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MOLECULAR CHARACTERISATION OF SWEET POTATO (*Ipomoea batatas* (L.) Lam) ACCESSIONS AND WILD RELATIVES USING SSR MARKERS

by

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(2010-09-115)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

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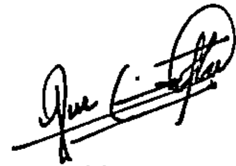
KERALA, INDIA

2015

DECLARATION

I hereby declare that this thesis entitled **"MOLECULAR CHARACTERISATION OF SWEET POTATO (*Ipomoea batatas* (L.) Lam) ACCESSIONS AND WILD RELATIVES USING SSR 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, associate ship, fellowship or other similar title, of any other university or society.



Place: Vellayani

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Produce tuber reduce hunger

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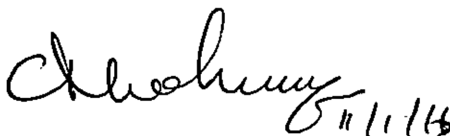

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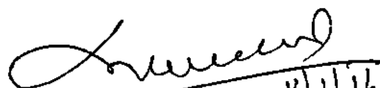

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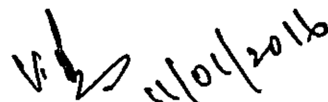

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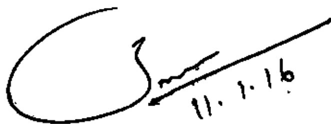

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Dedicated to my loving parents and sweet sister....

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LIST OF ABBREVIATIONS AND SYMBOLS USED

Hr	hour
Min	minute
Fig.	Figure
et al.	And other co workers
SDS	Sodium Dodecyl Sulphate
°C	Degrees centigrade
%	percentage
cm	Centimeter
Sec	Seconds
PCR	Polymerase Chain Reaction
Taq	*Thermus aquaticum*
µl	micro litre
µg	micro gram
μl^{-1}	per mico litre
ml	milli litre
rpm	rotation per minute
nm	nano meter
ng	nano gram
OD	optical density
A_{260}	Absorbance at 260 nano meter

A_{280}	Absorbance at 280 nano meter
bp	base pair
V	volt
MS	micro soft
W	watt
AD	After Death
BC	Before Christ
Etc	Etcetera

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Introduction

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) an economically important crop in tropical, subtropical and warm temperate regions (Mervat *et al.*, 2009), is grown in more than 110 countries on an area estimated at 9 million hectares, yielding 140 million tons per year. It is the third most important root or tuber crop after potato and cassava (Firon *et al.*, 2013). Over 97 per cent of the world output of sweet potato is from developing countries, it is because of its high nutritional contents to prevent malnutrition and ensure food security. In India, the annual production of sweet potato is 13 lakh tons (Indiastat, 2015).

Sweet potato is the most important food crop in the genus *Ipomoea.* There are thousands of sweet potato varieties cultivated all around the world, with high phenotypic variation. This high diversity is due to its self- incompatibility, high level of cross compatibility and hexapolyploidy. This diverse germplasm is important for subsequent analysis and improvement of sweet potato for desired traits. In India, there are about 60 *Ipomoea* species which include shrubby *I. carnea* to small flowered creeper *I. eriocarpa.* The knowledge about relationship between wild relatives are important for mining new genes, which may play crucial role in further development of germplasm or varieties for resistance to pathogen, increased protein content *etc.*

It is difficult to reveal the phylogenetic relationship between sweet potato and its wild relatives only on the basis of morphological, fertility and cytological differences. In recent years, the development of molecular markers made it easier to identify the wild relatives of sweet potato (Roullier *et al.*, 2013a).Molecular markers like Random amplified polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR) *etc.* are used for diversity analysis in sweet potato (Koussao, 2014).

Simple sequence repeats (SSRs) or microsatellite markers have been used to study genetic diversity, phylogenetic relationships, evolutionary processes, quantitative trait loci and classification in many crops. SSR markers are preferred over other markers due to their simplicity, effectiveness, abundance, codominant nature, easy to assay, wide distribution throughout the genome and reproducibility (Powell *et al.*, 1996).

Genetic improvement of sweet potato is important, because constant environmental changes results in evolution of new type of insects and diseases. So, breeders need a high genetic divergent material including the wild ancestry to meet the requirement. Although each diversity study dataset provides valuable information about the relationship of sweet potato, its wild relatives and their origin, no approaches have yet been elucidated to substantiate their relationship and origin.

In the present study, simple sequence repeats (SSR) were used to analyse the genetic variation, population structure and phylogenetic variation among selected sweet potato accessions from Central Tuber Research Institute (CTCRI) and wild species collected from different parts of India, mainly from Kerala.

Review Of Literature

2. REVIEW OF LITERATURE

Sweet potato

Sweet potato (*Ipomoea batatas* (L.) Lam) is a clonally propagated autohexaploid (2n = 6X = 90), dicotyledon plant that belongs to the family Convolvulaceae (morning glory family). It was described as the seventh most important food crop in the world (Okorie and Onyeneke, 2012 and Dantata *et al.*, 2010). The crop is native to tropical parts of America, but is now cultivated throughout tropical and warm temperate regions. The principal producers are China, Indonesia, Vietnam, India, Japan, Philippines, Korea, Bangladesh, Taiwan and most of the other Asian countries (FAO, 2015). China remains the leading sweet potato producer and contributes about 85% of world production (FAO, 2015). In India, the major sweet potato producing states are Odisha, West Bengal and Uttar Pradesh (IndiaStat, 2015).

There are many cultivars of sweet potato each with its own characteristics of size, shape, colour, storage life, levels of nutrition and suitability for processing. Sweet potato has the shortest growing cycle of the root crops grown in the tropics. A single plant may produce 2 to 8 tubers ranging in length from a few to 15cm; tuber may be spindle-shaped or spherical and weigh from 50g to 500g. Sweet potato crop is mostly vegetatively propagated, but can also be propagated *via* seeds (Schultheis *et al.*, 1994). The crop is normally harvested when the vines and leaves have turned yellow, generally about 4 months after planting, it is considered one of the least risky vegetables to grow, good production in poor soils, low incidence of diseases, high nutrient content, low cost, cultivated mainly by small scale farmers, and use as a staple food in many areas of the world (Roesler *et al.*, 2008). Tubers may have a smooth or irregular surface and the skin and the flesh may range from almost pure white through cream, yellow, orange and very deep purple (Onwueme, 1978).

Nevertheless, little information is available about sweet potato genetic variability and diversity (Souza, 2000).

Distribution of sweet potato across the world

In Central America, domestication of the sweet potato might have started at least 5000 years ago, and in South America, Peruvian sweet potato remnants dating as far back as 8000 BC have been found (Ugent and Peterson, 1988). Sweet potato was cultivated in Cook Islands in 1000 AD, and it may have been brought to Central Polynesia around 700 AD and from there sweet potato spread across Polynesia to Hawaii and New Zealand (Van Tilburg, 1994 and Bassett *et al.*, 2004).

From the New World, where it was already well established, sweet potato was spread to the Old World through numerous routes. The best known and most documented were with the European explorers. With the aid of explorers such as Columbus, the "batata line" of sweet potato was introduced to Europe from West Indies after the first voyage in 1492. During the 16th century, Portuguese explorers then brought them to Africa, Brazil, and India, while direct transfer of the plant was done by Spanish trading galleons from Mexico to the Philippines. The "camote line", which is named for its Mayan roots, represents a direct transfer of Mexican sweet potatoes traded between Mexico, Acapulco, and Manila also during the 16th century. From there the crop was introduced to the Far East countries of China and Japan. Currently, there is a small amount of evidence for pre historic dispersal of sweet potato. This information was proposed by both O'Brien (1972) and Yen (1982); their claim is based on the similarities between the Incan Quechua language word for sweet potato, "kumar" and the Polynesian word "kumara". Recent studies on the assessment of genetic diversity using AFLP suggested that the pre historic introduction in Oceania could be done from Mesoamerican sweet potato through natural dispersal (Rossel *et al.*, 2001).

Importance of sweet potato

Sweet potato is a crop with a significantly unrealized potential, produces more food per hectare than wheat, rice, or cassava, which makes it an important food security crop (schafleitner *et al.*, 2010). The chemical composition of sweet potatoes varies greatly according to genetic and environmental factors. The tuberous roots are highly nutritive, due to their elevated starch levels, which is considered to be a good source of dietary fiber (Vimala *et al.*, 2011), minerals (especially calcium and iron) and vitamins such as C and B-complex vitamins (Low *et al.*, 2007, Nedunchezhiyan *et al.*, 2010, Wang *et al.*, 2010 and Fonseca *et al.*, 2008). Some varieties contain coloured pigments such as β-carotene and anthocyanin, these pigments have antioxidant properties possessing health promoting attributes such as ability to fight cancer, protect against night blindness, delay aging and prevent liver injury (Yamakawa, 1998 and Hou *et al.*, 2001).

Sweet potatoes are utilized as food and livestock feed all over the world (Goncalves *et al.*, 2012). Have a long history as life saver; the Japanese used them when tropical storm destructs their rice fields. Sweet potato saved millions from famine- plagued China in early 1960's and in Uganda, where a virus ravaged cassava crops in 1990's. In the tropics the fresh roots are commonly boiled, fried or roasted and eaten as a carbohydrate constituent of the diet. Roots of some of the cultivars are used for the preparation of beverage, paste, powder, alcohol drink and natural colour (Islam and Jalaluddin, 2004, Hu *et al.*, 2003 and Nedunchezhiyan *et al.*, 2012). Reports on the production of non-alcoholic beverage from sweet potato are available in the literature (Wireko-Manu *et al.*, 2010). Recent attention has been paid to the nutritional value of the leaves, which can contain as much as 27% protein. In Africa, both the tuber and the leaves are consumed, it is found to be high in nutritional value. In Northern Cameroon sweet potato plays an important role in rural food security; dried chips are stored for use during the hungry period when the stocks of the staples sorghum and millet are depleted (Kure *et al.*, 2012). In Asia, particularly Japan,

Taiwan and South Korea, sweet potato is widely used as animal feed. In the USA, one third of sweet potato production is dehydrated and processed for animal feed. Furthermore, some sweet potato cultivars are grown as ornamental vines or leafy vegetables.

Origin of sweet potato

Besides sweet potatoes economic importance, the botanical origin, timing and geographical location(s) of its domestication remain unclear (Roullier *et al.*, 2013a). Based on the morphological features of sweet potato and other *Ipomoea* species in the wild, the centre of origin consider being somewhere near the Yucatan Peninsula of Mexico and the mouth of Orinoco river in Venezuela (Srisuwan *et al.,* 2006 and Austin, 1987). Later high diversity in Central America revealed by the molecular works, while considering the abundance of crop wild relative species of sweet potato Central America is the primary centre of diversity and most likely be the centre of origin (Huang and Sun, 2000). Secondary center of sweet potato diversity outside of the America are in China, South East Asia, New Guinea and East Africa (Austin, 1983, 1988 and Yen, 1982). Among the *Ipomoea* species within the genus series *batatas,*13 are consider to be closely related to sweet potato (Austin,1987), but the exact wild relative of this plant is not yet identified. On the basis of morphological studies (Austin, 1987) and several molecular - genetic studies among the *Ipomoea* species appeared to indicate that the diploid *I. trifida* is the closest wild relative of sweet potato (Roullier *et al.*, 2013, Jarret and Austin, 1994, Rajapakse *et al.*, 2004, and Srisuwan *et al.*, 2006), there by pointing to this species most likely be the progenitor of sweet potato.

Several hypotheses have been put forwarded by the scientific community to explain the origin of sweet potato. Magoon *et al.* (1970) proposed that sweet potato consists of three genomes of closely related taxa, in contrast with earlier theories that suggested an allohexaploid origin (Gustafsson and Gadd, 1965). Magoon *et al.* (1970)

also presented evidence for an autopolyploid origin, from a single diploid ancestor, based on the occurrence of a high frequency of tetravalent and bivalent pairing during meiosis, and the presence of penta and hexavalent configurations.

The second hypothesis was put forwarded by Nishiyama (1971) and that was sweet potato thought to originate from diploid *I. leucantha*, from which a tetraploid *I. littoralis* derived by polyploidization. The hybridization between *I. leucantha* (2x) and *I. littoralis* (4x) might have generated triploid *I. trifida*, which on doubling the chromosome set became a hexaploid. Further natural selection and domestication of those *Ipomoea* wild species may give birth to the *I. batatas*.

In 1987, Austin proposed a hypothesis based on the morphological study, which was natural hybridization between I. *trifida* and *I. triloba* result in the generation of wild ancestors of sweet potato somewhere between Mexico and Venezuela. In another hypothesis regarding the origin of sweet potato Kobayashi (1984) reported that, the autopolyploidization of *I. trifida*, with ploidy level ranging from diploid to hexaploid, and cultivar sweet potato may derive from this polyploidy *Ipomoea* species (Shiotani, 1987).

The use of the techniques of Restriction Fragment Length Polymorphism (RFLPs) of genomic DNA (Jarret *et al.*, 1992), Random Amplified Polymorphic DNAs (RAPDs) (Jarret and Austin, 1994) and microsatellites, Buteler *et al.* (1999) revealed the close relationship between *I. trifida* and *I. batatas*. Recent cpDNA data also indicated that *I. trifida* is most likely one of the diploid progenitors of hexaploid *I. batatas* (Haung and Sun, 2000). Molecular based studies suggested that the *I. batatas* hexaploid genome may be composed of two closely related genomes and the third one from a more distant relative (Buteler *et al.*, 1999 and Magoon *et al.*, 1970).

In spite of these entire hypotheses, there are several *Ipomoea* species with different ploidy level from diploid to hexaploid (mostly in 4x) show high similarity to *I. batatas*, but their taxonomical status is still highly disputed and they are poorly

consider by the scientific community, so lesser importance given to the genetic characterization. Bohac *et al.* (1993) collected *Ipomoea* species from different parts of Central America (Ecuador, Colombia, Guatemala and Mexico), those accessions were initially identified as *I. trifida* but on later taxonomical analysis, most of them are wild *I. batatas*. Whether these could represent any undomesticated forms of *I. batatas* has not been investigated. However the discovery of wild tetraploid *I. batatas* gives a clue that, other than the hexaploid cultivar sweet potato in the field there may be existence of true wild population of sweet potato with lower ploidy levels, and from which the cultivars would have domesticated (Roullier *et al.*, 2013a).

Roullier *et al.* (2013a) studied the origin of sweet potato based on the non-coding chloroplast and nuclear ITS sequences, and nuclear SSRs. This work supported the autopolyploid origin of sweet potato, there was no contribution given by the *I. triloba* to the genome of domesticated sweet potato, based on SSR marker the *I. triloba* was more distant related than *I. trifida*. They do not get any kind of data that support interspecific hybridization, thereby no evidence for allopolyploid origin. *I. trifida* and *I. batatas* are closely related, but they were not share the similar haplotypes, so *I. trifida* cannot consider as the direct progenitor of *I. batatas*. Based on the chloroplast lineage studies, two chloroplast lineages of *I. batatas* were identified and show more divergence with each other than either does with *I. trifida*. This data points towards that *I. batatas* have at least a double origin from polymorphic population of its ancestor. Apart from the autopolyploid multiple origin of sweet potato, that also argue that there is a polyploid true wild relative exist and that should share a common ancestry with present diploid *I. trifida*, that might be extinct or may simply not have been collected. This study is also fail to identify the ancestor of *I. batatas* but proposed that the domestication event takes place independently in South America and the Caribbean/Central America from the tuber bearing wild ancestor. Later these gene pools come into contact along human movements and give secondary origin.

***Ipomoea* species and Crop wild relatives of sweet potato**

Species that are genetically related to domesticated crops, forages, medicinal herbs, and other useful plants but are undomesticated themselves are known as Crop Wild Relative (CWR). Sweet potato also has crop wild relatives belong to the genus *Ipomoea.*

The genus *Ipomoea* comprises the largest number of species within the family Convolvulaceae which are widely grown as ornamentals because of their showy and beautiful flowers. This genus is dominated by twining or climbing woody or herbaceous plants that often have heart-shaped leaves and bell-shaped flowers (Austin, 1997). They occur in the tropics of the world although some species also reach temperate zones (Cao, 2005). Throughout the world; *Ipomoea* is usually estimated to contain more than 600 species (Mabberley, 2008) in which over half of them are concentrated in the Americans and Asian countries (Judd *et al.,* 2002), sweet potato is the only member of the genus *Ipomoea* whose roots are edible (Hahn, 1977 and Date and Eronico, 1987). There are more than 60 *Ipomoea* species present in the Indian sub-continent (Bellum, 2012) where as in Kerala about 35 *Ipomoea* species are identified (Sasidharan, 2015). The taxonomic relationships between sweet potato and its wild relatives have not yet been fully elucidated (Hu *et al.*, 2003).

The genus has four ploidy level *viz.*, the cultivated sweet potato, *I. batatas,* a hexaploid (2n=6x=90); *Ipomoea tiliaceae,* very similar to batatas, a tetraploid (2n=4x=60); *I. cordatotriloba, I. lacunosa* and *I. triloba,* all diplods (2n=30) (Austin, 1988); and *I. trifida,* a wild species with different cytotypes such as diploids, tetraploids and hexaploids (Shiotani *et al.*, 1991).

The pollen morphology of the Convolvulaceae is known to be highly diverse and of taxonomic importance (Telleria and Daners, 2003). Hallier (1893) recognized the usefulness of pollen characters as being palynologically and taxonomically important and divided the family into subfamily 'Echinoconiae' on the basis of

distinct spiny pollen. Within the genus there is a high variability, probably because of its high ploidy level, which needs to be preserved and studied to contribute with future plant breeding programs (Austin, 1987).

Need of crop wild relative

Some varieties are genetically more homogenous and therefore more vulnerable to pathogens (biotic stress) and adverse environmental conditions (abiotic stress). Wild relatives and crop landraces are important gene resources for improving resistance to and increasing genetic heterozygosity and large chromosome number (Veasey *et al.*, 2008).

