTRITIONAL AND HEALTH IMPORTANCE

Sweet potato is a crop with enormous potential to wipe out malnutrition from a whole country itself and this is already proven in many underdeveloped countries. The high starch levels in storage roots make it highly nutritious enough to be a staple food and also it can be utilized as a live stock feed all over the world (van Jaarsveld *et al.*, 2005). Sweet potato generally contains a variety of minerals such as iron, calcium, zinc, potassium, manganese, and sodium along with vitamin C (Antial *et al.*, 2006). Orange fleshed sweet potato (OFSP) has received much attention due to its ability to deliver high amount of β-carotene which enhances vitamin A production in body. A 100g of OFSP can deliver about 2400g of β-carotene which is necessary for daily routine activity and it has reduced the deficiency of vitamin A among the countries of sub Saharan Africa (Tumwegamire *et al., 2004*). van Jaarsveld *et al.* (2006) investigated the carotenoid content withholding capacity of OFSP after boiling and they observed 92% of retention when it boiled for 20 min.

Sporamin is the major storage protein found in sweet potato which accounts for about 80% of the total protein (Maeshima *et al.*,1985) and leaves are

also eatable as it is a good source of anti oxidants (Hue *et al.,* 2012), as well as the tremendous source of polyphenolics. When compared to other vegetables, a higher level of anthocyanins and phenolic acids such as caffeic, mono, di and tricaffeoylquinic acids can be seen in the leaves of sweet potato (Islam, 2014). So the leaves have gained recent attention and in Africa, leaves are also consumed along with the tubers and also it contains approximately 27% protein (Kure *et al.*, 2012). A recent study revealed non-alcoholic beverage can also be made from sweet potato (Wireko-Manu *et al.*, 2010).

2.6. GERMPLASM COLLECTION AND CONSERVATION

Genetic resource once considered unlimited are fast eroding as modern cultivars replace traditional cultivars over large areas and natural habitats of wild relatives of cultivated species are destroyed (Stamp *et al.* 2012). Genetic variation must be conserved and effectively utilized to combat new pest and diseases and to produce better adapted varieties for changing environments. The most important components of managing *ex situ* germplasm include well established procedures for collection/assembly, characterization, conservation and sound scientific approaches for effective utilization of conserved germplasm (Upadhyaya *et al.*, 2008). The fundamental objective of plant genetic resources is to capture the maximum amount of genetic variation in the smallest number of samples (Marshall and Brown, 1975)

2.7. GERMPLASM CHARACTERIZATION

Adequate characterization for morphological and agronomic traits is necessary to facilitate the utilization of germplasm by breeders. The development of efficient strategies depends on the extent of the information on the type of genetic variation in target taxa populations and the distribution in the target geographic region (Marshall and Allard, 1970). The major goal 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.

A large series of studies have been undertaken to study diversity, domestication, evolution and phylogeny of PGR, largely selected from gene bank collections. Early studies considered morphological and cytogenetic characters. Various other techniques and molecular markers have been applied subsequently (Kovach and Mc Couch, 2008). Until recently, amplified fragment length polymorphism (AFLP) or simple sequence repeats (SSR) were the molecular markers of choice for DNA fingerprinting of crop genomes (Allender and King, 2010). Owing to their amenability to systematic development and HT (high throughput) detection, SNP markers are increasingly being applied to study genetic diversity in germplasm collections of up to several hundreds of accessions.

2.8. PROBLEMS IN CONSERVATION

A germplasm constitutes samples of many cultivars from geographically different areas. Unfortunately, if the samples collected from different regions are of same cultivar that will leads to duplication of accessions in the germplasm (Huaman, 1992). The duplicates may show morphological resemblance in the field causing certain confusion on the samples and affect total maintenance of accessions. There are so many known and unknown reasons behind the duplication. One such reason is the economic importance of a species. Often the propagules of such species are exchanged between different regions of the world through institutes and farmers. When each propagule comes in a new place, they receive new individual names. Finally these genotypes come under a single germplasm and become duplicates (Moura *et al.*, 2013). Germplasm collections invariably contain duplicate accessions, both within and between genebanks. These redundancies are a burden for curators because they do not contribute to the diversity in the collection, but do require gene bank budget

for maintenance. Thus, both from a genetic and economic point of view, identification and elimination of redundancies should be an important gene bank objective (van Treuren *et al.*, 2001).

It is estimated that 50% of the accessions maintained in the gene banks world -wide are duplicates. Duplicates between gene banks occur for various reasons. One obvious cause is the exchange of accession between gene banks and acquisition of same accessions by several genebanks. Often material obtained for comparison in national variety testing trial was also deposited in the National gene bank; dividing a collected sample between participating centres in the collection expedition (Knuppfer *et al.*, 1997)

Duplicates within collections occur when by mistake a variety or an accession has been incorporated in the collection more than once. It might even be possible that the next generation of an accession has been incorporated in the collection more than once. It might even be possible that the next generation of an accession within a gene bank receives a new unique gene bank identification number and thus could be considered as new but duplicated accession.

One area that is often given low priority in the management of many germplasm collections is the elimination of duplicate and genetically redundant accessions. Eliminating duplicates is an effective method of reducing germplasm maintenance costs without losing valuable genetic resources (Greene and Pedrson, 1996). Presence of duplicates increases the cost of maintenance of accessions and slows down the process of evaluation of desirable traits for plant breeding programme (Huaman, 1992). It will also leads to expense of labour and wastage of plantation area. The success of a germplasm lies in maximum variability and minimum redundancy. As the duplicates make the germplasm more redundant, they must be eliminated (Naik *et al.*, 2006).

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2.9. DUPLICATION AMONG WORLD GENEBANKS

Duplication is being extensively observed among genebanks worldwide. Diederichsen (2009) has suggested that the more un-coordinated duplication can be seen within and among the germplasm collections of most of the gene banks that emerged after 1960s. Fowler (2007) reported that between 1984 and 1996, there is an increase in the number of gene bank accessions worldwide that ranges from 2 million to 6.5 million. The major reason was the presence of duplicates and "hyper-inflation" was the term used by him to depict this increment and the word means large amount of redundant accessions. He also pointed out that the germplasm was maintained in a "totally unco-ordinated and unknown way". The expense will be huge for maintaining a large germplasm. So the genebanks are often forced to rationalize their contents in order to make efficient germplasms. The first step to achieve this aim is to identify the duplicates within and among the germplasm collections (van Hintum and Visser, 1995). Rationalization efforts were being started efficiently from the 1980's when Frenkel, (1984) came up with the idea of developing "core collection" in the germplasm. A core collection is a reference status of the whole diverse collection and it forms the base resource to represent each identified set of a collection (van Hintum *et al.*, 2000).

Diederichsen (2009) assessed the number of duplicates within 339 *Avena sativa* L. accessions of the Canadian National Seed Gene bank which conserves about 27,000 accessions of this genus and the elimination of duplicates resulted in an exclusion of about 33% of the accessions. Similar effort was made by van Hintum and Visser (1995) among barley accessions maintained in the Center for Genetic resources, the Netherlands (CGN). They compared a set of 100 accessions inside the CGN barley collections and among the CGN and three other European barley collections. They found that 61% of accessions in all the four collections were probable duplicates. The four collections were from CGN, John Innes Institute (JII) in United Kingdom, Braunschweig Genetic Resources Centre

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(BGRC) and The Institute of Plant Genetics and Crop Plant Research (IPK) both in Germany.

Del Rio *et al.* (2006) compared the levels of similarity between duplicated accessions from two major gene banks of potato. One from the US potato gene bank (UPSG) of U.S.A. and the other from International Potato Center (CIP). They analysed the genetic similarity between suspected duplicate sets held in both the gene banks, because those accessions were originated from the dispersed descendants of a single mother germplasm collection. So they proceeded with an assumption that in spite of different resident locations, there will be genetic identity between the accessions maintained in the two gene banks. They used molecular markers for this analysis.

2.10. DUPLICATION AMONG SWEET POTATO GERMPLASMS

The largest collection of sweet potato (over 8018 accessions) is preserved in the International Potato Center (Rossel *et al.*, 2009). It is famous by Spanish abbreviation "CIP", launched in 1971 by a declaration of Peruvian government. CIP germplasm comprises of collections from so many countries and research centers. The major intend of this global center is to wipe out malnutrition in the developing countries by the production and conservation of roots and tuber crops. So several research programmes are being developed there for improving the quality and quantity of the roots and tuber crops and such information is being delivered to the countries where food scarcity is there. Scientists from such countries are also trained there for a better welfare of poor people (Pallais, 1991).

According the annual report of CIP in 1988, there were duplicates in the sweet potato germplasm collection from Peru and the duplicated accessions represented 66% of the total collection (Rossel *et al.*, 2009). The elimination of duplicates decreased the number of Peruvian sweet potato collection from 1939 to 909 (Mok *et al.*, 1997). During the time period between 1990 and 1996, more than 1200 Indonesian sweet potato accessions were there in CIP. About 40% of those were found as duplicates upon evaluation (Mok and Schmiediche, 1999).

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2.11. 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).

In sweet potato several morphological characterizations have been done all over the world for diversity analysis (Afuape *et al.*, 2011; Laurie *et al.*, 2013 and Placide *et al.*, 2015) and duplicate identification in the germplasm. Duplicates can be identified by morphological characterization in sweet potato, but being asexually propagated, the crop is amenable to mutation as well as it shows high variability in phenotypical and morphological traits. So the morphological characterization in sweet potato is quite difficult when compared to other tuber crops (Huaman *et al.*, 1999; Woolfe, 1992). The crop differs in various traits such as size, colour and shape of leaf and root as well as in their intensities also. The storage root skin colour and more frequently flesh colour are most amenable to somatic mutation in sweet potato (Huaman *et al.*, 1999).

Descriptors of sweet potato were developed by Huaman (1988) in the CIP, Peru and it is being used traditionally for characterization in sweet potato. IBPGR has descrbed morphological characterisation as "the recording of characters which are heritable, expressible in all environments and can be seen easly with naked eye (IBPGR, 1985; CIP *et al.*, 1991).

But morphological markers are often providing less genetic information (Rao, 2004) and also morphological traits are highly vulnerable to the environmental conditions (Gepts, 1993; Prakash *et al.*, 1996). So they cannot provide a scrupulous assessment regarding duplication in germplasm as it holds samples from different locations. Therefore morphological characterization is

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often harmonized with molecular characterization as the molecular marker does not change according to the environment and possess greater accuracy in locating the genes in its exact place (Westman and Kresovich, 1997).

2.12. MOLECULAR MARKERS

Molecular markers do their duty by finding the variation in the DNA level and exihibit adequate polymorphism to distinguish between genotypes (Kumar *et al.*, 2009). For assessing genetic variation, molecular characterisation of germplasm, has become a routine process, and has revolutionized agricultural field by the employment of more precise, quick and cheap molecular markers (Spooner *et al.*, 2005). Molecular markers have been proved to be a powerful tool in various genetic analyses because of their mere simplicity, easy conduct, and moreover unaffected by environmental influences even during plant growth stage and it assess immeasurable pool of genetic variability (Gepts, 2002). Molecular analysis can complement traditional approaches towards identifying duplications. Interpretation of molecular data is, however, by no means straightforward because various genetic relationships among potential duplicates may occur (van Treuren *et al.*, 2001).

During the last few decades, distinct and different molecular techniques have been developed for a variety of purposes including assessment of genetic variation (Karp *et al.*, 1996), genetic linkage map construction (Klein *et al.*, 2000), diversity assessment and core collection development (Zhang *et al.*, 2000).

Random Amplified Polymorphic DNA (RAPD) were the first one of PCR-based markers and were developed by Williams *et al.* (1990) and Welsh and McClelland (1990) in the same year independently. RAPD involves random amplification of fragments with single primer from an arbitrary nucleotide sequence and the primers are of 10 nucleotide length (decamer) (Kumar and Gurusubrahmanyan, 2011) and it does not require prior target sequence knowledge (Wiliams *et al.*, 1990).

Amplified Fragment Length Polymorphism (AFLP) is a technique based on PCR amplification which possess high multiplex ratio and described for the first

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time by Vos *et al.* (1995). The procedure includes the selective amplification of specific restriction fragments after the digestion of total genomic DNA (Lin and Kuo, 1995). So it combines both the integrity of restriction fragment length polymorphism (RFLP) and the advancement of PCR based methods. Moreover it does not require the prior knowledge of the sequence and are reproducible as well as produced in large numbers (Hoda *et al.*, 2012). The production of AFLP is comparatively expensive and is dominant in nature (Lin *et al.*, 1996).

Simple Sequence Repeats (SSR) markers or microsatellites are tandem short repeats of about 1-6 base pairs. It is co-dominant and the technique requires the prior knowledge of the flanking sequences. Thus the primers should be of 20-25 bp length in order to amplify the SSR regions by PCR (Spooner *et al.*, 2005). The development of SSR is rather expensive and time consuming (Wang *et al.*, 2009).

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

2.13. Inter Simple Sequence Repeats (ISSR)

Inter Simple Sequence Repeats (ISSR) are one of the arbitrary, PCR based dominant markers amplified with the use of single primer sequence. It is based on the amplification of regions between 100 and 300 bp, between two adjacent microsatellites that lies in opposite direction (Zietkiewicz *et al.*, 1994). The marker system developed from ISSR consists of single primer based reaction and the primer sequences will be the repeats of di- or tri nucleotide bases and it is being successfully used among a wide variety of plants including both cultivated and natural populations (Wolfe *et al.*, 1998). ISSR primers can rectify the difficulties in finding a prior sequence knowledge unlike while using those of SSR and also this modified SSR-dependent marker system can produce very much heritable fingerprinting patterns (Liu and Wendel, 2001).

ISSR requires low start up costs and does not possess reproducibility problems like RAPD. The high reproducibility of ISSR markers is mainly attributed by the use of its longer primer sequences. Also it possess effective high multiplex ratio as the microsatellites are abundant and ever-present in the genome of eukaryotes. These two major features make this marker system inexpensive and less time spending even when the bands are resolved in PAGE gels (Goulao *et al.*, 2001). Being a dominant marker, it cannot differentiate genotypes into heterozygote and homozygote. But it can produce multiple locus sites from every PCR reaction (Goulao and Oliveira, 2001).

Inter-SSR fingerprints have been proved as highly versatile tool, with varying applications such as to study genome organization (Pakinskiene *et al.*, 2000), gentic diversity and genetic identity analysis (Fernandez *et al.*, 2002), species polymorphism studies (Joshi *et al.*, 2000), genome mapping, population studies and cultivar identification (Melo *et al.*, 2011).

2.14. MOLECULAR MARKERS IN DUPLICATE IDENTIFICATION

Molecular markers have been found effective in detecting duplicates as well as rationalizing of germplasm in tuber crops such as potato (van Treuren *et al.*, 2004), taro (Mace *et al.*, 2006), as well as in other crop germplasms *viz.* apple (Gross *et al.*, 2012), mulberry (Naik and Dandin, 2006), *Citrus* (Fang and Roose, 1997), all over the world.

A core collection of cassava (*Manihot esclenta cranz.*) comprising of 630 accessions was subjected for characterization using SSR, isozymes and AFLP to evaluate the variability and redundancy of accessions. The molecular markers generated an estimated detail of allelic frequency and diversity of the core collection. The presence of duplicates (1.34%) was revealed by genotyping with isozyme and AFLP and this analysis enabled them to develop a core collection with very less redundant material (Chavarriaga-Aguirre *et al.*, 1999).

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In order to analyse the amount of redundancy in *Dioscorea spp.* collection from Nigeria, Mignouna *et al.* (2005) identified the duplicates among the 65 accessions including wild and cultivated yam species using DS-PCR and RAPD. A total of 37 accessions which were coming under 12 duplication groups were identified in the study.

Ipek *et al.* (2003) determined the duplicates in the garlic germplasm by assessing the genetic diversity among 45 garlic clones and three *Allium longicuspis clones* by using RAPD, AFLP along with isozymes. They found that AFLP was more efficient among the three in detecting polymorphism and thereby finding duplicates among the garlic clones.

Duplicates in the germplasm collection of *Oryza sativa* in International Rice Research Institute (Philippines) had been identified by Virk *et al.* (1995). They used RAPD and it enabled them to discriminate both putative and true duplicates in the germplasm. About 80,000 accessions of rice were there in IRRI at that time and they selected 44 samples as set of suspected and true duplicates. They carried morphological characterisation also along with RAPD technique and found that PCR-based marker techniques are more reliable for germplasm management.

Zhang *et al.* (2009) had conducted the molecular characterisation of cacao (*Theobroma cacao* L.) for identifying duplicated accessions in order to reduce the redundancy of its germlasm maintained in Costa Rica by using 15 Simple Sequence Repeat loci, in capillary electrophoresis. After this, they came to a conclusion that the germplasm was contaminated with high amount of redundant genetic material.

Upadhyay *et al.* (2013) have identified duplicates among 317 grape vine germplasm in the National Active Grape Germplasm site in India using 25 microsatellite markers. They got only 254 accessions with unique genotypes and rest 63 of those as duplicates by the analysis of microsatellite data. They concluded that microsatellite analysis will help them for creating a non-redundant grape vine germplasm.

2.15. ISSR reveals on duplicates

There are only few studies that stress on molecular marker mediated duplicate identification alone, rather it comes as a part of diversity analysis. So most of the times, duplicates are identified at the same time when genetic variability is analysed.

Ten coriander genotypes were characterized using ISSR by Melo *et al.* (2011) in order to evaluate genetic similarity, thereby finding the duplicates present in the germplasm. The study was conducted using 29 oligonucleotide sequences of ISSR and generated a total of 227 fragments. The highest band number generated is 16 by UBC 897. The calculated similarity obtained ranges from 52 to 75%. The study suggested that an efficient DNA polymorphism was detected in ISSR profiles of coriander.

Costa *et al.* (2017) analysed the genetic diversity among *Desmanthus spp.* accessions to eliminate the duplicates as well as in finding variability within the genotypes using ISSR and morphological traits. *Desmanthus* spp. accessions are maintained in the forage legumes gene bank of Universidade Federal Rural de Pernambuco (UFRPE) in Brazil. A similar study was conducted by Rocha *et al.* (2012) using nine ISSR primers among one of a special cultivar named 'uba' mango tree cultivated widely in Zona da Mata region of Brazil in order to identify the genetic similarity as well as the variability among 102 accessions.

2.16. Molecular marker studies in sweet potato

During the last decade plenty of molecular information has been revealed by different molecular markers and used for various kinds of analyses in sweet potato germplasm (Soegianto *et al.*, 2011).

Rossel *et al.* (2009) conducted a duplicate analysis study based on AFLP along with morphological markers among the Peruvian sweet potato collection maintained at CIP. About 360 accessions were taken for the study of which 197 were identified as duplicates. They used 33 morphological descriptors and seven AFLP primer sets for morphological and molecular characterization. A similar

study revealed the efficiency of AFLP marker when 97 sweet potato accessions were characterized using 10 primer combinations and gave a total of 202 clear polymorphic bands. It was found to be an efficient tool to analyse the genetic diversity as well as in finding genetic similarity among sweet potato accessions maintained in the germplasm collection of Tanzania (Elameen *et al.*, 2008).

SSR analysis of 112 sweet potato accessions in Burkina Faso with the aim to develop a core collection identified 5 duplicates using 28 SSR primer pairs and these markers were more elucidative in differentiating the accessions while eleven duplicates were identified by eight morphological markers (Koussao *et al.*, 2014). Wang *et al.* (2011) have been developed EST derived SSR markers in sweet potato. As the development of SSR markers is quite time spending and labour demanding, they used EST database to derive SSR markers at at a very cheap rate. Thus they were able to to harvest high amount of genetic SSR marker resources for sweet potato. The efficiency of SSR to characterize sweet potato germplasm has been proved by several researchers (Zhang *et al.*, 1999, Karuri *et al.*, 2010, Yada *et al.*, 2010, Veasey *et al.*, 2008).

RAPD technique has been applied to several aspects of sweet potato research such as linkage mapping (Thompson *et al.*, 1997), cultivar detection (Connolly *et al.*, 1994) and DNA finger printing (McGregor *et al.*, 2001). RAPD analysis of sweet potato accessions from chilli and all over the world corroborated the high genetic variability existing in sweet potato genome. 28 cultivars were selected from all over the world and 14 from Chile alone. 18 RAPD primers produced 124 highly polymorphic bands in those 28 cultivars. The characterisation of 14 accessions from Chile displayed complete uniformity with very little genetic variation and these might have originated from a single cultivar (Sagredo *et al.*, 1998).

DNA amplification finger printing (DAF) technique employed in 73 sweet potato accessions collected from all over the world and America, revealed reliable fingerprinting profiles with the use of seven octamer primers and found to be an effective tool in finding duplicates in sweet potato germplasm as it showed high genetic polymorphism in the sweet potato collection (He *et al.*, 1995). Another

marker system called Selective Amplification of Microsatellite Polymorphic Loci (SAMPL) also employed in sweet potato (Tseng *et al.*, 2002).

2.17. ISSR in sweet potato

ISSR finds a lot of applications in crop plants and its utility in sweet potato has been reported by several researchers (Hu *et al.*, 2003, Huang and Sun, 2000, and Rout *et al.*, 2010). The higher reliability and polymorphic ability of ISSR with respect to other marker system such as RAPD has been reported by fewer researchers (Moisan *et al.*, 2001, Nagaoka and Ogihara, 1996, Goulão *et al.*, 2001).

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

Moulin *et al.* (2012) studied the genetic relationship among 78 sweet potato cultivars in Brazil, which were collected from traditional farmers and characterized using ISSR along with RAPD.

Another study was conducted by Hu *et al.* (2003) in order to analyse the genetic diversity as well as the relationship between 34 sweet potato accessions and its wild relatives using selected eight ISSR primers. Cluster analysis grouped most of the sweet potato accessions based on their geographical origin. 70 polymorphic and reproducible ISSR bands were obtained using 22 SSR primers. Among the 70 ISSR bands, a linkage was observed between two simplex ISSR markers, which indicate ISSR markers are sufficient for fingerprinting in cultivated sweet potato and its wild relatives.

MATERIALS AND METHODS

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3. MATERIALS AND METHODS

The study entitled "Identification of duplicates in the germplasm of sweet potato (*Ipomoea batatas* (L.) Lam.) using morphological and molecular markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2016-2017. This section accounts for procedures and experimental materials used in the study.

3.1 PLANT MATERIALS

Ipomoea batatas accessions collected from various geographic locations are conserved in the sweet potato germplasm repository of ICAR-CTCRI, from which 50 accessions were selected for the present study which includes three sets of morphologically similar or suspected duplicate accessions. 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 April 2016. Weeding and intercultural operations were carried out as per standard procedure. The details of the accessions and their geographical origin are given in Table 1.

3.2 MORPHOLOGICAL OBSERVATION

Descriptors listed by the International Plant Genetic Resources Institute (IPGRI) were used to take morphological observations (CIP *et. al.*, 1991) using 20 descriptors were listed of which 18 are vine and leaf characters and two are storage root traits. The observations on leaf and vine were made after 45 days of planting. The tuber characters were recorded after the harvest.

Plate 1: Sweet potato field with plants at full growth stage

Table 1. Passport data of the sweet potato accessions used for the study 44

Sl.No	Acc. No.	Country of origin	Cultivar name	Type	Locality of collection	Year of collection	Donor/ collector name
1	S-236	India	Vella	LR	Kasargode	1965	CTCRI
2	S-256	India	IB14	LR	Coorge, Karnataka	1965	CTCRI
3	S-295	India	ND-135	LR	West Bengal	1966	CTCRI
4	S-298	India	ND-153	LR	West Bengal	1971	CTCRI
5	S-731	India	HT0 -35	HET	Kerala	1982	CTCRI
6	S-1026	Nigeria	TIS9068 (13)	INT	Nigeria	1987	IITA Nigeria
7	S-203	India	RED	LR	Kuppad, Kerala	1965	CTCRI
8	S-625	India	5X-20	PENT	Kerala	1982	CTCRI
9	S-651	India	5X-58	PENT	Kerala	1982	CTCRI
10	S-729	India	HTO-33	HET	Kerala	1982	CTCRI
11	S-733	India	HTO-40	HET	Kerala	1982	CTCRI
12	S-737	India	Mut-2	MUT	Kerala	1983	CTCRI
13	S-738	India	Mut-1	MUT	Kerala	1983	CTCRI
14	S-739	India	Mut-4	MUT	Kerala	1983	CTCRI
15	S-740	India	Mut-5	MUT	Kerala	1983	CTCRI
16	S-747	India	Mut-10	MUT	Kerala	1983	CTCRI
17	S-755	India	Mut-18	MUT	Kerala	1983	CTCRI
18	S-772	India	Goa Local	LR	Goa	1983	CTCRI
19	S-776	India	Basthar-1	LR	Madhya-pradesh	1983	CTCRI
20	S-1700	India	OPS1	OP	Trivandrum	2015	CTCRI
21	S-1701	India	RNK-2015-1	LR	Sirsi, Karnataka	2015	CTCRI
22	S-1650	India	SP-12-SP (SARSE-12)	LR	Joida Karnataka	2014	CTCRI
23	S-1702	India	RNK-2015-2	LR	Sirsi, Karnataka	2015	CTCRI
24	S-1596	India	AF2	LR	Assam	2014	Assam farmers
25	S-1606	India	SD-10	OP	Trivandrum	2012	CTCRI

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26	S-1703	India	RSM-2015-1	LR	Katel,Joida Karnataka	2015	CTCRI
27	S-1704	India	JAS-9-White	LR	Waynad	2015	CTCRI
28	S-1705	India	RSM-2015-4	LR	DeliyeJoida Karnataka	2015	CTCRI
29	S-1607	India	Varun-progeny (SV-1-2014)	OP	Trivandrum	2012	CTCRI
30	S-1603	India	SD-11	OP	Trivandrum	2012	CTCRI
31	S-1609	CIP,Peru	526/7	OP	CIP	2012	CIP
32	S-1401	India	TVM-local	LR	Trivandrum	2006	CTCRI
33	S-1652	India	SP-18-SP(SARSE-18)	LR	Joida Karnataka	2014	CTCRI
34	S-1653	India	SARSE-13	LR	Joida Karnataka	2014	CTCRI
35	S-1706	India	RSM-2015-5	LR	Wagabande, Joida Karnataka	2015	CTCRI
36	S-1707	India	RSM-2015-2	LR	KatelJoida Karnataka	2015	CTCRI
37		India	S.Arun	Released variety	Trivandrum		CTCRI
38	S-1654	India	Sp-7-SP	LR	Joida Karnataka	2014	CTCRI
39	S-1610	CIP,Peru	665/4	OP	CIP	2012	CIP
40	S-1655	India	ASSAM-1-2014	LR	Assam	2014	CTCRI
41	S-1708	India	RSM-2015-3	LR	Deliye,Joida Karnataka	2015	CTCRI
42	S-1709	India	RSM-2015-6	LR	Wagabande, Joida Karnataka	2015	CTCRI
43	S-1656	India	SWARNA-AP	Released variety	Arunachal Pradesh	2014	CTCRI
44	S-1710	India	JAS-10-PINK	LR	Wynad	2015	CTCRI

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45	S-1657	India	SARSE-7	LR	Joida Karnataka	2014	CTCRI
46	S-1611	India	SD-24	OP	Trivandrum	2012	CTCRI
47	S-1612	India	SD-29	OP	Trivandrum	2012	CTCRI
48	S-1613	India	SD-39	OP	Trivandrum	2012	CTCRI
49	S-1614	India	SD-53	OP	Trivandrum	2012	CTCRI
50	S-1615	India	SD-55	OP	Trivandrum	2012	CTCRI

*LR-land race;OP-open pollinated MUT-Mutant,PENT-Pentaploid;INT-International;HET-Heteroploid

3.3 MORPHOLOGICAL DATA ANALYSIS

The morphological data were recorded by phenotypic observation of the selected accessions. Each character and traits were recorded by assigning certain numerical value to them as per the IPGRI descriptor states of sweet potato (CIP *et. al.,* 1991) as shown in Table No. 2. All the recorded morphological data were tabulated in excel sheet for further statistical analysis. Morphologically similar or suspected duplicates are depicted in Plate 2, 3 and 4.

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

Sl.No:	Characters	Descriptor states
1	Twining	Non-twining-0,Slightly twining-3,Moderately twining-5,Twining-7,Very- twining-9
2	Plant type	Erect (<75 cm)-3, Semi-compact (75 – 150 cm)-5, Spreading (151 – 250 cm)-7, Extremely spreading (>250 cm)-9
3	Ground cover	Small-3, Medium-5, High-7
4	Internode diameter	Very thin (< 4mm) -1, Thin (4 - 6 mm) -3, Intermediate (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

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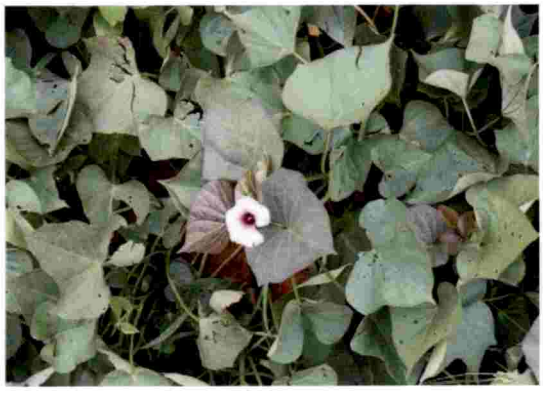
7	Secondary vine color	Absent-0, Green base-1, Green tip-2, Green nodes-3, Purple base-4, Purple tip-5, Purple nodes-6
8	Vine tip pubescence	None-0, Sparse-3, Moderate-5, Heavy-7, Very heavy-9
9	General leaf outline	Rounded-1, Reniform-2, Cordate -3, Triangular-4, Hastate-5, Lobed-6, Almost divided-7,
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
19	Predominant skin color	White-1, Cream-2, Yellow-3, Orange-4, Brownish orange-5, Pink-6, Red-7, Purple red-8, Dark purple-9
20	Predominant flesh color	White-1, Cream-2, Dark cream-3,Pale yellow-4, Dark yellow-5, Pale orange-6, Intermediate orange-7, Dark orange-8, strongly pigmented with anthocyanins-9

Plate 2. Morphologically similar accessions



Sree Arun

SV-1-2014

S-740

S-739

S-729

S-651

Plate 3 : Morphologically similar accessions

S-203

S-1026

S-731

S-776

S-256

S-236

Plate. 4. Morphologically similar accessions

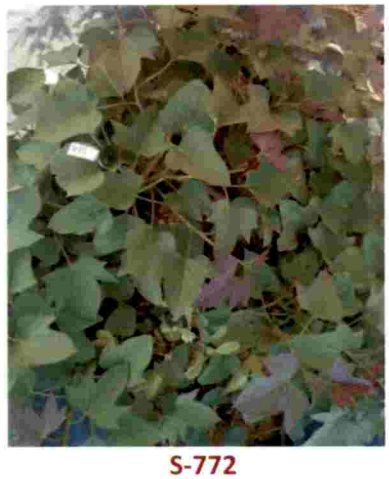
S-738

S-740

S-739

S-747

S-755



S-772

3.4.4 Diversity parameters

Percentage distribution of traits and Shannon's diversity index (H) are calculated. Percentage distribution of traits across the 50 accessions was estimated by calculating the proportion of accessions expressing particular trait as percentage.

Shannon's diversity index (H), a commonly used diversity index to measure species diversity also called as Shannon -Weaver index. It was calculated using the following formula:

$$H = -\sum [pi \times ln(pi)]$$

Where,

Pi = Proportion of a particular character i.e. number of individuals with a character/total number of individuals.

3.4.1 Cluster Analysis

To bring out the patterns of similarity and dissimilarity, data were subjected to UPGMA method of clustering based on similarity coefficients. Morphometric analysis was performed based on R statistical package 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.

3.4.3 Principal Component Analysis

The tabulated data was subjected to multivariate analysis by numeric taxonomic techniques using the principal component analysis (Sneath and Sokal, 1973). PCA was performed to analyse the contribution of traits in separating accessions into different groups and also to compare the clustering of accessions with respect to each cluster in a dendrogram.

3.5 DNA EXTRACTION

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3.5.1 Sample collection

Fresh and tender young leaves of sweet potato accessions were collected from the germplasm of CTCRI, Thiruvananthapuram. The leaf samples were collected in small plastic bags and brought to the lab in an ice box.

3.5.2 CTAB Method

DNA was extracted from fresh tender and young leaf samples using CTAB method of DNA extraction for sweet potato.

Leaves collected were weighed out; about 80-120 mg was ground in a pre-cooled mortar and pestle to a fine powder using liquid nitrogen. Pre-warmed CTAB extraction buffer (2 ml) containing β-mercaptoethanol was added to the powdered mixture and ground once more along with a pinch of PVP. The homogenised lysate was transferred into 2.0 ml eppendorf tubes and incubated at 65°C for 30 minutes with frequent shaking. The mixture was then centrifuged at 12,000 rpm for 10 minutes at 4°C. The resultant supernatant was transferred to another fresh 2.0 ml eppendorf tube. Equal volume of chloroform: isoamyl alcohol (24:1) were added and mixed by gentle inversion. The mixture was then centrifuged at 10000 rpm for 10 minutes at 4°C and the supernatant was transferred to another fresh 2 ml tube. In order to remove RNA contamination 8 µl of RNase was added and the mixture was incubated at 37° C for 1hr. This mixture was further extracted in an equal volume of chloroform: isoamyl alcohol (24:1) by centrifugation at 10000 rpm for 10 minutes at 4° C. The supernatant was then transferred into a fresh 1.5 ml tube. The extracted supernatant was diluted by adding 0.33 volume of ice-cold isopropanol and mixed by inversion in order to precipitate the DNA. The diluted extract was then centrifuged at 10000 rpm for 10 minutes at 4° C. The supernatant was discarded without disturbing the pellet. The retained pellet was washed with 70% chilled ethanol by centrifugation at 10000 rpm for 15 minutes at 4° C. The pellet was air dried in RT after discarding the supernatant. Finally the air dried pellet was dissolved in an appropriate amount of TE buffer (100-150 µl)

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

3.6 ANALYSIS OF THE EXTRACTED DNA

3.6.1 Checking the quality of DNA using Agarose Gel Electrophoresis

The quality of the DNA was checked by agarose gel electrophoresis in 0.8 % agarose. Clean, dry gel casting tray was set up with combs to make a mold and placed on a horizontal flat surface. 0.8 % agarose was dissolved in freshly prepared 1X TBE in a conical flask and melted by boiling for 1-2 minutes. About 1µl of ethidium bromide (EtBr) was added to the molten gel immediately after the flask attained a hand bearable temperature. Immediately after the addition of EtBr, the molten gel was poured into the casting tray and was allowed to solidify. After the 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 submerged. 5µl of extracted DNA sample was mixed with 3µl 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, M/S Syngene).

3.6.2 Quantification of DNA

The extracted DNA was quantified using Thermo Scientific NanoDrop™ 1000 Spectrophotometer. Both the quality and quantity were assessed with the help of this equipment with high accuracy. Nanodrop requires only a small amount (1µl) of sample to calibrate different measurements such as the concentration of isolated DNA (ng/µl), absorbance at 260nm, 280nm 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.

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3.7 MOLECULAR CHARACTERISATION

3.7.1 Dilution of samples for primer screening

DNA samples were diluted with nuclease free water to a concentration of 10ng/µl based on the data obtained from DNA quantification.

3.7.2 Primer dilution

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

3.7.3 PRIMER SCREENING

The diluted DNA samples of two sweet potato accessions were taken for primer screening. Initially primer screening was done with the following 21 ISSR primers:

UBC 808, UBC 809, UBC 811, UBC 817, UBC 824, UBC 825, UBC 827, UBC 847, UBC 848, UBC 849, UBC 851, UBC 860, $(ACC)_6Y$, $(GA)_9AT$ and $(GA)_9AC$, UBC 11, UBC 807, UBC 14, UBC 836, UBC 818, UBC 891

3.7.3.1 PCR amplification

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

Table 3. PCR

Components	Stock concentration	Required concentration	Volume for one reaction (20µl)
Taq buffer (With 25 Mm $MgCl_2$)	10x	1x	2 µl
$MgCl_2$	25 mM	2.5 mM	(From buffer)
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
Final volume made up to 20 µl with d H_20			20 µl

3.7.3.2 PCR conditions

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

Lid -105°C

Initial denaturation - 94°C – 5 min

Denaturation - 94°C – 30 sec

Annealing - 56.3°C – 1 min

Extension - 72°C – 10 min

} 35cycles

Final extension - 72 °C – 10 min

Hold - 4 °C

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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 softwere (M/s. 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 4.

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

Sl. No.	Primer Name	Sequence	Annealing temp.(°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	$(ACC)_6Y$	ACCACCACCACCACCACCY	56.3°C
10	$(GA)_9AT$	GAGAGAGAGAGAGAGAGAT	56.3°C
11	$(GA)_9AC$	GAGAGAGAGAGAGAGAGAC	56.3°C

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

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3.8 ISSR ANALYSIS OF SWEET POTATO ACCESSIONS

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

3.8.1 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 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.

3.8.1.1 Gel preparation

2.6 g of agarose was weighed out and transferred into a 250 ml conical flask. 130 ml 1X TBE buffer was added to it and and the solution was boiled in microwave oven till the agarose completely dissolved in buffer. The solution was allowed to cool to which 1µl/ml ethidium bromide 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.

3.8.1.2 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. 11µl of each sample was loaded into the wells, along with 2µl each of 100 bp and 1Kb ladders at both the end wells for reference. The gel was allowed to run at 100V for about 1 to 1.5 hr. The bands were visualized under UV transilluminator and documented using gel documentation system.

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3.8.2 Gel profile visualization

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

3.9 MOLECULAR DATA ANALYSIS

3.9.1 Scoring of bands

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

3.9.2 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.

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

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

3.9.3 Cluster Analysis

The binary data prepared was used to generate a dendrogram which grouped the 50 accessions, on the basis of Nei genetic distance (Nei and Li, 1979) using unweighted pair group method with arithmetic average (UPGMA) (Sneath

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and Sokal, 1973) was generated 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).