Wild species of *Ipomoea* are an important reservoir of useful genes and may provide a new approach for genetic improvement (Komaki, 2001). Most of them are weedy in nature but they may play an important role in providing new genes, such as those for resistance to various diseases and insects. Many wild *Ipomoea* species possess agriculturally desirable traits. For example, resistances to sweet potato weevil (*Cylas* spp.), scab (*Elsinoe batatas* (Saw.), Viegas and Jenkins), and black rot (*Ceratocystis fimbriata* Ell.et Halst.) have been found in *I. trifida* and *I. littoralis* (Iwanaga, 1988). Bohac *et al.*(1995) note that the germplasm used in sweet potato breeding programs represents only a fraction of the genetic diversity available in the wild and that there is a need to increase the genetic diversity in the crop to meet future needs.

Genetic diversity analysis

Genetic diversity is the variation of genes within species or sequence variation within species or which is the heritable variation within and between populations of organisms. Information on genetic diversity and relationships among and between plant varieties are of importance for classification and conservation of germplasm resources, that helps plant breeders for studying the evolutionary ecology of

populations (Vigouroux *et al.,* 2005) and for the selection of parents for hybridization (Adebisi *et al.,* 2001). A sound crop improvement depends upon the magnitude of genetic variability in the base population (Afuape *et al.,* 2011).

Genetic diversity analysis can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification (Vigouroux *et al.,* 2005). Without determining the diversity reliably, it would not be possible to identify molecular markers or qualitative trait associations (Baig *et al.,* 2009). A better knowledge of germplasm diversity is critical for developing new varieties and useful for conducting basic research into the biology of a crop plant. Sweet potato is the oldest vegetables known to mankind, having been in cultivation since about 10,000 years ago (Ugent and Peterson, 1988), the crop has evolved into many morphotypes, creating broad genetic diversity for most of the desirable agronomic traits. The successful conservation and genetic improvement of sweet potato is also dependent on the level of knowledge on germplasm diversity (Yada and Tukamuhabwa, 2010). By having a high genetic variability, sweet potatoes can be selected for numerous purposes (Silva*et al.*, 2011).

In crop plants genetic variability or diversity can be evaluated based on morphological, biochemical and molecular characteristics.

Morphological characterization

Morphological characterization has been used extensively on various crop plants diversity analysis in many places of the world (Li *et al.*, 2009 and K'Opondo, 2011).Despite the environmental influences on plant morphology, this direct inexpensive and easy to use method of estimations was perceived as the strongest determinant of the agronomic value and taxonomic classification of plants (Li *et al.*, 2009).

In sweet potato, morphological characterization is the first step in the assessment of diversity (Karuri *et al.*, 2010), this tool has been used successfully to analyse genetic diversity necessary for the germplasm conservation, to reduce accession number by identification of genotypes [in many parts of the world, different accessions are given similar names or same accessions have different names and leading difficulties in precise identification of genotypes (Ahiakpa *et al.*, 2013)] and elimination of duplicates, to enhance crop breeding (Tairo *et al.*, 2008; Karuri *et al.*, 2009 and Yada *et al.*, 2010), to studies the genetic diversity patterns and correlation with characteristics of agronomic importance (Elameen *et al.*, 2011 and Karuri *et al.*, 2010).

In sweet potato collections morphological characterisation is not an easy task because there is a great diversity in morphological and phenotypic traits, such as root size and shape, resistance to diseases, leaf shape and skin colour (Woolfe, 1992), this is mainly due to the asexual propagation of the crop, (Huaman *et al.*, 1999). There are so many works done in sweet potato genetic diversity analysis based on morphological characters that are shown in the Table.1. These works confirmed that sweet potato is a crop with high genetic polymorphism, also represented in the great diversity observed in morphological and phenotypical traits (Woolfe, 1992). Seed micro and macro-morphological characters (Khalik, 2013); morphometric and quantitative characteristics of mature cotyledon (Ogunwenmo, 2003) and morphological variations (Mondal *et al.*, 2006) were utilized for identification of *Ipomoea* taxa. All these result shows than there is high diversity exist in genus *Ipomoea*.

Morphological approach has certain limitations since genetic information provided by morphological characters is often limited and expression of quantitative traits is subject to strong developmental and environmental influences (Rao, 2004). Therefore, morphological characterization is often complemented with other methods of characterization to ensure precise identification of true genetic identities of

accessions in any collection (Sossah *et al.*, 2014). Morphological traits and biochemical markers have been employed in sweet potato germplasm studies (Ritschel and Huaman, 2002). General plant morphological character states as well as RAPD fragment phenotypes were employed for the *Ipomoea* diversity analysis and revealed higher polymorphism in morphological and RAPD profile (Das, 2011).

Morphological traits alone cannot provide a thorough assessment of genetic diversity since morphological characters may represent limited loci within the whole genome (Gepts, 1993). If trait expression is environmentally unstable or difficult to evaluate, molecular markers become more useful than traditional phenotypic evaluations (La-Bonte, 2002).

Biochemical Methods

The use of biochemical markers based on total seed, tuberous root protein and enzyme by SDS- PAGE technique has proven to be a reliable, yet inexpensive method of developing genetic markers for identification and genetic analyses of several plant species, as they reveal differences between storage proteins or enzymes encoded by different alleles at a single (allozymes) or more gene loci (isozymes) (Oppong-Konadu *et al.,* 2005 and Rao, 2004). Electrophoretic profiles of total proteins and isozymes have been used in genetic diversity studies in sweet potato and to identify duplicates in germplasm collections (Kennedy and Thompson, 1991 and Saha *et al.,* 2000).Sweet potato accessions were evaluated for genetic diversity based on leaf and tuberous proteins showed marked variations in the electrophoregrams of total proteins gave an indication of intense variability among the accessions (Sossah *et al.,* 2014).

Seed proteins of *Ipomoea* species (Pragati *et al.,* 2013 and Khalik *et al.,* 2012) showed high diversity in the genus, from number of bands to the intensity of bands. Khalik *et al.* (2012) also found out a close relationship between *Ipomoea purpurea*

(L.) Roth of section *Pharbitis* (Choisy) Griseb (sub-genus *Ipomoea*) and species of the sub-genus *Quamoclit.*

Analysis of a combination of molecular and phenotypic or biochemical data provide a powerful tool for establishing core collections, which facilitate more efficient germplasm curation. RAPD and isozyme analysis have been used for cultivar identification (Kennedy and Thompson, 1991 and Connolly *et al.*, 1994). Further RAPD analysis of the same *Ipomoea* sample gave additional information that *I. cairica* (L.) sweet show high divergence from other samples. There are some diversity works done in sweet potato and *Ipomoea* accessions based on their protein compositions of leaf, tuber, seed *etc.* that is shown in the Table 1.

Molecular Methods

Molecular markers work by highlighting differences (polymorphisms) within a nucleic sequence between different individuals. These differences include insertions, deletions, translocations, duplications and point mutations. Molecular analysis of germplasm has become a valuable tool for genetic germplasm characterization (Karuri *et al.*, 2009) that distinguishes between accessions that may have similar morphological and agronomical traits (Goncalves *et al.*, 2008). That have been used to analyze many crops for different reasons including genetic diversity assessment (Ipek *et al.*, 2003,; Xu *et al.*, 2003 and Zhang *et al.*, 2000) and effective in evaluating genetic variation within species (Powell *et al.*, 1996; Shim *et al.*, 2000 and Truren *et al.*, 2001), genetic linkage map construction (Klein *et al.*,2000 and Kriegner *et al.*, 2000), core collection establishment and collection management (McGregor *et al.*, 2002 and Zhang *et al.*, 2000), trait identifications; associations (Ghislain *et al.*, 2001), and sequencing (Kim *et al.*, 1997) etc.

Molecular markers are the most efficient markers for genetic diversity studies in many species (Rakoczy-Trojanowska and Bolibok, 2004) including sweet potato (Zhang *et al.*, 2000) has shown significant and critical application in the assessment

and conservation of genetic variation of sweet potato (Veasey *et al.,* 2008). To date, several molecular genetic techniques that have been used to analyze genetic diversity.

During the last decade a lot of molecular information has been accumulated and used for genetic diversity assessment on sweet potato germplasm (Soegianto *et al.,* 2011), such as Random Amplified Polymorphic DNA (RAPD) (Gickuki *et al.*, 2003), DNA Amplification Fingerprinting (DAF) (He *et al.*, 1995), Microsatellites or Simple Sequence Repeats (SSR) (Hu *et al.,* 2004), Inter-Simple Sequence Repeats (ISSR) (Hu *et al.*, 2003), Amplified Fragment Length Polymorphism (AFLP) (Bruckner, 2004), and Selective Amplification Of Microsatellite Polymorphic Loci (SAMPL) (Tseng *et al.*, 2002). The sweet potato molecular marker studies summarized in the Table 1.

Random Amplified Polymorphic DNA (RAPD)

RAPD were the first of the PCR-based markers and were developed independently by Welsh and McClelland (1990) and Williams *et al.* (1990). RAPD is a modification of the PCR in which a single, short and arbitrary primer (10-mers), able to anneal and prime at multiple locations throughout the genome, can produce a spectrum of amplification products that are characteristics of the template DNA (Kumar and Gurusubramanian, 2011).

RAPD technique has been effective in the first method in detecting the diversity of population in various types of specimens (Carvalho *et al.*, 2013). The RAPD technique has been applied in several aspects of sweet potato research, such as, cultivar identification (Connolly *et al.,* 1994), diversity assessment (Jarret and Austin, 1994), and detection of intra-clonal variation (Villordon and La- Bonte, 1995). RAPD has been demosntrated to be a useful tool to determine linkage mapping in sweet potato (Thompson *et al.,* 1997).

Genetic variability analysis of sweet potato using 15 RAPD primers showed 100% polymorphism (Zhang *et al.,* 1998). Analysis of 28 sweet potato cultivars from all over the world showed polymorphic bands with all 18 RAPD primers, results confirmed that sweet potato exhibits high genetic variation (Sagredo *et al.,* 1998). In other study on sweet potato, a total of 150 bands were scored and 145 were polymorphic using 18 RAPD primers (Moulin *et al.,* 2012); genetic diversity and structure of 52 sweet potato accessions from Brazil using seven RAPD markers showed that, the collection had a high level of polymorphism (Silva *et al.*, 2014).

The genetic diversity of larger collections of sweet potato and others *Ipomoea* species of the same genus, collected all over the world for establishing taxonomic as well as evolutionary relationship in *Ipomoea* species (Jarret and Austin, 1994 and He *et al.*, 1995). Phylogeny of sweet potato and its wild species was analysed involving morphological variation, crossing ability and RAPD pattern of sweet potato and its closely related species was done by Katsumi (2001). Interspecific genetic relationships were although established among *I. lacunose, I. ramoni, I. trichocarpa* and *I. triloba* (Jones and Deonier, 1965) using RAPD marker long back but not much of work have been done in various Indian varieties of sweet potato except genome variation and RAPD marker variation in interspecific level (Dhillon and Ishiki, 1999).

Amplified Fragment Length Polymorphism (AFLP)

AFLP was first described by Zabeau and Vos (1993) and Vos *et al.* (1995), as a technique based on the detection of genomic restriction fragments by PCR amplification and can be used for DNAs of any origin or complexity. That has been reliably used for determining genetic diversity and phylogenetic relationship between closely related genotypes (Hill *et al.*, 1996). AFLP analysis combines both the reliability of restriction fragment length polymorphism (RFLP) and the convenience of PCR-based fingerprinting methods. AFLP markers are generally dominant and do

not require prior knowledge of the genomic composition. Those are produced in great numbers and are reproducible (Abdel-Mawgood, 2012).

In several reports, the efficiency of AFLP (Vos *et al.*, 1995) to identify and characterize the genetic diversity of sweet potato has been proved by several groups (Zhang *et al.*, 2000, Fajardo *et al.*, 2002, Huang *et al.*, 2002 and Zhang *et al.*, 2004). AFLP analysis of 97 sweet potato accessions using 10 primer combinations gave a total of 202 clear polymorphic bands, which were found to be an efficient tool to characterize the genetic diversity and relationships of sweet potato accessions in the germplasm collection in Tanzania (Elameen *et al.*, 2008), China (Liu *et al.*, 2012) and tropical America (Zhang *et al.*, 2000). Sixty nine sweet potato cultivars from 4 geographical regions (including 13 Countries) of Latin America were randomly sampled and fingerprinted using 8 AFLP markers (Zhang *et al.*, 2000). Furthermore, the origin and dispersal of sweet potato was revealed using RAPD (Gichuki *et al.*, 2003) and AFLP (Rossel *et al.*, 2001 and Zhang *et al.*, 2004) markers.

Inter- Simple Sequence Repeats (ISSR)

The Inter-Simple Sequence Repeats (ISSR) are arbitrary, dominant markers amplified by PCR in the presence of one primer complementary to a target microsatellite. Each band corresponds to a DNA sequence delimited by two inverted microsatellites (Abdel-Mawgood, 2012). ISSR does not require prior knowledge of the genome sequence; it leads to multilocus, highly polymorphous patterns (Reddy *et al.*, 2002 and Li *et al.*, 2008), is an inexpensive genotyping technique based on variation in the regions between microsatellites.

ISSR markers have successfully been used to study the genetic diversity and genotype identity of many crops (Reddy *et al.*, 2002) including sweet potato (Huang and Sun 2000, Hwang *et al.*, 2002, Hu *et al.*, 2003 and Li *et al.*, 2008). The genetic relationships between cultivated sweet potato and its wild relatives were estimated using ISSR (Huang and Sun 2000 and Hu *et al.*, 2003) markers, and the genetic

diversity among sweet potato landraces was evaluated using AFLP, ISSR and RAPD (He *et al.*, 2005a, 2006) markers. In North East India with the help of ISSR marker determination of genetic relationship among 21 *Ipomoea* species were achieved (Rout *et al.*, 2010).

Simple Sequence Repeats (SSR)

Simple Sequence Repeat (SSR) markers are repeats of short nucleotide sequences, usually equal to or less than six bases in length, that vary in number (Reddy *et al.*, 2002), distributed throughout the genome consisting of short tandem repeats of mono-, di-, tri-, tetra-, penta- and hexa-nucleotides (Tautz and Schlotterer, 1994), and are highly polymorphic because of the high mutation rate affecting the number of repeated units. It is suggested that the variation or polymorphism of SSRs are a result of polymerase slippage during DNA replication or unequal crossing over (Levinson and Gutman, 1987).

SSR markers are also called microsatellites. It require a small amount of DNA for scoring and polymorphisms of this length can be easily detected on high-resolution gels (eg., sequencing gels) by running PCR-amplified fragments obtained using a unique pair of primers flanking the repeat (Weber and May, 1989). Microsatellites have some advantages, such as technical simplicity, relatively low cost, high genetic resolution power, and being highly polymorphic. Moreover, SSR are reliable and easy to score (Gupta and Varshney, 2000). SSRs demonstrate a high degree of transferability between species, as PCR primers designed to an SSR within one species frequently amplify a corresponding locus in related species, enabling comparative genetic and genomic analysis

The most widely used molecular marker procedures for population genetic analysis of plants during the past few years are the SSR markers or microsatellites (Veasey *et al.*, 2008; Li *et al.*, 2009; Karuri *et al.*, 2010; and Yada *et al.*, 2010). These markers are frequently used to assess genetic variation within and between

populations (Vigouroux *et al.,* 2005), this is because of their high levels of allelic variation and their co-dominant character, which means that deliver more information per unit assay than any other marker system (Rakoczy-Trojanowska and Bolibok, 2004)

Several SSR markers have been developed for sweet potato (Jarret and Bowen, 1994, Hu *et al.*, 2004 and Buteler *et al.,* 1999) these have been further screened and applied in paternity analysis and its wild relative species (Buteler *et al.,* 1999). SSR markers used successfully for determining the genetic relationship between cultivars derived from hybrid or polycross breeding programs (Hwang *et al.,* 2002) and for analyzing the genetic diversity of sweet potato landraces from Brazil (Veasey *et al.,* 2008). SSR markers also used determine the genetic diversity of a sweet potato collection from Kenya (Karuri *et al.*, 2009) and diversity of sweet potato landraces from Kenya, Uganda, and Tanzania (Gichuru *et al.,* 2006). Genetic relationship of 192 high yielding disease resistant sweet potato accessions from Uganda revealed by 10 fluorescent labeled SSR markers and show high degree of genetic diversity, these studies helping to identify the duplicates in the population (Yada *et al.,* 2010). A recent molecular study (Roullier *et al.,* 2011) analyzed 329 landraces collected from Mexico to Peru by nuclear and chloroplast microsatellite markers and the result supported the existence of two geographically restricted gene pools of sweet potato (North and South American gene pools). The diversity study of New Guinea sweet potato accessions showed moderate diversity compared to North America (Roullier *et al.,* 2013b)

Comparative studies in plants have shown that SSR markers, which are single locus markers with multiple alleles, are more variable than other markers and provide an effective means for discriminating between genotypes (Powell *et al.*, 1996).

Table 1. Different markers used for sweet potato diversity analysis

Accessions used	Reference
Morphological Characterization	
1939-accessions –CIP, Peru	Huaman *et al.* (1999)
18-Ipomoea species	Ogunwenmo (2003)
4-Ipomoea species – Prana (floral morphology)	Terada *et al.* (2005)
10-Ipomoea species - Bangladesh	Mondal *et al.* (2006)
74-Accessions -Vale do Ribeira, Brazil.	Veasey *et al.* (2007)
30-Acessions - Ethiopia	Tsegaye *et al.* (2007)
21-Accessions -Nigeria	Afuape *et al.* (2011)
120-Sweet potato wild relatives - Indonesia	Waluyo *et al.* (2011)
12-Ipomoea species -India	Das (2011)
50 Ipomoea species – North America	McDonald *et al.* (2011)
7-Ipomoea species- Nigeria	Jayeola and Oladunjoye (2012)
15-Ipomoea species - Nigeria	Folorunso (2013)
15-Species – Egypt (seed morphology)	Khalik *et al.* (2013)
Biochemichal Markers	
12-Species – India (pollengrain enzyme)	Das and Mukherjee (1995)
12-Ipomoea species –West Bengal, India	Das and Mukherjee (1997)
10-Ipomoea species - Egypt (seed protein)	Khalik *et al.* (2012)
9-Ipomoea species -India	Pragati *et al.* (2013)
18-Ipomoea species	Sossah *et al.* (2014)
Cytogenetic Approaches	
10 cultivar,11 wild ipomoea species (FISH)	Srisuwan *et al.* (2006)
Nuclear DNA ITS Analysis	
40-Ipomoea species	Miller and Rausher (1999)
36-Ipomoea species	Miller *et al.* (2004)

Cont...