RESULTS

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4. RESULTS

The study entitled "Identification of duplicates in the germplasm of sweet potato (*Ipomoea batatas* (L.) Lam.) using morphological and molecular markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2016-2017 are described in this chapter.

4.1 MORPHOLOGICAL CHARACTERIZATION

Fifty 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 three sets of morphologically similar or suspected duplicates. Habitats of accessions selected for the study are shown in plate 5 and 6. Leaf lobe variability of accessions is depicted in plate 7.

4.1.1 Diversity indices

Percentage distribution of traits as well as Shannon's wiever diversity index was calculated using the appropriate formulas. Both the parameters were tabulated (Table. 5, Plate 8).

Table 5. Frequency distribution and Shannon index of morphological traits

Sl. No:	Characters	Observed Traits	Frequency distribution	Shannon index
1	Twining	Non- twining	100%	0
2	Plant type	Erect	12%	0.9288
		Semi compact	66%	
		Spreading	20%	
		Extremely spreading	2%	
3	Ground cover	Small	18%	1.152
		Medium	36%	
		High	42%	
		Extreme	4%	

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4	Vine internode length	Very short	16%	1.054
		Short	62%	
		Intermediate	18%	
		Long	7%	
		Very long	9%	
5	vine internode diameter	Thin	2%	0.764
		Intermediate	8%	
		Thick	14%	
		Very thick	76%	
6	Predominant vine colour	Green	56%	1.475
		Green with few purple spots	10%	
		Green with many purple spots	4%	
		Green with many dark- purple spots	12%	
		Mostly purple	8%	
		Mostly dark purple	4%	
		Totally purple	2%	
		Totally dark purple	4%	
7	Secondary vine colour	Absent	46%	1.27
		Green base	2%	
		Green tip	24%	
		Purple tip	6%	
		Purple nodes	22%	
8	Vine tip pubescence	None	38%	1.079
		Sparse	48%	
		Moderate	10%	
		Heavy	4%	
9	General leaf outline	Reniform	12%	1.64
		Cordate	8%	
		Triangular	18%	
		Hastate	24%	
		Lobed	32%	
		Almost divided	6%	
10	Type of leaf lobes	Very slight (teeth)	14%	1.316
		Slight	18%	
		Moderate	36%	
		Deep	32%	
11	Number of leaf lobes	Central tooth without lateral lobe	12%	1.249
		3 Lateral lobes	14%	
		5 Lateral lobes	54%	
		7 lateral lobes	18%	
		9 lateral lobes	2%	

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12	Shape of central leaf lobe	Toothed	14%	1.733
		Triangular	12%	
		Semi-circular	2%	
		Semi-elliptic	38%	
		Elliptic	14%	
		Lanceolate	4%	
		Linear (narrow)	14%	
		Linear (broad)	2%	
13	Mature leaf size	Small	16%	0.53
		Medium	82%	
		Large	2%	
14	Abaxial leaf vein pigmentation	Green	44%	1.146
		Purple spot at base of main rib	16%	
		Purple spots in several veins	2%	
		Main rib partially purple	10%	
		Main rib mostly or totally purple	2%	
		All veins mostly or totally purple	24%	
		Lower surface and veins totally purple	2%	
15	Mature leaf color	Green	96%	0.167
		Green with purple edge	4%	
16	Immature leaf color	Green	44%	1.34
		Green with purple edge	28%	
		Slightly purple	4%	
		Mostly purple	8%	
		Purple both surfaces	16%	
17	Petiole length	Very short	42%	1.07
		Short	34%	
		Intermediate	24%	
18	Petiole pigmentation	Green	44%	1.25
		Green with purple near leaf	8%	
		Green with purple at both ends	36%	
		Green with purple spots throughout petiole	6%	
		Totally or mostly purple	6%	
19	Predominant skin color	Cream	30%	1.118
		Yellow	2%	
		Orange	4%	
		Brownish orange	2%	
		Pink	58%	
		Purple red	2%	
		Dark purple	2%	

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20	Predominant flesh color	White	58%	1.185
		Cream	24%	
		Dark cream	6%	
		Pale orange	8%	
		Dark orange	2%	
		Strongly pigmented with anthocyanins	2%	

4.1.2 Cluster analysis

Cluster analysis using R statistical package generated a dendrogram as shown in (fig.1). UPGMA Cluster analysis using 20 variables separated all the 50 accessions into 6 major clusters and two outliers SD-29 and SD-53 at a Euclidean distance of 1.The pattern of clustering of accessions is described in Table 6. Distantly located accessions including outliers and their peculiar morphological appearance that differs from others are depicted in plate 9 and 10.

Table 6. The clustering pattern of different accessions

Clusters	Sub-Clusters	Accessions
Cluster 1	1A	731,1026,203,295,256,236
	1B	740,739,738,RSM-2015-3,737
Cluster 2	2A	RSM-2015-6, SP-7-SP
	2B	RSM-2015-2,RNK-2015-1,SARSE-7,TVM-Local,776
Cluster 3	3	625,298
Cluster 4	4A	RSM-2015-4,ASSAM-1-14,526/7,SD-10,AF2,OPS1,733,729,651
	4B	Swarna-AP,665/4,RSM-2015-1,755,772,747,RSM-2015-5,SARSE-13,SP-18-SP,RNK-2015-2
Cluster 5	5A	S. Arun, Varun progeny (SV-1-2014), JAS-9 white,SP-12-SP
	5B	SD-39,SD-24,SD-11,
Cluster 6	6	SD-55, Jas-10-pink
Cluster 7	Outlier	SD-29
Cluster 8	Outlier	SD-53

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4.1.3 Principal component analysis (PCA)

Principal component analysis was done considering all the 20 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 66.2% of the variability among the characters studied (Table 7.).

PC1 accounted for 31% of the variation. The first principal component had predominant vine colour and secondary vine colour, abaxial vein pigmentation and petiole pigmentation with high values. The second principal component accounted for 19% of the variation which included leaf lobe shape and leaf lobe number, shape of central leaf, lobe and immature leaf colour, etc. The third principal component accounted for 15.6% of the variation which included vine tip pubescence as highly loaded character. The trait included in PC1, PC2 and PC3 was the shape of central leaf lobe. Predominant vine colour and petiole pigmentation was the trait included in both PC1 and PC2. Hence predominant vine colour, shape of central leaf lobe and petiole pigmentation were important in distinguishing the accessions. The PCA plot is illustrated in (fig. 2).

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Table7. Principal component analysis in 50 accessions of sweet potato

(Highly loaded variables in combined analysis given in boldface)

Variables	PC1	PC2	PC3	PC4	PC5
Twining	0.000	0.000	0.000	0.000	0.000
Plant type	-0.002	-0.124	-0.037	-0.032	0.024
Ground cover	-0.087	-0.019	-0.021	-0.179	-0.096
Vine internode length	-0.114	-0.231	-0.038	-0.173	-0.028
Vine internode diameter	-0.035	0.008	-0.021	-0.122	-0.039
Predominant vine colour	**0.362**	-0.191	-0.028	**0.452**	**-0.549**
Secondary vine colour	**0.680**	-0.028	0.141	-0.12	**0.551**
Vine tip pubescence	0.112	-0.247	0.**898**	-0.077	-0.138
General outline of leaf	0.050	0.110	0.159	0.17	-0.031
Leaf lobes type	0.092	**0.431**	0.130	-0.161	-0.249
Leaf lob number	0.037	**0.285**	-0.022	-0.343	0.-186
Shape of central leaf lobe	0.198	**0.419**	0.168	-0.203	-0.214
Mature leaf size	0.004	-0.006	-0.022	-0.120	0.040
Abaxial leaf vein pigmentation	**0.343**	-0.074	-0.063	-0.275	0.025
Mature leaf colour	-0.002	-0.005	0.006	-0.010	0.018
Immature leaf colour	-0.214	**0.235**	0.021	0.175	0.292
Petiole length	-0.121	-0.297	-0.043	-0.572	-0.082
Petiole Pigmentation	**0.362**	-0.234	-0.141	-0.064	-0.284
Tuber predominant skin colour	-0.097	-0.006	-0.267	-0.174	0.196
Tuber predominant flesh colour	-0.043	-0.416	-0.028	-0.006	0.082
Eigen values	0.230	0.142	0.115	0.057	
Percent variation	31.242	19.276	15.699	7.696	
Cumulative percentage	31.242	50.518	66.217	73.913	

Fig. 2: PCA plot of morphological variables

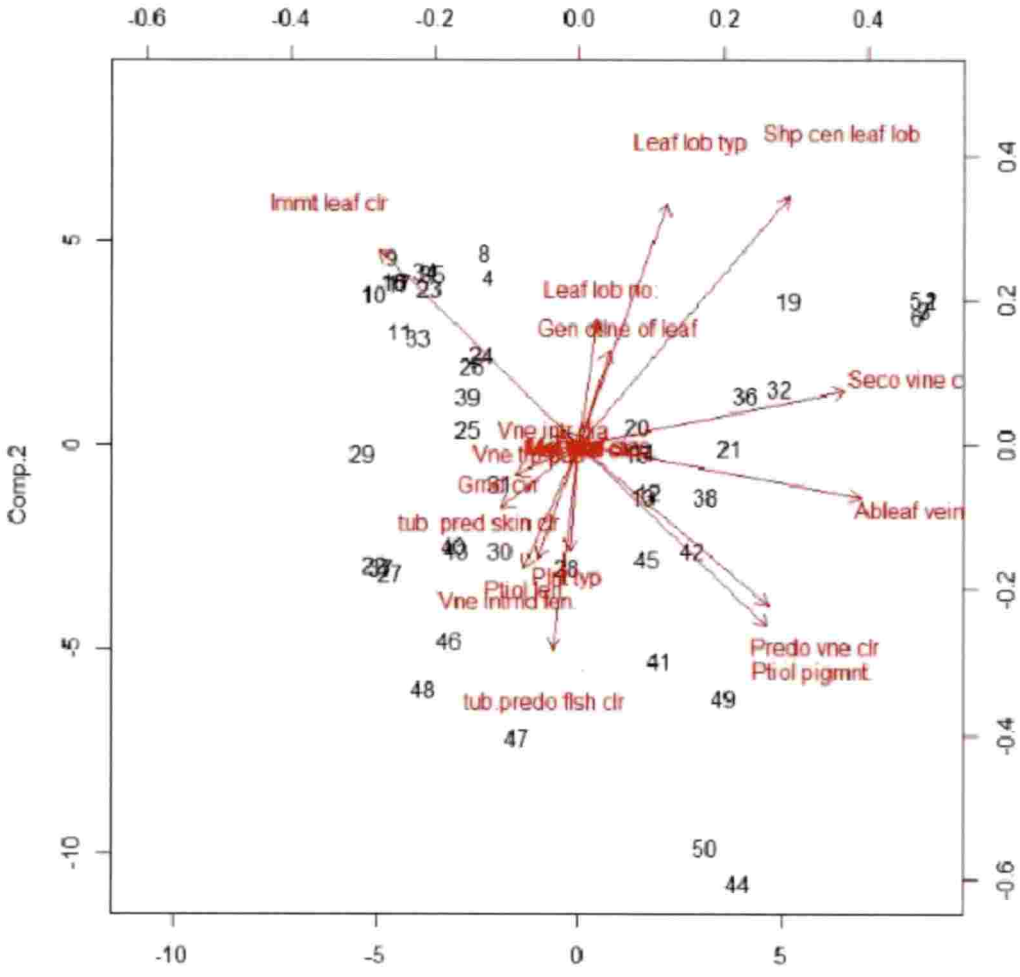


Plate 5. Habitats of different accessions used in the study


SARSE-7


RNK-2015-1


SP-7-SP


665/4


SP-12-SP


AF2


SP-12-SP


SD-10


JAS-10-pink


RSM-2015-2


JAS-9-white


RSM-2015-3


ASSAM-1-2014


RSM-2015-4


SWARNA-AP

Plate 6. Habitats of different accessions used in the study

526/7

SD-11

RSM-2015-6

TVM-LOCAL

SD-55

SD-39

SD-29

SD-24

737

S-738

625

747

S-755

S-298

SD-53

Plate 7. Variability in leaf characters

S-203

S-755

S-236

S-256

S-729

S-298

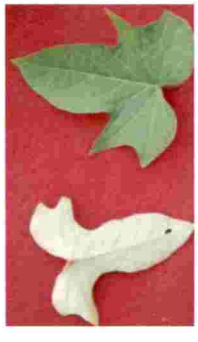
S-731

S-739

S-772

S-651

S-1026



S-738

S-747

S-625

S-776

Plate 8: Frequency distribution of major variables

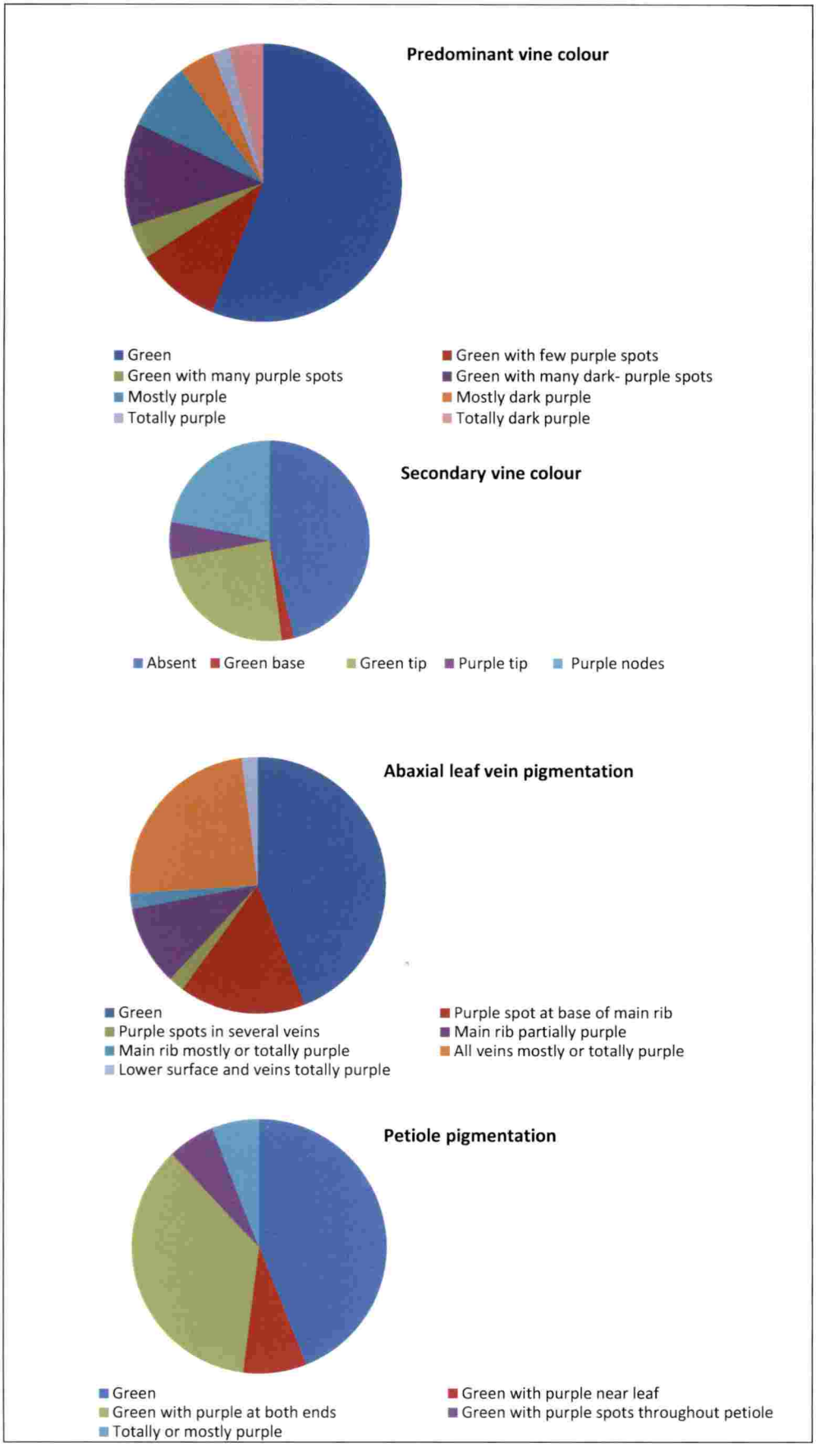
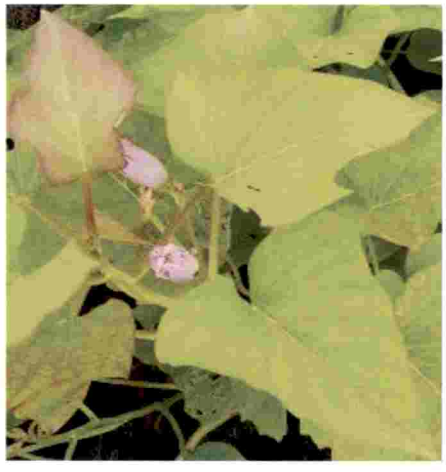


Plate 9. Leaf lobe characteristics of outliers and other distantly - located accessions

SD-29

SD-29- Habit

SP-12-SP



SD-39

Plate 10. Leaf lobe characteristics of outliers and other distantly located accessions

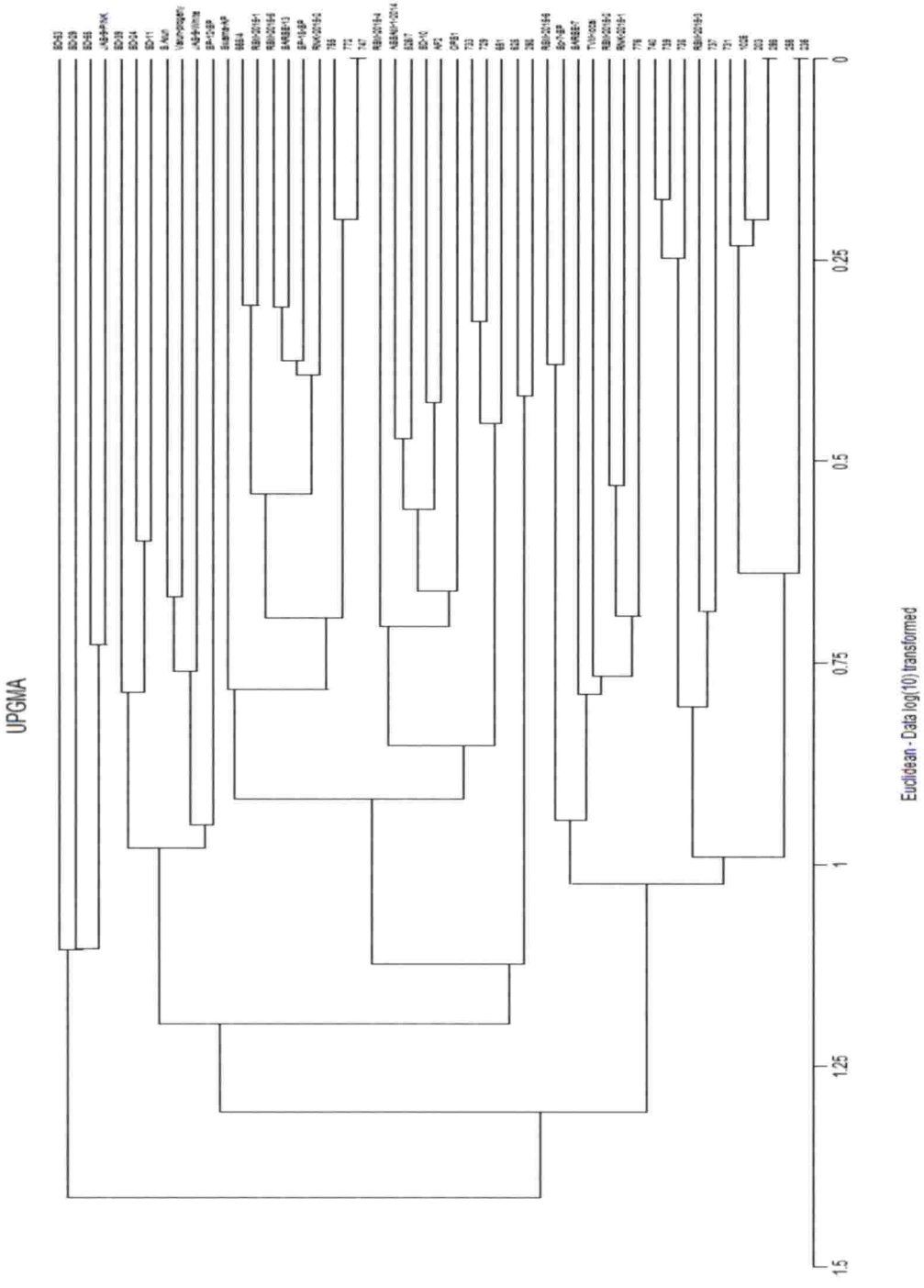
JAS-9-WHITE

SARSE 7

Swarna AP

RSM-2015-4

Fig.1: Dendrogram grouping of accessions based on morphological characters



4.2 MOLECULAR DATA ANALYSIS

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4.2.1 DNA isolation and quantification

DNA of 50 accessions was isolated using the CTAB method of DNA isolation. The quality of isolated DNA was checked in 1% agarose gel (Plate 11 and 12). The quantity as well as purity of the DNA obtained from spectrophotometric readings are included in Table 8. The concentrations of isolated DNA ranges from 76.8 ng/µl to1681.8 ng/µl with high purity.

Table 8. Spectrophotometric readings of DNA isolated using CTAB method.

Sl. No.	Sample Name	A260/A280	Concentration (ng/µl)
1	236	1.91	208.8
2	256	1.79	100.7
3	1026	2.03	345.9
4	203	2.18	1681.8
5	298	2.08	348.7
6	731	2.09	572.4
7	625	2.02	265.5
8	737	1.98	412.0
9	Varun Progeny	2.03	175.5
10	RSM-2016-6	1.97	150.4
11	SARSE-7	1.96	222.3
12	SP-18-SP	2.03	253.2
13	526/7	2.03	240.7
14	SARSE-13	2.04	170.5
15	Tvm local	1.93	205.2
16	Jas-10- Pink	1.92	132.0
17	RNK-2015-1	1.94	335.5
18	Jas-9- White	2.02	381.0
19	RSM-2015-3	1.98	350.4

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20	RSM-2015-5	2.02	638.4
21	SP-12-SP	2.02	239.4
22	OPS 1	1.85	110.8
23	738	1.99	434.0
24	739	2.03	491.5
25	SD-24	2.11	250.0
26	SD-29	1.84	273.3
27	SD-39	1.98	76.8
28	295	2.12	398.8
29	288	2.05	375.8
30	651	2.01	170.0
31	729	2.02	146.5
32	Arun	1.95	159.9
33	RSM-2015-1	2.06	114.4
34	RSM-2015-2	2.02	257.2
35	SD-11	1.98	503.5
36	665/4	2.08	224.3
37	AF2	2.01	932.3
38	740	2.05	235.8
39	747	2.02	330.4
40	755	1.99	509.8
41	SD-10	1.99	236.5
42	SWARNA-AP	1.94	487.5
43	SD-53	1.98	180.9
44	SD-55	1.88	338.3
45	776	1.91	249.1
46	ASSAM-1-2014	1.73	179.1
47	772	1.66	237.1
48	SP-7-SP	1.98	468.3
49	RNK-2015-2	1.81	134.2
50	RSM-2015-4	1.96	103.4

Plate (11 & 12): Checking the quality of DNA of 50 accessions (1% Agarose gel electrophoresis)

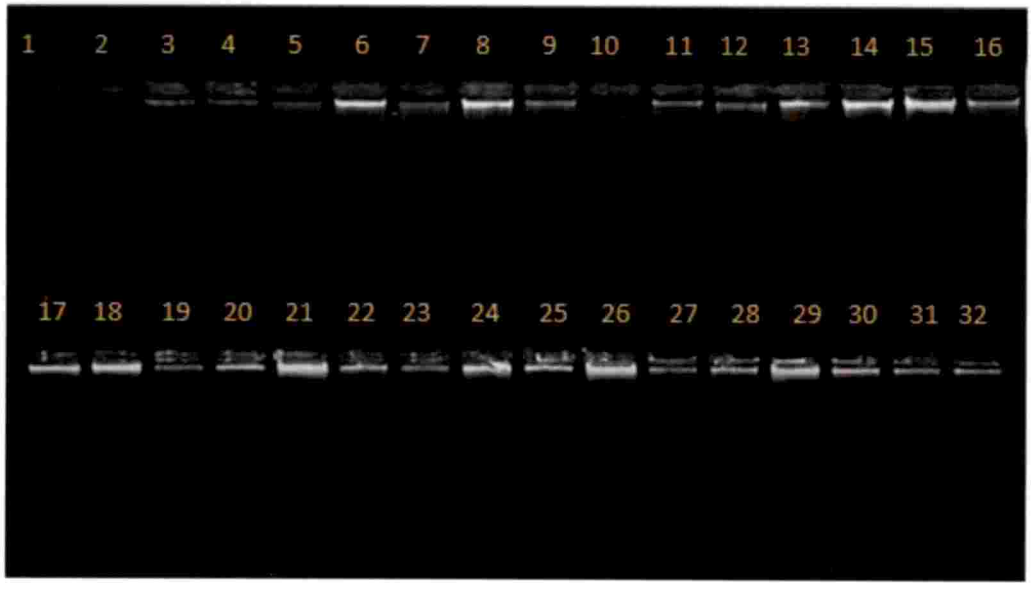

Plate: 11

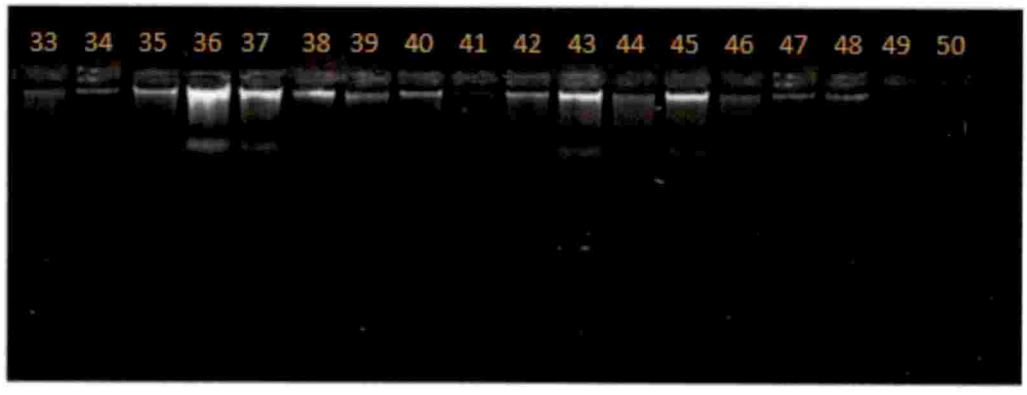

Plate: 12

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4.2.2 ISSR analysis of sweet potato accessions

After preliminary primer screening using 21 primers 11 primers gave clear and reproducible bands which were selected for PCR amplification. After the completion of PCR using selected primers the amplicons were resolved in 2% agarose gel and the obtained amplicons were shown in Plate 13 to Plate 24.

4.3 ANALYSIS OF MOLECULAR MARKER DATA

4.3.1 ISSR profile

Using 11 ISSR primers (Plates 13-24) a total of 162 bands were generated with an average of 14.7 bands per primers. Total number of bands per ISSR primer ranged from 9 (UBC 818) to 18 (UBC 860 and (GA)9AC). 100% polymorphism was obtained with all the primers (Table 9).

4.3.2 Cluster analysis

The UPGMA dendrogram created using Jaccards similarity coefficient separated the accessions into two principal clusters and an outlier at a coefficient 0.61. (Figure 3). The outlier was SD-29. The first principal cluster comprised of 40 accessions which were assembled in 4 groups as shown in the Table 10. The second principal cluster comprised of nine accessions in 3 groups. In this principal cluster S-236 and S-256 grouped together with 100% similarity. So this set can be considered as duplicates which were also found as duplicates in morphological analysis.