Accessions used	Reference
13-Accessions including 3 cultivar and 10 Ipomoea species – China	Li-Zuan *et al.* (2014)
Chloroplast DNA Analysis	
3- Ipomoea species – North America	McDonald and Mabry (1992)
417 - Cultivars- New Guinea	Roullier *et al.* (2013)
Randomly Amplified Polymorphic DNA (RAPD)	
26 Accessions – Oceania, Peru, Phillippines, US8 Ipomoea species	Jarret and Austin (1994)
6 Cultivars - 36 primers	Connolly *et al.* (1994)
9 Cultivars - New Zealand	Harvey *et al.* (1997)
28 Cultivars –cip, peru, 14 cultivars - Chille	Sagredo *et al.* (1998)
36 Cultivars- South America and Papua New Guinea	Zhang *et al.* (1998)
110 Accession – both cultivar and wild accessions	Komaki *et al.* (1998)
28 Cultivar and 4 ipomoea wildspecies	Hu *et al.*(2003)
74 Varieties - 23 countries	Gichuki *et al.* (2003)
12 Ipomoea species- India	Das (2011)
10 Ipomoea species	Khalik *et al.* (2012)
27 Cultivars – Kenya (RAPD and SCAR markers)	Lee *et al.* (2015)
Inter-Simple Sequence Repeat (ISSR)	
I. trifida, I. ramosissima, I. umbraticola, I. triloba, I. triloba and *I. batatas*	Huang and Sun (2000)
21 Ipomoea species including 3 cultivars - India	Rout *et al.* (2010)
59 Cultivars – Brazil (RAPD and ISSR)	Moulin *et al.* (2012)
34 Cultivars – Japan	Hu *et al.* (2003)
Cultivars and wild species	Hu *et al.* (2004)
DNA Amplification Fingerprinting (DAF)	
73 Accessions - USA, New Guinea., Tetraploid- *I. batatas* (*I. triloba*)	He *et al.* (1995)

Cont...

Accessions used	Reference
30 cultivars	Prakash *et al.* (1996)
42 Accessions - Guangdong, Fuji and Japan	Wang *et al.* (1998)
21 Ipomoea species – North East India	Rout*et al.* (2010)
240 Sweet potato accessions - China	Kai *et al.* (2014)
Amplied Fragment Length Polymorphism (AFLP)	
69 cultivars - CIP, Peru	Zhang *et al.* (2000)
141 Accessions - PNG	Fajardo *et al.* (2002)
13 Species of Ipomoea	Huang *et al.* (2002)
80 Accessions - Pacific region & Latin America	Zhang *et al.* (2004)
775 Accessions - USA	Bruckner (2004)
97 Cultivars – Tanzania	Elameen *et al.* (2008)
Selective Amplification of Microsatellite Polymorphic Loci (SAMPL)	
22 elite cultivars - Taiwan	Tseng *et al.* (2002)
Simple Sequence Repeat (SSR)	
Cultivars – China, Japan, Taiwan	Hwang *et al.* (2002)
78 Sweet potato accessions- Brazil	Veasey *et al.* (2008)
192 Cultivar accessions – Uganda	Yada *et al.* (2010)
89 Cultivars –Kenya	Karuri *et al.* (2010)
417 Cultivars- New Guinea	Roullier *et al.* (2013b)
112 Sweet potato accessions - Burkina Faso,Ghana	Koussao *et al.* (2014)
167 Cultivars - Puerto Rico, USA	Rodriguez-Bonilla *et al.* (2014)

Materials And Methods

3. MATERIALS AND METHOD

3.1 Plant Materials

Twenty sweet potato (*Ipomoea batatas*) varieties/ cultivar's leaf samples (Table 2) were collected from ICAR-Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram (Plate1A) and wild *Ipomoea species* were collected from Kerala and Tamil Nadu region. The collection was done based on their morphological characters.

Collected seeds, stem cuttings and whole wild plants were propagated in ICAR-CTCRI farm. The plants were maintained in pot as well as in the field for observing there phenotypic characters. The plants were identified based on their observed phenotypic characters and conformation was done with help of taxonomist. Many weedy species in the collection show morphological similarity to *Ipomoea* species were excluded from the study after taxonomical identification.

A total of 23 wild *Ipomoea species* were used for this study (Table 3) (Plate 1 B). Apart from *Ipomoea species* two *Merremia species* (Table 3) were included in the study, show high morphological similarity to *Ipomoea species*. In total, forty- five samples were used for this study.

Table 2. Sweet potato cultivated accessions selected for diversity study.

Sl. No.	Name of the accession	Code
1	Pusa Safed	S_1
2	Gautham	S_2
3	Kishan	S_3
4	H-42	S_4
5	Samrat	S_5
6	ST-14	S_6
7	Kankan Aswin	S_7
8	Sree Arun	S_8
9	Sree Ratna	S_9
10	Sree Nandhini	S_{10}
11	Sree Varun	S_{11}
12	Sree Kanakha	S_{12}
13	Gowri	S_{13}
14	ST-13	S_{14}
15	S1	S_{15}
16	Pusa Red	S_{16}
17	Pale yellow	S_{17}
18	Sankar	S_{18}
19	Pink- white	S_{19}
20	CO3-4	S_{20}

Table 3. Sweet potato wild species collected for diversity study.

Sl. No.	Common name	Wild species	Location	Code
1	Moon flower vine	*Ipomoea alba* L.	Thrissur	S_{21}
2	Water spinach	*Ipomoea aquatica* Forssk.	Nagercoil, Thrissur	S_{22}
3	Coastal morning glory	*Ipomoea cairica* (L.) Sweet	Trivandrum	S_{23}
4	Pink morning glory (whole flower colour is pink)	*Ipomoea carnea* Jacq.	Trivandrum	S_{24}
5	Pink morning glory (whole flower colour is deep pink)	*Ipomoea carnea* Jacq.	Trivandrum	S_{25}
6	Pink morning glory(corolla pink and purple corolla tube)	*Ipomoea carnea* Jacq.	Assam	S_{26}
7	-	*Ipomoea capitellata* Choisy.	Madurai	S_{27}
8	Tiny morning glory	*Ipomoea eriocarpa* R. Br.	Madurai	S_{28}
9	Scarlet morning glory	*Ipomoea hederifolia* L.	Palakkad	S_{29}
10	Blue morning glory	*Ipomoea indica* (Burm f.) Merr.	Thrissur	S_{30}
11	-	*Ipomoea violacea (macrantha)* L.	Nagercoil	S_{31}
12	Giant potato	*Ipomoea mauritiana* Jacq.	Trivandrum	S_{32}
13	Ivy morning glory	*Ipomoea nil* L.	Thrissur	S_{33}
14	Lesser Glory	*Ipomoea obscura* (L.) Ker-Gawl.	Trivandrum	S_{34}
15	Goat's foot morning glory	*Ipomoea pes-caprae* (L.) R. Br.	Thrissur	S_{35}
16	Tiger foot morning glory	*Ipomoea pes-tigridis* L.	Trivandrum	S_{36}
17	Common morning glory	*Ipomoea purpurea* (L.) Roth	Thrissur	S_{37}
18	Cardinal climber (red flower)	*Ipomoea quamoclit* L.	Trivandrum	S_{38}
19	Cardinal climber (pink flower)	*Ipomoea quamoclit* L.	Assam	S_{39}
20	Purple heart glory	*Ipomoea sepiaria* Roxb.	Nagercoil	S_{40}
21	-	*Ipomoea trifida* (Kunth) G. Don	CTCRI	S_{41}
22	little bell morning glory	*Ipomoea triloba* L.	Trivandrum	S_{42}
23	Lavender moon vine	*Ipomoea turbinata* Lag.	Palakkad	S_{43}
24	Alamo vine	*Merremia dissecta* (Jacq.) Hallier f.	Nagercoil	S_{44}
25	Grape leaf wood rose	*Merremia vitifolia* Burm f.	Trivandrum	S_{45}

Plate 1. (A) Field view of sweet potato cultivars used for the study

I. alba

I. aquatica

I. cairica

I. carnea

I. capitellata

I. eriocarpa

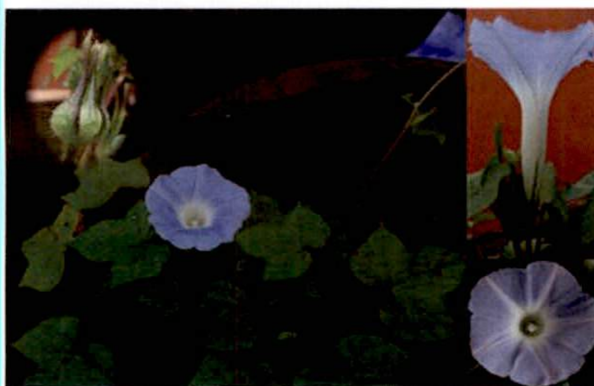
Plate 1. (B) Wild *Ipomoea* and *Merremia* species collected for the study(Cont.)

I. hederifolia

I. indica

I. violacea

I. mauritiana



I. nil

I. obscura

Plate 1. (B) Wild *Ipomoea* and *Merremia* species collected for the study (Cont.)

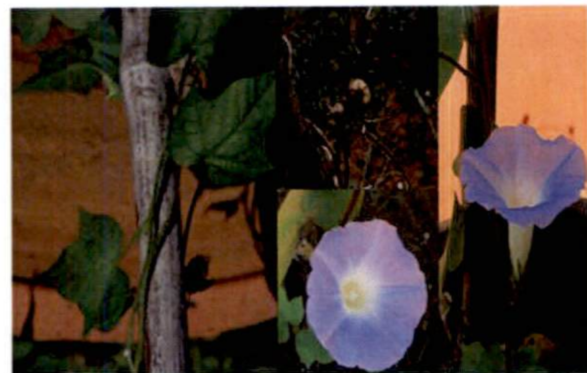
I. pes-caprae

I. pestigridis

I. purpurea

I. quamoclit

I. sepiaria



I. trifida

Plate 1. (B) Wild *Ipomoea* and *Merremia* species collected for the study (continued)

I. triloba

I. turbinata

M. dissecta

M. vitifolia

Plate 1. (B) Wild *Ipomoea* and *Merremia* species collected for the study

3.2 DNA Extraction

DNA isolation was done using SDS Dellaporta *et al.*, (1983) methodology with some modification.

- One gram of tender leaf tissue was transferred into clean, sterile mortar. The tissue was freezed rapidly in liquid nitrogen and 50 mg PVP was added into it, ground to fine powder using pestle.
- The frozened fine powder was transferred into 20 ml centrifuge tube containing 15 ml of extraction buffer, 20 µl β-mercaptoethanol. Placed the tubes in ice bucket. 1ml of 20% SDS was added and mixed thoroughly by vigorous shaking then the tubes were incubated at 65 °C for 1hr in a water bath (Memmert) and the tubes were mixed at every 10min interval.
- 5ml of 5M potassium acetate was added to the tubes and mixed well, incubated at 4 °C for 20 min.
- The mixture was centrifuged (Sigma) at 12000 rpm for 20 min under 4 °C.
- The upper layer was transferred to new eppendorf tubes, and the volume made upto 2ml by adding 1ml isopropanol, Kept the tubes at -20 °C for 30mins. Tubes were mixed gently by inverting. The DNA was appeared as white thread.
- Centrifuged the tubes at 12000 rpm for 15mins.The pellets were retained after discarding the supernatant.
- The pellets were redissolved in 500µl of TE buffer; 3µl of Rnase was added and kept for incubation at 37°C for 1hr.
- 1ml of chloroform: isoamyl alcohol mixture (24:1) was added, mixed thoroughly and centrifuged at 12,000 rpm for 15 mins.
- Aqueous phase was transferred to new eppendorf tube and 500µl ice-cold ethanol and 10µl sodium acetate were added. Mixed well and the tubes were incubated at -20 °C for 2hrs.

- Centrifuged the tubes at 10,000 rpm for 5mins and after discarding supernatant, the pellets were saved.
- To that pellet 500µl of 70% ethanol was added and centrifuged for 30-40 sec and washing repeated for one more time.
- Discarded the ethanol and dried the pellet to eliminate alcohol completely.
- The pellets were dissolved in 50-100µl sterile distilled deionized water and stored at -20 °C.

3.2.1 DNA quality checking using agarose gel electrophoresis

Agarose gel electrophoresis was done to check the quality of DNA

- The sides of the clean, dry gel casting tray was sealed with a tape to make a mold and placed in a horizontal flat surface.
- The comb was placed over the casting tray, agarose (0.8%) was added to freshly prepared 1X TBE in the beaker and melted the agarose was boiled and melted completely.
- The solution was allowed to cool and ethidium bromide (EtBr) was added
- Immediately after the addition of EtBr, the solution was poured into the casting tray.
- After solidification of agarose, the comb and tape were removed and placed the tray containing gel in electrophoresis unit.
- 1X TBE was added to the electrophoresis unit until submerged the gel.
- 2µl of DNA samples were pipette onto a clean non-adsorbing surface (cello tape) and mixed well with 4µl of loading dye.
- The DNA - dye mixtures were added to the corresponding wells.
- Connected the gel apparatus unit to the power pack and the gel was run at 85V for 30 minutes.
- The bands were visualized and documented in a gel documentation system (alpha imager, Alpha Innotech Corp).

3.2.2 Quantification of DNA

DNA Quantification was carried out by UV Spectrophotometer (Systronics)

- The Equipment was calibrated with distilled water as blank.
- 10µl of DNA sample was added to the quartz cuvette and made up the volume to 1ml with distilled water.
- The absorbance of the solution were taken at wavelengths of 260 and 280nm.
- The ratio A260/ A280 was calculated and the DNA concentration calculated using the relationships for double stranded DNA;

OD at 260 nm = 50µg / ml.

Concentration of DNA/ml of sample = OD (A260/ A280) × number of dilution × 50.

- Working stock of the DNA samples were prepared of about 100µl with concentrations of 10ng/µl.

3.3 Polymerase Chain Reaction (PCR)

3.3.1 Simple Sequence Repeats (SSR) Primers

SSR primers have both forward and reverse primer. For this study we used 12 SSR primers (Table.4). Among that two primers were fluorescently labeled IB R12 and IB S18.

Table 4. SSR primers used for the present study

Sl. No.	Primer name	SSR motif	Primer sequence	No. of bases	Annealing temperature	Source
1	Ib255	$(CT)_{14}$	F: CGTCCATGCTAAAGGTGTCAA R: ATAGGGGATTGTGCGTAATTTG	21 22	57.5	Buteler *et al.* (1999)
2	GDAAS0047	$(TTC)_{7}$	F: TTCTGACCTGCGAAATCG R: TGGACTTCCTCTGCCTTG	18 18	50.1	Wang *et al.* (2011)
3	GDAAS0049	$(AT)_{12}$	F: GTTCAAGATCAACAACCAGAG R: GCCAATCCTCCAACTTTC	21 18	50.1	Wang *et al.* (2011)
4	IBSSR05	$(GA)_{27}$	F: GGGTTCAACCTTTGCTCTTAAAATC R: TCATCCGCCATTTCCGTGAG	25 20	54.5	Hu *et al.* (2004)
5	IBSSR21	$(CA)_{14}$	F: AAACAACCAACGGGTCTTTGC R: CTCTAGGGTCGCCATAAAAATCAC	21 24	54.5	Hu *et al.* (2004)
6	Ib242	$(CT)_{3}$ CA $(CT)_{11}$	F: GCGGAACGGACGAGAAAA R: ATGGCAGAGTGAAAATGGAACA	18 22	59.5	Buteler *et al.* (1999)
7	Ib248	$(CT)_{9}$ $(CT)_{8}$	F: GAGAGGCCATTGAAGAGGAA R: AAGGACCACCGTAAATCCAA	20 20	57.5	Buteler *et al.* (1999)
8	IB R12*	$(CAG)_{5}$A	F: GATCGAGGAGAAGCTCCACA R: GCCGGCAAATTAAGTCCATC	20 20	59.5	Yada *et al.* (2010)
9	IB S18*	$(TAGC)_{4}$	F: CTGAACCCACAGCACAAG R: GGGAAGTGACCGGACAAGA	19 19	59.5	-
10	GDAAS0542	$(AG)_{7}$ $(TTGAA)_{4}$ $(TTC)_{5}$	F: CTGTTCGCTCATAGATAATCATCG R: GTTCTCTCCCATACTTCAATTTCC	24 24	49.3	Wang *et al.* (2011)
11	GDAAS0809	$(AGA)_{8}$	F: CGGGTTATGCTTGGTTCTCC R: TGTGGACGAGGATGCTGTG	20 19	49.3	Wang *et al.* (2011)
12	Ib 3/31	-	F: TTCCCTTTCCTTTCCTTCCC R: ACCCCAAATCCCAACTCCA	20 19	59.5	Tseng *et al.* (2002)

* - Fluorescent labelled primers

3.3.2 The polymerase chain reactions (PCR) for amplification of DNA were conducted in PCR tubes and the reaction mixture was prepared for 10µl as follows:

DNA (10ng/µl)	:	2.0 µl
Primer (2µm)	:	1.0 µl
dNTPs (40)	:	0.1 µl
Taq polymerase (3U)	:	0.1 µl
Taq Assay Buffer (10X)	:	1.0 µl
Sterile distilled water	:	5.8 µl
Total	:	10 µl

The PCR mixtures were vortexed for few seconds. Then the amplification was carried out in a Thermal cycler (BioRad C1000™ Thermal Cycler). The PCR amplification conditions were consisted of six steps as follows;

Step		Temperature	Time	Specification	
1	:	94^0C	5 minutes	Initial denaturation	
2	:	94^0C	1 minute	Denaturation	Run for 30 cycles
3	:	59.5^0C^*	2 minutes	Annealing	
4	:	72^0C	2 minutes	Extension	
5	:	72^0C	5 minutes	Final extension	
6	:	4^0C	for infinity	Hold	

*Annealing temperature of each SSR primers were followed based on the Table 4.

3.4 Polyacrylamide Gel Electrophoresis (PAGE)

PCR amplified products were separated using PAGE (polyacrylamide gel electrophoresis) gel. The PCR products (3-4μl) were run in a 5-6 % per cent denaturing polyacrylamide gel electrophoresis (PAGE) at 100 watts for 1-2 hours and amplified fragments were resolved by silver staining (Panaud *et al.*, 1996).

3.4.1 PAGE gel casting: (Sequi-Gen® GT, BIO-RAD sequencing gel, USA)

- The small glass plates were soaked in 2% Sodium hydroxide solution overnight and then the plates were cleaned using scrubber in tap water and again in distilled water.
- Both the small and large plates were again wiped with absolute alcohol.
- 2 ml of repellent was applied on large plate and spread uniformly with Kim wipes.
- 2 ml of bind saline was applied on small plate and spread uniformly
- Vaseline was applied to both the sides of the spacers.
- The spacers were placed with rubber adapter on either sides of the large plate and the small plate was placed on top of the large plate in such a way that it was seated uniformly on the edges and sides.
- Then the plates were clamped using side lever clamp set

3.4.2 Gel matrix preparation and gel casting

- 100 ml of a 6% polyacrylamide denaturation solution was taken in a conical flask and 500 μl of 10 % ammonium per sulphate (APS) solution and 50 μl of TEMED (Tetra methyl ethylene diamine) were added and mixed well.
- The plates were kept in slanting position in such a way that the gel matrix flows freely into the plates without air bubble.
- The solution was poured between the plates with the help of 100 ml syringe. After the matrix spreads uniformly throughout the plate, the comb was placed
- The plates were left 1 hours for polymerization.

3.4.3 Sample loading and gel running (electrophoresis)

- After polymerization, comb was removed and the gel setup was mounted on a electrophoresis apparatus.
- After flushing the wells with running buffer (1X TBE), the gel was pre-run for 15-30 minutes.
- To the 20 µl PCR product, 8 µl gel loading dye added and from this 3 µl sample loaded
- 3 µl of DNA (each PCR reaction) and 2 µl of loading dye were taken for sample preparation. Samples were denatured at 95°C for 5 minutes and snap cooled on ice.
- After flushing the wells again, the denatured DNA samples were loaded onto the gel.
- The electrophoresis was resumed and allowed to proceed at 100 watts (constant) 1-2 hours based on SSR product size (bp)
- Finally plates were dismantled from the electrophoresis apparatus for silver staining.

3.4.4 Silver staining for PAGE gel

3.4.4.1 Staining of gel

- After careful removal of the small plate with gel from the assembly, the gel with plate was stained with washing treatments of various solutions. Silver staining was done in following steps:

3.4.4.2 Fixing

- Gel was soaked in fixer for 15 minutes or till the dye disappears with mild shaking.
- Then washed twice in double distilled water for 5 minutes.

3.4.4.3 Staining

- Gel was soaked in staining solution for 15 minutes with mild shaking followed by brief wash in double distilled water for 10 seconds.

3.4.4.4 Developing

- Gel was soaked in developer for 3-5 minutes or till bands appeared.
- Again gel was soaked in fixer for 5 minutes to stop the reaction followed by washing with double distilled water for 2-5 minutes.
- Gel documentation
- Then the gel was dried and scanned using a computer scanner.

3.5 SSR Fragment analysis using Genetic Analyzer

- The DNA amplification was done (as section 3.3) using fluorescent labeled SSR primers, IB R12 and IB S18.
- The PCR products were dilute with sterile deionized water in the ratio of 1:10.
- 0.5µl diluted samples plus 0.5µl Liz 600 size standard were pipette into the corresponding wells of 96 welled sample plate.
- Then pipetted 9.0µl of Hi-dye™Formamide added to all samples.
- Plate wells were covered with septa strip and briefly centrifuged the plate (Mix Mate 96, Eppendorff)
- Plate assembly was done by placing the sample plate on plate base and upper part of sample plate above the septa was covered using plate retainer.
- The plate was placed in Autosampler in the Genetic Analyzer (Applied Biosystems -3500).
- The fragment analysis for the SSR primers were set in the Genetic Analyzer and the samples were run in the machine. The data file collected at the end of the programme.

3.6 Data collection and analysis

- The clearly defined SSR bands from PAGE were scored manually using scale and rulers, the marker size scored range from 100bp to 1000bp.
- The scored data saved as binary matrix, for the presence of band as (1) or absence (0).
- For all the *Ipomoea* samples and sweet potato varieties, amplification failure samples bands were scored as 9 and the data entered in MS Excel sheets.
- From the Genetic Analyzer the data was scored based on the coloured peak of the each fluorescent labelled primer using the standard peaks.
- The data from genetic analyzer was converted as binary matrix similarly as done in PAGE.
- Both the PAGE and Genetic Analyzers data were combined and formed a single data matrix.
- The data was analyzed for similarity matrix, PCA (Principle Component Analysis) and dendrogram using NTSYS-pc software.
- Polymorphism Information Content (PIC) and Heterozygocity (He) value was calculated using PIC Calculator.

Results

4. RESULT

4.1 Accessions used for the study

A total of 45 *Ipomoea* and *Merremia* accessions including 20 sweet potato cultivars, 23 wild *Ipomoea* species and two wild *Merremia* species were taken for the genetic diversity study. All the wild species were identified based on morphological characters and the molecular characterization was done using 12 sweet potato SSR primers.

4.1.1Morphological characters observed for identification of wild *Ipomoea* species

The flowers of *Ipomoea* are normally bell shaped and are of different sizes and colours like red, blue, yellow and purple. *Ipomoea* wild species identification was done based on qualitative characters like leaf shape, plant type, flower shape, presents of seeds, stem colour and appearance. The recorded data is shown in the Table 5 and there was a high morphological variability in the flower, leaves, seeds and seed capsules of the species are shown in (Plate 2A, 2B and 2C).

4.2Isolation of genomic DNA

DNA was isolated from 20 sweet potato accessions and 25 wild relatives using modified extraction protocol of Dellaporta *et al.* (1983).

4.2.1Quality analysis of DNA

The quality of DNA was checked using 0.8% agarose gel to ensure the high quality of DNA for SSR marker studies (Plate 3). The results, showed genomic DNA of all the studied genotypes were intact with no protein and RNA contamination. The observed genomic DNA bands with an expected size range of 100 to 300 bp.

I. alba | *I. cairica* | *I. carnea* | *I. capitellata*

I. eriocarpa | *I. hederifolia* | *I. indica* | *I. violacea*

I. mauritiana | *I. nil* | *I. obscura* | *I. pes-caprae*

Plate 2 (A). Variations observed in flower colour and flower shape of sweet potato wild relatives (Cont.)

I. pestigridis *I. purpurea* *I. quamoclit* (red) *I. sepiaria*

I. trifida *I. triloba* *I. turbinata* *M. dissecta*

M. vitifolia *I. carnea* *I. quamoclit* (pink) *I. carnea*

Plate 2 (A). Variations observed in flower colour and flower shape of sweet potato wild relatives

I. alba *I. aquatica* *I. cairica* *I. carnea*

I. carnea *I. capitellata* *I. eriocarpa* *I. hederifolia*

I. indica *I. violacea* *I. mauritiana* *I. nil*

Plate 2 (B). Variations observed in leaves of sweet potato wild relatives (Cont.)

I. obscura *I. pes-caprae* *I. pestigridis* *I. purpurea*

I. quamoclit *I. sepiaria* *I. trifida* *I. triloba*

I. turbinata *M. dissecta* *M. vitifolia* *I. quamoclit*

Plate 2 (B). Variations observed in leaves of sweet potato wild relatives

I. alba *I. carnea* *I. capitellata* *I. eriocarpa*

I. hederifolia *I. violacea* *I.. nil* *I. obscura*

I. pestigridis *I. purpurea* *I. quamoclit* *I. sepiaria*

I. trifida *I. triloba* *I. turbinata* *M. dissecta*

Plate 2 (C). Variation observed in capsules and seeds of sweet potato wild relatives

Table. 5. Morphological characters observed for *Ipomoea* wild species.

Sl. No.	Scientific Name	Morphology characters
1	*Ipomoea alba*	Climbing plant with hairless twinning green stems, leaves are simple, entire and born on long stalks having heart-shape with pointed tips. Large funnel shaped flowers have very long narrow greenish-white floral tube and five large white spreading lobes with white centre. Flowers open at night and usually wither by early the next morning. Mature capsules split open to release four hairless, brownish coloured seeds.
2	*Ipomoea aquatica*	Semi aquatic floating plant grown in ponds and banks of water bodies with hairless, hollow, fleshy stems, leaves are simple, entire and borne on stalks having lanceolate shape with pointed tips. Medium funnel shaped flowers have narrow inner purple coloured corolla tube and five pinkish white spreading lobes. Flowers open at early morning, solitarate or cymes. Mature seeds are grey in colour. Normally rooting occurs at nodes.
3	*Ipomoea cairica*	Climbing plants with hairless slender stems, Leaves are simple, entire having very distinctive 5-7 finger-like lobes. Flowers are relatively large, funnel shaped purplish-pink with whitish flower tube have a darker purple centre. Flowers open in the morning. Small capsule turn brown as they mature and contain four seeds, are partly covered in long silky hairs.
4	*Ipomoea carnea*	Bushy herb with thick woody hairless stem, sometimes the younger twigs show twinning property. Leaves are simple, entire with elongated heart shaped with dominant veins on green leaf lamina. Funnel shaped flowers seen as cymes with pink or white or pinkish purple colour with dark purple centre. The capsules have rigid coating with 3 to 4 seed in it. The hairy seeds are with dark brown colour.
5	*Ipomoea capitellata*	Climbing plant with green hairy brownish stem, leaves are simple, entire, and trilobed with pale hairs on both sides. Funnel shaped flowers with white colour. Flowers are open in early morning. The sepals are unequal, hairy outside and overlapped. The capsules are large and haired. Seeds are with dark brown to black in colour.

Sl. No.	Scientific Name	Morphology characters
6	*Ipomoea eriocarpa*	Climbing plant slightly haired slender stem. Leaves are simple, entire, having pale coloured hairs on both the leaf surfaces with elongated heart shape and acuminate apex. Very small funnel shaped, almost sessile flower with pink petals and dark centre, the sepals are with outer hairy nature, unequal and overlapped. In capsules the sepals persistent at the base. The seeds are grey in colour.
7	*Ipomoea hederifolia*	Climbing plant with hairless twinning stem. Leaves are simple, entire, thin, and trilobed with angles and narrow acuminate apex, the veins are dominantly seen on the leaf surface. The flowers are funnel shaped with deep blood red colour having orange red at the centre. The sepals are green, unequal, small and overlapped. The capsules are round in shape with small beak at the top, contain 3-4 seeds. The seed are dark brown in colour with slightly rough surface.
8	*Ipomoea indica*	Climbing plant with non- hairy stems and heart-shaped or three-lobed leaves. Funnel shaped flowers are relatively large, deep blue sepals and purple corolla are long and thin, The blue colour fade and by afternoon the whole flower become purple in colour. The sepals are unequal, overlapped and slightly hairy outside. It does not produce viable seeds (capsules are generally not seen).
9	*Ipomoea mauritiana*	Climbing plant with non hairy stems, leaves are simple, entire lobed with shining surfaces and dominant veins. Funnel shaped flowers with pink or pinkish red petals and dark purple centre. The major specialty of this plant is, it's one of the tuber bearing species with the taproot as large creamy storage tuber. The stem or leaf produce milky excretion when there is a wound.
10	*Ipomoea nil*	Climbing plant with twinning hairy stems and heart-shaped or three-lobed leaves. Its funnel shaped flowers are relatively small with blue corolla lobes with white inner edges and centre, its strongly curved sepals are unequal, long, thin, and overlapped. It produces capsules containing four to six hairless seeds. The seeds are black coloured with smooth surface.

Sl. No.	Scientific Name	Morphology characters
11	*Ipomoea obscura*	Climbing plant with twinning hairless stem. Leaves are simple, entire, heart shaped with dark green colour and dominant veins. The flowers are small funnel shaped one with cream colour corolla and dark purple centre. The sepals are small round green coloured. It produces capsules with small beak at the top. The seeds are dark brown in colour.
12	*Ipomoea pes-caprae*	The creeper plant seen near coastal areas with strong hairless stem. Leaves are simple, entire, bilobed and fleshy with dominant veins. Flowers are funnel shaped solitarate with pink corolla and purple coloured centre, petals more deeply lobed than other collected species. They produce capsule with thick covering. The brown coloured seeds are covered throughout with brown coloured hairs.
13	*Ipomoea pes-tigridis*	It is a twining, herbaceous, hairy, annual vine, all parts being more or less covered with rather long, spreading, pale, or brownish hairs. The leaves are somewhat rounded in outline, palmately lobed, with apex acuminate and hair on both surfaces. The 5-lobed leaf resembles tiger's paw. Funnel shaped flowers occur in axillary head, usually only one opening at a time. The sepals are green and hairy. The flowers are white with white centre. The capsule is large and haired, with dark brownish seeds.
14	*Ipomoea purpurea*	Climbing herb has hairy younger stems and elongated heart-shaped with small basal lobes or trilobed leaves. Its flowers are relatively large, with dark pink petals and white corolla tubes and sepal outlining, its sepals are moderately long and hairy outside. It produces capsules with 3 to 4 seeds. The seeds are black and velvety.
15	*Ipomoea quamoclit*	Climbing plants with thin, hairless stem. The leaves are simple but feathery (pinnately lobed) lake appearance. Flowers are funnel shaped, small one with either deep red colour with slightly whit- red corolla tube or dark pink in colour with slightly whitish pink corolla tube. Sepals are fused with the corolla. It produce capsule with elongated shape and beak at the top. The seeds are black, elongated and hairless.

Sl. No.	Scientific Name	Morphology characters
16	*Ipomoea sepiaria*	Climbing herb with thin, hairless deep purple coloured twining younger stems. Leaves are simple, small, entire, fleshy heart shaped. Flowers are small with pink corolla and deep red centre. Sepals are fused to petals, non hairy, unequal and overlapped. It produce capsule with globular shape and grey coloured hairless seeds
17	*Ipomoea trifida*	Climbing herb with haired stems. Leaves are simple, entire, angled, trilobed one with acuminate apex. The leaves surfaces are covered with small pale coloured hairs. Flowers are funnel shaped medium sized with light lavender coloured corolla lobes and white corolla. Flowers are usually soliterate; sepals are long, unequal and overlapped with hairy outside. They produce capsules with 3 to 4 seeds. The seeds are black, smooth, large viable ones.
18	*Ipomoea triloba*	Climbing herb with hairless stem. Leaves are simple, entire, heart shaped to tri lobed one with acuminate apex. The flowers are small funnel shaped one, soliterate, sepals are deep pink in colour with small corolla tube, and the cente is deep purple coloured. Sepals are thin and green in colour. They produce viable capsules with globular shape. The seeds are small, smooth and black in colour.
19	*Ipomoea turbinata*	Climbing herb with hairless, fleshy, prickly stems. Leaves are simple, entire, heart shaped, fleshy one with acuminate apex. The flowers are funnel shaped with pale purple colour and deep purple at centre. The flower is usually soliterate, bloom in the evening and withered by next morning. They produce large capsule with 4 seeds. The seeds are with large shiny tough surface and black in colour.

Sl. No.	Scientific Name	Morphology characters
20	*Ipomoea violacea (macrantha)*	Climbing plant with dark green hairless stems. Leaves are simple, entire, heart shaped, thick fleshy with acuminate apex. Flowers are with long stalk, funnel shaped with pale yellow colour. Produce large capsules with shiny surface have 2-3 seeds per capsule. The seeds are with large shiny tough surface and black colour.
21	*Merremia dissecta*	ıts with sparsely hairy (i.e. puberulent) stems and five to aves, but each segment is also further lobed or divided. Its flowers arge, its large sepals are relatively long, and it often ıles containing four hairless seeds.
22	*Merremia vitifolia*	Twinning plants with hairy brown shaded stems. The leaves are large, 5 lobed with hairy surface. The young leaves are brown in colour and later on maturation turned to dark green colour. The leaf resembles the grape vine leaves. Flowers are funnel shaped, yellow in colour and seen as clusters. Generally the seeds were not seen.

4.2.2Quantification and dilution of DNA

The DNA quantity of each sample was determined by the absorbance recorded at 260nm and 280 nm wavelengths in a spectrophotometer. The 260:280 reading ratio was calculated and the DNA was found to be intact with no RNA or Protein contamination. The DNA concentration of different samples varied from 1800- 2500 ng μl^{-1}. After the quantification, DNA was diluted to 10 ng μl^{-1}.

4.3 Molecular Characterization

4.3.1 PCR amplification using SSR primers

The 45 genotypes were molecular characterized by 12 SSR primers. The summarized overall primer PCR amplified result was showed in the Table 6.

Table 6. Summary of SSR primer amplified product.