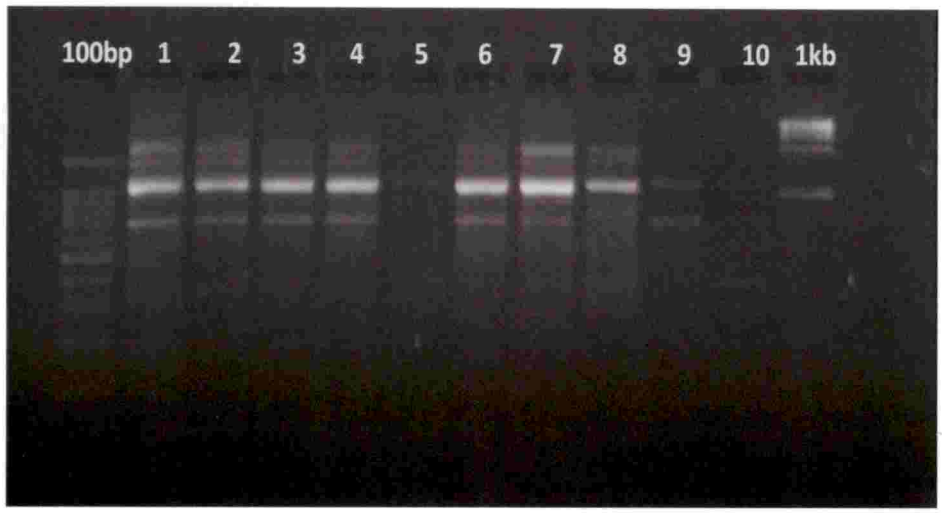

Plate 13: ISSR profile of 2% agarose gel using primer UBC 808

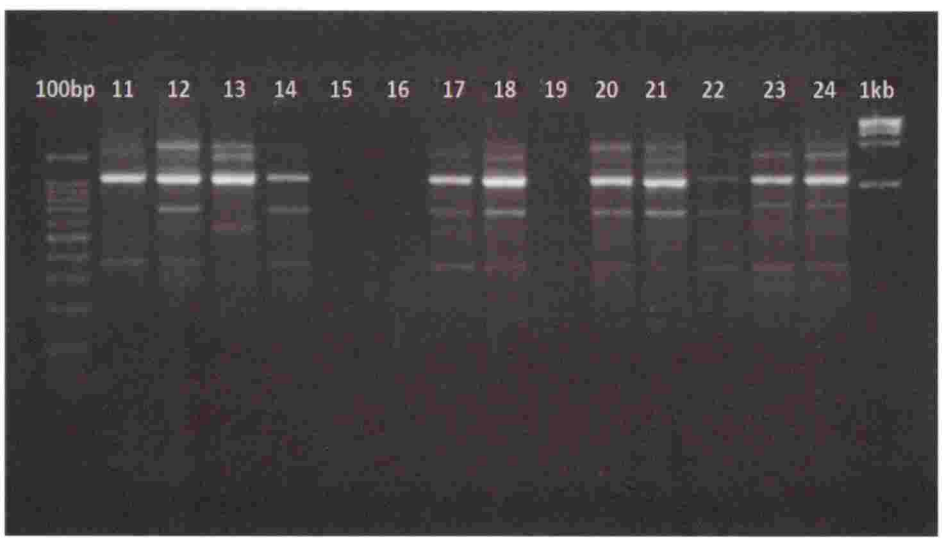

Plate 14: ISSR profile of 2% agarose gel using primer UBC 808

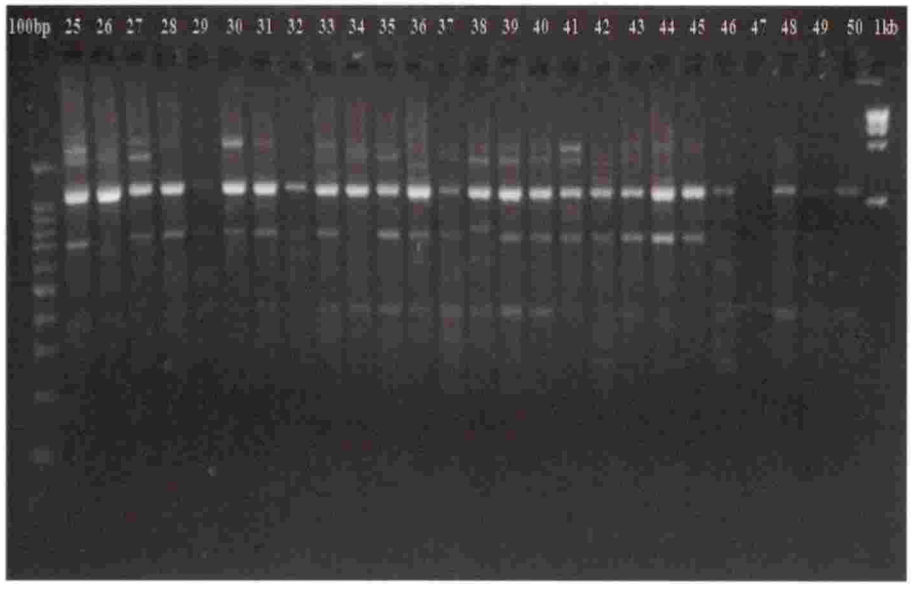

Plate 15: ISSR profile of 2%agarose gel using primer UBC 808

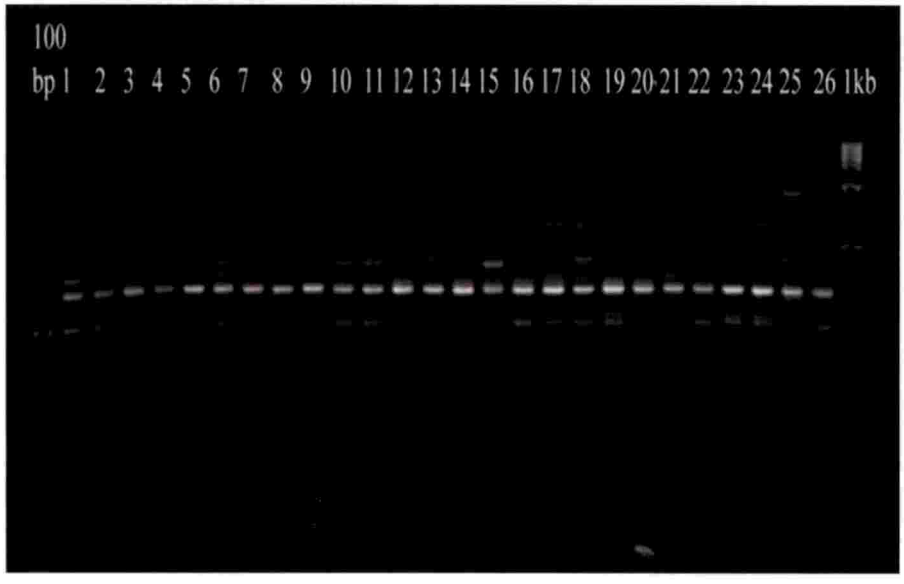

Plate 16: ISSR profile of 2% agarose gel using primer UBC 809

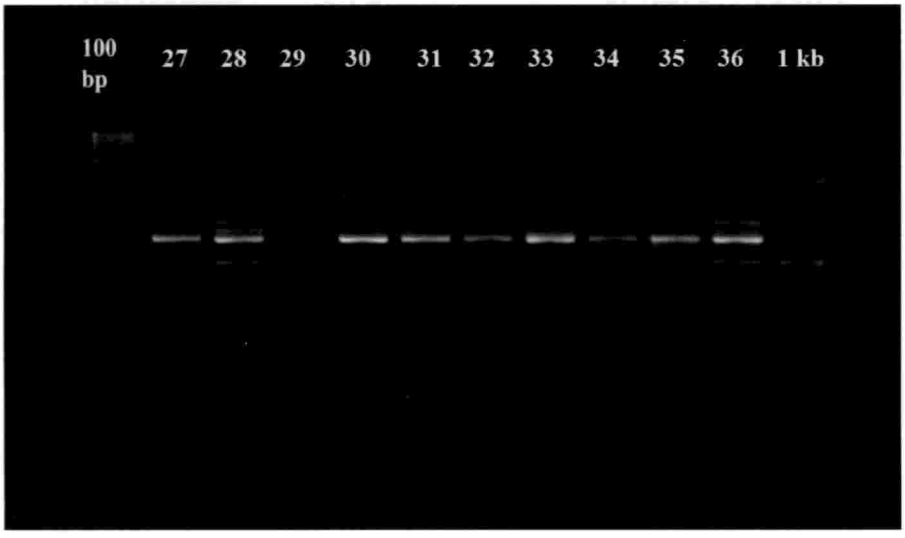

Plate17 : ISSR profile of 2% agarose gel using primer UBC 809

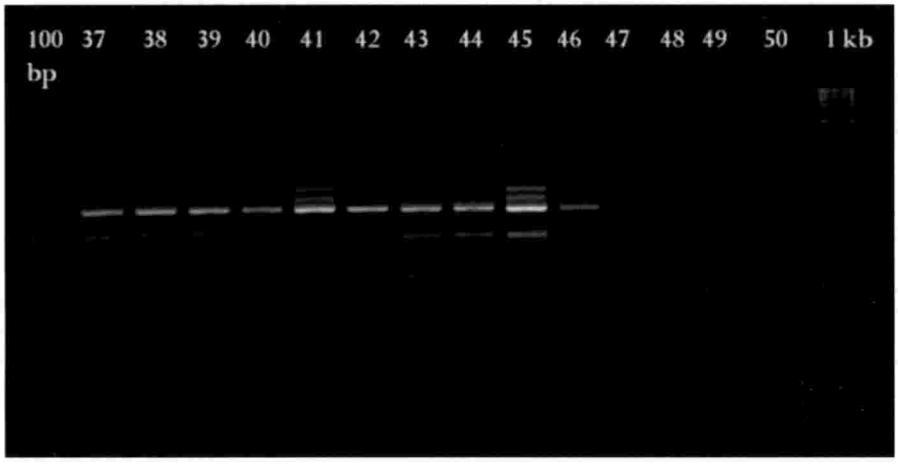

Plate 18: ISSR profile of 2% agarose gel using primer UBC 809

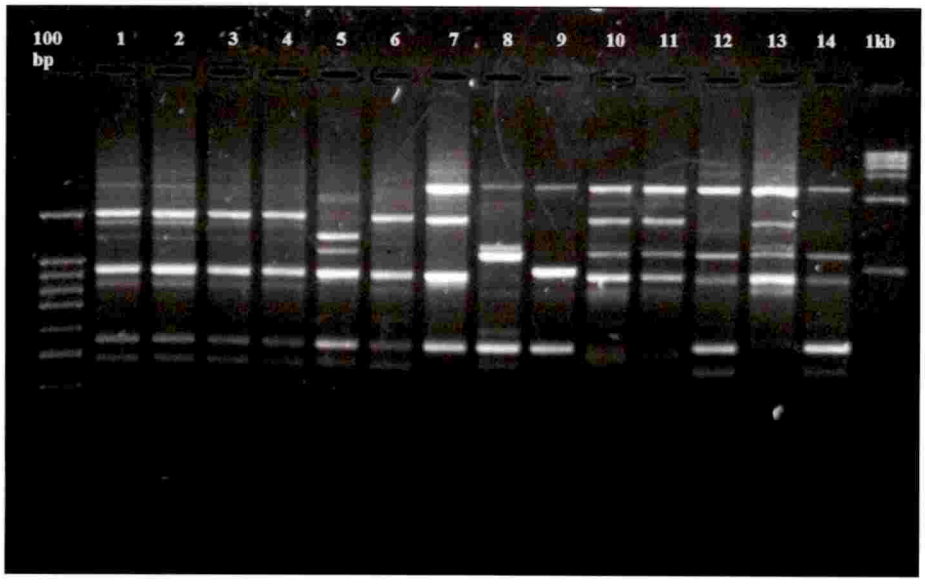

Plate 19: ISSR profile of 2% agarose gel using primer UBC 825

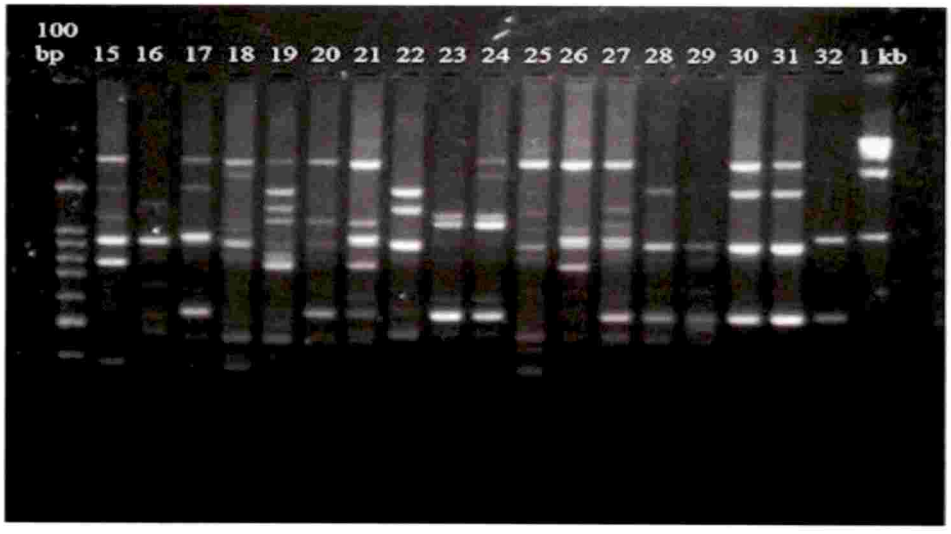

Plate 20: ISSR profile of 2% agarose gel using primer UBC 825

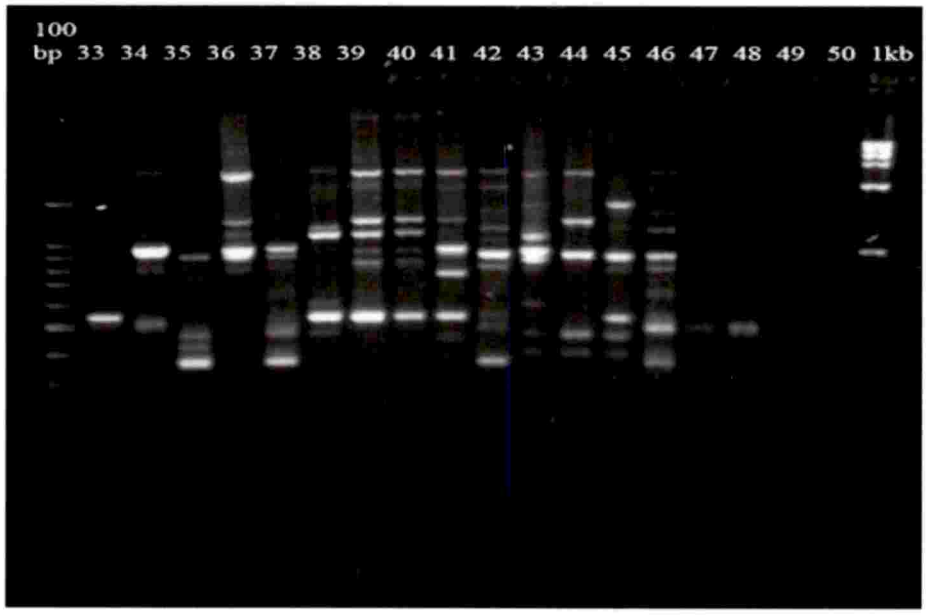

Plate 21: ISSR profile of 2% agarose gel using primer UBC 825

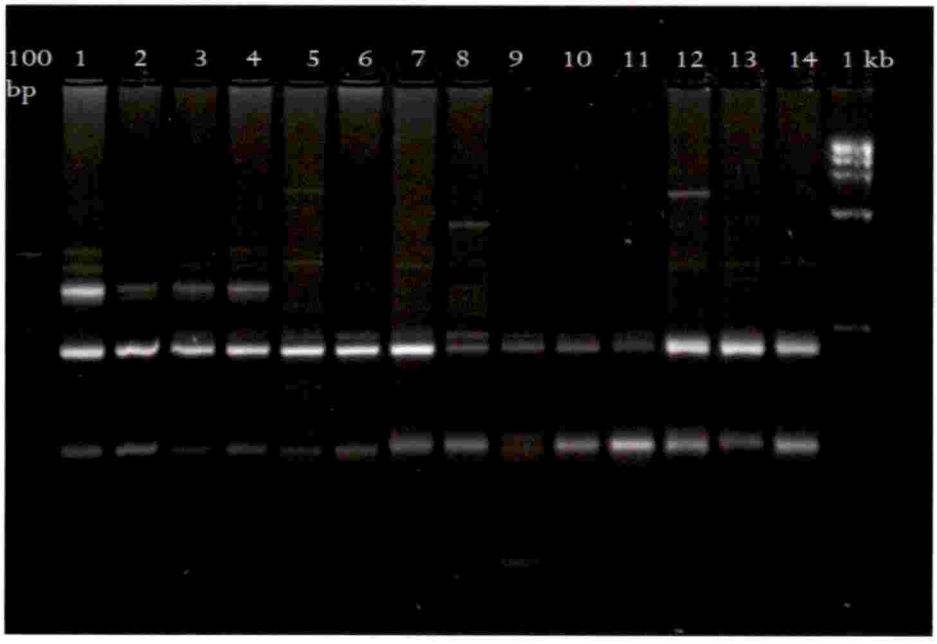

Plate 22: ISSR profile of 2% agarose gel using primer UBC 860

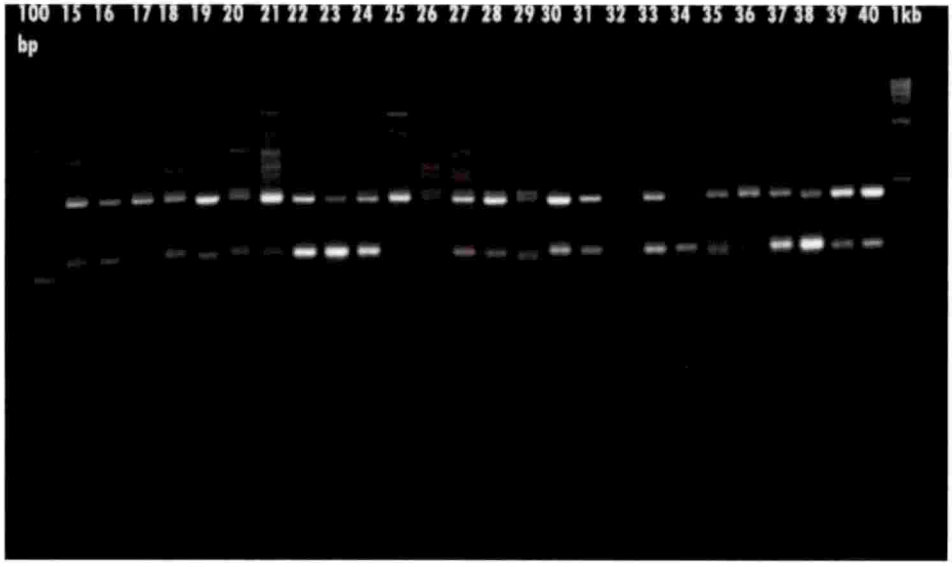

Plate 23: ISSR profile of 2% agarose gel using primer UBC 860

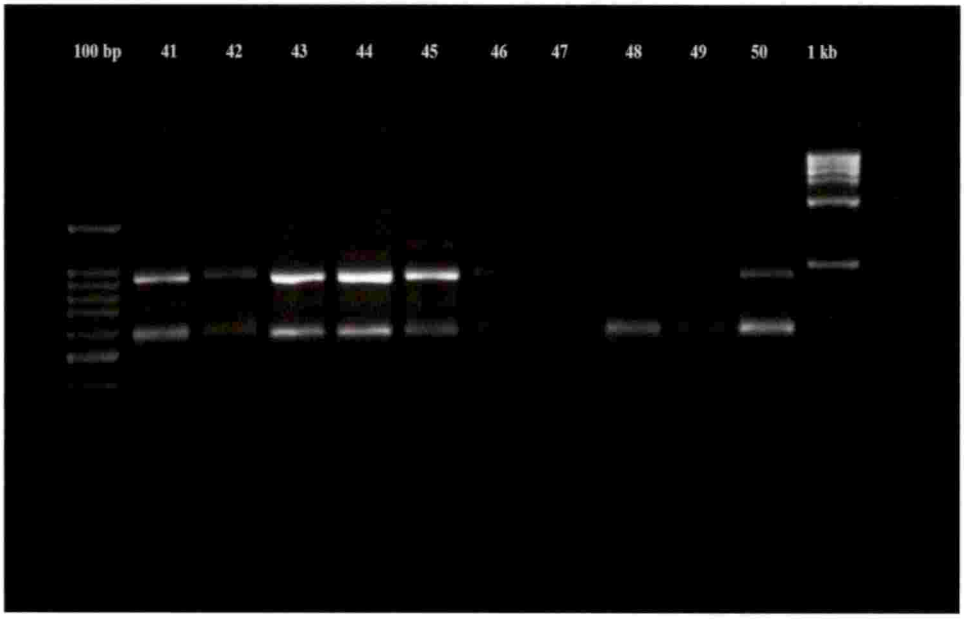

Plate 24: ISSR profile of 2% agarose gel using primer UBC 860

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Table9. Polymorphism detected in 50 accessions of sweet potato with 11 primers

Primer	Sequence	Total no. of bands	Polymorphism	
			No.of Bands	%
UBC 807	AGAGAGAGAGAGAGAGT	17	17	100
UBC 808	AGAGAGAGAGAGAGAGC	11	11	100
UBC 809	AGAGAGAGAGAGAGAGG	15	15	100
UBC 818	CACACACACACACACAG	9	9	100
UBC 825	ACACACACACACACACT	15	15	100
UBC 827	ACACACACACACACACG	10	10	100
UBC 847	CACACACACACACACARC	16	16	100
UBC 860	TGTGTGTGTGTGTGTGRA	18	18	100
(ACC)6Y	ACCACCACCACCACCACCY	16	16	100
(GA)9AT	GAGAGAGAGAGAGAGAGAT	17	17	100
(GA)9AC	GAGAGAGAGAGAGAGAGAC	18	18	100
	Total	162	162	100
	Mean	14.7	14.7	

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Table 10. Accessions grouped based on ISSR markers

Clusters	Sub-Clusters		Accessions
Outlier			**SD-29**
Cluster 1	1A	A	737,740
		B	Varun progeny, Arun, JAS-10-pink, RSM-2015-2, 772, sp-7-sp, RNK-2015-2, RSM-2015-4,288,OPS1,SD-11,RSM-2015-1,Sarse13,738,RSM-2016-6,Sarse-7,Tvm-local,RSM-2015-3,Swarna-AP,Assam-1-2014,625,RNK-2015-1,295,AF2,SD-55,776,SD-10
	1B		747, 755 ,SD-53
	IC		SP-18-SP,739
	ID		JAS -9-white,RSM-15-5,SP-12-SP,SD-24,665/4,SD-39
Cluster 2	2A		298,526/7
	2B		651,729
	2C		236,256,203,1026

4.3.3 Genetic relationships based on similarity matrix

An estimate of genetic relationship was depicted from the marker data using Jaccards simiality coefficient (Table 11). Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 100%- 52%. The maximum similarity of 100% was observed between S-236 and S-256. A 96% similarity was observed between S-203 and S-236. JAS-10-pink and RSM-2015-2 showed 95% similarity. Similarly 772 and SP-7-SP showed 95% similarity. The least similar accessions were SD-39 and S-298(52%).A similarity coefficient of 0.53 was observed between S. Arun and JAS-10-pink, JAS-9-white and 526/7, SP-12-SP and 526/7, ASSAM-1-2014 and S-1026. SD-29

was different from all the remaining accessions by a similarity coefficient of 0.61. Within each principal cluster there was lot of intraclusteral variation.

4.3.4 Mantel's Test

Mantel's test has been performed to check whether there is a correlation between morphological and molecular aspects of the accessions. The Mantel statistic (r) value obtained as 0.034 and its significance value corresponds to a value of 0.305. There is no correlation found between morphological and molecular data of the accessions as here the significance value is above 0.05. If and only if the significance value is below 0.05, there can be significant correlation between the morphological and molecular data.

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1	1.00																									
2	1.00	1.00																								
3	0.80	0.88	1.00																							
4	0.96	0.10	0.91	1.00																						
5	0.65	0.65	0.73	0.07	1.00																					
6	0.85	0.85	0.87	0.85	0.71	1.00																				
7	0.69	0.69	0.67	0.71	0.73	0.75	1.00																			
8	0.58	0.58	0.64	0.61	0.65	0.67	0.75	1.00																		
9	0.65	0.65	0.64	0.64	0.67	0.74	0.76	0.75	1.00																	
10	0.65	0.65	0.62	0.64	0.66	0.72	0.77	0.76	0.88	1.00																
11	0.67	0.67	0.66	0.67	0.64	0.78	0.88	0.74	0.82	0.89	1.00															
12	0.64	0.64	0.66	0.65	0.67	0.68	0.78	0.73	0.68	0.79	0.72	1.00														
13	0.67	0.67	0.67	0.67	0.69	0.65	0.69	0.64	0.64	0.66	0.64	0.67	1.00													
14	0.62	0.62	0.62	0.63	0.73	0.71	0.76	0.77	0.86	0.82	0.79	0.75	0.65	1.00												
15	0.61	0.61	0.62	0.62	0.62	0.69	0.74	0.71	0.82	0.82	0.81	0.66	0.64	0.76	1.00											
16	0.63	0.63	0.63	0.62	0.67	0.73	0.73	0.73	0.93	0.89	0.82	0.67	0.64	0.87	0.83	1.00										
17	0.62	0.62	0.62	0.64	0.64	0.69	0.81	0.78	0.81	0.75	0.76	0.69	0.59	0.79	0.74	0.8	1.00									
18	0.61	0.61	0.61	0.61	0.61	0.64	0.73	0.66	0.64	0.66	0.70	0.66	0.53	0.67	0.67	0.64	0.76	1.00								
19	0.60	0.60	0.59	0.59	0.60	0.66	0.65	0.73	0.80	0.81	0.74	0.66	0.62	0.80	0.76	0.84	0.72	0.68	1.00							
20	0.59	0.59	0.66	0.61	0.62	0.67	0.71	0.73	0.70	0.70	0.72	0.72	0.58	0.73	0.74	0.73	0.75	0.71	0.66	1.00						
21	0.63	0.63	0.61	0.64	0.57	0.63	0.62	0.59	0.67	0.64	0.65	0.62	0.53	0.67	0.61	0.64	0.67	0.65	0.66	0.74	1.00					
22	0.63	0.63	0.62	0.62	0.67	0.72	0.73	0.73	0.87	0.84	0.80	0.69	0.63	0.84	0.77	0.87	0.79	0.69	0.84	0.67	0.64	1.00				
23	0.60	0.60	0.61	0.62	0.64	0.69	0.74	0.82	0.80	0.77	0.74	0.69	0.59	0.85	0.71	0.80	0.79	0.68	0.77	0.70	0.63	0.79	1.00			
24	0.58	0.58	0.57	0.59	0.59	0.62	0.70	0.77	0.74	0.70	0.69	0.70	0.59	0.73	0.64	0.70	0.70	0.62	0.68	0.67	0.62	0.69	0.82	1.00		
25	0.61	0.61	0.64	0.61	0.64	0.66	0.68	0.68	0.68	0.67	0.65	0.66	0.62	0.68	0.61	0.67	0.67	0.67	0.64	0.67	0.64	0.70	0.68	0.67	1.00	
26	0.62	0.62	0.62	0.63	0.66	0.66	0.67	0.60	0.62	0.60	0.62	0.61	0.62	0.60	0.61	0.61	0.62	0.62	0.59	0.61	0.61	0.58	0.58	0.62	0.67	1.00

Table 11: NTSYS-pc similarity coefficicents between 50 accessions.