Total number of primers tested	12
Total number of polymorphic bands	84
Total number of monomorphic bands	3
Total number of bands	87
Size range of amplified products (in bp)	105 to 393
Percentage polymorphism	94.5%

Out of the 16 SSR primers tested, 12 SSR primers (IB 255, IB 242, GDAS0047, GDAS0049, IB 3/31, IB 248, IB SSR 05, IB SSR 21, GDS0542, GDAS0809, IBS12 and IBR 18) were selected for the diversity analysis (Plate 4.). None of the primer was monomorphic and six unique bands were observed for

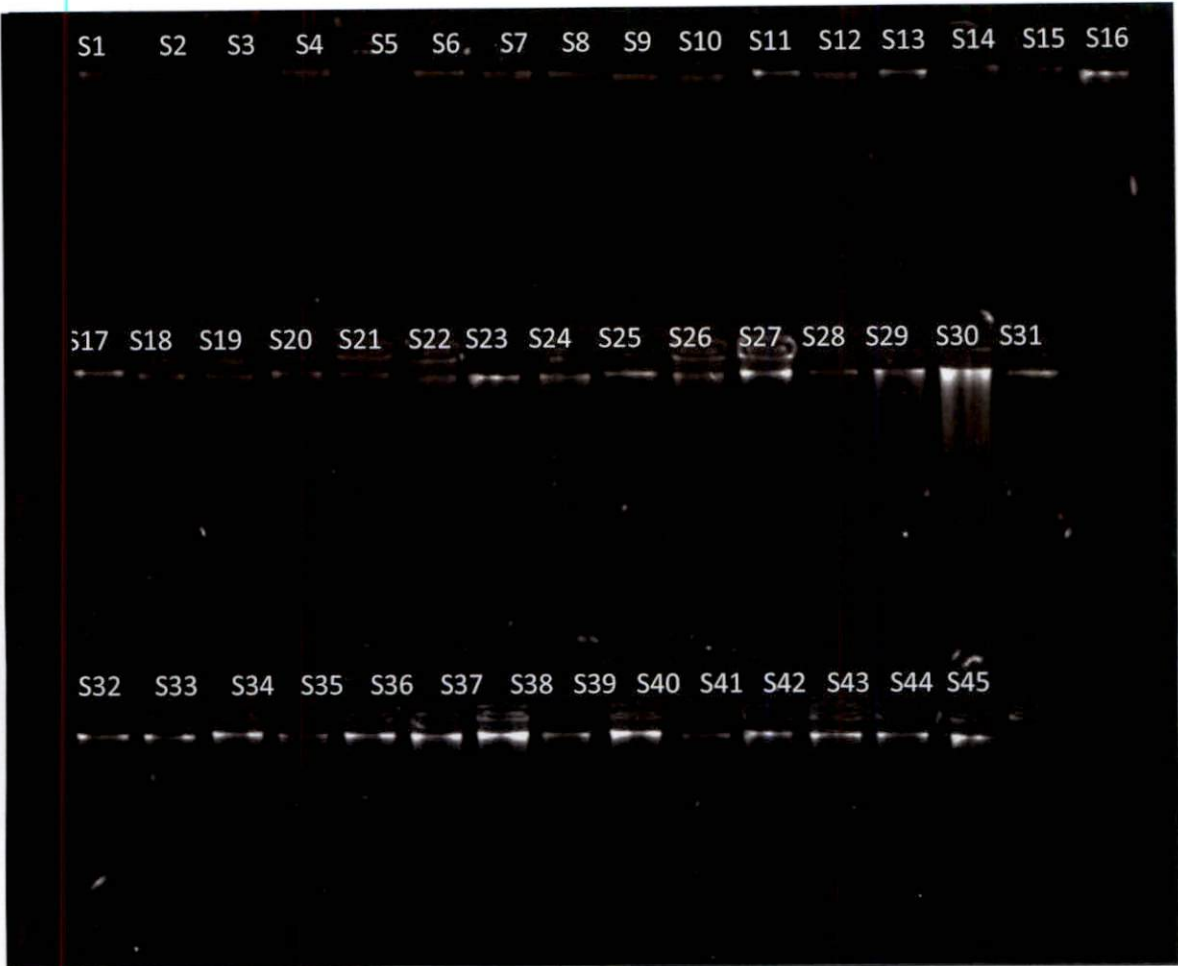


Plate 3. Quality analysis of 45 genomic DNA using 0.8% agarose gel

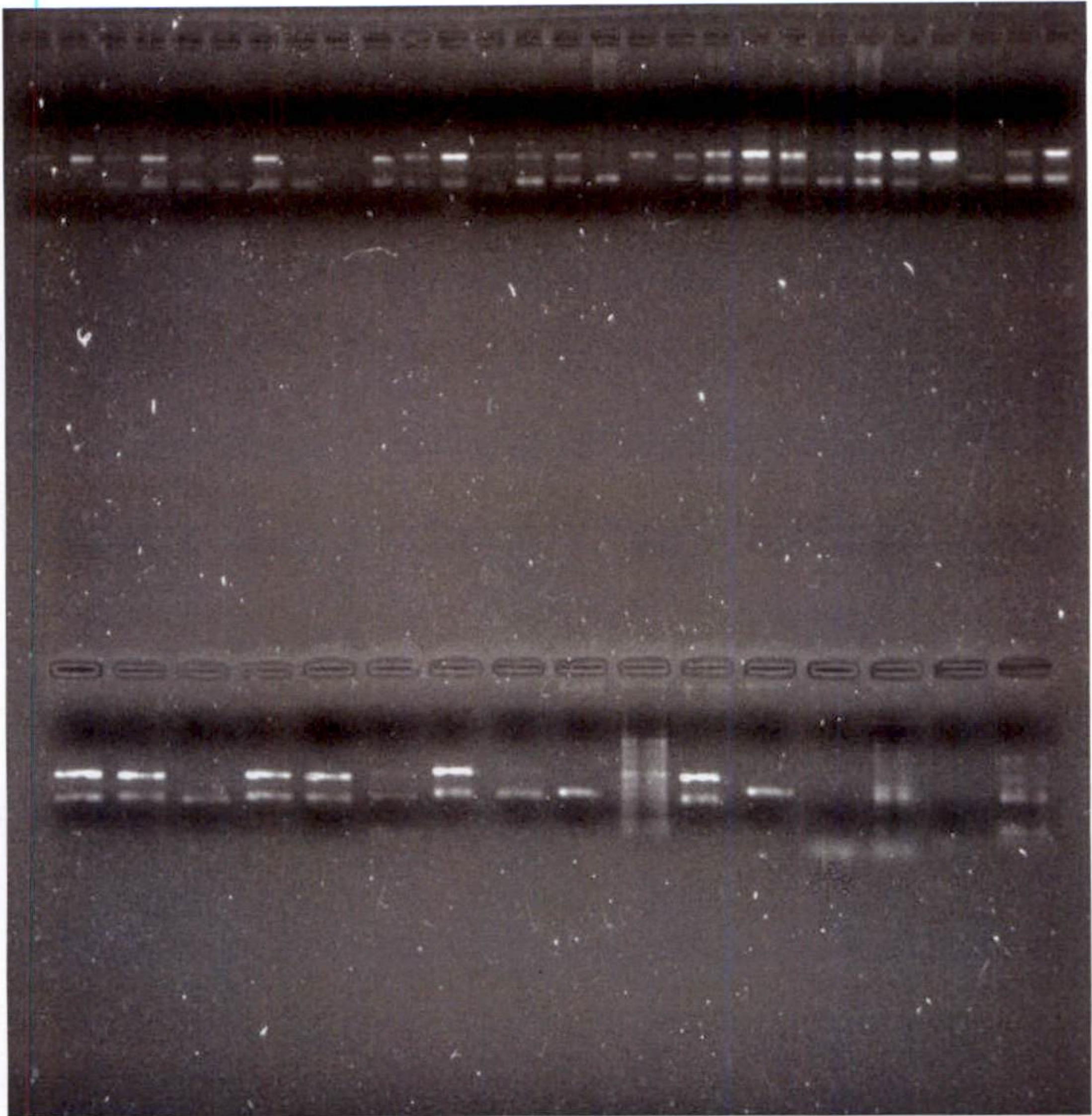


Plate 4. PCR amplified products of SSR primers checked in 2% agarose gel

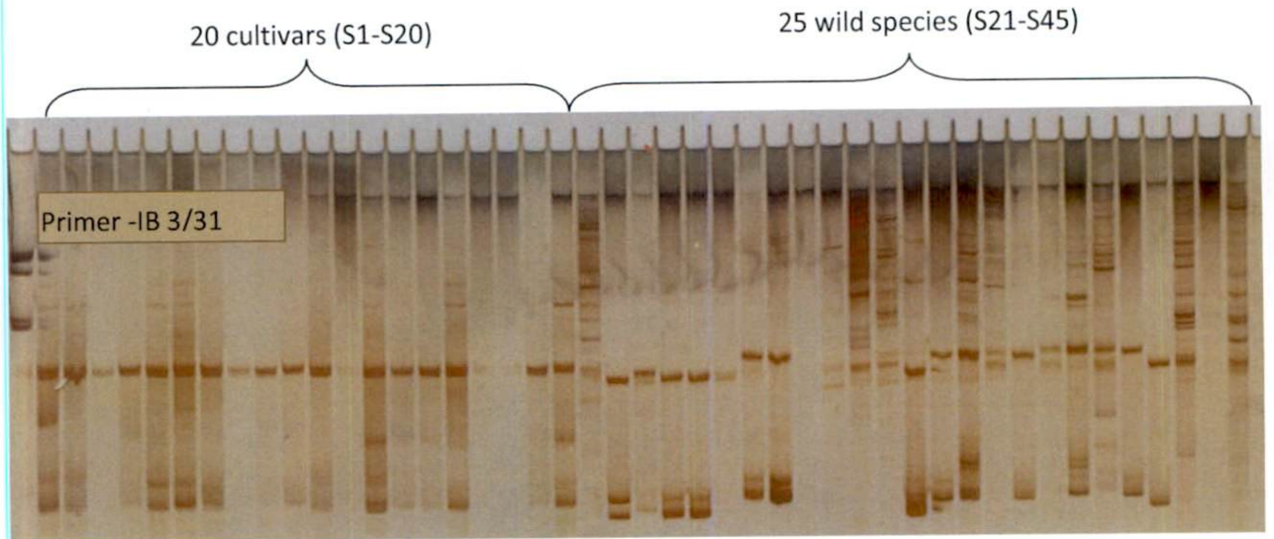
primers IB 242, GDAS0047, GDS0542, IB 3/31 and GDAS0809. A total of 87 loci were amplified that exhibited 94.5% polymorphism. The primer wise analysis of 10 SSR markers were done in PAGE (Plate 5) and that of two fluorescent labeled SSR primers (IBS12 and IBR 18) were analysed for fragment analysis in Genetic Analyzer (Fig. 1).

The PCR products generated from SSR analysis was used to determine the interrelationship between 20 sweet potato cultivar and 25 wild relatives as well as the intra relationships within cultivar accessions and wild accessions. All the SSR primers produced amplified fragments not universally present, that is, high level of polymorphism in DNA amplification profile was observed (Table 7). The number of polymorphic loci ranged from 2 to 13 per primer with an average of 6 polymorphic loci per primer and all these amplified products size ranged from 105 - 393bp. Primer IB SSR05 (Plate 5) produced least number of amplified products where as GDAS0047 primer produced highest number of amplified products. All the primers except IB SSR 05 and GDAS0809 had 100% polymorphism. The summary of SSR primer amplification showed in table 6.

IB 255 - Six amplified loci were obtained and the amplicons size varied from 210 bp to 245 bp. No unique band was observed. This primer pair showed 100% polymorphism.

IB 242- Seven amplified loci were obtained and the amplicons size was 190 bp to 240 bp. One unique band was there for sample *I. obscura*. Showed 100% polymorphism

GDAS0047 –Thirteen amplified loci were obtained and one unique band was observed for *M. vitifolia*. The amplicons size varied from 218 bp to 315bp, were showed 100% polymorphism



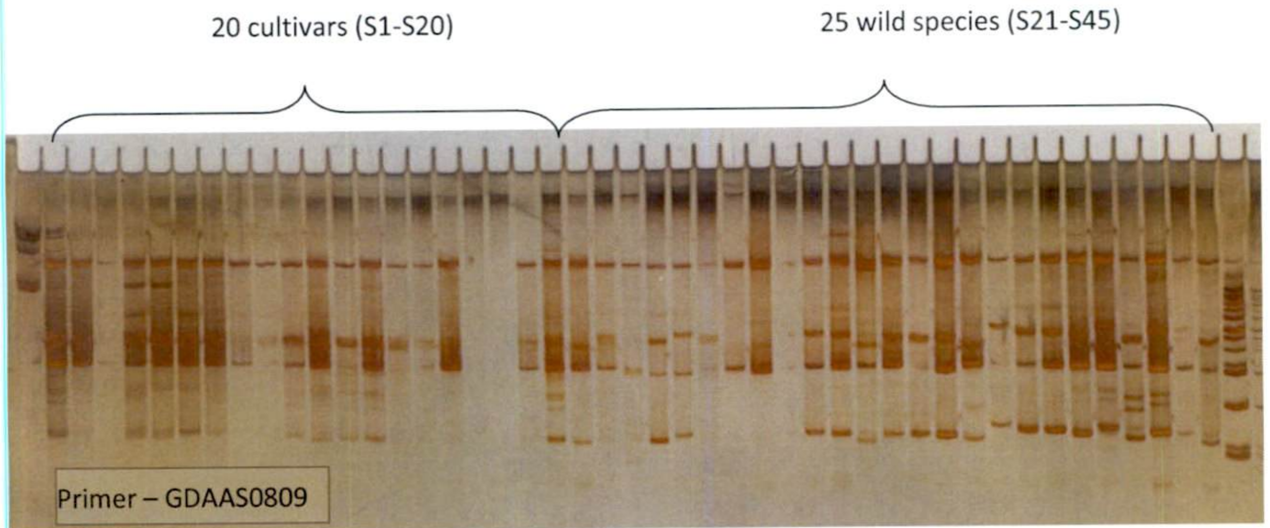


Plate 5. SSR banding profile on poly acrylamide gel (cont.)

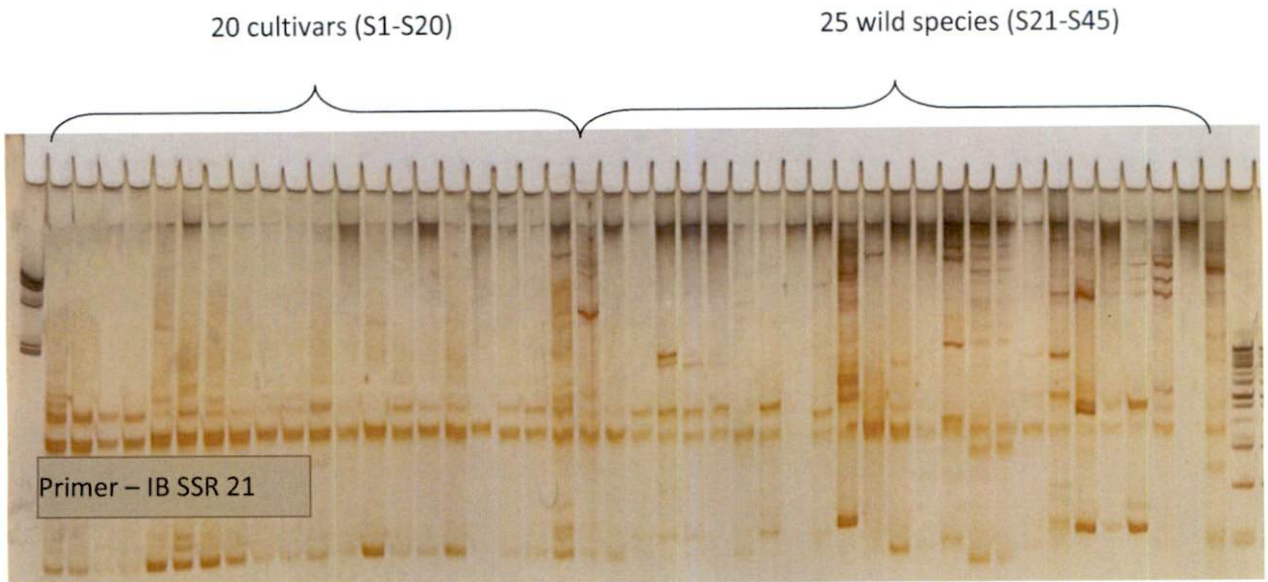


Plate 5. SSR banding profile on poly acrylamide gel (cont.)

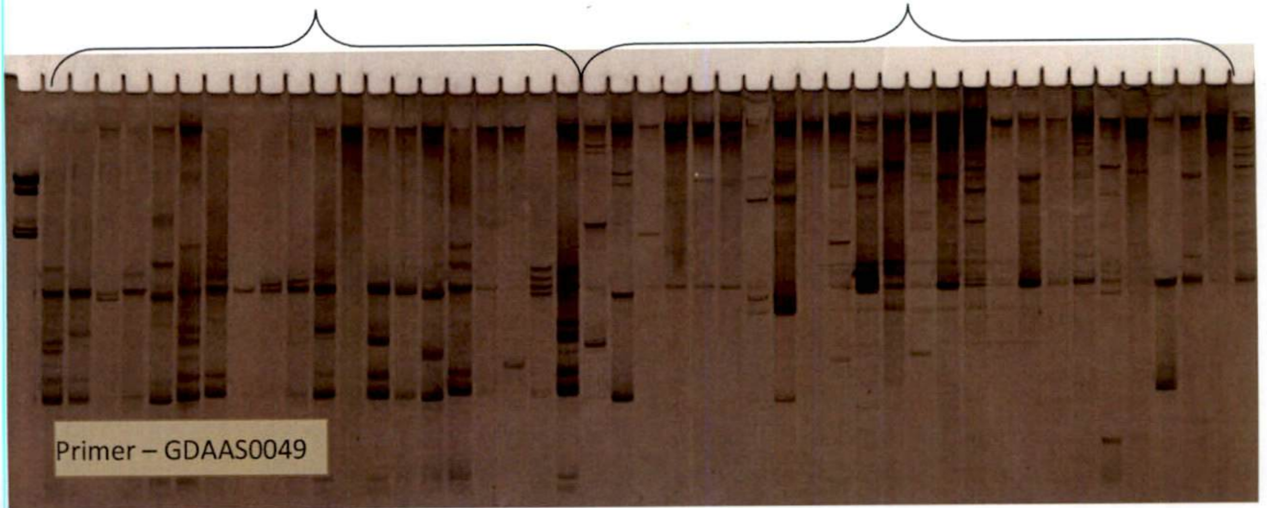
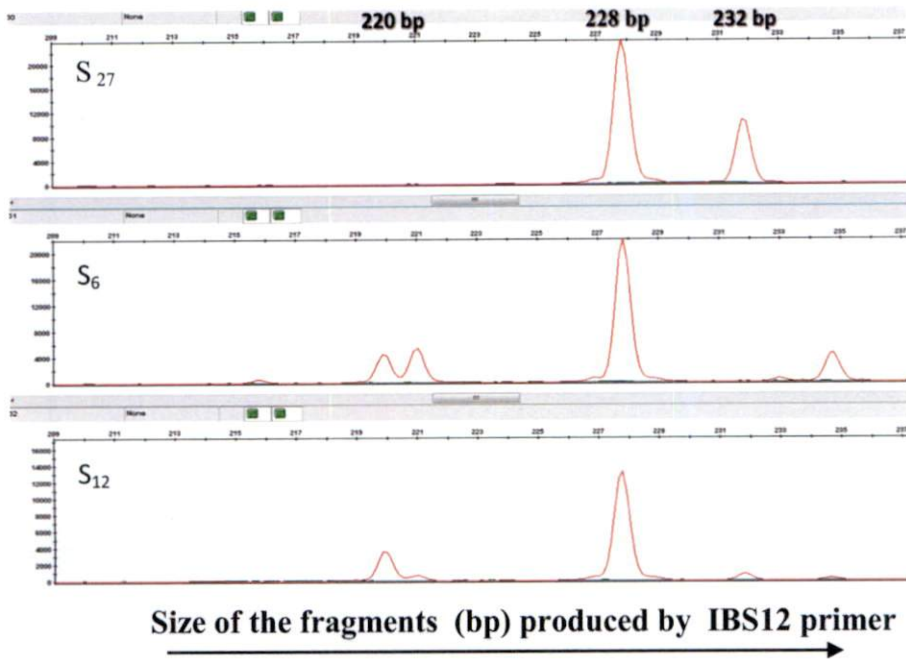


Plate 5. SSR banding profile on poly acrylamide gel



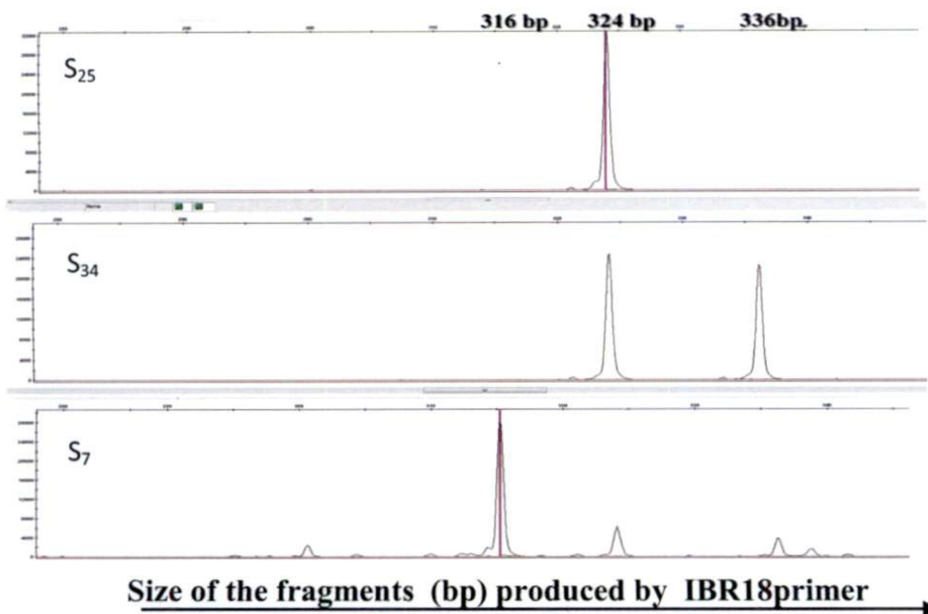


Fig. 1. Peaks generated on fragment analysis of genotypes using IBS12 and IBR 18

GDAS0049 – Ten amplified loci were obtained. Showed 100% polymorphism. No unique bands were obtained and the amplicons size varied from 245bp to 320bp.

IB 3/31 – Six amplified loci were obtained. One unique band was observed for *I. triloba*. The amplicons size varies from 227 bp to 235 bp, showed 100% polymorphism

IB 248- Ten amplified loci were obtained for the primer and showed 100% polymorphism. No unique band was observed and the amplicons size varies from105bp to 142bp.

IB SSR 05 – Three amplified loci were obtained and showed 67% polymorphism. No unique band was observed. The amplicons size varied from155bp to 170bp.

IB SSR 21 – Five amplified loci were obtained and showed 100% polymorphism. No unique band was observed and the amplicons size varied from 331bp to 393bp.

GDS0542 - Eight amplified loci were obtained and showed 100% polymorphism. Two unique bands were observed, one for *I. pes capre* and other for *M. vitifolia*. The amplicons size varied from232bp to 242bp.

GDAS0809 - Six amplified loci were obtained and showed 67% polymorphism. One unique band for I. purpurea was observed. The amplicons size varied from 289 bp to 368bp.

IBS12 – Seven amplified loci were obtained and showed 100% polymorphism. No unique bands were observed. The amplicons size varied from 300bp to 340bp.

IBR 18 – Six amplified loci were obtained and showed 100% polymorphism. No unique band was observed. The amplicons size varied from 200bp to 250 bp.

Table 7. Summary of Polymorphic bands produced on SSR amplification

Serial No.	Primer Name	Total loci	Polymorphic loci	Polymorphic per cent (%)	Amplified product size (bp)
1	IB 255	6	6	100	210-245
2	IB 242	7	7	100	190- 240
3	GDAAS0047	13	13	100	218 – 315
4	GDAAS0049	10	10	100	245- 320
5	IB 3/31	6	6	100	227 -235
6	IB 248	10	10	100	105-142
7	IB SSR 05	3	2	67	155-170
8	1B SSR 21	5	5	100	331-393
9	GDAAS0542	8	8	100	232-242
10	GDAAS0809	6	4	67	289 -368
11	IBR12	7	7	100	200-240
12	IBS 18	6	6	100	300-340

4.3.2 Relationship among sweet potato cultivar and wild relatives using SSR

Based on the SSR amplification profile on PAGE and graph peaks from Genetic Analyzer were scored into binary data as input for data analysis. The SSR binary data were used to calculate the genetic relationship between cultivars and its wild relatives by NTsys-PC software, relationship revealed by similarity index (Table. 8) (Fig. 2), dendrogram (Fig. 3) and Principle Coordinate Analysis (PCA) analysis (Fig. 4, 5). Polymorphism of marker was analyzed by Polymorphism Information Content (PIC) and heterozygosity values (Table 9).

4.3.2.1 Similarity index

Similarity index values based on Jaccard's Similarity Coefficients obtained for each pair wise comparison among 45 accessions of sweet potato and its wild relatives based on SSR marker binary data is given in Table 8. The similarity coefficient values ranged from 0.37 - 0.96. Among the 45 accessions of sweet potato, lowest similarity index (0.37) was observed between sweet potato variety Sankar and *Merremia dissecta*, the highest was observed between two *I. carnea* species. Based on similarity coefficient data a frequency graph was plotted in Fig.2. From the graph high similarity coefficients were between 0.60 and 0.85. The bell shaped graph showed that there was a high degree of polymorphism exists in the genus *Ipomoea.*

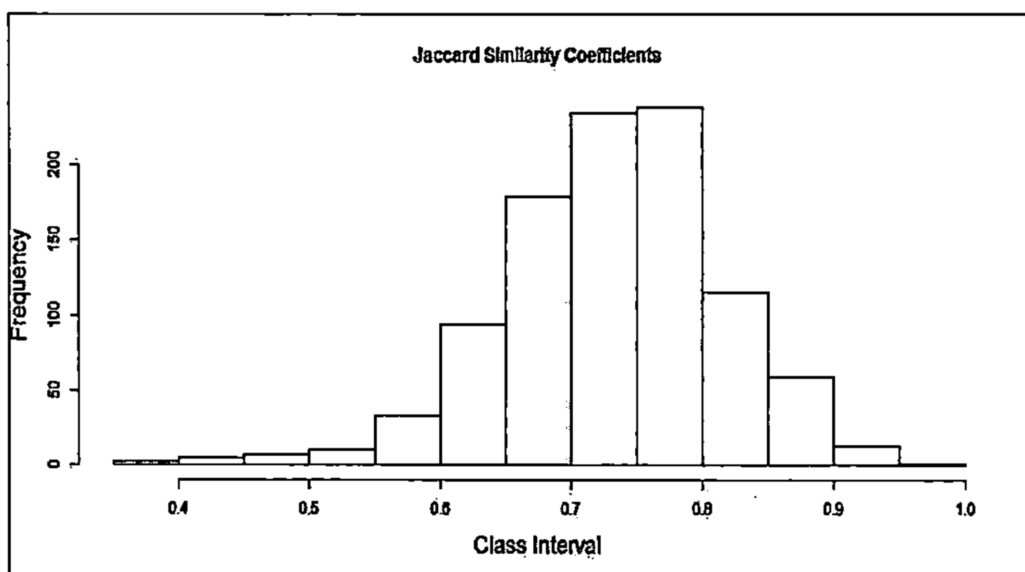


Fig. 2. Frequency distribution of SSR marker based on similarity estimates

Table 8. Similarity coefficient matrix of 20 cultivars, 23 *Ipomoea* species and 2 *Merremia* species

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45
S1	1																																												
S2	0.85	1																																											
S3	0.77	0.79	1																																										
S4	0.79	0.85	0.92	1																																									
S5	0.83	0.77	0.7	0.73	1																																								
S6	0.8	0.85	0.78	0.85	0.77	1																																							
S7	0.83	0.84	0.75	0.85	0.84	0.83	1																																						
S8	0.79	0.76	0.9	0.83	0.78	0.83	0.83	1																																					
S9	0.77	0.83	0.78	0.84	0.78	0.8	0.87	0.86	1																																				
S10	0.8	0.86	0.74	0.8	0.82	0.84	0.93	0.83	0.94	1																																			
S11	0.84	0.94	0.75	0.82	0.71	0.83	0.87	0.79	0.86	0.9	1																																		
S12	0.8	0.83	0.81	0.83	0.77	0.84	0.87	0.84	0.83	0.87	0.84	1																																	
S13	0.86	0.87	0.83	0.84	0.83	0.83	0.87	0.84	0.88	0.87	0.85	0.84	1																																
S14	0.86	0.87	0.78	0.82	0.83	0.85	0.9	0.82	0.88	0.82	0.83	0.9	0.91	1																															
S15	0.77	0.9	0.79	0.84	0.8	0.83	0.85	0.77	0.83	0.9	0.83	0.84	0.86	0.86	1																														
S16	0.87	0.84	0.83	0.85	0.79	0.83	0.84	0.8	0.8	0.82	0.78	0.83	0.87	0.87	0.83	1																													
S17	0.77	0.79	0.91	0.89	0.77	0.77	0.77	0.84	0.84	0.79	0.79	0.79	0.82	0.79	0.75	0.77	1																												
S18	0.73	0.78	0.85	0.9	0.76	0.83	0.83	0.83	0.84	0.8	0.78	0.82	0.76	0.79	0.83	0.85	0.85	1																											
S19	0.76	0.86	0.78	0.85	0.79	0.84	0.85	0.78	0.87	0.88	0.83	0.84	0.8	0.87	0.91	0.82	0.73	0.84	1																										
S20	0.83	0.86	0.73	0.83	0.82	0.86	0.86	0.78	0.78	0.84	0.83	0.77	0.8	0.8	0.83	0.79	0.82	0.79	0.84	1																									
S21	0.65	0.71	0.71	0.72	0.63	0.64	0.67	0.7	0.72	0.69	0.72	0.71	0.68	0.72	0.75	0.64	0.7	0.72	0.69	0.67	1																								
S22	0.59	0.63	0.72	0.7	0.74	0.72	0.71	0.76	0.73	0.72	0.68	0.69	0.7	0.7	0.76	0.69	0.74	0.76	0.7	0.65	0.7	1																							
S23	0.63	0.71	0.74	0.79	0.69	0.73	0.73	0.82	0.79	0.76	0.72	0.74	0.72	0.72	0.75	0.71	0.73	0.83	0.74	0.71	0.72	0.76	1																						
S24	0.61	0.69	0.77	0.79	0.64	0.73	0.71	0.77	0.75	0.71	0.68	0.71	0.7	0.7	0.75	0.71	0.73	0.79	0.74	0.69	0.7	0.82	0.77	1																					
S25	0.62	0.68	0.78	0.78	0.65	0.7	0.63	0.78	0.74	0.63	0.69	0.67	0.71	0.69	0.71	0.72	0.77	0.8	0.7	0.63	0.69	0.81	0.78	0.84	1																				
S26	0.64	0.67	0.79	0.8	0.67	0.71	0.63	0.82	0.74	0.67	0.65	0.63	0.74	0.69	0.71	0.74	0.79	0.78	0.7	0.67	0.67	0.79	0.76	0.85	0.96	1																			
S27	0.63	0.69	0.77	0.79	0.67	0.73	0.71	0.82	0.75	0.71	0.68	0.73	0.7	0.7	0.72	0.74	0.73	0.85	0.71	0.69	0.72	0.74	0.84	0.79	0.83	0.83	1																		
S28	0.5	0.5	0.68	0.7	0.65	0.7	0.6	0.85	0.55	0.55	0.5	0.6	0.6	0.65	0.65	0.68	0.83	0.75	0.5	0.55	0.45	0.65	0.75	0.6	0.75	0.8	0.9	1																	
S29	0.6	0.63	0.73	0.71	0.65	0.63	0.65	0.78	0.71	0.65	0.67	0.7	0.69	0.67	0.67	0.68	0.77	0.78	0.63	0.59	0.67	0.72	0.71	0.76	0.83	0.79	0.71	0.75	1																
S30	0.59	0.57	0.68	0.65	0.64	0.6	0.62	0.72	0.63	0.6	0.63	0.61	0.65	0.59	0.6	0.57	0.7	0.67	0.57	0.57	0.65	0.7	0.68	0.68	0.74	0.72	0.7	0.8	0.78	1															
S31	0.59	0.67	0.67	0.77	0.6	0.69	0.69	0.72	0.72	0.69	0.68	0.74	0.68	0.65	0.72	0.64	0.64	0.76	0.74	0.69	0.72	0.69	0.77	0.75	0.74	0.71	0.79	0.65	0.71	0.68	1														
S32	0.63	0.69	0.73	0.79	0.64	0.71	0.73	0.77	0.79	0.71	0.7	0.74	0.75	0.7	0.75	0.71	0.64	0.76	0.69	0.67	0.75	0.77	0.84	0.77	0.75	0.79	0.77	0.65	0.69	0.7	0.75	1													
S33	0.72	0.73	0.77	0.77	0.73	0.73	0.78	0.85	0.82	0.78	0.77	0.73	0.72	0.8	0.77	0.73	0.84	0.73	0.74	0.73	0.66	0.69	0.77	0.78	0.77	0.82	0.76	0.7	0.74	0.74	0.68	0.76	1												
S34	0.54	0.64	0.64	0.7	0.62	0.62	0.62	0.68	0.72	0.67	0.63	0.64	0.68	0.65	0.73	0.63	0.7	0.73	0.69	0.62	0.7	0.72	0.77	0.77	0.74	0.74	0.72	0.6	0.69	0.65	0.7	0.75	0.73	1											
S35	0.64	0.69	0.83	0.79	0.65	0.67	0.67	0.75	0.71	0.67	0.65	0.67	0.67	0.65	0.7	0.74	0.73	0.8	0.7	0.67	0.67	0.64	0.78	0.67	0.71	0.74	0.87	0.85	0.64	0.67	0.73	0.73	0.69	0.62	1										
S36	0.63	0.67	0.7	0.72	0.63	0.64	0.67	0.75	0.7	0.67	0.68	0.63	0.68	0.63	0.65	0.64	0.73	0.76	0.64	0.64	0.63	0.69	0.77	0.75	0.74	0.74	0.85	0.75	0.67	0.65	0.79	0.7	0.73	0.7	0.79	1									
S37	0.7	0.72	0.78	0.78	0.72	0.74	0.77	0.84	0.78	0.74	0.76	0.75	0.84	0.76	0.73	0.74	0.81	0.78	0.7	0.63	0.68	0.75	0.78	0.8	0.84	0.84	0.8	0.75	0.76	0.76	0.73	0.77	0.9	0.72	0.73	0.8	1								
S38	0.57	0.63	0.74	0.76	0.63	0.7	0.63	0.76	0.74	0.63	0.67	0.65	0.69	0.64	0.69	0.68	0.77	0.8	0.63	0.63	0.69	0.81	0.76	0.8	0.83	0.77	0.83	0.7	0.75	0.76	0.76	0.74	0.72	0.75	0.77	0.78	0.78	1							
S39	0.55	0.62	0.74	0.72	0.62	0.67	0.67	0.75	0.7	0.67	0.63	0.61	0.65	0.61	0.63	0.64	0.79	0.76	0.64	0.64	0.7	0.8	0.73	0.79	0.8	0.8	0.79	0.73	0.74	0.73	0.72	0.73	0.73	0.77	0.7	0.77	0.77	0.84	1						
S40	0.69	0.7	0.74	0.76	0.68	0.72	0.72	0.85	0.78	0.75	0.73	0.73	0.71	0.74	0.69	0.7	0.84	0.78	0.7	0.68	0.71	0.73	0.76	0.76	0.77	0.8	0.83	0.83	0.77	0.74	0.78	0.74	0.77	0.71	0.74	0.78	0.76	0.79	0.78	1					
S41	0.63	0.69	0.82	0.78	0.67	0.73	0.7	0.8	0.73	0.67	0.7	0.7	0.73	0.67	0.73	0.75	0.76	0.79	0.72	0.66	0.64	0.73	0.77	0.78	0.86	0.86	0.83	0.8	0.77	0.73	0.69	0.79	0.81	0.72	0.79	0.73	0.89	0.77	0.78	0.7	1				
S42	0.71	0.77	0.86	0.85	0.74	0.72	0.72	0.84	0.81	0.73	0.76	0.76	0.77	0.77	0.83	0.77	0.81	0.84	0.79	0.75	0.79	0.73	0.77	0.81	0.83	0.83	0.79	0.77	0.84	0.74	0.75	0.85	0.83	0.75	0.76	0.71	0.79	0.8	0.81	0.79	0.81	1			
S43	0.62	0.68	0.73	0.78	0.63	0.72	0.75	0.75	0.76	0.72	0.69	0.73	0.69	0.69	0.71	0.68	0.75	0.75	0.7	0.7	0.8	0.8	0.76	0.78	0.72	0.74	0.71	0.55	0.63	0.69	0.74	0.78	0.63	0.71	0.67	0.67	0.69	0.77	0.76	0.77	0.64	0.76	1		
S44	0.43	0.6	0.53	0.63	0.6	0.61	0.64	0.56	0.64	0.56	0.56	0.52	0.68	0.64	0.6	0.63	0.67	0.57	0.56	0.52	0.6	0.6	0.6	0.6	0.61	0.68	0.64	0.53	0.64	0.76	0.56	0.64	0.73	0.75	0.56	0.56	0.64	0.68	0.72	0.72	0.68	0.73	0.56	1	
S45	0.7	0.76	0.78	0.82	0.71	0.69	0.73	0.79	0.79	0.74	0.77	0.71	0.79	0.75	0.77	0.71	0.79	0.76	0.73	0.71	0.77	0.73	0.77	0.75	0.73	0.77	0.7	0.55	0.74	0.72	0.77	0.82	0.78	0.73	0.75	0.7	0.77	0.78	0.75	0.78	0.72	0.85	0.76	0.76	1