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Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
27	0.63	0.63	0.63	0.65	0.52	0.64	0.69	0.63	0.70	0.64	0.64	0.62	0.56	0.63	0.61	0.66	0.66	0.61	0.62	0.67	0.71	0.65	0.64	0.65	0.71	0.68
28	0.69	0.69	0.67	0.70	0.70	0.74	0.75	0.71	0.79	0.77	0.73	0.64	0.59	0.76	0.74	0.77	0.79	0.67	0.70	0.66	0.58	0.77	0.72	0.62	0.61	0.58
29	0.65	0.65	0.67	0.64	0.71	0.76	0.72	0.74	0.87	0.84	0.77	0.67	0.64	0.83	0.76	0.87	0.75	0.64	0.77	0.69	0.62	0.83	0.79	0.68	0.64	0.62
30	0.65	0.65	0.66	0.66	0.67	0.70	0.72	0.61	0.65	0.62	0.63	0.64	0.61	0.61	0.59	0.63	0.67	0.59	0.55	0.64	0.54	0.62	0.59	0.62	0.58	0.62
31	0.69	0.69	0.69	0.70	0.67	0.78	0.74	0.69	0.78	0.74	0.76	0.65	0.67	0.79	0.70	0.80	0.76	0.61	0.70	0.67	0.59	0.70	0.74	0.66	0.66	0.62
32	0.64	0.64	0.64	0.63	0.65	0.74	0.72	0.74	4.30	0.85	0.78	0.66	0.60	0.85	0.76	0.53	0.79	0.62	0.80	0.69	0.67	0.87	0.81	0.72	0.67	0.60
33	0.64	0.64	0.67	0.66	0.63	0.74	0.72	0.73	0.83	0.79	0.80	0.71	0.63	0.83	0.75	0.86	0.79	0.62	0.75	0.77	0.71	0.81	0.79	0.71	0.69	0.58
34	0.63	0.63	0.63	0.62	0.66	0.73	0.74	0.74	0.91	0.89	0.82	0.69	0.64	0.88	0.80	0.95	0.79	0.65	0.82	0.71	0.67	0.86	0.82	0.72	0.70	0.63
35	0.66	0.66	0.66	0.65	0.69	0.73	0.67	0.69	0.85	0.78	0.74	0.62	0.67	0.83	0.75	0.85	0.72	0.65	0.78	0.64	0.61	0.85	0.78	0.67	0.69	0.59
36	0.60	0.60	0.60	0.62	0.61	0.66	0.67	0.64	0.74	0.69	0.68	0.58	0.54	0.71	0.67	0.73	0.70	0.66	0.66	0.67	0.70	0.70	0.69	0.69	0.72	0.64
37	0.62	0.62	0.61	0.64	0.66	0.69	0.74	0.74	0.76	0.76	0.74	0.67	0.56	0.76	0.72	0.75	0.78	0.70	0.71	0.66	0.62	0.78	0.76	0.67	0.66	0.61
38	0.64	0.64	0.63	0.65	0.61	0.69	0.69	0.82	0.78	0.79	0.74	0.71	0.63	0.80	0.73	0.80	0.74	0.65	0.74	0.69	0.66	0.80	0.82	0.72	0.65	0.58
39	0.59	0.59	0.56	0.61	0.63	0.63	0.74	0.67	0.73	0.74	0.72	0.69	0.59	0.77	0.66	0.71	0.77	0.71	0.69	0.70	0.71	0.74	0.74	0.70	0.66	0.58
40	0.63	0.63	0.62	0.65	0.61	0.66	0.72	0.70	0.73	0.74	0.76	0.70	0.64	0.75	0.67	0.74	0.76	0.66	0.70	0.70	0.67	0.75	0.74	0.70	0.65	0.58
41	0.59	0.59	0.59	0.61	0.68	0.66	0.74	0.70	0.77	0.75	0.70	0.65	0.58	0.75	0.69	0.75	0.80	0.66	0.68	0.69	0.64	0.72	0.73	0.66	0.62	0.59
42	0.61	0.61	0.62	0.61	0.61	0.69	0.69	0.69	0.83	0.80	0.76	0.66	0.82	0.82	0.72	0.85	0.72	0.61	0.71	0.62	0.60	0.77	0.75	0.70	0.64	0.56
43	0.61	0.61	0.54	0.60	0.58	0.64	0.72	0.69	0.75	0.75	0.75	0.69	0.61	0.75	0.72	0.72	0.75	0.66	0.67	0.66	0.61	0.74	0.74	0.69	0.66	0.58
44	0.64	0.64	0.63	0.66	0.66	0.73	0.75	0.77	0.79	0.79	0.79	0.70	0.62	0.79	0.79	0.80	0.76	0.70	0.74	0.74	0.61	0.82	0.75	0.69	0.67	0.62
45	0.69	0.69	0.64	0.68	0.67	0.74	0.75	0.71	0.85	0.80	0.77	0.67	0.59	0.81	0.76	0.84	0.80	0.70	0.75	0.67	0.64	0.82	0.77	0.69	0.67	0.58
46	0.55	0.55	0.53	0.57	0.61	0.60	0.67	0.70	0.80	0.79	0.72	0.64	0.60	0.81	0.71	0.80	0.74	0.63	0.75	0.59	0.58	0.74	0.79	0.68	0.63	0.54
47	0.61	0.61	0.61	0.60	0.66	0.70	0.70	0.73	0.90	0.87	0.79	0.65	0.62	0.85	0.79	0.95	0.76	0.61	0.83	0.69	0.65	0.85	0.80	0.70	0.66	0.59
48	0.62	0.62	0.61	0.61	0.64	0.70	0.70	0.73	0.89	0.85	0.79	0.66	0.61	0.85	0.75	0.91	0.76	0.62	0.79	0.69	0.65	0.85	0.82	0.70	0.66	0.57
49	0.62	0.62	0.58	0.61	0.64	0.68	0.67	0.69	0.84	0.80	0.74	0.65	0.62	0.78	0.70	0.83	0.74	0.59	0.72	0.62	0.60	0.79	0.74	0.67	0.62	0.57
50	0.64	0.64	0.61	0.64	0.70	0.69	0.72	0.72	0.85	0.85	0.77	0.66	0.66	0.79	0.74	0.86	0.74	0.64	0.77	0.65	0.62	0.82	0.77	0.66	0.62	0.58

Taxa	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
27	1.00																							
28	0.63	1.00																						
29	0.67	0.83	1.00																					
30	0.61	0.71	0.62	1.00																				
31	0.64	0.73	0.75	0.75	1.00																			
32	0.68	0.79	0.86	0.64	0.77	1.00																		
33	0.72	0.74	0.83	0.63	0.81	0.84	1.00																	
34	0.69	0.75	0.85	0.63	0.80	0.91	0.86	1.00																
35	0.65	0.77	0.85	0.62	0.80	0.83	0.79	0.86	1.00															
36	0.69	0.65	0.66	0.59	0.68	0.74	0.74	0.75	0.67	1.00														
37	0.64	0.81	0.74	0.62	0.69	0.79	0.75	0.78	0.75	0.65	1.00													
38	0.66	0.72	0.74	0.61	0.71	0.79	0.80	0.81	0.75	0.68	0.74	1.00												
39	0.62	0.66	0.64	0.66	0.70	0.73	0.74	0.74	0.66	0.73	0.69	0.75	1.00											
40	0.65	0.63	0.66	0.59	0.72	0.74	0.77	0.75	0.66	0.70	0.70	0.72	0.86	1.00										
41	0.64	0.75	0.68	0.67	0.71	0.78	0.76	0.79	0.69	0.68	0.78	0.71	0.76	0.75	1.00									
42	0.64	0.70	0.78	0.61	0.77	0.82	0.81	0.85	0.77	0.69	0.73	0.77	0.70	0.71	0.71	1.00								
43	0.62	0.70	0.66	0.64	0.67	0.75	0.74	0.76	0.70	0.68	0.75	0.72	0.77	0.71	0.76	0.67	1.00							
44	0.66	0.75	0.74	0.64	0.72	0.78	0.79	0.80	0.75	0.69	0.80	0.75	0.74	0.76	0.77	0.71	0.77	1.00						
45	0.66	0.83	0.79	0.69	0.73	0.85	0.78	0.83	0.79	0.71	0.80	0.78	0.73	0.68	0.80	0.75	0.79	0.84	1.00					
46	0.58	0.74	0.74	0.59	0.69	0.79	0.74	0.82	0.77	0.65	0.79	0.73	0.69	0.67	0.72	0.82	0.73	0.72	0.76	1.00				
47	0.65	0.74	0.88	0.59	0.75	0.91	0.85	0.92	0.83	0.70	0.74	0.79	0.69	0.70	0.74	0.81	0.71	0.77	0.82	0.80	1.00			
48	0.65	0.75	0.87	0.61	0.75	0.91	0.85	0.92	0.83	0.72	0.77	0.79	0.70	0.71	0.76	0.80	0.72	0.77	0.83	0.83	0.95	1.00		
49	0.59	0.78	0.82	0.69	0.72	0.88	0.77	0.83	0.81	0.66	0.77	0.75	0.67	0.65	0.75	0.75	0.75	0.74	0.82	0.83	0.88	0.91	1.00	
50	0.61	0.79	0.84	0.64	0.72	0.87	0.79	0.86	0.82	0.67	0.78	0.79	0.71	0.69	0.77	0.74	0.75	0.76	0.82	0.79	0.88	0.91	0.91	1.00

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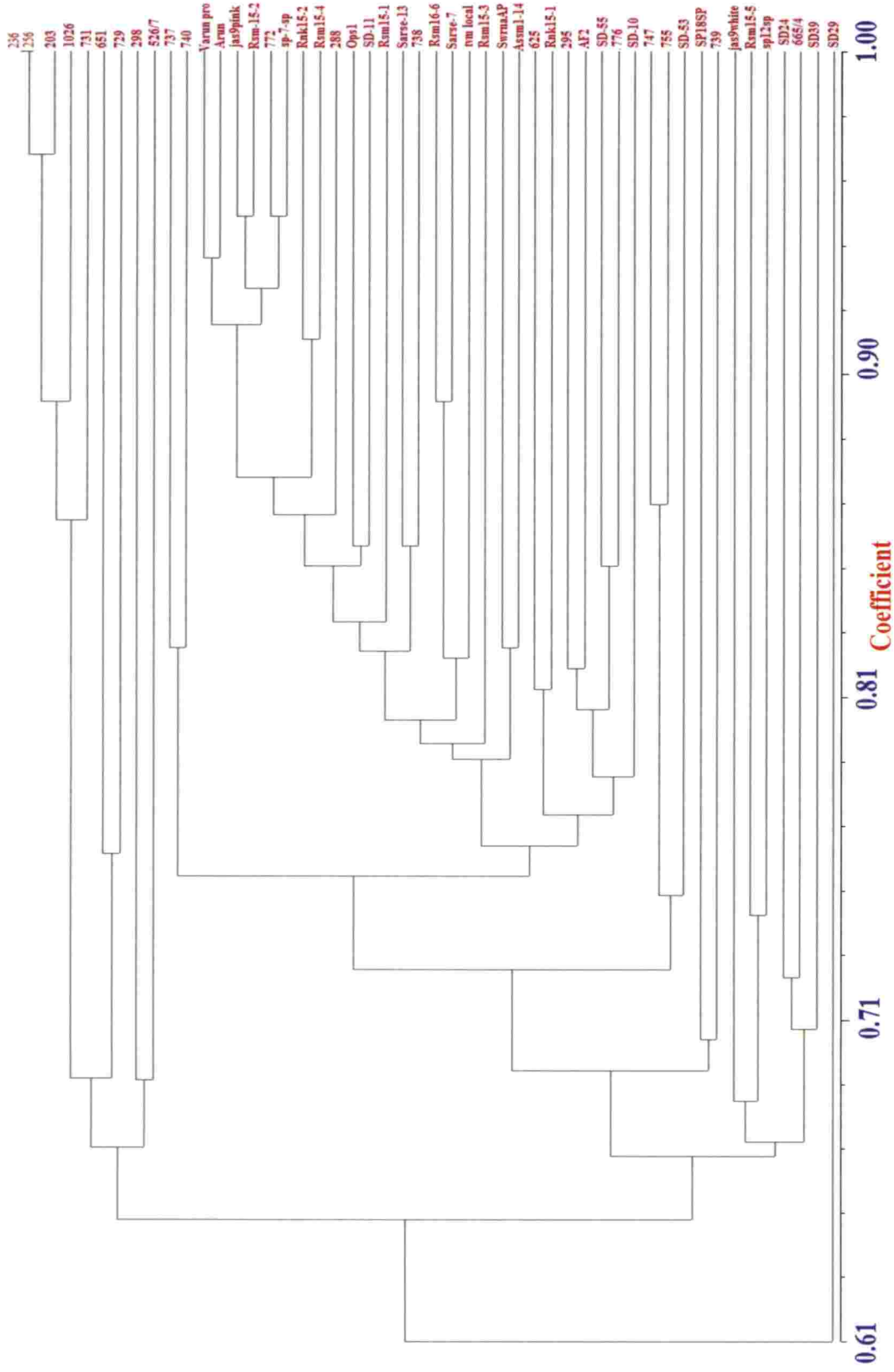
Fig. 3: Cluster dendrogram of 50 accessions based on Hierarrarchial clustering.



DISCUSSION

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5. DISCUSSION

Sweet potato (*Ipomoea batatas* (L.) Lam) belongs to Convovulaceae family. They are herbaceous perennial plants and *Ipomoea batatas* is the only species with edible roots. 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 poor people in 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 within India and different parts of the world. Often the acquisition and collection of new accessions by different collectors from different places will cause accidental invasion of duplicates in the germplasm. Duplicates are considered as the redundant material in every germplasm and often it hampers the variability generation within the germplasm. So duplicates must be eliminated. The only way to identify the duplicates is germplasm characterisation.

In the present study, identification of duplicates in the sweet potato accessions were carried out using morphological and molecular markers and the characterization highlighted the broad genetic base of the crop.

5.1 MORPHOLOGICAL CHARACTERISATION

Twenty morphological characters were recorded using IPGRI descriptors. Three sets of similar or suspected duplicates were included in the accessions selected for the study. Slight variability in few characters even within the suspected duplicate sets was observed. Secondary vine colour, vine tip pubescence and mature leaf size were the main variable characters observed within the suspected duplicate sets. Characters such as leaf lobes type, general outline of leaf, shape of central leaf lobe and leaf lobe numbers were observed similar and unique within the suspected duplicate sets of accessions. This is in contrast to that observed in the morphological analysis done by Tairo *et al.* (2008)

where the mature leaf colour was found as the common character among the sweet potato accessions studied.

STATISTICAL ANALYSIS

In the present study, the UPGMA cluster analysis using 20 variables separated the selected fifty sweet potato accessions into eight clusters at a Euclidean distance of 1. Among the eight clusters, 4th cluster was having maximum number of accessions. Thus the similarity co efficient value in this study is low in contrast to that obtained by Karuri *et al.* (2010). They evaluated 89 sweet potato accessions morphologically and the dendrogram based on morphological data separated the accessions into two major clusters at a Euclidean distance of 6.98. Among the 34 characters studied, the major characters that separated the accessions into two major clusters in their study were the shape of the central leaf lobe and the general outline of leaf. In the present study based on the principal component analysis, the major grouping of accessions was based on secondary vine colour. The present study obtained a higher similarity co efficient of 1.0 compared to Tairo *et al.* (2008) which is 0.52 in a duplicate identification study among sweet potato.

In the present study, two sets of duplicates were identified in the first cluster and one set in the fourth cluster with each set consisting of 2 accessions. Thus the study identified three sets of duplicates. This is very low value as compared to Huaman *et al.* (1999) where, the identification of duplicates in the Peruvian sweet potato collections in CIP reduced from 1939 to 673 and the duplicate accessions per cultivar ranged from 1 to 99. They used only 21 descriptors.

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

PRIMER SCREENING FOR PCR

ISSR primer screening was done in the sweet potato accessions in this study for selecting primers that gave a clear and good gel profile. A similar screening

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was done by Basha and Sujatha (2007) to select primers with clear and good banding pattern. They screened a total of 100 ISSR primers. Out of which, 48 primers gave fair amplification products. Mao and Fang (2014) also screened ISSR primers. They investigated 100 UBC primers and they selected only 11 out of the total primers as they gave considerable clear and good bands. In this study, a total of 21 ISSR primers were screened. Out of which, only 11 primers were found suitable for characterization as they provided good and clear banding patterns which only were chosen for further analysis.

ISSR ANALYSIS OF SWEET POTATO ACCESSIONS

ISSR analysis is quick and simpler than most of the markers 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 809, UBC 818, UBC 825, UBC 827, UBC 847, UBC 860, (ACC)6Y, (GA)9AT and (GA)9AC.

Usually ISSR will detect high level of polymorphism compared to other marker system and it has been reported by several researchers (Nagaoka and Ogihara, 1996, Goulão *et al*., 2001). The present study also revealed the very high polymorphism of ISSR primers as all the selected ISSR primers provided a 100% polymorphic gel profile and obtained an average number of 14.7 bands per locus and this is high when compared to those obtained in a study conducted in sweet potato by Quiang *et al.* (2009) where they obtained 13.8 ISSR bands on an average as well as only 89.6% of polymorphism.

A higher value of mean band number per primer was obtained by He *et al.* (2007). They used 14 ISSR primers and obtained a mean band number of 17 per primer and a total of 239 polymorphic bands were generated. This is also high when compared to the present study where only 162 polymorphic bands were obtained in total. They concluded that the detected higher polymorphism in the

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sweet potato accessions was due to the fact that all of them were collected from the secondary centers of the crop diversity.

In the present study, the highest band number of 18 were obtained from two of 11 primers studied and they were UBC 860 and $(GA)_9AC$. The lowest number of polymorphic bands (9) was obtained from the primer UBC 818. So the number of polymorphic bands in the study ranged from 9 to 18 which is high in contrast to that obtained in the study conducted by Moulin *et al.* (2012) among sweet potato accessions where the value ranged from 4 to 11 by using 19 ISSR primers and generated a total of 135 bands which is also low when compared to the present study.

CLUSTER ANALYSIS

Using the molecular scoring data, hierarchical clustering was done based on UPGMA using Jaccard's similarity coefficient and the fifty accessions were divided into two principal cluster and one outlier at a similarity coefficient of 0.61 of which the both principal clusters were further divided into sub clusters.

The first principal cluster consisted of 40 accessions in many sub clusters which indicates high intra clusteral variability indicating the variability in the sweet potato accessions selected for the study. Moulin *et al.* (2012) obtained a dendrogram from a study of 44 sweet potato accessions based on molecular data and found that the clustering and geographical location of the accessions were quite unrelated as accessions were grouped in same cluster regardless of their geographical location. They obtained highly genetically distinct accessions from dendrogram as they did not fit into any of the cluster formed. Similarly the present study also obtained a highly genetically distant accession that existed as an outlier in the dendrogram (SD-29). Similar result was obtained in the dendrogran generated from morphological data also where SD-29 and SD-55 stood as different outliers. SD-29 might have stood apart may be due to the peculiar shape of the leaf which is different from all the other accessions (Plate 7).

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The second principal cluster comprised of one set of two accessions with 100% similarity, indicating this as a duplicate set. These accessions were identified as duplicate set in morphological analysis also. Along with the duplicates, this principal cluster comprised a total of nine asscessions in two sub clusters. Most of the accessions in the first sub cluster possessed a dissected leaf shape.

SIMILARITY MATRIX

Pair wise comparison of accessions indicated genetic similarity between accessions ranging from a maximum of 100% - 52%. . The similarity coefficient between the duplicates 1 indicating 100% similarity between them. This means that dissimilarity or variability ranged from 0- 48 % .This indicates that low to moderate variability exist between the selected accessions.

Among the 50 sweet potato accessions studied, the lowest similarity index of 1.0 was obtained between, S-236 and S-256 wheras the lowest similarity index (0.52) was obtained between SD-39 and S-298.