4.3.2.2 Cluster based Dendrogram

Similarity matrices of 20 accessions, 23 *Ipomea* species and two *Merremia* species based on Jaccard's similarity coefficient were used for UPGMA (Unweighted Pair Cluster Method with Arithmetic mean) cluster analysis of SSR data to generate the dendrogram (Fig. 3) by NTsys software. All the 45 accessions were clustered into three major clusters (cluster-A, cluster-B and cluster-C) at a similarity coefficient of 0.74. The genotypes in each clusters varied from 2 to 28. The cluster-A includes 15 cultivars. The cluster-B consist of five cultivars *viz*, Kishan, Sree Arun, light green, H-42 and Sankar, 22 *Ipomoea* species and one *Merremia* species that was *Merremia vitifolia*. The B cluster again sub-divided into seven sub clusters at 0.77 similarity coefficient and were named sub cluster 1, 2, 3, 4, 5, 6 and 7 respectively. The first sub-cluster consists of 5 cultivar genotypes, 7 *Ipomoea* species and one Merremia species. The second sub-cluster consist only two *Ipomoea* species that were *I. cairica* and *I. mauritiana*. Third sub-cluster had a cluster of two *I. quamoclit* species and *I. aquatica*. Fourth sub-cluster consists of five *Ipomoea* species. *I. capitellata*, *I. eriocarpa*, *I. pes-caprae*, *I. sepiaria* and *I. pes- tigridis*. Fifth sub-cluster had two members *I. hederifolia* and *I. indica*. Sixth sub-cluster with *I. alba* and *I. turbinata* and the seventh sub-cluster contains only one member that was *I. violacea*. The third cluster-C was an out cluster which contains only two samples, *Ipomoea obscura* and *Merremia dissecta*.

4.3.3 Principle Coordinate Analysis (PCA) Analysis

Two dimentional and 3-D principle co-ordinate analysis was performed for 20 sweet potato accessions and 25 wild relatives (Fig. 4, 5). The 20 cultivars were clustered separately from the *Ipomoea* and *Merremia* species. The *M. dissecta* was showed high level of variation from cultivars. In the 2- D PCA plot the cultivars were grouped together and wild species were grouped separately two clusters. In 3-D PCA plot the cultivars were grouped into two which were a small group of five cultivar and other group with15 cultivars. Wild species were also grouped together into one group.

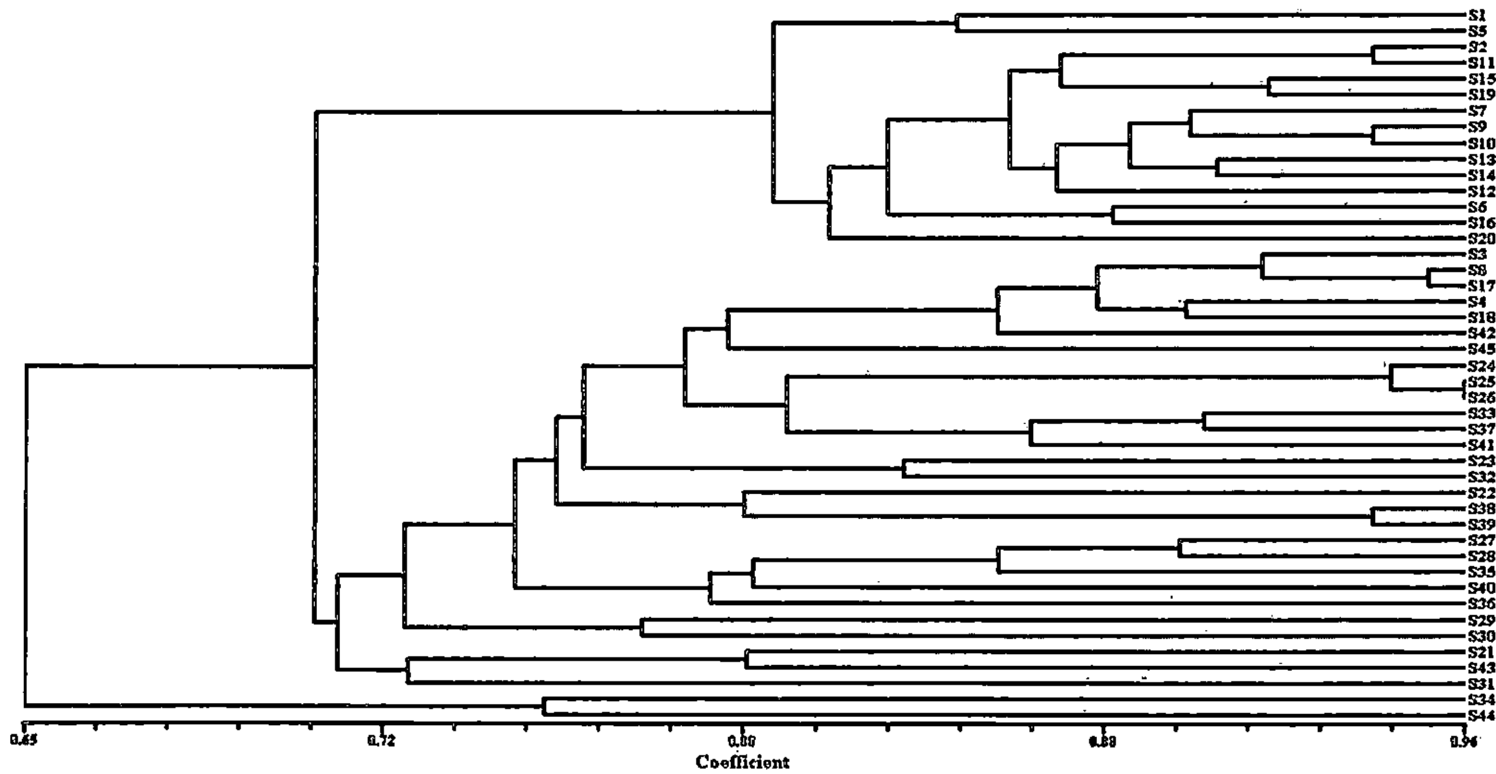


Fig. 3. Cluster analysis based dendrogram of 20 cultivars (S1-S20), 23 *Ipomoea* species (S21-S43) and 2 *Meremia* species (S44-S45)

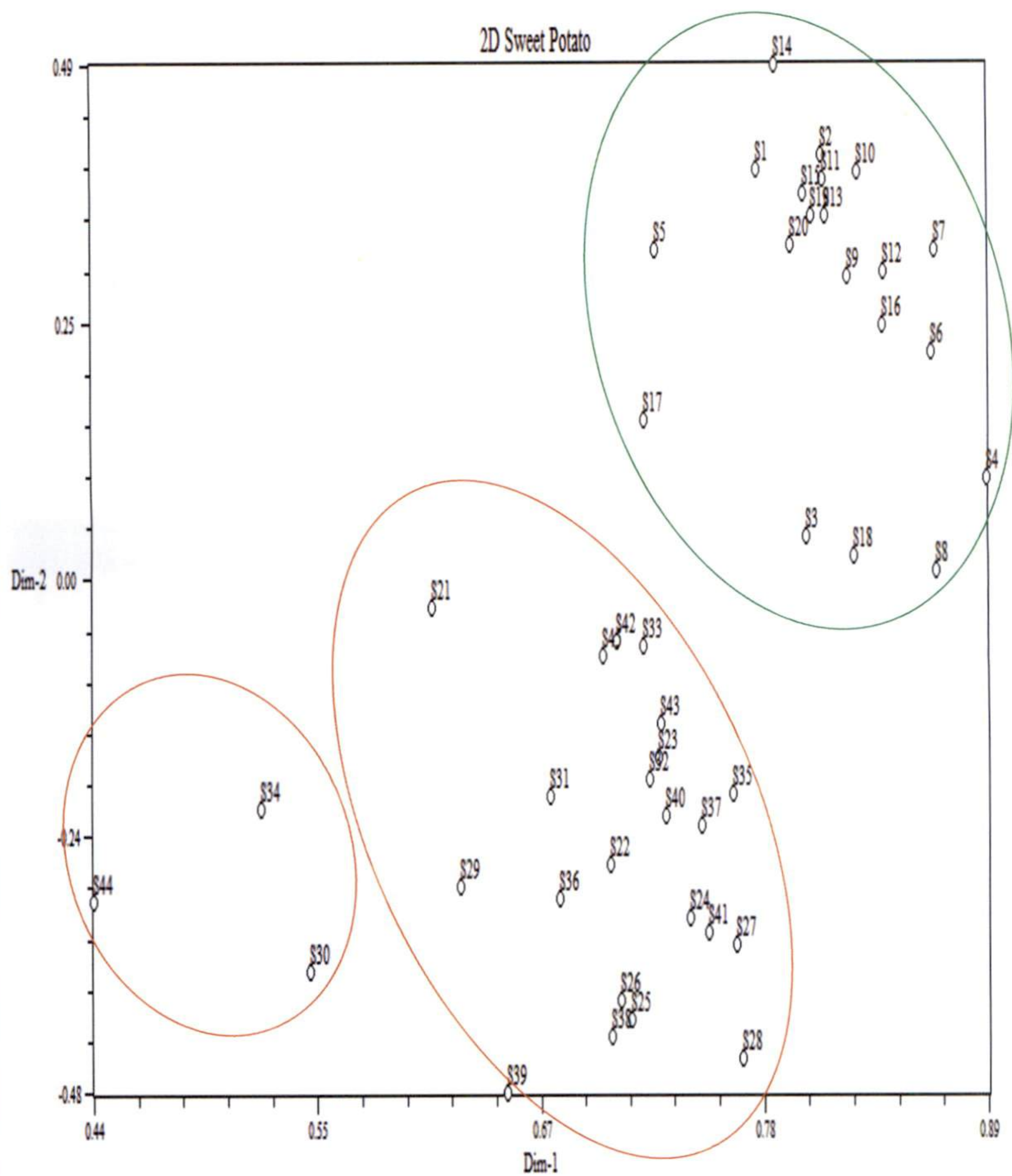


Fig. 4. 2-Dimentional Principle Co-ordinate Analysis plot of 45 accessions

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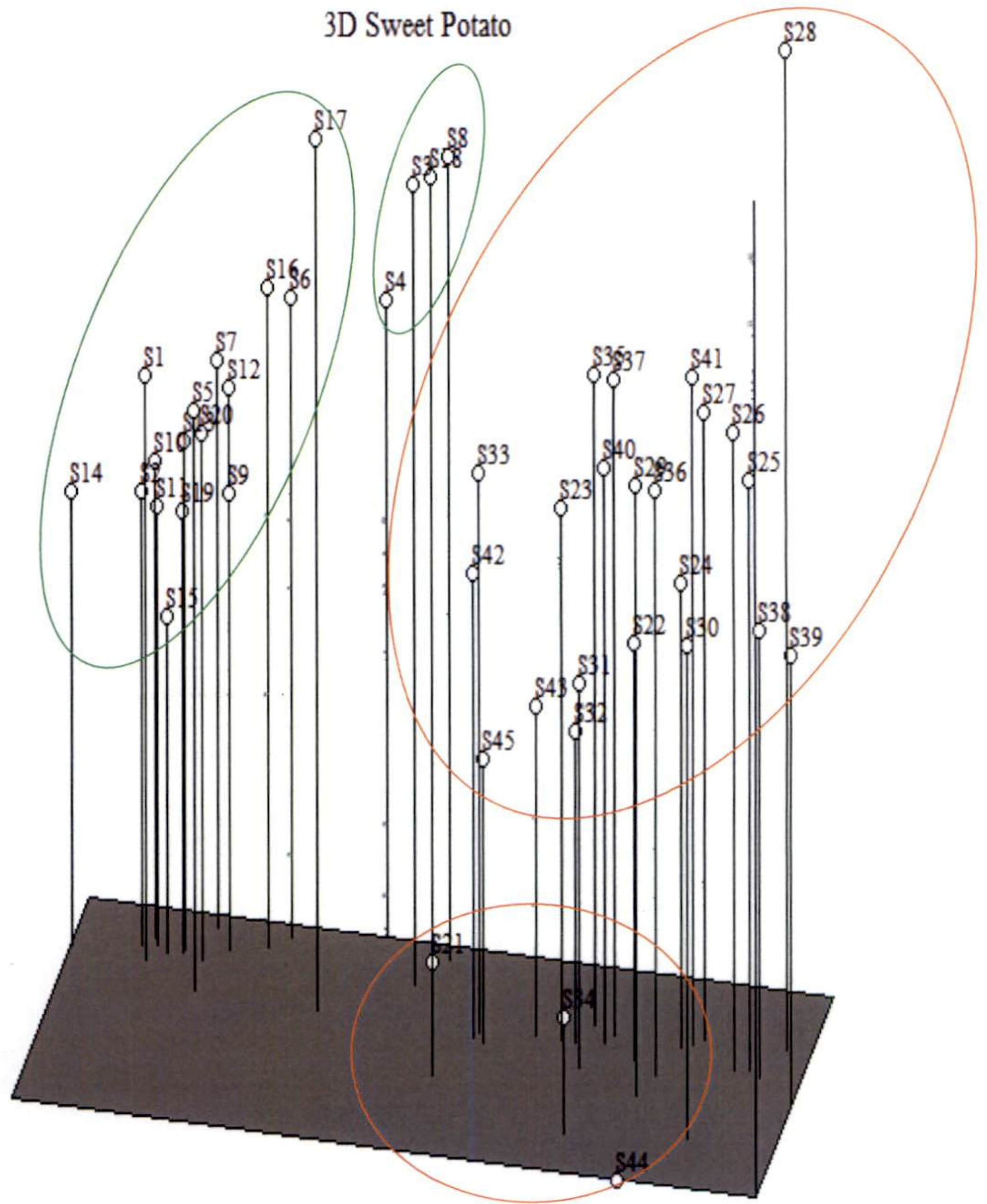

Fig. 5. 3-Dimentional Principle Coordinate Analysis plot of 45 accessions

4.3.4 Polymorphism Information content (PIC) and Heterozygocity (He)

Binary data scored from wet lab were directly used for find out the PIC and He values (Table 9) using PIC calculator (www.liv.ac.uk/~kempsj/pic.html). The PIC values ranges from 0.26 to 0.85 with an average of 0.67. The lowest PIC value was showed by the primer IB SSR 05 and highest value by GDAAS0047. Similarly the heterozygosity (He.) value ranges from 0.27 to 0.87 with same above mentioned primers. The average He value was 0.7.

Table 9. PIC value observed for primers

Serial No.	Primer Name	Heterozygosity (He.)	PIC value	No. of alleles
1	IB 255	0.7475	0.7054	6
2	IB 242	0.6625	0.6028	7
3	GDAS0047	0.8656	0.851	13
4	GDAS0049	0.7638	0.7466	10
5	IB 3/31	0.608	0.5692	6
6	IB 248	0.8009	0.7758	10
7	IB SSR 05	0.2768	0.2592	3
8	1B SSR 21	0.6759	0.6202	5
9	GDS0542	0.713	0.6782	8
10	GDAS0809	0.7466	0.7046	6
11	IBSSR12	0.8093	0.7812	7
12	IB SSR 18	0.7674	0.7333	6

Discussion

5. DISCUSSION

The analysis of molecular genetic diversity in sweet potato accessions and wild relatives are of critical importance for the conservation of genetic diversity and proper utilization of valuable genes present in diverse cultivars as well as wild relatives in crop improvement. Conservation and sustainable use of genetic resources is essential to meet the demand for future food security (Karuri *et al.* 2010).Even though there exist many wild species related to sweet potato and a number of interspecific hybridization projects have been initiated, characterization of excellent wild germplasm and creation of interspecific hybrids are still scarce (Cao *et al.* 2009) because most of the previous molecular work focused only on economically important hexaploid sweet potato accessions, their origin, evolution and relationship with putative progenitor (Das, 2011).