SUMMARY

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6. SUMMARY

The study entitled "Identification of duplicates in the germplasm of sweet potato (*Ipomoea batatas* (L.) Lam.) using morphological and molecular markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2016-2017. The objective of the study was to identify the duplicates present in the sweet potato germplasm based on morphological and molecular characteristics. Fifty accessions of sweet potato which mainly included three sets of morphologically similar or suspected duplicates 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 and some of storage root characters of sweet potato based on the IPGRI descriptors (CIP *et. al.*,1991) for sweet potato. The percentage distribution of each trait for a particular character was obtained 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 R statistical package. The dendrogram resulted in the separation of accessions into six principal clusters and two outliers at Euclidean distance 1. Three sets of duplicates were observed. The first three principal components accounted for 66.2 % of the variability and the highly loaded characters in the three principal components were predominant vine colour and secondary vine colour, abaxial vein pigmentation and petiole pigmentation (PC1), leaf lobe shape and leaf lobe number, shape of central leaf, lobe and immature leaf colour (PC2) vine tip pubescence (PC3) that contributed to the clustering of the sweet potato accessions.

After morphological analysis, molecular analysis was done by DNA isolation procedures. Fresh young leaf samples were collected from the field was

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used for studies. DNA was isolated using CTAB method. The quality of the obtained DNA was good with a A_{260}/A_{280} ratio of 1.79–2.18.

A total of 21 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. All the primers showed 100% polymorphism and the number of bands ranged from 9 to 18 with an average mean value of 14.7 bands per primer. The highest number of bands (18) were produced by two primers (UBC 860 and (GA) 9AC). Using marker data, NTSYS PC Version 2.02 program generated a dendrogram, which grouped the accessions on the basis of Jaccards similarity coefficient. Thus the fifty accessions were grouped into two principal clusters and one outlier at 0.61 on similarity index scale. The first principal cluster comprised of 40 accessions which were grouped in many sub-clusters and there was lot of intra clusteral variation of intra clusteral variation. The second principal cluster comprised of nine accessions, in which one set of duplicates comprising of two accessions S-236 and S-256 were grouped together. The outlier was SD-29. 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 the identified duplicates (1.0) and least similarity coefficient of 0.52 was observed between SD-39 and S-298. The molecular clustering of sweet potato accessions showed a wide range of intraclusteral variability between accessions. Although an asexually propagated crop, the self incompatibility, out crossing nature and hexaploidy exhibited by sweet potato might have been contributed to the high diversity. Despite higher number of duplicates were observed morphologically, only two true duplicates were identified in this study. This indicates that there is not much duplications in the selected germplasm. More specific markers may help in eliminating duplicates and developing a core collection.

REFERENCE

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7. REFERENCE

Afuape, S. O., Okocha, P. I., and Njoku, D. 2011. Multivariate assessment of the agromorphological variability and yield components among sweet potato (*Ipomoea batatas (L.) Lam*) landraces. *Afr. J. Plant Sci.* 5(2): 123-132.

Aguoru, C. U., Uhia, P., and Olasan, J. O. 2015. Varietal characterisation and taxonomic evaluation of sweet potato *(Ipomoea batatas*) using macro-and micro morphological evidence. *Open Access Libr. J.* 2(08). Available: http://dx.doi.org/10.4236/oalib.1101757. [30 November 2017].

Ahiakpa, J. K., Kaledzi, P. D., Adi, E. B., Peprah, S., and Dapaah, H. K. 2013. Genetic diversity, correlation and path analyses of okra *(Abelmoschus spp.(L.) Moench)* germplasm collected in Ghana. *Int. J. Dev. Sustain* 2(2): 1396-1415.

Allender, C. J. and King, G. J. 2010. Origins of the amphiploid species Brassica napus L. investigated by chloroplast and nuclear molecular markers. *BMC Plant Biol.* 10(1): 54p.

Antial, B. S., Akpanz, E. J., Okonl, P. A., and Umorenl, I. U. 2006. Nutritive and Anti-Nutritive Evaluation of Sweet Potatoes. *Pak. J. Nutr.*, 5(2): 166-168.

Austin, D. F. 1988. The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. In: Gregory, P. (ed.), *Exploration, maintenance, and utilization of sweet potato genetic resources*. International Potato Center, Lima, Peru, pp. 27-60.

Basha, S. D. and Sujatha, M. 2007. Inter and intra-population variability of Jatropha curcas (L.) characterized by RAPD and ISSR markers and

development of population-specific SCAR markers. *Euphytica*, 156(3): 375-386.

Bourke, R. M. 1985. Sweet potato (*Ipomoea batatas*) production and research in Papua New Guinea. *Papua New Guinea J. Agric. For. Fish.* 33(3/4): 89-108.

Bovell-Benjamin, A. C. 2007. Sweet potato: a review of its past, present, and future role in human nutrition. *Adv. Food Nutr. Res.* 52: 1-59.

Brown, A. H. D. 1989. Core collection: A practical approach to genetic resources management. *Genome* 31: 818-824.

Buteler, M. I., Jarret, R. L., and LaBonte, D. R. 1999. Sequence characterization of microsatellites in diploid and polyploid Ipomoea. *Theor. Appl. Genet.* 99(1-2): 123-132.

Chavarriaga-Aguirre, P., Maya, M. M., Tohme, J., Duque, M. C., Iglesias, C., Bonierbale, M. W., Kresovich, S. and Kochert, G., 1999. Using microsatellites, isozymes and AFLPs to evaluate genetic diversity and redundancy in the cassava core collection and to assess the usefulness of DNA-based markers to maintain germplasm collections. *Mol. Breed.* 5(3): 263-273.

CIP, AVRDC, IBPGR. [International Potato Center, Asian Vegetable Research and Developement Center, International Board for Plant Genetic Resources], 1991. *Descriptors for Sweet Potato*. In: Huaman, Z. (ed.), International Board for Plant Genetic Resources, Rome, Italy. 52p.

Connolly, A. G., Godwin, I. D., Cooper, M., and DeLacy, I. H. 1994. Interpretation of randomly amplified polymorphic DNA marker data for fingerprinting sweet potato (*Ipomoea batatas L.*) genotypes. *Theor. Appl. Genet.* 88(3): 332-336.

Costa, J. C., Fracetto, G. G. M., Fracetto, F. J. C., Santos, M. V. F., and Júnior, M. L. 2017. Genetic diversity of *Desmanthus sp* accessions using ISSR markers and morphological traits. *Genet. Mol. Res.* 16(2): 1-9.

Del Rio, A. H., Bamberg, J. B., and Huaman, Z. 2006. Genetic equivalence of putative duplicate germplasm collections held at CIP and US potato genebanks. *Am. J. Potato Res.* 83(3): 279-285.

Diederichsen, A. 2009. Duplication assessments in Nordic Avena sativa accessions at the Canadian national genebank. *Genet. Resour. Crop Evol.* 56: 587-597.

Elameen, A., Fjellheim, S., Larsen, A., Rognli, O. A., Sundheim, L., Msolla, S., Masumba, E., Mtunda, K., and Klemsdal, S. S. 2008. Analysis of genetic diversity in a sweet potato *(Ipomoea batatas L.)* germplasm collection from Tanzania as revealed by AFLP. *Genet. Resour. Crop Evol.* 55(3): 397-408.

Elibariki, G., Njahira, M., Ndunguru, J., Hosea, K. M., and Wanjala, B. W. 2013. Genetic diversity and identification of duplicates in selected Tanzanian farmer preferred cassava landraces using Simple Sequence Repeat (SSR) markers. *Afr. J. Res.* 22: 3257-3258.

Fang, D. Q. and Roose, M. L. 1997. Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theor. Appl. Genet.* 95(3): 408-417.

FAOSTAT [Food and agriculture organisation statistical database]. 2014 [online] Available: http://faostat.fao.org [6 June 2017].

Fernandez, M., Figueiras, A., and Benito, C. 2002. The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and

104

genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet.* 104(5): 845-851.

Fongod, A. G. N., Mih, A. M., and Nkwatoh, T. N. 2012. Morphological and agronomic characterization of different accessions of sweet potatoes (*Ipomoea batatas*) in Cameroon. *Int. Res. J. Agric. Sci. Soil Sci.* 2(6): 234-245.

Fowler, C. 2007. Global dimensions of conserving crop diversity. In: *Seed Savers 2006 Harvest Edition*. Seed Savers Exchange, Decorah, pp. 33-42.

Frankel, O. H. 1984. Genetic perspectives of germplasm conservation. In: Arber, w. (ed.), *Genetic manipulation: Impact on man and society*. Cambridge University Press, Cambridge, pp. 161-170.

GCDT [Global Crop Diversity Trust] 2007. *Global strategy for ex-situ conservation of sweet potato genetic resources*. Global Crop Diversity Trust, Rome, p.59.

Gepts, P. 1993. The use of molecular and biochemical markers in crop evolution studies. *Evol. biol.* 27: 51-94.

Gepts, P. 2002. A comparison between crop domestication, classical plant breeding, and genetic engineering. *Crop Sci.* 42(6): 1780-1790.

Goulao, L. and Oliveira, C. M. 2001. Molecular characterisation of cultivars of apple (Malus× domestica Borkh.) using microsatellite (SSR and ISSR) markers. *Euphytica* 122(1): 81-89.

Goulão, L., Valdiviesso, T., Santana, C., and Oliveira, C. M. 2001. Comparison between phenetic characterisation using RAPD and ISSR markers and phenotypic data of cultivated chestnut (Castanea sativa Mill.). *Genet. Res. Crop Evol.* 48(4): 329-338.

105

Greene, S. L. and Pederson, G. A., 1996. Eliminating duplicates in germplasm collections: a white clover example. *Crop sci.* 36(5):1398-1400.

Gross, B. L., Volk, G. M., Richards, C. M., Forsline, P. L., Fazio, G., and Chao, C. T. 2012. Identification of "duplicate" accessions within the USDA-ARS National Plant Germplasm System Malus collection. *J. Am. Soc. Hortic. Sci.* 137(5): 333-342.

Gupta, M., Chyi, Y. S., Romero-Severson, J., and Owen, J. L. 1994. Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theor. Appl. Genet.* 89(7-8): 998-1006.

Hahn, S. K. 1977. Sweet potato. In: Alvium, T. and Kozlowski, T. T. (eds), *Ecophysiology of tropical crops*, pp. 237-248.

Hall, A., Bockett, G., and Nahdy, S. 1998. *Sweet potato postharvest systems in Uganda: Strategies, constraints and potentials.* International Potato Centre (CIP), Lima, Peru. 73p.

He, G., Prakash, C. S., and Jarret, R. L. 1995. Analysis of genetic diversity in a sweet potato *(Ipomoea batatas)* germplasm collection using DNA amplification fingerprinting. *Genome* 38(5): 938-945.

He, X. Q., Liu, Q. C., Ishiki, K., Zhai, H., and Wang, Y. 2007. ISSR analysis of genetic diversity and relationships among sweet potato (*Ipomoea batatas*) landraces in China. *Plant Genet. Resour. Newsl.* 150: 35-41.

Hoda, A., Sena, L., and Hykaj, G. 2012. Genetic diversity revealed by AFLP markers in Albanian goat breeds. *Arch. Biol. Sci.* 64(2): 799-807.

Hu, J., Nakatani, M., Lalusin, A. G., Kuranouchi, T., and Fujimura, T. 2003. Genetic analysis of sweet potato and wild relatives using inter-simple sequence repeats (ISSRs). *Breed. Sci.* 53(4): 297-304.

106

Huamán Z. 1988. Descriptors for the characterization and evaluation of sweet potato genetic resources. In: *Planning conference of exploration, maintenance and utilization of sweet potato genetic resources;* 23-27 February, 1987; International Potato Center, Lima, Peru, pp. 331-335.

Huaman, Z. 1992. Morphologic identification of duplicates in collections of *Ipomoea batatas. CIP Res. Guide.* 32p.

Huaman, Z. 1999. *Sweet potato Germplasm Management (Ipomoea batatas).* Training manual, International Potato Centre, Lima, Peru, 218p.

Huaman, Z., Aguilar, C., and Ortiz, R. 1999. Selecting a Peruvian sweet potato core collection on the basis of morphological, eco-geographical, and disease and pest reaction data. *Theor. Appl. Genet.* 98(5): 840-844.

Huang, J. C. and Sun, M. 2000. Genetic diversity and relationships of sweet potato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by InterSsimple Sequence Repeat (ISSR) and restriction analysis of chloroplast DNA. *Theor. Appl. Genet.* 100(7): 1050-1060.

Hue, S. M., Boyce, A. N., and Somasundram, C. 2012. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*'Ipomoea batatas'*). *Aust. J. Crop Sci.* 6(3): 375.

IBPGR (International Board for Plant Genetic Resources). 1985. *Oat descriptors.* International Board for Plant Genetic Resources, Rome, 21p.

ICAR-CTCRI [Indian Council of Agricultural Research - Central Tuber Crop Research Institute]. 2015. Collection, conservation, characterization and evaluation of germplasm of tropical root and tuber crops, *ICAR-CTCRI Annual report 2014-2015.* ICAR-CTCRI, Sreekariyam, Trivandrum, Kerala, p.29.

107

Ipek, M., Ipek, A., and Simon, P. W. 2003. Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. *J. Am. Soc. Hortic. Sci.* 128(2): 246-252.

Ishiguro, K., Toyama, J., Islam, M.S., Yoshimoto, M., Kumagai, T., Kai, Y., Nakazawa, Y. and Yamakawa, O., 2004. Suioh, a new sweet potato cultivar for utilization in vegetable greens. *Acta hortic.* 637: 339-345.

Islam, S. 2014. *Nutritional and Medicinal Qualities of Sweet potato Tops and Leaves*. Cooperative Extension Service, University of Arkansas at Pine Bluff, Jefferson, pp. 1-4.

Jana, R. K. 1982. Status of sweet potato cultivation in East Africa and its future. In: Villareal, R.L. and Griggs, T.D. (ed.), *Sweet potato.* Proceedings of the first International Symposium, March, 1981, Taiwan. Asian Vegetable Research and Development Center, Taiwan, pp. 23-27.

Jarret, R. L. and Austin, D. F. 1994. Genetic diversity and systematic relationships in sweet potato (*Ipomoea batatas (L.) Lam.)* and related species as revealed by RAPD analysis. *Genet. resour. crop evol.* 41(3): 165-173

Jarret, R. L., Gawel, N., and Whittemore, A. 1992. Phylogenetic relationships of the sweet potato [Ipomoea batatas (L.) Lam.]. *J. Am. Soc. Hortic. Sci.* 117(4): 633-637.

Jones, A. 1968. Chromosome numbers in the genus Ipomoea and related genera. *J. Heredity* 59:99-102.

Joshi, S. P., Gupta, V. S., Aggarwal, R. K., Ranjekar, P. K., and Brar, D. S. 2000. Genetic diversity and phylogenetic relationship as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus *Oryza. Theor. Appl. Genet.* 100(8): 1311-1320.

108

Karp, A., Seberg, O. L. E., and Buiatti, M. 1996. Molecular techniques in the assessment of botanical diversity. *Ann. Bot.* 78(2): 143-149.

Karuri, H. W., Ateka, E. M., Amata, R., Nyende, A. B., Muigai, A. W. T., Mwasame, E., and Gichuki, S.T. 2010. Evaluating diversity among Kenyan sweet potato genotypes using morphological and SSR markers. *Int. J. Agric. Biol.* 12(1): 33-38.

Khalik, K. A., El-Sheikh, M., and El-Aidarous, A. 2013. Floristic diversity and vegetation analysis of wadi Al-Noman, Mecca, Saudi Arabia. *Turkish J. Bot.* 37(5): 894-907.

Klein, P. E., Klein, R. R., Cartinhour, S. W., Ulanch, P. E., Dong, J., Obert, J. A., Morishige, D. T., Schlueter, S. D., Childs, K. L., Ale, M., and Mullet, J. E. 2000. A high-throughput AFLP-based method for constructing integrated genetic and physical maps: progress toward a sorghum genome map. *Genome Res.* 10(6): 789-807.

Knüpffer, H., Frese, L., and Jongen, M. W. M. 1997. Using central crop databases: searching for duplicates and gaps. In: *Central Crop Databases: Tools for Plant Genetic Resources Management.* Workshop Report, 13-16 October 1996; Budapest, Hungary, pp. 67-77.

Koussao, S., Gracen, V., Asante, I., Danquah, E. Y., Ouedraogo, J. T., Baptiste, T. J., and Vianney, T. M. 2014. Diversity analysis of sweet potato *(Ipomoea batatas [L.] Lam)* germplasm from Burkina Faso using morphological and simple sequence repeats markers. *Afr. J. Biotech.* 13(6): 729-742.

Kovach, M. J. and McCouch, S. R. 2008. Leveraging natural diversity: back through the bottleneck. *Curr. Opn. plant boil.* 11(2): 193-200.

Kumar, N. S. and Gurusubramanian, G. 2011. Random Amplified Polymorphic DNA (RAPD) markers and its applications. *Sci. Vis.* 11(3): 116-124.

109

Kumar, P., Gupta, V. K., Misra, A. K., Modi, D. R., and Pandey, B. K. 2009. Potential of molecular markers in plant biotechnology. *Plant Omics* 2(4): 141-162.

Kure, O.A., Nwankwo, L., Wyasu, G. 2012. Production and quality evaluation of garri- like product from sweetpotatoes. *J. Nat. Prod. Plant. Resour.* 2 (2): 318-321.

Laurie, S. M., Calitz, F. J., Adebola, P. O., and Lezar, A. 2013. Characterization and evaluation of South African sweet potato (*Ipomoea batatas (L.) LAM*) land races. *S. Afr. J. Bot. 85*: 10-16.

Lin, J. J. and Kuo, J. 1995. AFLPTM: a novel PCR-based assay for plant and bacterial DNA fingerprinting. *Focus* 17: 66-70.

Lin, J. J., Kuo, J., Ma, J., Saunders, J. A., Beard, H. S., MacDonald, M. H., Kenworthy, W., Ude, G. N., and Matthews, B. F. 1996. Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. *Plant Mol. Biol. Report.* 14(2): 156-169.

Liu, B. And Wendel, J. F. 2001. Inter Simple Sequence Repeat (ISSR) polymorphisms as a genetic marker system in cotton. *Mol. Ecol. Resour.* 1(3): 205-208.

Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F., and Tschirley, D. 2007. A food-based approach introducing orange-fleshed sweet potatoes increased vitamin A intake and serum retinol concentrations in young children in rural Mozambique. *J. Nutr.* 137(5): 1320-1327.

Mace, E. S., Mathur, P. N., Izquierdo, L., Hunter, D., Taylor, M. B., Singh, D., DeLacy, I. H., Jackson, G. V. H., and Godwin, I. D. 2006. Rationalization of taro germplasm collections in the Pacific Island region using simple sequence repeat (SSR) markers. *Plant Genet. Resour.* 4(3): 210-220.

110

Maeshima, M., Sasaki, T., and Asahi, T. 1985. Characterization of major proteins in sweet potato tuberous roots. *Phytochemistry* 24(9): 1899-1902.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer res.* 27: 209-220.

Mao, L. H. and Fang, Y. M. 2014. ISSR primer screening and preliminary evaluation of genetic diversity in *Haplocladium microphyllum*. *Biochem. Syst. Ecol.* 55: 107-111.

Marshall, D. R. and Allard, R.W. 1970. Isozyme polymorphisms in natural populations of *Avena fatua* and *A. barbata*. *Heredity* 25: 373-82.

Marshall, D. R. And Brown A. H. D. 1975. Optimum sampling strategies in genetic conservation. In: Frenkel, O. H. and Hawkes J. G. (eds), *Crop genetic resources for today and tomarrow.* International biological programme; London, Cambridge University Press. pp. 53-80.

Martins, G. V., Martins, L. S. S., Veasey E. A. And Lederman, I. E. 2012. Diversity and genetic structure in natural populations of Hancornia speciosa var. speciosa Gomes in northeastern Brazil. *Rev. Bras. Frutic.* 34: 1143-1153.

McGregor, C., Greyling, M., Banda, J., and Laurie, S. 2001. DNA fingerprinting of sweet potato (*Ipomoea batatas* L.): two case studies in africa. *Acta Hortic.* 546: 243-248.

Melo, R. D. A., Resende, L. V., Menezes, D., Beck, A. P. A., Costa, J. C. D., Coutinho, A. E., and do Nascimento, A. V. S. 2011. Genetic similarity between coriander genotypes using ISSR markers. *Hortic. Bras.* 29(4): 526-530.

Mignouna, H. D., Abang, M. M., Wanyera, N. W., Chikaleke, V. A., Asiedu, R., and Thottappilly, G. 2005. PCR marker-based analysis of wild and

cultivated yams (*Dioscorea spp.*) in Nigeria: genetic relationships and implications for ex situ conservation. *Genet. Resour. Crop Evol.* 52(6): 755-763.

Moisan-Thiéry, M., Le Hingrat, Y., and Kerlan, M. C. 2001. Potato cultivars identification using molecular markers. *Acta Hortic.* 546:471-477

Mok, I. G. and Schmiediche, P. 1999. Collecting, characterizing and maintaining sweet potato germplasm in Indonesia. *Plant genet. resour. Newsl.* pp. 12-18.

Mok, I. G., Zhang, D., and Carey, E. E. 1997. Sweet potato breeding strategy of CIP. In: *Proceedings of international workshop on sweet potato production system towards the 21st Century*; December 1997, International Potato Center, Lima, Peru, pp. 9-27.

Moulin, M. M., Rodrigues, R., Gonçalves, L. S. A., Sudré, C. P., and Pereira, M. G. 2012. A comparison of RAPD and ISSR markers reveals genetic diversity among sweet potato landraces *(Ipomoea batatas (L.) Lam.). Acta Sci. Agron.* 34(2): 139-147.

Moura, E. F., Fariansneto, J. T. D., Sampaio, J. E., Silva, D. T. D., and Ramalho, G. F. 2013. Identification of duplicates of cassava accessions sampled on the north region of Brazil using microsatellite markers. *Acta Amazon* 43(4): 461-467.

Nagaoka, T. and Ogihara, Y. 1997. Applicability of Inter Simple Sequence Repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. *Theor. Appl. Genet.* 94(5): 597-602.

Naik, S., Hampson, C., Gasic, K., Bakkeren, G. and Korban, S. 2006 Development and linkage mapping of E-STS and RGA markers for functional gene homologues in apple. *Genome* 49: 959-968.

112

Naik, V. G. and Dandin, S. B. 2006. Identification of duplicate collections in the mulberry (Marus spp.) germplasm using RAPD analysis. *Indian J. Genet. Plant Breed.* 66(4): 287-292.

Nei, M. and Li, W. H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natnl Acad. Sci.* 76(10): 5269-5273.

O'Brien, P. J. 1972. The sweet potato: its origin and dispersal. *Am. Anthropologist* 74(3): 342-365.

Onwueme, I. C. 1978. *The tropical tuber crops: yams, cassava, sweet potato, and cocoyams.* John Wiley and Sons, Chichester, 234p.

Ozias-Akins, P. and Jarret, R. L. 1994. Nuclear DNA content and ploidy levels in the genus Ipomoea. *J. Am. Soc. Hortic. Sci.* 119(1): 110-115.

Paäakinskiene, I., Griffiths, C. M., Bettany, A. J. E., Paplauskiene, V., and Humphreys, M. W. 2000. Anchored simple-sequence repeats as primers to generate species-specific DNA markers in Lolium and Festuca grasses. *Theor. App. Genet.* 100(3-4): 384-390.

Padmaja, G. 2009. Uses and nutritional data of sweet potato. In: Loebenstein G. and Thottappilly G. (eds), *The Sweetpotato*. Springer, Dordrecht. pp. 189-234.

Pallais, N. 1991. Origin of the International Potato Center. *HortSci.* 26(3): 230-323.

Placide, R., Shimelis, H., Laing, M., and Gahakwa, D. 2015. Phenotypic characterisation of sweet potato genotypes grown in East and Central Africa. *S. Afr. J. Plant Soil* 32(2): 77-86.

113

Prakash, C. S., He, G., and Jarret, R. L. 1996. DNA marker-based study of genetic relatedness in United States sweet potato cultivars. *J. Am. Soc. Hortic. Sci.* 121(6): 1059-1062.

Qiang, L. I., LI, X. Y., LI, H. M., Tang, Z. H., Ling, H. U., Cao, Q. H., Xie, Y. P., and Xin, W. A. N. G. 2009. Selection of parents for breeding edible varieties of sweet potato with high carotene content. *Agric. Sci. China* 8(10): 1166-1173.

Rao, N. K. 2004. Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotech.* 3(2): 136-145.

Reddy, M. P., Sarla, N., and Siddiq, E. A. 2002. Inter Simple Sequence Repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica* 128(1): 9-17.

Rocha, A., Salomão, L. C. C., Salomão, T. M. F., Cruz, C. D., and de Siqueira, D. L. 2012. Genetic diversity of 'Uba'mango tree using ISSR markers. *Mol. Biotech.* 50(2): 108-113.

Rohlf, F. J. 1998. *NTSYS-pc Numerical Taxonomy and Multivariate Analysis System Version 2.02*. (Reprint, 2004). Exeter Publishing Ltd, Setauket, New York, USA, 44p.

Rossel, G., Kriegner, A., and Zhang, D. P. 2001. From Latin America to Oceania: the historic dispersal of sweet potato re-examined using AFLP. In: *CIP program report* 2000; Peru. International Potato Center, Lima, Peru, pp. 315-321.

Rossel, G., Robles, R., and Tay, D. 2009. Identification of duplicate accessions within a sweet potato germplasm collection using morphological characterization and AFLP markers. In: *Fifteenth triennial ISTRC*

symposium; 2-6 November, 2009; Peru. International Society for Tropical Root Crops, Peru, pp. 25-30.

Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Bringas, V. M. F., Rossel, G., Tay, D., McKey, D., and Lebot, V. 2013. Disentangling the Origins of Cultivated Sweet Potato (*Ipomoea batatas (L.) Lam.*). *PLoS One* 8(5): 1-12.

Rout, G. R., Senapati S. K. and Aparajitha, S. 2010. Identification and phylogenetic analysis of Ipomoea species collected from eastern India using inter simple sequence repeat markers. *Int. J. Pharma. Bio Sci.* 1(4): 1-13.

Sagredo, B., Hinrichsen, P., López, H., Cubillos, A. and Muñoz, C. 1998. Genetic variation of sweet potatoes *(Ipomoea batatas L.)* cultivated in Chile determined by RAPDs. *Euphytica* 101(2): 193-198.

Sneath, P. H. A. and Sokal, R. R. 1973 *Numerical Taxonomy.* WH Freeman and Company, San Francisco, pp. 100-308.

Soegianto, A., Ardiarini, N. R., and Surgiarto, A. N. 2011. Genetic diversity of sweet potato (*Ipomoea batatas L.*) in East Java, Indonesia. *J. Agric. Food. Technol.* 9:179-183.

Spooner, D., van Treuren, R., and de Vicente, M. C. 2005. *Molecular markers for genebank management.* IPGRI Technical Bulletin No. 10, International Plant Genetic Resources Institute, Rome, Italy, 136p.

Srisuwan, S., Sihachakr, D., and Siljak-Yakovlev, S. 2006. The origin and evolution of sweet potato (*Ipomoea batatas Lam.*) and its wild relatives through the cytogenetic approaches. *Plant Sci.* 171(3): 424-433.

115

Stamp, P., Messmer, R., and Walter, A. 2012. Competitive underutilized crops will depend on the state funding of breeding programmes: an opinion on the example of Europe. *Plant breed.* 131(4): 461-464.

Tairo, F., Mneney, E., and Kullaya, A. 2008. Morphological and agronomical characterization of sweet potato [*Ipomoea batatas (L.) Lam.*] germplasm collection from Tanzania. *Afr. J. Plant Sci.* 2(8): 077-085.

Thompson, P. G., Hong, L. L., Ukoskit, K., and Zhu, Z. 1997. Genetic linkage of randomly amplified polymorphic DNA (RAPD) markers in sweet potato. *J. Am. Soc. Hortic. Sci.* 122(1): 79-82.

Tsegaye, E., Dechassa, N., and Sastry, E. D. 2007. Genetic variability for yield and other agronomic traits in sweet potato. *J. Agron.* 6(1): 94-99.

Tseng, Y. T., Lo, H. F., and Hwang, S. Y. 2002. Genotyping and assessment of genetic relationships in elite polycross breeding cultivars of sweet potato in Taiwan based on SAMPL polymorphisms. *Bot. Bull. Acad. Sinica* 43(2): 99-105.

Tumwegamire, S., Kapinga, R., Zhang, D., Crissman, C., and Agili, S. 2004. Opportunities for promoting orange-fleshed sweet potato as a mechanism for combat vitamin-A deficiency in Sub-Saharan Africa. *Afr. Crop Sci. J.* 12(3): 241-252.

Ugent, D., Peterson, L. W., Pozorski, S., and Pozorski, T. 1982. Archaeological potato tuber remains from the Casma Valley of Peru. *Econ. Bot.* 36: 182-192.

Upadhyay, A., Aher, L. B., Shinde, M. P., Mundankar, K. Y., Datre, A., and Karibasappa, G. S. 2013. Microsatellite analysis to rationalize grape germplasm in India and development of a molecular database. *Plant Genet. Resour.* 11(3): 225-233.

116

Upadhyaya, H. D., Gowda, C. L. L., and Sastry, D. V. S. S. R. 2008. Plant genetic resources management: collection, characterization, conservation and utilization. *J. SAT Agric. Res.* 6:1-16.

van Hintum, T. J., Brown, A. H. D., Spillane, C., and Hodkin, T. 2000. *Core collections of plant genetic research.* In: IPGRI Technical Bulletin No. 3. International Plant Genetic Resources Institute, Rome, Italy, 48p.

van Jaarsveld, P. J., Faber, M., Tanumihardjo, S. A., Nestel, P., Lombard, C. J., and Benadé, A. J. S. 2005. β-Carotene–rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with the modified-relative-dose-response test. *Am. J. Clin. Nutr.* 81(5): 1080-1087.

van Jaarsveld, P. J., Harmse, E., Nestel, P., and Rodriguez-Amaya, D. B. 2006. Retention of β-carotene in boiled, mashed orange-fleshed sweet potato. *J. Food Compos. Anal.* 19(44): 321-329.

van Treuren, R., Magda, A., Hoekstra, R., and van Hintum, T. J. 2004. Genetic and economic aspects of marker-assisted reduction of redundancy from a wild potato germplasm collection. *Genet. Resour. Crop Evol.* 51(3): 277-290.

van Treuren, R., van Soest, L. J. M., and van Hintum, T. J. 2001. Marker-assisted rationalisation of genetic resource collections: a case study in flax using AFLPs. *Theor. Appl. Genet.* 103(1): 144-152.

van Hintum, T. J. and Visser, D. L. 1995. Duplication within and between germplasm collections. *Genet. resour. crop Evol.* 42(2): 135-145.

Veasey, E. A., Borges, A., Rosa, M. S., Queiroz-Silva, J. R., Bressan, E. D. A., and Peroni, N. 2008. Genetic diversity in Brazilian sweet potato (*Ipomoea batatas (L.) Lam.*, Solanales, Convolvulaceae) landraces assessed with microsatellite markers. *Genet. Mol. Boil.* 31(3): 725-733.

117

Virk P. S., Newbury H. J., Jackson M .T., and Ford-Lloyd B, V. 1995. The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theor. Appl. Genet.* 90: 1049-1055.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Lee, T. V. D., Hornes, M., Friters, A., Pot, J., Paleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic acids Res.* 23(21): 4407-4414.

Walker, T., Thiele, G., Suárez, V., and Crissman, C. C. 2011. *Hindsight and foresight about sweet potato production and consumption.* International Potato Center, Lima, Peru, 52p.

Wang, X., Rinehart, T. A., Wadl, P. A., Spiers, J. M., Hadziabdic, D., Windham, M. T., and Trigiano, R. N. 2009. A new electrophoresis technique to separate microsatellite alleles. *Afr. J. Biotech.* 8(11): 2432-2436.

Wang, Z., Li, J., Luo, Z., Huang, L., Chen, X., Fang, B., Li, Y., Chen, J., and Zhang, X. 2011. Characterization and development of EST-derived SSR markers in cultivated sweet potato (*Ipomoea batatas*). *BMC plant Boil.* 11(1): 139p.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic acids Res.* 18(24): 7213-7218.

Westman, A. L. and Kresovich, S. 1997. Use of molecular marker techniques for description of plant genetic variation. *Biotech. Agric. ser.* pp. 9-48.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids Res.* 18(22): 6531-6535.

Wireko-Manu, F. D., Ellis, W. O., and Oduro, I. 2010. Production of a non-alcoholic beverage from sweet potato (*Ipomoea batatas L.*). *Afr. J. Food Sci.* 4(4): 180-183.

118

Wolfe, A. D., XIANG, Q. Y., and Kephart, S. R. 1998. Assessing hybridization in natural populations of Penstemon (*Scrophulariaceae*) using hypervariable Inter Simple Sequence Repeat (ISSR) bands. *Mol. Ecol.* 7(9):1107-1125.

Woolfe, J. A. 1992. *Sweet potato: an untapped food resource*. Cambridge University Press. Newyork, USA, 643p.

Wu, K. S., Jones, R., Danneberger, L., and Scolnik, P. A. 1994. Detection of microsatellite polymorphisms without cloning. *Nucleicacids Res.* 22(15): 3257-3258.

Yada, B., Tukamuhabwa, P., Wanjala, B., Kim, D. J., Skilton, R. A., Alajo, A., and Mwanga, R. O. 2010. Characterization of Ugandan sweet potato germplasm using fluorescent labeled Simple Sequence epeat markers. *HortScience* 45(2): 225-230.

Yen, D. E. 1982. Sweet potato in historical perspective. In: Villareal, R. L. and Griggs, T. D. (ed.), *Sweet potato.* Proceedings of the first International Symposium, Asian Vegetable Research and Development Center, Shanhua, Tainan. pp. 17–30.

Zhang, D. P., Carbajulca, D., Ojeda, L., Rossel, G., Milla, S., Herrera, C., and Ghislain, M. 1999. Microsatellite analysis of genetic diversity in sweet potato varieties from Latin America. *CIP Program report* 2000. pp. 295-301.

Zhang, D., Cervantes, J., Huamán, Z., Carey, E., and Ghislain, M. 2000. Assessing genetic diversity of sweet potato (*Ipomoea batatas (L.) Lam.*) cultivars from tropical America using AFLP. *Genet. Resour. Crop Evol.* 47(6): 659-665.

119

Zhang, D., Mischke, S., Johnson, E. S., Phillips-Mora, W., and Meinhardt, L. 2009. Molecular characterization of an international cacao collection using microsatellite markers. *Tree Genet. Genomes* 5(1): 1-10.

Zhang, D., Rossel, G., Kriegner, A., and Hijmans, R. 2004. AFLP assessment of diversity in sweet potato from Latin America and the Pacific region: Its implications on the dispersal of the crop. *Genet. Resour. Crop Evol.* 51(2): 115-120.

Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* 20(2): 176-183

APPENDICES

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8. APPENDIXES

APPENDIX I

A) CTAB Extraction Buffer

Tris -HCL	1M	
EDTA(pH=8)	0.25M	
NaCl	5 M	
CTAB	2%	
β-mercaptoethanol	0.2% (v/v)	Added freshly prior to grinding
PVP	1% (w/v)	
PEG	1%(w/v)	
Distilled water		

APPENDIX II

B) TE BUFFER (10X)

Tris – HCl(pH 8.0)	10 mM
EDTA	1 mM

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APPENDIX III

C) TBE Buffer (10 X)

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

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

ABSTRACT

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IDENTIFICATION OF DUPLICATES IN THE GERMPLASM OF SWEET POTATO (*Ipomoea batatas* (L.) LAM.) USING MORPHOLOGICAL AND MOLECULAR MARKERS

By

BABITHA BABU

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Abstract of the thesis

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9. ABSTRACT

The study entitled "Identification of duplicates in the germplasm of sweet potato (*Ipomoea batatas* (L.) Lam.) using morphological and molecular markers" was carried out at the Division of Crop Improvement, ICAR-Central Tuber Crops Research Institute, Sreekariyam, Thiruvananthapuram during 2016-2017. The objective of the study was to identify duplicates in the sweet potato germplasm based on morphological and molecular markers. Identification and elimination of these common redundant materials will enhance the germplasm viability.

Fifty accessions were selected for the study. The study was divided into two phases - morphological and molecular analysis. Morphological analysis was performed by using twenty descriptors as provided by IPGRI (CIP *et. al.*, 1991). The recorded data were analyzed statistically by various tools such as PCA and cluster dendrogram. Cluster dendrogram identified three sets of morphological duplicates and the accessions were separated into six principal clusters and two outliers at a Euclidean distance of 1. The PCA analysis revealed predominant vine colour and secondary vine colour, abaxial vein pigmentation and petiole pigmentation as the major factors that contributed to the clustering of the sweet potato accessions.

After morphological analysis, molecular analysis was performed. The genomic DNA was isolated using CTAB method which gave good quality DNA. 11 ISSR primers were used for screening of fifty accessions. After the final PCR using selected primers, the product was resolved in 2% agarose and polymorphic bands were obtained. All the primers showed 100% polymorphism and the number of bands ranged from 9 to 18 with a mean value of 14.7 bands per primer. Using the molecular scoring data, UPGMA clustering was done and the whole fifty accessions were divided mainly into two principal clusters and one outlier. The first principal cluster comprised of 40 accessions which were grouped into many subclusters and there was lot of intraclusteral variation. The second

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principal cluster consisted of 9 accessions and this principal cluster comprised of two true duplicates which were also found similar in morphological characterization. The outlier was different from all the other accessions and may be due to the peculiar leaf shape which is not seen in other accessions selected in the study. SD-29 was different from all the remaining accessions by a similarity coefficient of 0.61.The similarity between the different accessions ranged between 52-100%. The duplicates S-236 and S-256 were 100% similar. The least similar accessions were SD-39 and S-298 (52%). Thus it can be inferred that a 48% variability or diversity existed within the selected accessions which can be considered as a moderate diversity.

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. Only two duplicates were identified. In future more specific markers may be used for core collection development and to eliminate duplicates.

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