SSR markers were widely applied to analyse genetic diversity in several economically important crops including sweet potato. Most of the previous works done on diversity analysis in sweet potato using SSR markers showed high diversity in the population and also helped to identify the duplicates (Koussao *et al.* 2014 and Karuri *et al.* 2010). This made them the most apt tool for genetic diversity studies. Sweet potato is a hexaploid crop and, therefore produces different band patterns. In the present study also they produced highly polymorphic bands in a range of 2 to 13 alleles per locus with an average of 7.25 alleles per locus. This result confirms the extraordinary discriminatory capacity of SSR primers as reported in previous studies on sweet potato accessions by Gichuri *et al.* 2006.Tumwegamire *et al.* (2011) analyzed 92 African sweet potato accessions with 26 SSR markers and found a mean value of 6.1 alleles per locus ranging from 2 to 11. Similarly, Gwandu *et al.* (2012) analyzed 57 sweet potato genotypes in Tanzania with 4 SSRs and found higher number of alleles, fluctuating from 11 to 22.Rodriguez-Bonilla*et al.* (2015) analysed 137 Puerto Rico sweet potato cultivars with 23 SSR primers and found 4 to 25 alleles per locus with a mean value of 11.08 alleles per locus. However, Hwang *et al.* (2002)

obtained only few polymorphic bands in sweet potato accessions that ranging 1 to 4 alleles per locus. This study showed 94.5% average polymorphism with 84 polymorphic loci. Considering the average value of polymorphic loci we can conclude that there is high diversity exists within the cultivars, within wild relatives and between cultivars and wild relatives. One possible reason for the high degree of variation among the genotypes of accessions may be related to the mating system of sweet potato; a cross pollinating and hexaploid species (Ozias-Akins and Jarret, 1994).

All the cultivars and wild genotypes showed some common bands on amplification using primers IB SSR05 and GDAS0809, these three SSR loci were recorded as species specific as they occurred in all selected accession. These may be developed into species specific probes in future for identification purposes. There were no common bands for all wild relatives. In almost all primers *I. carnea* species with different flower colour (S24, S25 and S26) showed unique banding pattern. Similarly, in the case of *I. quamoclt* species with different flower colour (S38 and S39). But the morphologically similar *I. capitellata* and *I. pes-tigridis* differing in leaf shape showed similarities in GDAS0047,GDAS0049,IB 3/31 and IBSSR12 primer profiles but showed different banding pattern in all other primers. *I. triloba* showed similar banding pattern to the cultivar banding pattern, where as *I. trifida* rarely showed same banding pattern with cultivars, the morphology of *I. trifida* which was taken for study was also do not show much similarity with the cultivars instead they showed high phenotypic similarity towards *I. nil*. *Merremia dissecta* was produce entirely different banding pattern than rest of the accessions. In most of the primers, the wild relatives showed highly divergent banding pattern than that shown by cultivar accessions. Rare alleles were observed in several genotypes probably due to the high rate of mutation in SSR loci (Henderson and Petes, 1992).

The similarity matrix constructed for all the possible pair-wise comparison value showed a wide range from 0.37 to 0.96. The lowest similarity index was

obtained for the pair-wise comparison of *M. dissecta* and cultivar Sankar. Similarly, the highest value similarity index was showed by *I. carnea* species with pink colour flower collected from Kerala and Arunachal Pradesh. The maximum value of similarity index indicated that there were no duplicate accession present in the study. If there were any duplicates, the pair- wise similarity coefficient value becomes 1.00 (Veasey *et al.* 2007) which was not observed in the present study. From the similarity matrix, the *I. trifida* showed the maximum similarity index of 0.89 with *I. purpurea* and showed less similarity with the cultivars. Cultivar S8 and S17, *I. carnea* species with different flower colour of pink and light pink, showed high pair wise similarity index of 0.95. Different flower coloured *I. quamoclit* showed a pair wise similarity index value of 0.94. Lowest similarity index value of 0.37 and 0.4 was shown between *M. dissecta*, S18 and *I. eriocarpa*, *I. obscura* respectively. The bell shaped similarity frequency graph shows a high degree of polymorphism within the accessions. The range of similarity matrix value 0.37 to 0.95 also showed a high range of polymorphism that was comparable to many previous works in sweet potato germplasm studies using SSR markers, where the coefficient of similarity ranged from 0.4 to 0.938 in Brazil (Veasy *et al.* 2008) and in China (Li *et al.* 2009).

Based on the SSR markers, a high level of genetic variation was observed among 45 accessions as exhibited in the dendrogram and that was comparable to the Rout *et al.* (2010) studies on 21 accessions of North Eastern Indian *Ipomoea* species and few cultivars using ISSR markers. Considering a cutoff point at a distance of 0.70 in the dendrogram, three clusters were found. This result allowed us to distinguish the *I.obscura* and*M. Dissecta* species accessions of C group as a divergent cluster. The *M.dissecta* showed high divergence from all other accessions. These results are supported by the previous work on phylogenetic analysis of *Ipomoea* species of America based on floral morphology by McDonald *et al.*, 2011, where *M. dissecta* showed high divergence from *Ipomoea* species and this result also supported the present taxonamical grouping of this species based on the pollengrain structure. The

high dissimilarity of *I.* obscura obtained in this study is comparable to the previous work of Rout *et al.* (2010), where the ISSR analysis showed a high divergence of *I. obscura* from the cultivar as well as wild genotypes. This divergence was also supported by *Ipomoea* species seed protein analysis (Pragati *et al.*, 2013) results where it produced more number of seed protein profile than other species. This result opposed the previous studies by Folorunso (2013), where *I. obscura* and cultivar of sweet potato were placed in a group as glandular trichomes based on the foliar micro morphological characters. The first cluster A, consisted of cultivars that showed a high degree of divergence from all other wild species.

This SSR marker study showed that there was high genetic similarity between the 15 cultivar genotypes compared to other 5 cultivar genotypes and wild species in other cluster. This indicated the morphological similarity of cultivars included in the first cluster. This was comparable to previous studies on sweet potato accessions using ISSR and RAPD (Moulin *et al*, 2012). Koussao *et al.*, 2014 studies using SSRs showed distinct cluster for cultivars with less separation in the cluster. Cluster B included both the cultivars and wild genotypes. The remaining five cultivars were included in the sub cluster of the second cluster along with the wild species. Rout *et al.* (2010) also got a similar pattern of clustering while characterizing the wild relatives and cultivars using ISSR marker.

He *et al.* (2006) indicated that the high level of genetic diversity found in sweet potato accessions could be due to spontaneous mutations, which are quite common in this species, along with selection and geographic and environmental factors, which makes populations of this species an important genetic resource. The first sub cluster showed a cluster of 5 cultivars along with 8 wild species, including *I. triloba* and *I. trifida*, which were considered as the putative parents of cultivated sweet potato (Austin, 1987). And this result supports the allopolyploid origin of sweet potato. This close relationship of *I. triloba* and *I. trifida* with cultivars is comparable to all the previous works which showed high relationship between these species and

cultivars (Roullier *et al.*, 2013a, McDonald *et al.*, 2011 and Hu *et al.*, 2003). Among the 8 wild species, *I. triloba* exhibited more similarity with cultivars by grouping along with the cultivars. This data highlighted the postulate of Austin (1987) based on the morphological study that natural hybridization between *I. trifida* and *I. triloba* leads to the generation of wild ancestor of sweet potato cultivar. Recent studies by Roullier *et al.* (2013a) made a conflict with Austin (1987) where ITS sequence did not show any kind of common sequence between *I. triloba* and *I.* trifida while SSR marker clustering placed the cultivar and these species in separate clusters. The results of current investigation is supported by foliar morphological studies of McDonald *et al.* (2011), where the cultivars showed more similarity towards *I. triloba* than that with *I. trifida.* On further sub-clustering the *I. trifida* was placed along with *I. nil and I. purpurea.* These also showed high morphological similarity of hairy stem, hairy leaf surface and attractive flowers but this result was against the foliar morphological studies by McDonald *et al.* (2011), where *I.* nil and *I. purpurea* were placed far away from cultivar and *I. trifida.*

The *I. nil* and *I. trifida* flowers were blue white in colour. All the *I. carnea* species also showed high degree of similarity with each other. *M. vitifolia* showed negligible divergence with the cultivar and wild *Ipomoea* species than with *M. dissecta* species. This result suggests that *M. vitifolia* is more suited to be included in the *Ipomoea* genus than Merremia genus, and this result is against the current classification of *Ipomoea* and *Merremia* based on the pollen grain structure and shape. The third sub cluster of cluster B accomodated *I. quamoclit* species. On the fourth sub cluster *I. capitellata* and *I. pes-tigridis* showed high similarity coefficient but these differed only with respect to leaf lobes. On the sixth sub cluster *I. alba* and *I. turbinata* were placed together. They showed high morphological similarity even in the blooming of flower that occurs after sunset. The member of seventh cluster also showed high morphological similarity with the sixth subcluster in the shape of the flower. These result supported the current classification of *Ipomoea* species.

Eleven out of the 12 SSR markers revealed a high discriminatory power (PIC value of greater than 0.5) and hence were highly informative. The low PIC value of 0.27 for IB SSR05 was due its highly monomorphic nature of the primer. Higher PIC value of primer GDAAS0047 and average PIC value were comparable to 30 SSR markers used for the sweet potato germplasm characterization (Koussao, 2014). The average PIC value of 12 SSR markers had also supported the high power of polymorphism. Higher value of Heterozygosity of primers also showed the higher level of heterozygosity. High level of observed heterozygosity of primers, cultivar genotypes and wild genotypes could be attributed to the out breeding nature of sweet potato, where the proportion of heterozygous loci is likely to be high. It has also been reported that self- incompatibility in the flowers results in allogamy, increasing genetic heterozygosity (Thompson *et al.*, 1997).

Two dimensional and three dimentional PCA scatter plot gave high differentiation of 20 cultivars and 25 wild genotypes. In 2D PCA scatter plot all the cultivars were clustered together as far from the two axes. All the wild species were clustered into two clusters near the first dimensional axis. *M. dissecta* was placed on the second dimensional axis, show high genetic distance from the other cultivars and wild relatives similar to three dimensional PCA scatter plot. This result is supported by the previous studies of Tairo *et al.* (2008) where PCA was used to study the genetic variation among Tanzanian landraces. Similarly Waluyo *et al.* (2011) showed variability among the sweet potato crop wild relatives on morphological based PCA analysis.

These results revealed many intra and inter-relationship between cultivars and wild genotypes. The present study also established the efficacy of SSRs in differentiating the individual genotypes than morphological studies.

Summary

6. SUMMARY

Present study entitled "Molecular characterization of sweet potato (*Ipomoea batatas* (L.) Lam) accessions and wild relatives using SSR markers", was done with the objective to analyses the extent of genetic variation and phylogenetic relationship among selected accessions of sweet potato accessions and wild relatives at molecular level using SSR markers. The material consists of 20 sweet potato genotypes, 23 *Ipomoea* species and 2 *Merremia* species. The study was carried out on Central Tuber Research Institute (CTCRI), Thiruvananthapuram, Kerala.

The following are the summary of findings made on this study:

- Isolated DNAs from all the 45 genotypes were shown good quality with no RNA or protein contamination. Genetic polymorphism among the sweet potato cultivars and wild relatives were assessed using 12 SSR primers. All the primers used for the study was polymorphic in nature. All the primers except IB SSR 05 and GDAAS0809 had 100% polymorphism and a total number of 87 loci were amplified that exhibit 94.5% polymorphism that was helpful for the genetic diversity analysis. The number of polymorphic bands ranges from 2 to 13 and the product size was ranges from 105 to 393 bp.
- The similarity coefficient values of 45 genotypes ranged from 0.37- 0.96. Lowest similarity index (0.37) was observed between sweet potato variety Sankar and *Merremia dissecta*, the highest was observed between two *I. carnea* species. Most similarity coefficients were between 0.60 and 0.85.
- Dendrogram based on similarity coefficient values shows SSR primers clustered the 20 cultivars, 23 *Ipomoea* species and *Merremia* species into three major clusters (A, B, C)at 70 per cent similarity levels. Among the clusters, the number of accommodated genotypes varied from 2 to 28. The cluster A contains only cultivars, cluster B consists of both cultivar and wild species and the cluster C contains only wild species.

- In PCA scatter plot the 20 cultivars were clustered separately from the wild species. In the 2-D PCA scatter plot the cultivars were grouped together and wild species were grouped separately two clusters. In 3-D PCA plot the cultivars were grouped into two and wild species were also grouped together into one group.
- The PIC values for 12 SSR primers range from 0.26 to 0.85 with an average of 0.67. The lowest PIC value was showed by the primer IB SSR 05 and highest value by GDAAS0047. Similarly the Heterozygosity (He.) value ranges from 0.27 to 0.87 with same above primers. The average He value was 0.7.
- The result thus obtained showed a high genetic diversity within and between the cultivars and wild species under the molecular investigation by SSR markers. The wild relatives of sweet potato are appear to be potential source of untapped genetic resource for crop improvement.

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Appendices

8. APPENDIX

APPENDIX I

Reagents for DNA isolation

1. Extraction Buffer

Tris (pH 8.0)	-	100mM
EDTA (pH-8.0)	-	5Mm
NaCl	-	500mM
β-mercaptoethanol	-	2 % (v/v) freshly added prior to DNA extraction
PVP	-	2% (w/v) freshly added prior to DNA extraction

2.SDS -20%

3. Potassium Acetate -500mM

4.Resuspension Buffer / TE Buffer (1X)

Tris (pH-8.0) - 100mM

EDTA (pH-8.0) - 50mM

5.Sodium Acetate – 300mM

6. Chloroform isoamyl alcohol mixture (24:1)

7. Ethanol – 70 %

8.RNase

10 mg/ml (RNase A was dissolved in TE buffer and boiled for 15 minutes at 100°C to destroy DNase and stored at –20°C).

9. TE buffer (10X)

Tris- HCl (pH 8.0) - 10 mM

EDTA - 1 Mm

Final volume made upto 100ml with distilled water.

APPENDIX II

Solutions for Agarose gel electrophoresis

1. TBE Buffer (10X)

Tris base - 107 g

Boric acid - 55 g

0.5 M EDTA (pH 8.0) - 40 ml

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

2. Ethidium bromide

Weigh 10mg of ethidium bromide powder (sigma- aldrich) and dissolve in 1ml of distilled water.

3. Loading dye

Formamide – 50ml

Xyline cyanol – 50mg

Bromophenol blue – 50mg

EDTA(0.5M) – 1 ml

APPENDIX III

Reagents and solutions for Poly Acrylamide Gel Electrophoresis

1. Bind saline

Absolute ethanol (99.5%) – 497.5ml

Acetic acid (0.5%) – 2.5 ml

Bind saline () - 1 µl

2. 40% acrylamide

Acrylamide -38g

Bis – acrylamide -2g

Final volume made upto 100ml using distilled water.

3. Ammonium Persulfate

0.25g APS was weighed in foil covered eppendorff tube and 1 ml distilled water was added. Vortexed well.

4. 6% Polyacrylamide gel containing 7 M urea

42g urea was dissolved in a beaker containing 10 ml TBE buffer (10X) and 15 ml distilled water by heating in a microwave oven for 30-40s. 15 ml acrylamide solution (19:1) was filtered and added to a measuring cylinder followed by the melted urea solution. The final volume was made up to 100 ml using distilled water and stored in dark till use. 60 µl TEMED (1:10) and 600 µl APS (100 mg/ml) was added and mixed just before casting the gel.

5. Empty well dye

Loading dye (Appendix III.4) - 50µl

Sterile distilled water - 50µl

6. 100bp marker

100bp marker - 5µl

Loading dye - 40µl

Sterile distilled water - 55µl

Final volume made up to 100µl.

7.Fixer

Acetic acid	200 ml
Distilled water	1800 ml

8. Silver stain

2 g silver nitrate dissolved in distilled water to a final volume of 2000 ml and 3 ml formaldehyde added.

9. Developer

60g sodium carbonate was dissolved in distilled water to a final volume of 2000 ml and stored at -20°C. 3ml formaldehyde and 400µl sodium thiosulphate (10 mg/ml) was added and mixed thoroughly before use.

Abstract

MOLECULAR CHARACTERISATION OF SWEET POTATO (*Ipomoea batatas* (L.) Lam) ACCESSIONS AND WILD RELATIVES USING SSR MARKERS

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Abstract of the thesis
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9. ABSTRACT

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the major tuber crops in Asian countries including India, yet the interrelationship and genetic variability among the cultivar and its crop wild relatives remain unclear. The present study utilised 12 simple sequence repeats (SSR) marker for analyse the degree of genetic diversity and relationship within and between 20 sweet potato accessions, 23 wild *Ipomoea* species and two *Merremia* species.

The SSR primers produced 84 polymorphic alleles within the range of 105-393 bp. That showed an average polymorphism of 94.5%. The average PIC value of 0.67 showed that all the primers were polymorphic in nature. Similarity coefficient based dendrogram and PCA scatter plot clearly differentiated all the cultivars and wild species. This dendrogram helped to identify the close relationship of cultivars with *I. triloba* and *I. trifida* at a similarity index of 0.75.

The dendrogram based on the SSR molecular data shows high degree of genetic variability within and between hexaploid sweet potato and diploid wild species, whereas lower within the cultivars, which shows that there is a high level of genetic diversity exist within the *Ipomoea* species.

The results supported the current taxonomical position of *M. dissecta* as *Merremia* species whereas opposed the taxonomical position of *M. vitifolia* as *Merremia as* this study revealed more similarity to *Ipomoea* than *Merremia* species. The similarity coefficient varied from 0.37 to 0.96 indicating high variability in the genotypes that were included for the present investigation.